IRAMI ARAÚJO FILHO

BIODISTRIBUIÇÃO DO RADIOFÁRMACO PERTECNETATO DE SÓDIO APÓS CIRURGIA DO *SWITCH* DUODENAL.

Tese apresentada ao Programa de Pós-graduação em Ciências da Saúde, do Centro de Ciências da Saúde da Universidade Federal do Rio Grande do Norte, como requisito para obtenção do título de Doutor em Ciências da Saúde.

NATAL – RN
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Orientador: Prof. Dr. Aldo da Cunha Medeiros

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Prof.Dr. Aldo da Cunha Medeiros

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DEDICATÓRIA

À Amália, minha esposa, amiga fiel e verdadeiro amor, e aos meus filhos

Irami Neto e João, com carinho.

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SUMÁRIO

RESUMO

A cirurgia do desvio biliopancreático é uma das técnicas mistas utilizada no tratamento da obesidade mórbida. O *Switch* duodenal reduz o estômago a ¼ de sua capacidade e deixa apenas 50-100 cm de intestino delgado para nutrição e absorção. Isso leva a alterações estruturais, hormonais e bioquímicas que podem influenciar no resultado de exames cintilográficos de pacientes operados. Com o objetivo de avaliar a biodistribuição pós-operatória do pertecnetato de sódio ($Na^{99m}TcO_4$) em órgãos de diversos sistemas como cérebro, tireóide, coração, pulmão, fígado, baço, rim, estômago, duodeno, pâncreas, intestino delgado, bexiga, músculo e fêmur de ratos *Wistar* submetidos à técnica do *switch* duodenal, foram utilizados ratos com 3 meses de idade alocados aleatoriamente em: grupo tratado, submetido à cirurgia de desvio biliopancreático, técnica do *switch* duodenal (SD), o grupo controle (C) e o grupo submetido a uma simulação de cirurgia, denominado *sham* (S). No décimo dia de pós-operatório, 0,1 mL do pertecnetato de sódio ($Na^{99m}TcO_4$) foi injetado via plexo orbital (IV). Decorridos trinta minutos da administração do radiofármaco, os animais foram sacrificados e submetidos a toracolaparotomia mediana para retirada do fígado, baço, pâncreas, estômago, duodeno, intestino delgado, tireóide, pulmão, coração, rim, bexiga, músculo, fêmur e cérebro. A detecção da radioatividade foi feita com Contador Gama (Wizard, PerkinElmer - USA), automático. Calculou-se em seguida o percentual de atividade por grama de tecido (%ATI/g) do pertecnetato de sódio (Na^{99m} TcO_4) em cada órgão. Os valores da biodistribuição do pertecnetato no baço, pâncreas e músculo nos animais do grupo *switch* tiveram uma diferença significativamente maior do que

os dos grupos C e S ($p<0,05$). Na tireóide dos animais do grupo SD o pertecnetato teve menor captação em relação aos demais grupos, sendo estatisticamente significante quando comparada ao grupo S ($p<0,05$). A biodistribuição pulmonar nos animais do grupo SD só foi significativamente maior quando comparada a do grupo S ($p<0,05$). Não houve diferença significante, para os demais órgãos. Em conclusão, a técnica do desvio biliopancreático com *switch* duodenal alterou a biodistribuição pós-operatória do $Na^{99m}TcO_4$ em órgãos como tireóide, pulmão, baço, pâncreas e músculo de ratos operados, devido a prováveis alterações metabólicas e estruturais causadas por técnica cirúrgica mutilante. O trabalho teve um caráter multidisciplinar com a participação de pesquisadores de diferentes departamentos e laboratórios, como Núcleo de Cirurgia Experimental, Departamento de Cirurgia, Laboratório de Radiobiologia e o Serviço de Medicina Nuclear da Liga Norte-Riograndense contra o Câncer, atestando o caráter multidisciplinar da pesquisa.

Palavras chave: Desvio biliopancreático, *Switch* duodenal, Cirurgia bariátrica, Pertecnetato de sódio, Tecnécio, Biodistribuição, Pós-operatório, Ratos.

1. INTRODUÇÃO

A obesidade é considerada atualmente um problema de saúde pública. Estima-se que 1,1 bilhões de pessoas no mundo sejam obesas[1]. Caracteriza-se como obeso mórbido o paciente com Índice de Massa Corpórea (IMC = peso/altura²) maior que 40 ou que está 100 kg acima do peso ideal[2].

As conseqüências da obesidade incluem o diabetes *mellitus*, hipertensão arterial, acidente vascular cerebral, doenças articulares, apnéia do sono, asma, entre outras, os quais consomem altos custos em tratamento[3-7]. Condutas como mudanças de estilo de vida, assim como a terapia clínica têm sido desanimadoras na manutenção da perda ponderal devido a não aderência a programas dietéticos, exercícios físicos e efeitos colaterais de anorexígenos[8-10].

A Cirurgia Bariátrica, nas últimas quatro décadas, tornou-se eficaz no controle das co-morbidades, reduzindo o peso e a morbi-mortalidade[3]. Os procedimentos bariátricos são classificados em três grupos distintos: restritivo, disabsortivo e misto[6,10,11].

A banda gástrica horizontal, utilizada desde 1980, assim com a banda gástrica vertical são restritivas por criarem um pequeno reservatório gástrico de 10-15 ml com ajuda de um anel de silicone, reduzindo o volume do estômago, promovendo saciedade precoce [6,12]. Essas técnicas não mantêm perda de peso por longos períodos, sendo ineficazes nos superobesos (IMC>50) [10,13-15]. As técnicas disabsortiva e mista reduzem a capacidade gástrica e a absorção de nutrientes. No *bypass* gástrico é criado um pequeno reservatório na região da cárdia separado do restante do estômago por sutura, e neste anastomosado alça de jejuno de 100 cm com grampeador circular. Essa técnica é utilizada

como uma alternativa de re-intervenção nos pacientes com banda gástrica que não obtiveram bons resultados, ditos refratários[16,17]. A ocorrência de complicações diminui com a curva de aprendizagem dos cirurgiões[3]. Assim como o *bypass* gástrico em Y de Roux, a cirurgia de desvio biliopancreático foi utilizada por Hess no tratamento da obesidade mórbida, sendo atualmente conhecida como s*witch* duodenal[6]. O *switch* duodenal mantém 80% de perda ponderal a longo prazo, e está indicado em pacientes com IMC de 50 kg/m^2 [2,7,12]. Dificuldades técnicas são observadas, principalmente se realizada por vídeolaparoscopia[2,6]. Com a redução do estômago a ¼ de sua capacidade e apenas 50-100 cm de intestino delgado (canal comum) para absorção de nutrientes e minerais, relata-se a possibilidade de alterações estruturais, hormonais e bioquímicas, necessitando controle e reposição no pós-operatório[2,6]. Isso tem provocado a realização de pesquisas para identificar e quantificar tais mudanças[7,10,11,18].

A cintilografia com o emprego de radiofármacos é um dos meios mais utilizados no estudo de alterações orgânicas[19-23]. Desde a década de 60, o ^{99m}tecnécio vem sendo empregado em estudos na área biomédica devido a sua natureza física, química e econômica como um elemento biodistribuível, sem riscos de radiação excessiva[18,24]. Apesar de utilizado em ensaios clínicos e experimentais sobre agentes terapêuticos[25-32], função fagocitária[33,34], dentre outras, não há na literatura pesquisas que correlacionem sua atividade tecidual e a cirurgia bariátrica, como o desvio biliopancreático com *switch* duodenal.

Embasado no fato de ser uma operação mutilante, de importantes repercussões metabólicas, o primeiro trabalho anexado a essa tese, que serviu como um dos requisitos para a obtenção do grau de Doutor, teve como objetivo

avaliar a biodistribuição pós-operatória do pertecnetato de sódio ($Na^{99m}TcO_4$) em órgãos como cérebro, tireóide, coração, pulmão, fígado, baço, rim, estômago, duodeno, pâncreas, intestino delgado, bexiga, músculo e fêmur de ratos *Wistar* submetidos à técnica do desvio biliopancreático com *switch* duodenal, conhecida no meio cirúrgico como *switch* duodenal. Justifica-se a pesquisa partindo do princípio de que, uma vez ocorrendo alteração da biodistribuição desse radiofármaco em qualquer dos órgãos em estudo, os resultados podem levar à suspeita de que eventuais exames cintilográficos pós-operatórios podem produzir resultados falso-positivos ou falso-negativos, obrigando à repetição de exames e exposição de pacientes a radiações ionizantes.

Adicionalmente, outros 06 trabalhos (um clínico e seis experimentais) realizados no Hospital Universitário Onofre Lopes (UFRN) e no Núcleo de Cirurgia Experimental, estão anexados neste volume, publicados em periódicos com indexação internacional, fazendo parte da formação do autor como Doutor em Ciências da Saúde.

2. REVISÃO DA LITERATURA

O desvio biliopancreático com *switch* duodenal foi inicialmente realizado por Hess et al. (1998) no tratamento da obesidade mórbida[6]. Essa técnica incorpora dois procedimentos distintos: o desvio biliopancreático desenvolvido por Scopinaro[42] desde 1976 e aprimorada por DeMeester[2], quando idealizou o *switch* duodenal para redução de gastrite de refluxo alcalino após gastrectomias.

Consiste de uma gastrectomia longitudinal, abrangendo a grande curvatura, reduzindo o estômago em 75%, preservação do piloro e confecção de anastomose duodenoentérica (*switch*) com alça ileal após secção a 250 cm do ângulo de Treitz, terminando com uma anastomose íleoileal a 50 cm da válvula ileocecal, no homem. Este último segmento funciona como canal comum de nutrição e absorção.

Segundo Hess et al. (2005), a incorporação do *switch* duodenal ao desvio biliopancreático cria uma operação híbrida com benefícios restritivos e disabsortivos[36]. Isso faz dessa técnica uma das mais eficientes na perda de peso a longo prazo, variando entre 70-80% em algumas séries[2,8]. Estima-se que a técnica cirúrgica em questão possua uma mortalidade em torno de 0,5-1% e morbidez variável entre 10 – 20%, tendendo a cair com a curva de aprendizagem do cirurgião, após a realização de 200 procedimentos ou mais[37]. O *switch* duodenal é uma alternativa na falha de outros procedimentos bariátricos, quando por complicações cirúrgicas, há perda ponderal insatisfatória ou mesmo ganho de peso pós-operatório[38]. A gastrectomia vertical é responsável pela redução inicial do peso, uma vez que diminui o

volume do estômago. Com a retirada do fundo gástrico ocorre uma baixa nos níveis de grelina, hormônio orexígeno produzido nas células dessa região do estômago que é extirpada[39,40]. O desvio biliopancreático reduz a absorção de lipídios, proteínas e carboidratos, contribuindo para a manutenção do peso perdido a longo prazo. Além disso, diminui a resistência periférica à insulina. Portanto, há uma normalização da glicemia e dos níveis de colesterol, o que reflete sobre o controle de hipertensão arterial e diabetes mellitus associados à obesidade[41-43]. Silecchia et al. (2006) afirmam que mesmo realizado por videolaparoscopia, onde a dificuldade técnica é maior, obtem-se bons resultados no controle de co-morbidades em pacientes de alto risco[44,45].

Várias alterações metabólicas são atribuídas ao *switch* duodenal. As mudanças anatômicas resultantes da técnica provocam grande redução nas quantidades de nutrientes sistemicamente necessários ao paciente, acarretando deficiência energética, protéica e de minerais essenciais. Como conseqüência, a estrutura orgânica pós-operatória é modificada se não houver reposição e suplementação alimentar[46].

2.1 Deficiência protéico-calórica

Desnutrição protéico-calórica é a complicação mais séria da cirurgia bariátrica. Byrne estima uma prevalência entre 7-12% dentre os pacientes submetidos ao desvio biliopancreático[47]. Marceau et al. (2001) encontraram hipoalbuminemia em quase 20% dos casos[48]. Isso leva à diminuição da proteína visceral, atrofia muscular e edema, características da síndrome de Kwashiorkor. Devido a tais modificações, supõe-se que ocorram alterações em proteínas transportadoras assim como enzimas catalizadoras de reações[46].

A má absorção de gordura é o principal mecanismo resultante do desvio biliopancreático na redução do peso. Manifesta-se por esteatorréia pós-operatória e é devida ao pequeno comprimento do canal comum, cerca de 50cm[47]. Tem como desvantagem a redução na absorção de vitaminas lipossolúveis, importantes na homeostase[47].

2.2 Deficiência de Vitaminas lipossolúveis

2.2.1 Vitamina A

Alguns estudos têm demonstrado a deficiência de vitamina A após cirurgia bariátrica. Dolan et al. (2004) encontraram níveis reduzidos dessa vitamina em 61% dos pacientes submetidos ao switch duodenal[49]. Slater et al. (2004) encontraram uma deficiência similar de 69% em estudo multicêntrico[50]. A deficiência de vitamina A pode causar xeroftalmia, ceratose folicular, alopécia, além de cegueira noturna.

2.2.2 Vitamina E

A deficiência de vitamina E é fato incomum. Apesar de alguns estudos relatarem somente 5% de pacientes acometidos[49], em outros 96% dos pacientes tinham níveis normais desta vitamina anti-oxidante, embora a maioria estivesse em tratamento de reposição[50].

2.2.3 Vitamina K

Fenômenos hemorrágicos são incomuns em pacientes submetidos à cirurgia bariátrica. Estudos referem que mais de 50% dos pacientes operados sob a técnica do *switch* duodenal têm deficiência de vitamina k, sem manifestação clínica de sangramento[49,50].

2.2.4 Vitamina D e cálcio

O desvio biliopancreático é a operação bariátrica que mais altera a absorção de vitamina D e cálcio. Isso leva a um hiperparatireodismo secundário, redução de 25,$(OH)_2$D, osteomalácia e osteoporose. Tal fenômeno pode acometer até 60% dos pacientes operados[51]. Newbury et al. (2003) constataram que mesmo com suplementação vitamínica, 25,9% tinham hipocalcemia e 63,1% elevação do paratormônio após 32 meses de seguimento[52].

2.3 Deficiência de Vitamina B12 e Folato

Além das modificações anatômicas, uma secreção inadequada do fator intrínseco também contribui para a deficiência de vitamina B12. O fato deve-se à gastrectomia vertical realizada, o que reduz as células produtoras. As principais manifestações clínicas da deficiência de folato e vit. B12 são a anemia megaloblástica e as seqüelas neurológicas que incluem neuropatia periférica, parestesias e desmielinização do trato corticoespinhal[53].

2.4 Deficiência de Tiamina (Vitamina B_1)

A prevalência dessa hipovitaminose é baixa em decorrência da cirurgia bariátrica. Para Foster et al. (2005) ela pode se manifestar por polineuropatia, alteração do comportamento e miopatia[54]. Chang et al. (2004) encontraram somente 29 casos com deficiência em um total de 168.010 operados, e a principal manifestação foi a síndrome de Wernicke-Korsakoff que cursa com anormalidades oculomotoras, visuais, ataxia e confusão mental[55]. Outros ensaios enfatizam que o vômito pós-operatório seja o fator desencadeante[56,57].

2.5 Deficiência Mineral

2.5.1 Ferro

Os pacientes submetidos ao *switch* duodenal têm uma deficiência de ferro em torno de 20-49%[58]. Assim como o folato, a fisiopatologia desse fenômeno está relacionada ao desvio da secreção ácida do estômago e redução na superfície absortiva do duodeno e jejuno proximal. Embora a má absorção seja a principal razão, sangramento gastrointestinal assim como menstruação devem ser investigados na evolução pós-operatória[46]. Rabkin et al. (2004) relataram uma alta prevalência de anemia em mulheres após o desvio biliopancreático[59].

2.5.2 Magnésio

Marceau et al. (2002) não encontraram anormalidades significativas nos níveis de magnesemia no seguimento de 4 e 10 anos após o *switch* duodenal[60]. Entretanto, outros estudos revelaram 5% de hipomagnesemia, sem repercurssões clínicas[49].

2.5.3 Zinco

A alopécia é a principal manifestação clínica da deficiência de zinco pós-operatória. É um fenômeno comum e de patogênese controversa. Pode acometer até 36% dos pacientes, revertendo com a suplementação desse mineral[61]. Slater et al. (2004) afirmam que a hipozincemia pode acometer até 50% dos pacientes operados, estabilizando as alterações após 4 anos. Apesar de ter inúmeras funções na estrutura e imunologia orgânica, não tem havido manifestações clínicas significativas nos operados[50].

2.6 Cintilografia

A aplicabilidade da cintilografia é variada. Sua utilização nos diversos sistemas orgânicos pode sofrer influências diversas, que interferem no seu resultado. Exames cintilográficos pós-operatórios são realizados para diagnosticar sangramento digestivo oculto após transplante de pâncreas[62], refluxo gastresofágico após gastrectomia, trânsito intestinal assim como patência de anastomoses[63]. Leen (1999) refere-se à cintilografia como um método de alta sensibilidade e especificidade na pesquisa de metástases hepáticas de câncer de cólon[64]. A avaliação da função renal, hepática e pulmonar, após ressecções e transplantes, como também da função cardíaca após angioplastia, são outras aplicações desse exame[65-69]. A cintilografia é realizada por meio de gama câmera que capta energia de um determinado radionuclídeo na forma de fóton. Esse pulso luminoso é transformado em imagem de acordo com a distribuição do elemento radioativo no organismo[70].

Para ser utilizado em exame cintilográfico, o radiofármaco tem de emitir baixa radiação, toxicidade tolerável, ser estável e possuir meia vida suficiente para o preparo e realização do exame, sem causar maiores danos ou seqüelas[70].

2.7 ^{99m}tecnécio

O ^{99m}tecnécio é um dos radionuclídeos mais utilizados em medicina nuclear assim como em pesquisa básica. Ele preenche muitos dos critérios de um radionuclídeo ideal, possui baixa radiação, uma meia vida de 6h, sofrendo 10% de conversão interna, o que resulta em mínima dose para o paciente[71]. A forma química utilizada para leitura em gama câmera é o pertecnetato de sódio

($Na^{99m}TcO_4$) que necessita de redução iônica para a marcação de substâncias, células ou órgãos em estudo. Para tal processo a substância mais utilizada é o cloreto estanoso. Apesar disso, o $Na^{99m}TcO_4$ pode entrar ou sair do meio intracelular livremente, num processo de difusão passiva[71]. Há vários estudos sobre a interferência de drogas quimioterápicas[25-27], anestésicos[72], fitoterápicos e outras substâncias na biodistribuição do $Na^{99m}TcO_4$ em células sanguíneas e tecidos orgânicos. A terapia com drogas ou extratos obtidos de plantas medicinais pode alterar a marcação de células sangüíneas e resultar em efeitos inesperados[29-32].

Os dados da literatura demonstram que, apesar de utilizado em exames cintilográficos pós-operatórios, não se conhece a influência que a cirurgia bariátrica pode ter na atividade do $Na^{99m}TcO_4$ como radiofármaco. No presente trabalho procurou-se estudar, em modelo animal, a interferência da cirurgia do desvio biliopancreático com *switch* duondenal na biodistribuição do $Na^{99m}TcO_4$.

3. ANEXAÇÃO DOS ARTIGOS

3.1 ARTIGO I

Aceito para publicação no periódico

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(Qualis Internacional B)

Biodistribution of the Radiophamarceutical Sodium Pertechnetate after Biliopancreatic Bypass with a Duodenal Switch.

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ABSTRACT

Study with the purpose to examine the effects of duodenal switch (DS), regularly performed in morbidly obese patients, on biodistribution of sodium pertechnetate in several organs of rats. There was no early or late mortality in either rats groups. The values of percent radioactivity per gram of tissue (%ATI/g), showed no significant difference in liver, stomach, small bowel, duodenum, kidney, heart, bladder, bone and brain, when compared the DS rats with sham and controls rats. A postoperative significant increase in mean %ATI/g levels was observed in spleen, pancreas and muscle in group DS rats, as compared to group S and C rats ($p<0.05$). In the lung there was an increase and in thyroid a decrease in mean %ATI/g of DS rats, when compared to sham rats ($p>0.05$). In conclusion, the biliopancreatic diversion with duodenal switch in rats modified the biodistribution of sodium pertechnetate in tireóide, lung, pancreas, spleen and muscle.

Key words: Bariatric surgery, duodenal switch, sodium pertechnetate, biodistribution

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INTRODUCTION

Worldwide, it is estimated that more than 300 million people are obese (Haslam & James., 2005). Obesity, particularly abdominal obesity, is associated with increased risks of hypertension, diabetes, hyperlipidemia, sleep apnea, coronary heart disease, and stroke (Li et al., 2005). The accumulating evidence identifying obesity-related mortality and comorbidities is an important factor that has led to increased numbers of patients seeking treatment through bariatric surgery. This is a surgical procedure that reduces caloric intake by modifying the anatomy of the gastrointestinal tract and provides effective treatment for many patients with morbid obesity. Bariatric operations are classified as either restrictive or malabsorptive. Restrictive procedures limit intake by creating a small gastric reservoir with a narrow outlet to delay emptying. Malabsorptive procedures bypass varying portions of the small intestine where nutrient absorption occurs (DeMaria & Jamal., 2005). The biliopancreatic diversion with duodenal switch (DS) is a hybrid operation involving both components of weight loss surgery. In the DS, a lateral gastrectomy provides a restricted gastric volume, while excess fat absorption is limited by shortening the functioning length of the intestine. This involves diversion of the biliopancreatic secretions by partitioning the bowel into two limbs – an alimentary channel,

and the biliopancreatic (afferent) limb. These two limbs of small bowel are reconnected to form the common channel (Hess., 1998; Marceau et al., 1998). DS produces a sustained weight loss, with low side effects and without any increase in the perioperative morbidity and mortality rate, comparing to other bariatric operations (Biron et al., 2004; Rabkin., 2004; Anthone et al., 2003).

There are numerous complications that may arise following any of the bariatric surgical procedures that require understanding and delineation of the specific anatomy of the operation performed. These complications may include nutrient deficiencies or gastrointestinal pathology. Anastomotic leak and stricture commonly occurs (Schauer et al., 2000; DeMaria et al., 2002). The most frequently reported complication of gastric band placement is prolapse of stomach superiorly through the band producing obstruction at the band (O'Brien et al., 1999). Radiographs and scintigraphs may show an air fluid level in the gastric pouch, malposition, angulation of the band bands, problems with gastric emptying, and gastric obstruction (O'Brien et al., 2005).

The radiopharmaceuticals are frequently used in diagnostic procedures. Examinations of gastric emptying, patency of anastomoses, enterogastric reflux, hepatic and thyroid diseases, osteoporosis, metastasis, etc, frequently are carried after bariatric surgery (Kitabaiashi et al., 2002; Fonseca et al., 2000; Badiali et al., 2001; Obradovic et al., 2000). Several works have studied the relationship between chemotherapy, phytotherapy and other drugs with the biodistribution of sodium pertechnetate (Braga et al., 2000; Oliveira et al., 2002; Gomes et al., 1998; Ripoll-Hamer et al, 1995; Simões et al., 1997; Feliciano et al., 2002., Abreu et al., 2006., Santos et al., 1995). With regard to potential consequences of bariatric surgery in the biodistribution of radiopharmaceuticals, little or no study was published until now.

As DS is a restrictive and malabsorptive operation, of raised anatomical and metabolic repercussion, postoperative evaluation of patients through scintigraphy can be necessary. If the biodistribution of sodium pertechnetate to organs and tissues is modified as a result of bariatric surgery, scintigraphic examinations can be false-positive or false-negative, resulting in repetition of examinations with unnecessary exposition of patient to ionizing radiations. The purpose of this study was to examine the effects of DS, similar to that performed in morbidly obese patients, on biodistribution of sodium pertechnetate in several organs and tissues of rats.

MATERIAL AND METHODS

Male Wistar rats who were 12 weeks of age and weighing 328g ± 33g were obtained from Center of Experimental Surgery-UFRN, Brazil. Animals were housed in polypropilene cages for 1 week to acclimatize them to the study laboratory: 12-h light/dark cycle, room temperature of 25°C, and 50% relative humidity. Rats were allowed free access to water and standard rat chow (Labina, Purina®). The study was approved by the Institutional Animal Care Committee of the University Hospital-UFRN, Brazil and the international guidelines for the care and use of laboratory animals were followed throughout the study.

Rats were randomly divided into three groups: duodenal switch group (DS), control group (C), and sham-operated group (S). After 12 h of food deprivation, rats were anesthetized with a ketamine and xylazine mixture (200 mg: 5 mg, 0.1 ml/100 g, IM), the abdomen was shaved and prepared, and the operations were performed with aseptic technique. All the surgical procedures were performed by the same investigator, a well trained and experienced surgeon in animal surgery and three previous series of experiments were performed in sequence to develop the DS model. A single intramuscular dose of 75 mg/kg of ceftriaxone sodium (Roche, SP,Brazil) was given as antimicrobial prophylaxis 30 minutes before the surgical procedures.

In the DS rats (n=7) the surgery was performed via an upper 3cm midline incision. A sleeve 75% longitudinal gastrectomy was performed leaving a tabularized stomach. (Figure. 1). The duodenum was divided about 1 cm beyond the pylorus. The stump of

duodenum was closed with running sutures. The small bowel was divided at its midpoint, and the distal end (alimentary limb) was anastomosed to the proximal duodenum. The proximal end of the divided small bowel, now the distal end of the iliopancreatic limb, was anastomosed to the ileum 5cm from the ileocecal valve to create a 10cm common channel. (Figure. 1). The anastomosis were hand sewn using interrupted polypropilene 6-0 sutures (Prolene® - Ethicon), using a surgical microscope (DFV- M900, São Paulo, Brazil). The hydration was done with normal saline (10 ml) injected subcutaneously into the back of the rats for the first 2 postoperative days. Postoperatively pain was treated with tenoxicam (Roche Pharm., Brazil); 0.5 mg/kg was given im to the rats once a day for 3 days.

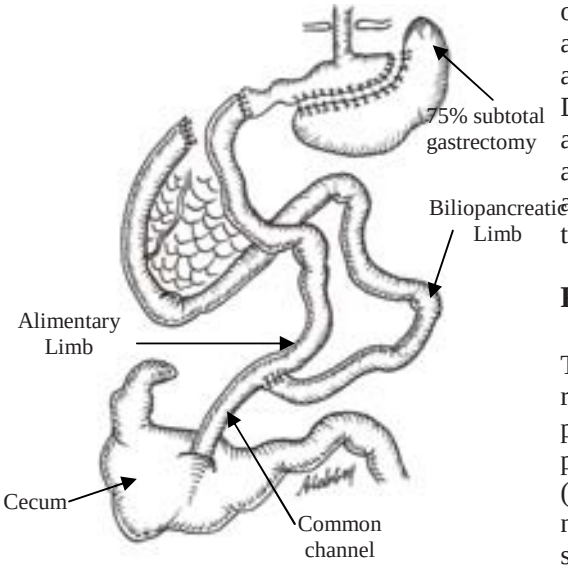


Figure 1 - The biliopancreatic diversion with duodenal switch operation.

Rats were allowed to drink and eat 24 h after surgery. Liquid diet (Nestogeno, Nestlé, SP-Brazil, 1 cal/g) was provided for the first 2 days, followed by ground Purina Labina chow. The sham rats (n=7) were submited to a laparotomy and soft malipulation of stomach, duodenum and small bowel. The control rats (n=7) were not operated. Body weight, using a digital scale (Filizola®, São Paulo, Brasil), and operative complications were evaluated daily for 10 days.

On the 10th day all the animals were anaesthetized again, and injected with 0.lmL of $Na^{99m}TcO_4$ IV in the orbital plexus, corresponding to radioactive activity of 0.66MBq. After 30 minutes, the animals were killed by lethal dose of anesthetic. Samples of the liver, spleen, stomach, small intestine, duodenum, pancreas, kidney, heart, lung, thyroid, bladder, muscle, bone (femur) and brain were harvested. The samples were washed in 0.9% saline , weighed on a high-precision digital scale (Bel-Mark 160-II Itália®) and subjected to radioactivity detection using a 1470 Wizard™ Gamma Counter- Perkin-Elmer, with automatic correction of radiation decline. The percentage of radioactive activity/g (%ATI/g) of each organ was calculated by dividing the activity/g of the tissue by the total activity administered to each animal.

Data are expressed as mean ±SD. Statistical analysis was performed using one-way analysis of variance and the Tukey test as appropriate. *P* values <0.05 were considered to be statistically significant.

RESULTS

There was no early or late mortality in either rats groups. The values of all sodium pertechnetate biodistribution, expressed as percent radioactivity per gram of tissue (%ATI/g), are shown in Table 1. There was no significant difference in %ATI/g in liver, stomach, small bowel, duodenum, kidney, heart, bladder, bone and brain, when compared the DS rats with S and C rats. A postoperative significant increase in mean %ATI/g levels was observed in spleen, pancreas and muscle in group DS rats, as compared to group S and C rats ($p<0.05$). Looking at each group separately, in the lung there was an increase and in thyroid a decrease in mean %ATI/g of DS rats, when compared to sham rats ($p>0.05$).

Table 1 - Results of the percentage of radioactivity/g of organs and tissues(%ATI/g).

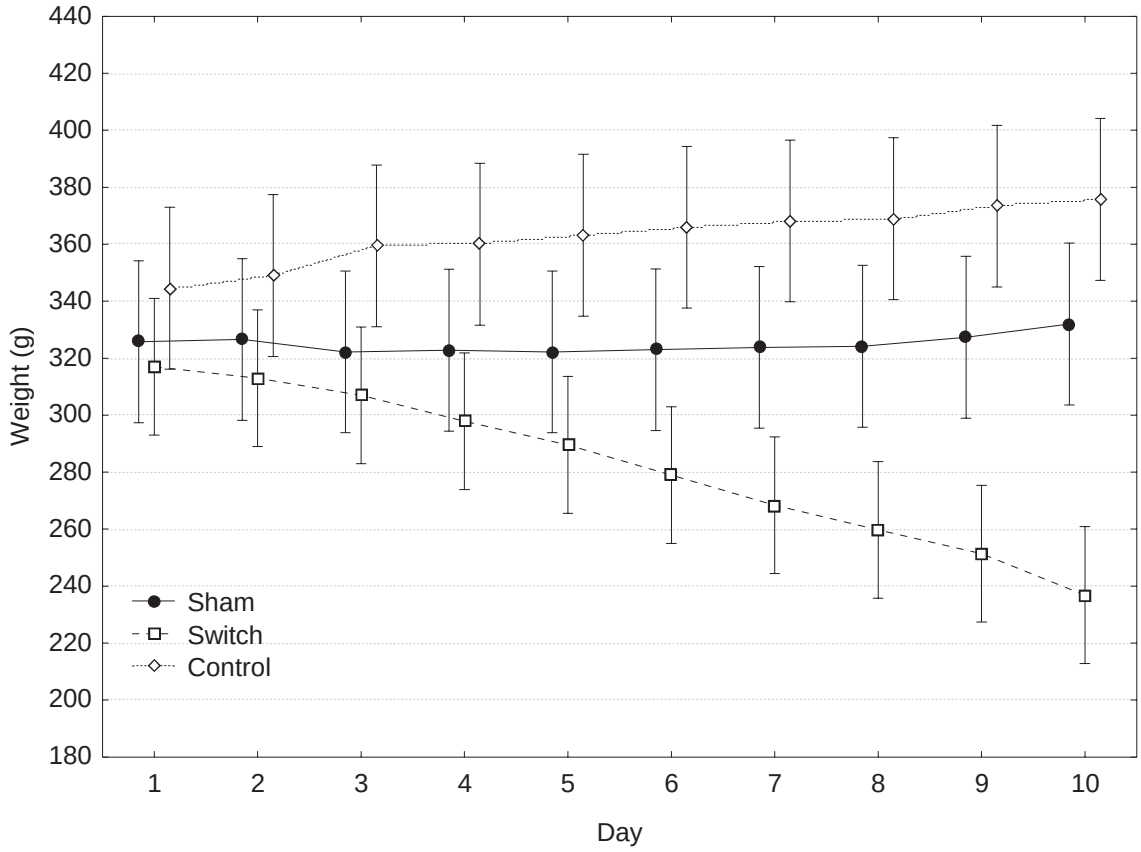
Organs	*%ATI/g*			
	Switch	C	*Sham*	*P*[(1)]
Liver	0.42 ± 0.103	0.35 ± 0.069	0.35 ± 0.097	0.256
Spleen[(2)]	0.26 ± 0.079[ab]	0.18 ± 0.028[a]	0.17 ± 0.045[b]	0.016
Stomach	3.39 ± 1.321	2.98 ± 1.507	4.23 ± 0.941	0.205
Small bowel	0.19 ± 0.052	0.19 ± 0.052	0.26 ± 0.109	0.209
Duodenum	0.65 ± 0.541	0.44 ± 0.088	0.61 ± 0.484	0.633
Pancreas[(2)]	0.27 ± 0.089[ab]	0.14 ± 0.045[a]	0.15 ± 0.078[b]	0.006
Kidney	0.53 ± 0.157	0.42 ± 0.075	0.36 ± 0.174	0.115
Heart	0.28 ± 0.131	0.27 ± 0.061	0.16 ± 0.079	0.050
Lung[(2)]	0.44 ± 0.075[a]	0.39 ± 0.143	0.28 ± 0.075[a]	0.030
Thyroid[(2)]	1.48 ± 2.070[a]	3.60 ± 1.578	4.44 ± 1.143[a]	0.010
Bladder	0.30 ± 0.115	0.33 ± 0.094	0.32 ± 0.173	0.929
Muscle[(2)]	0.09 ± 0.028[ab]	0.06 ± 0.018[a]	0.05 ± 0.026[b]	0.008
Bone	0.14 ± 0.032	0.14 ± 0.032	0.14 ± 0.050	0.958
Brain	0.03 ± 0.033	0.01 ± 0.003	0.01 ± 0.007	0.244

Mean ± standard deviation

1. P-value.
2. Groups identified by the same letter differ significantly. Significance 0.05 using the Tukey test.

Preoperative and postoperative data of weight loss of DS rats are summarized in Figure 2. Before treatments, there were no significant differences between groups in terms of weight. The operative time for the sham operation was equivalent to that of DS. Postoperatively in group DS there was a significant decrease in body weight during the 10 days of observation attributed to the effects of operation ($p<0.05$). Body weight in the C and S groups gradually increased by day 10, when the rats were euthanized (Figure 2). So, the differences in the mean weight of DS rats at the end of the 10 days were significant, when compared to C and sham rats ($p<0.05$).

Figure 2 - Effect of duodenal switch, sham and control on body weight in rats during the observation period.



Body weight decreased significantly in the duodenal switch group vs the sham and control, $P<0.05$. No difference was observed between sham and control rats.
In day 1 (operative day) no difference was observed among all the rats weights, meaning that the groups were uniform.

DISCUSSION

Obesity has become an epidemic condition and in the United States, the percentage of adults who are obese increased from 15.3% in 1995 to 23.9% in 2005. Approximately 4.8% are considered to be extremely or morbidly obese. Worldwide, it is estimated that more than 300 million people are obese (Ogden et al., 2006; Haslam & James., 2005).
Obesity results in a major risk for serious diseases, including diabetes mellitus, cardiovascular disease, hypertension, dyslipidemia, degenerative arthritis, certain forms of cancer and respiratory problems and may result in socioeconomic and psychosocial impairment (Ali et al., 2006). Therefore many weightlowering therapies, such as dietary and pharmaceutical regimens completed with physical exercise, have been proposed. However, almost 95% of morbidly obese (BMI ≥40 kg/m2) patients fail to achieve acceptable long-term weight loss with any form of non-operative treatment Ali et al., 2006). Hence, great efforts have been made to achieve better results using surgery. The bariatric operations have proved very successful and cost effective in achieving marked and maintained weight loss. In spite of good results, bariatric surgery may cause anatomic and metabolic complications (DeMaria et al., 2002). The diagnostic of these side effects may require image exams such as radiography and scintigraphy.
To understand and explore the relationship between bariatric surgery and biodistribution

of radiopharmaceuticals to organs and tissues, a well-characterized and reproducible animal model was used. To achieve this purpose, this is a report of the experience with a biliopancreatic diversion with duodenal switch rat model.

Technetium- ^{m}99 (^{m}99Tc) is the most used radioisotope in nuclear medicine as well as in basic research. It has a low mean life (6 h), low radiation and a low doses is needed for diagnostic procedures (Braga et al., 2006). It has been used in vivo and in vitro under the form of sodium pertechnetate, in the study of diseases, drugs, chemotherapics and phytoterpics that interfere in its biodistribution (Oliveira et al, 2002; Amorim et al., 2003). Pertechnetate labelled red blood cells and leukocytes have been used in the study of drugs, and in the evaluation of the mononuclear system (Palestro et al., 2006).

Postoperative scintigraphic exams are accomplished to diagnose digestive bleeding (Bingener-Casey et al., 2002), gastroesofageal reflux (Adachi et al., 1999), as well as anastomoses patency (Blachar., 2004). Diagnosis of cancer metastasis (Leen., 1999), and postoperative chances in kidney, liver, lung, heart and other organs are done using scintigraphy with radiotracers and pertechnetate (Aktas et al., 2005; Chalela., 1999).

In the present work the DS did not affect the biodistribution of sodium pertechnetate in liver, stomach, small bowel, duodenum, kidney, heart, bladder, bone and brain. The stomach is commonly examined in the postoperative of bariatric surgery to diagnose mainly leaks, because leakage from the reservoir or the connecting tube is a late complication of bariatric surgery. In small leaks the escape rate of a radio-contrast agent may be low, and hence these leaks may be overlooked on radiography. By contrast, using scintigraphy, the slowly diffusing 99^{m}Tc-pertechnetate is re-absorbed by peritoneal blood vessels and subsequently absorbed into the gastric mucosa, because of high gastric affinity for pertechnetate. This fact may explain because, in this study, biodistribution of pertechnetate was not affected in stomach. It has been suggested a higher accuracy of scintigraphy as compared with radiography in the assessment of leakage in bariatric surgery. (Van DenBossche et al., 2002). The liver, small bowel, duodenum, kidney, heart, bladder, bone and brain have a few affinity for pertechnetate and we did not find alteration in biodistribution of this radiopharmaceutical in them. So, if a scintigraphy is to be done to study anyone after bariatric surgery, false results certainly are not expected.

The biodistribution of pertechnetate showed elevated in spleen and this organ may be the target of future scintigraphs. Splenectomy during exploratory laparotomy after bariatric surgery significantly increases morbidity and mortality. Peters et al, (1990) related that six of 200 patients having primary or revisional vertical banded gastroplasty for morbid obesity or failure of previous bariatric surgery had splenic injury. Eventual scintigraphy in these patients should be interpreted with caution.

Obese patients often complain of dyspnea despite not having demonstrable lung disease It has been hypothesized that increased chest wall mass along with increased abdominal size imparts a restrictive ventilatory defect, which then imposes an increased work of breathing (Sahebjani, 1996). The bariatric procedures are performed in morbidly obese patients who tend to have reduced chest wall compliance, reduced lung volume, less functional residual capacity, and increased physiologic intrapulmonary shunt during mechanical ventilation (Damia et al., 1988). Therefore, morbidly obese patients may be at risk for intraoperative and postoperative complications, which may be diagnosed by scintigraphy. Together, pulmonary emboli, anastomotic leaks, and respiratory failure account for 80 percent of all deaths in the first 30 days following bariatric surgery (Virji & Murr, 2006). As in this study the biodistribution of pertechnetate was higher in lungs of operated rats then in controls, the interpretation of eventual lung exams (Giordano et al., 1997) in patients has to be with caution.

In the past five years, several confirmed cases of pancreatic disorders occurred in persons who had undergone bariatric surgery (Service et al., 2005). Some patients presented with repeated episodes of symptoms of profound postprandial neuroglycopenia associated with endogenous hyperinsulinemic hypoglycemia and demonstration of diffuse beta-cell hypertrophy and hyperplasia in resected

pancreatic tissue. A plausible explanation, with broader implications, is that bariatric surgery results in long-term stimulation of beta-cell growth and activity by gut hormones (glucagon-like peptide 1) that are perturbed as a result of the altered gastrointestinal transit. These metabolic disorders may explain the high radioactivity observed in pancreas of DS operated rats (D'Alessio & Vahl, 2004). Moreover, at least in rodents, GLP-1 triggers beta-cell neogenesis and proliferation while inhibiting apoptosis (Brubaker & Drucker., 2004).

In this study the %ATI/g in thyroid was decreased in DS rats compared to sham rats. Paradoxically, a great percentage of obese patients have hypothyroidism, that is improved after bariatric surgery (Raftopoulos et al., 2004). The reduction in the %ATI/g in thyroid can be related to the postoperative energy deficiency, knowing that half of the small bowel is defunccionalyzed and the stomach is highly reduced. Steps et al had attributed this phenomenon to a reduction of the transport of sodium pertechnetate to the thyroid gland, as happen with iodine in malnourished patients (Passos et al., 2000; Passos et al., 2002). Further studies are necessary to explain these findings.

Bariatric surgery has become the treatment of choice for morbid obesity and it greatly changes the body composition for years following surgery. The lean body mass (muscle) is significantly reduced in the postoperative period (Tanner et al., 2002). In this study we found a paradoxical relationship between weight loss, muscle loss, and increased muscle %ATI/g. So that, questions remain with regard to the physiological mechanisms and pathophysiological consequences of duodenal switch, and biodistribution of radiopharmaceuticals is one of them. It has been related that the polyneuropathy and miopathy after bariatric surgery are resultant of the deficiency of B12 vitamin, tiamin and vitamin D respectively (Koffman et al., 2006). The highly significant muscular captation of sodium pertechnetate in DS rats may be a consequence of muscle inflammation for vitamin D deficit (Plotnikoff & Quigley., 2003).

In conclusion, the data of this study permits the conclusion that the biliopancreatic diversion with duodenal switch in rats modified the biodistribution of sodium pertechnetate in tireóide, lung, pancreas, spleen and muscle.

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RESUMO

Estudo objetivando de examinar efeitos da operação switch duodenal (SD) na biodistribuição do pertecnetato de sódio em vários órgãos de ratos. Não ocorreu mortalidade precoce e tardia nos animais operados. Os valores do percentual de radioatividade/grama de tecido (%ATI/g) mostraram nenhuma diferença significante no fígado, estômago, intestino, duodeno, rim, coração, bexiga, osso e cérebro, comparando-se o grupo SD com sham e controle. Aumento significante na média de %ATI/g foi observado no baço, pâncreas e músculo (grupo SD), comparado com os grupos sham e controle (p<0,05). No pulmão houve aumento e na tireóide diminuição no %ATI/g nos ratos SD, comparados com os sham e controles (p>0,05). Em conclusão, a operação denominada derivação biliopancreática com switch duodenal em ratos modificou a biodistribuição de pertecnetato de sódio na tireóide, pulmão, pâncreas, baço e músculo, podendo significar que a interpretação de exames cintilográficos nesses órgãos deve ser feita com cautela .

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3.2 Artigo II

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(Qualis Internacional C)

Biodistribuição do radiofármaco pertecnetato de sódio ($Na^{99m}TcO_4$) em ratos submetidos a ressecção extensa de intestino delgado

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ABSTRACT

Purpose: To evaluate the biodistribution of sodium pertecnetate ($Na^{99m}TcO_4$) in organs and tissues, the morphometry of remnant intestinal mucosa and ponderal evolution in rats subjected to massive resection of the small intestine. **Methods:** Twenty-one Wistar rats were randomly divided into three groups of 7 animals each. The short bowel (SB) group was subjected to massive resection of the small intestine; the control group (C) rats were not operated on, and soft intestinal handling was performed in sham rats. The animals were weighed weekly. On the 30th postoperative day, 0.l mL of $Na^{99m}TcO_4$, with mean activity of 0.66 MBq was injected intravenously into the orbital plexus. After 30 minutes, the rats were killed with an overdose of anesthetic, and fragments of the liver, spleen, pancreas, stomach, duodenum, small intestine, thyroid, lung, heart, kidney, bladder, muscle, femur and brain were harvested. The biopsies were washed with 0.9% saline, weighed and the percentage of radioactivity per gram of tissue (%ATI/g) was determined using Gama Counter Wizard^{TM} 1470, PerkinElmer. Biopsies of the remaining jejunum were analysed by HE staining to obtain mucosal thickness. Analysis of variance (ANOVA) and the Tukey test for multiple comparisons were used, considering $p<0.05$ as significant. **Results:** There were no significant differences in %ATI/g of the $Na^{99m}TcO_4$ in the organs of the groups studied ($p>0.05$). An increase in the weight of the SB rats was observed after the second postoperative week. The jejunal mucosal thickness of the SB rats was significantly greater than that of C and sham rats ($p<0.05$). **Conclusion**: In rats with experimentally-produced short bowel syndrome, an adaptive response by the intestinal mucosa reduced weight loss. The biodistribution of $Na^{99m}TcO_4$ was not affected by massive intestinal resection, suggesting that short bowel syndrome is not the cause of misleading interpretation, if an examination using this radiopharmaceutical is indicated.

Key words: Short bowel. Syndrome. Intestine. Surgery. Sodium pertechnetate. Biodistribution. Rats

RESUMO:
Objetivo: Avaliar em modelo animal com ressecção extensa do intestino delgado a biodistribuição de pertecnetato de sódio ($Na^{99m}TcO_4$) em órgãos e tecidos, a evolução ponderal e a morfometria da mucosa do intestino delgado remanescente. **Métodos:** Vinte e um ratos *Wistar* foram aleatoriamente divididos em três grupos de 7 animais cada. O grupo intestino curto (IC) foi submetido a ressecção extensa do intestino delgado, o grupo controle (C) não foi operado e o grupo *sham* foi submetido a leve manipulação cirúrgica das alças intestinais.Todos foram pesados semanalmente. No 30º dia pós-operatório foi administrado 0,l ml de $Na^{99m}TcO_4$ aos animais dos três grupos, IV no plexo orbital, com atividade radioativa média de 0,66MBq. Após 30 minutos os ratos foram mortos e retirados fragmentos do fígado, baço, pâncreas, estomago, duodeno, intestino delgado, tireóide, pulmão, coração, rim, bexiga, músculo, fêmur, e cérebro. As amostras foram lavadas com solução salina 0,9%, pesadas e submetidas ao Contador Gama 1470, Wizard^{TM} Perkin-Elmer para se determinar o percentual de atividade radioativa por grama (%ATI/g) de cada órgão. Biópsias do jejuno foram submetidas a análise da espessura da mucosa (coloração HE). Utilizou-se avaliação estatística paramétrica (ANOVA) e teste de Tukey, considerando $p<0,05$ como significante. **Resultados**: Não houve diferenças significantes da %ATI/g nos órgãos dos grupos estudados ($p>0,05$). Verificou-se acentuada redução inicial de peso, em seguida um aumento do peso dos animais tratados a partir da segunda semana de observação e aumento da espessura da mucosa jejunal do grupo IC, comparado com os demais. **Conclusão**: Em ratos com síndrome do intestino curto, uma adaptação na espessura da mucosa contribuiu para reversão na perda de peso inicial e para que a biodistribuição do $Na^{99m}TcO_4$ não fosse afetada pela ressecção extensa do intestino, sugerindo que o intestino curto não é causa de interpretações duvidosas, quando exame cintilográfico com este radiofármaco estiver indicado.

Descritores: Síndrome do Intestino curto. Cirurgia. Intestino. Pertecnetato de sódio. Biodistribuição. Ratos.

Introduction

Radioisotopes are used in nuclear medicine for diagnostic and therapeutic purposes. The labelling capacity of these isotopes for the plasmatic proteins is well known, and their bioavailability and pharmacokinetics can be modified by drugs and diseases[1,2].

Among the most useful artificial radioisotopes, technetium (^{99m}Tc), in the chemical form of sodium pertechnetate ($Na^{99m}Tc0_4$), is the most important. Its use in nuclear medicine is due to the emission of gamma energy with a wave length of 140 KeV, enabling it to take advantage of the scintillation detectors and obtain enhanced image quality; this is known as scintigraphy[3]. It has a short life, is low cost, and is obtained from a molybdenum generator ($^{99}Mo/^{99m}Tc$).[3] It is easily distributed through the vascular fluid, intersticial space with uptake by the salivary glands, thyroid, stomach, intestines and other organs[3]. It is rapidly eliminated by the urine and when incorporated to specific substances, produces organ images of different densities and functions[4].

Experimental studies carried out with the labeling of red blood cells with ^{99m}Tc identified important biological effects, in addtion to alterations in the labeling process[5,7]. Starch et al carried out a study with mitomicin-C that described alterations in the labelling of red blood cells[6]. The literature reports that several natural drugs reduce the power of labelling red blood cells with ^{99m}Tc[7]. An experimental study with Vincristin, used in oncology protocols, showed an interaction of this drug with ^{99m}Tc in several organs[8].

However, there are no reports of research studying the biodistribution of radiopharmaceuticals after surgical procedures. In the present study, we used an experimental model of massive resection of the small intestine, characterizing the short bowel syndrome, which results in unsuitable water and nutrient absorption, causing malnutrition[9,10].

In spite of the short bowel syndrome, the intestine can be adapted through physiologic, cellular and molecular mechanisms[9]. In some patients, dilation and lengthening of the remnant small intestine occur as a phenomenon of functional adaptation. Surgical techniques have been reported that attempt to lengthen this intestinal segment. Such procedures are complex and frequently ineffective, and call for assessments of their efficacy[11]. Recently, new therapeutic methods, such as isolated small intestine transplantation or combined with liver transplantation, have been an alternative for cases of hepatic failure due to total parenteral nutrition in the treatment of short bowel syndrome[12].

Given the anti- absorptive effect of the operation, with great repercussions on the metabolism, radioisotope images may be necessary in the postoperative, in order to control the series of pathological conditions resulting from short bowel syndrome. Scintigraphy can be used in the postoperative of intestinal resections to assess the morphology and metabolism of several organs. Under these conditions, it becomes relevant to study the biodistritubion of $Na^{99m}Tc0_4$ in specific organs and tissues.

Therefore, the present paper aims to study, in an animal model of massive resection of the small intestine, the biodistribution of $Na^{99m}TcO_4$ in several organs by means of scintigraphic evaluation in the postoperative period. We also evaluated the ponderal evolution of the animals after the operation, as well as the mucosal morphometry of the remnant small intestine..

Methods

Twenty-one Wistar rats weighing 265g±31g were used. They were supplied by the vivarium of the Nucleus for Experimental Surgery of the Federal University of the Rio Grande do Norte, Brazil. All the animals were weighed and observed in individual cages with water and food (Labina ® Purina) *ad libitum* and acclimated in the laboratory for 7 days. They were maintained under temperature control (21ºC), air humidity(60 – 70 %) and lighting (12/12 hours light / dark cycle) and handled in accordance with the Animal Experimentation Code of Ethics (Council for International Organizations of Medical Sciences) and the rules of the Brazilian College of Animal Experimentation. They were randomly divided into three groups: the experimental group rats , denominated short bowel, (SB, n = 7) were subjected to massive resection of the small intestine (90%); control group rats were not operated on (C, n=7) and the third group underwent a simulated operation, called Sham (n=7). Rats were fasted overnight before surgery, and anesthetized with sodium pentobarbital (20mg/Kg intraperitoneal) and ketamine (20mg/Kg intramuscular); they were operated on under sterile conditions.

A 3cm midline laparotomy was performed and intestinal transections were done 5 cm above the ileocecal junction and 5 cm from the duodenojejunal transition. With the aid of a surgical microscope (DF Vasconcelos, São Paulo, Brazil), interrupted sutures of 6-0 prolene (Ethicon®, Brazil) were used for bowel anastomosis. The animals typically have a small bowel length of 100 cm, and accordingly, residual length was 5 cm of jejunum and 5 cm of ileum (10 cm), corresponding to 90% of resection. After surgery, the abdomen was closed with interrupted sutures of 4-0 nylon suture (Ethicon®). The animals were allowed water immediately after surgery and food on the second postoperative day. The sham rats were subjected to a 3cm medium laparotomy and mild manipulation of the small bowel. The rats were weighed weekly with a digital scale (Filizola® São Paulo, Brazil) and observed for 30 days.

On the 30th day all the animals were anaesthetized again, and injected with 0.lml of $Na^{99m}TcO_4$ IV in the orbital plexus, corresponding to radioactive activity of 0.66MBq. After 30 minutes, the animals were killed by lethal dose of anesthetic. Samples of the liver, spleen, pancreas, stomach, duodenum, small intestine, thyroid, lung, heart, kidney, bladder, muscle, femur and brain were harvested. The samples were washed in 0.9% saline , weighed on a high-precision digital scale (Bel-Mark 160-II Itália®) and subjected to radioactivity detection using a 1470 Wizard^{TM} Gamma Counter- Perkin-Elmer, with automatic correction of radiation decline. The percentage of radioactive activity/g (%ATI/g) of each organ was calculated by dividing the activity/g of the tissue by the total activity administered to each animal.

Samples with 2cm of jejunum were harvested 2 cm below the anastomosis. After washed in 0.9% saline, the excised tissues were fixed in 10 % buffered formalin for 48 h, dehydrated and embedded in paraffin. Sections cut at 5μm thickness were stained with hematoxilyn and eosin and morphology was assessed by an observer, who was unaware of the tissue origin. For the morphometric study of intestinal mucosa, Media Cybernetics – LP, USA, Image Pro-Plus software was used with an Olimpus BX-50 microscope fitted with a digital (Samsung®) video camera. The video camera transferred the image from the microscope to the computer screen. The measurement of the mucosal thickness was delimited with a computer mouse from the apex of the villus to the muscularis mucosae and was expressed in microns (μm). The analysis was made under 100x magnification using specimens in which the villi and the crypts were perpendicular to the muscularis mucosae.

For the analysis of the different values related to post-surgical weight loss, to the measurements of total mucosal thickness, and to the biodistribution of sodium pertechnetate of the different groups, parametric variance (ANOVA) was used. For the multiple comparisons, the Tukey test was used. A significance level of 5% ($p<0.05$) was established.

Results

All the animals survived the surgical procedures. Table 1 shows the results of the differences in %ATI/g among groups SB, C and sham. We observed an increase in the biodistribution of $Na^{99m}TcO_4$ in the thyroid and duodenum of SB rats, when compared to control rats. However, since the standard deviation was high, there was no significant difference ($p>0.05$). In the stomach, an apparent tendency for reduced biodistribution of the %ATI/g occurred in the SB rats, compared to sham rats, but without statistical significance ($p>0.05$). In several organs the percentages of radioactive activity (%ATI/g) had very similar values among the groups, without significant differences (Table 1).

TABLE 1 – Biodistribution of $Na^{99m}TcO_4$ in the organs of the respective groups.

Organs	*%ATI/g*			
	SB	C	Sham	ANOVA (1)
Liver	0.35 ± 0.089	0.36 ± 0.079	0.39 ± 0.113	0.794400
Spleen	0.22 ± 0.090	0.18 ± 0.031	0.19 ± 0.044	0.565470
Estomach	2.58 ± 0.730	2.72 ± 0.614	4.11 ± 1.793	0.116180
Small bowel	0.28 ± 0.107	0.20 ± 0.052	0.28 ± 0.130	0.690700
Duodenum	1.73 ± 1.814	0.41 ± 0.062	1.13 ± 1.719	0.378723
Pancreas	0.16 ± 0.063	0.14 ± 0.055	0.18 ± 0.125	0.811183
Kidney	0.41 ± 0.086	0.42 ± 0.082	0.36 ± 0.187	0.738872
Heart	0.17 ± 0.075	0.27 ± 0.057	0.17 ± 0.084	0.076831
Lung	0.35 ± 0.105	0.38 ± 0.125	0.31 ± 0.058	0.581337
Thyroid	5.35 ± 1.979	3.71 ± 1.256	3.80 ± 1.058	0.187603
Bladder	0.39 ± 0.114	0.33 ± 0.109	0.27 ± 0.139	0.309546
Muscle	0.07 ± 0.028	0.06 ± 0.019	0.05 ± 0.035	0.570391
Femur	0.16 ± 0.055	0.14 ± 0.036	0.15 ± 0.050	0.760950
Brain	0.02 ± 0.013	0.01 ± 0.003	0.03 ± 0.027	0.482193

Mean ± Standard deviation

3. P- from analysis of variance (ANOVA).

The results of the test did not show statistically significant differences ($p>0.05$), for all the variables. %ATI/g, percent radioactivity per gram of tissue.

The weight of SB rats decreased during the first and second weeks of survival and, after that, their weight gradually increased until the 30th postoperative day, when it was nearly the same as the C and SHAM rats, which continually increased their weight over time (Figure 1). The differences in the mean weight of SB rats at the end of the second week were significant, when compared to C and SHAM rats ($p<0.05$).

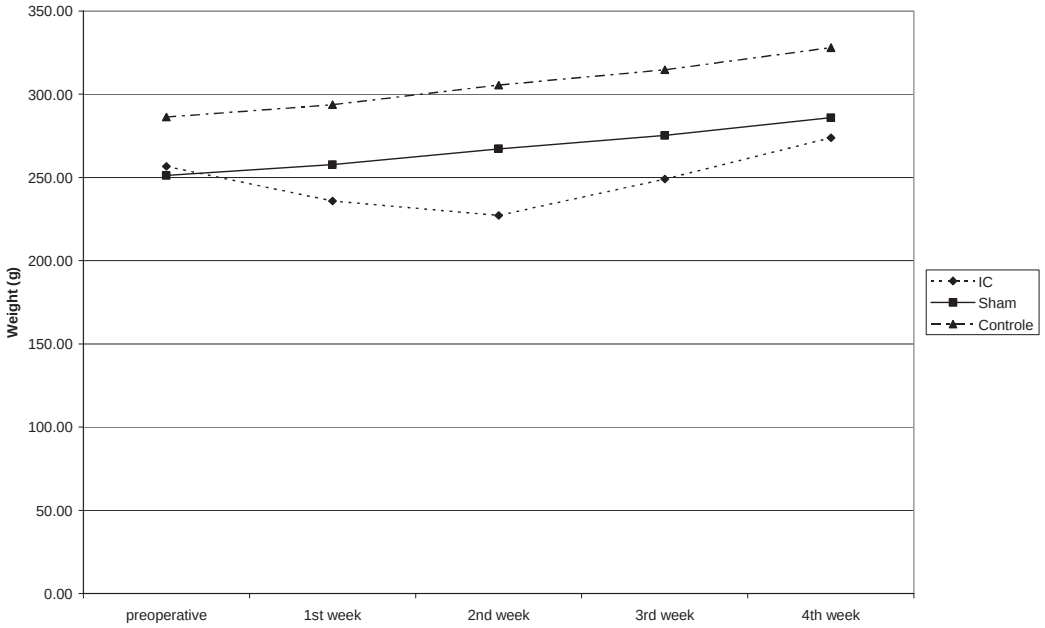

FIGURE 1 – Mean weight of rats in each group and postoperative period.

The presence of an increase in intestinal mucosa thickness was detected in all IC rats, when compared to C and SHAM rats until the end of the observation period, as seen in table 2 ($p<0.05$) and figure 2.

TABLE 2 – Jejunal mucosa thickness of rats and their respective groups.

Variable	Groups			
	SB	C	Sham	*ANOVA* (1)
Mucosal thickness in µm(2)	334.34 ± 25.9^{ab}	194.40 ± 39.0^{a}	194.22 ± 33.2^{b}	0.0000

Values expressed in Mean ± Standard deviation

(1) P-value of analysis of variance (ANOVA).

(2) Groups identified with the same letter differ significantly at a level of 5% (Tukey test).

SB = Short bowel; C = control.

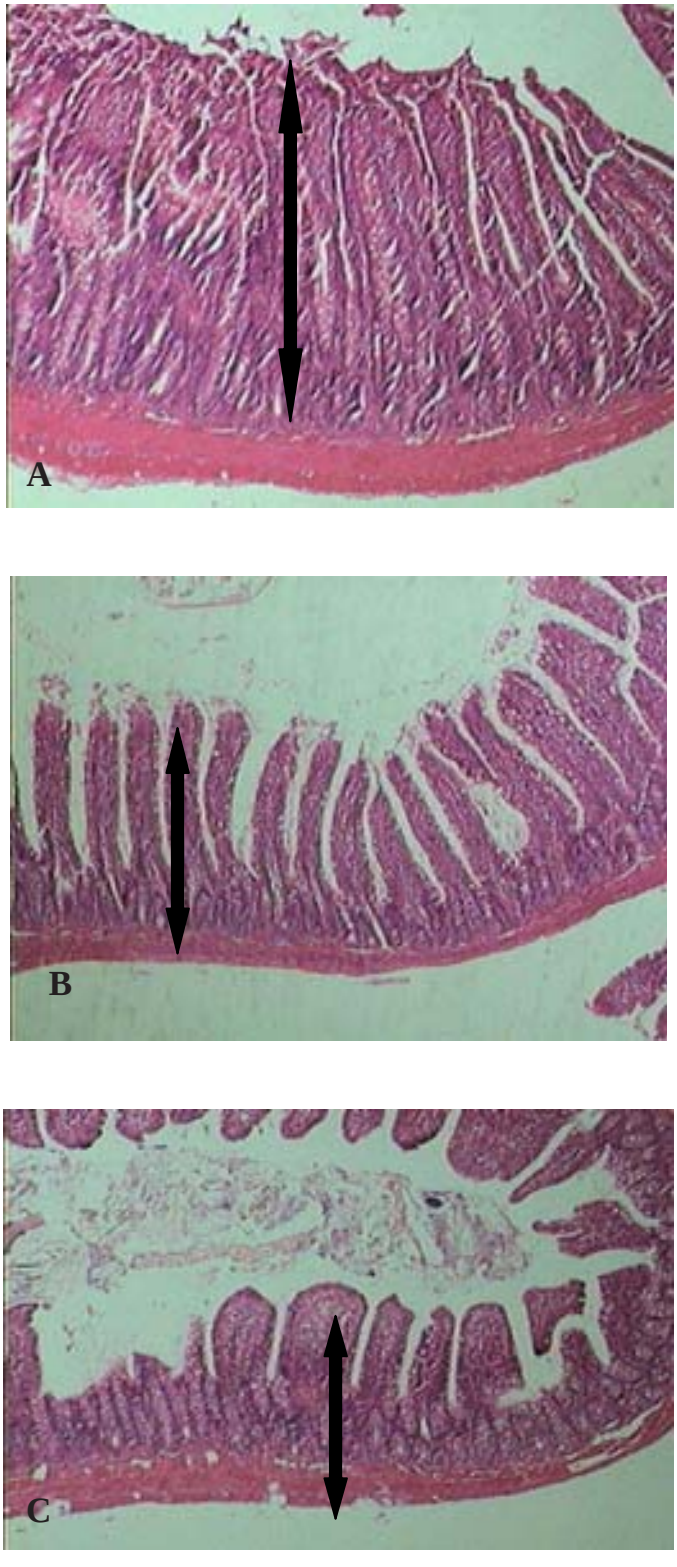

FIGURE 2 - Small intestine morphology and mucosal cell proliferation on day 30 in short bowel (A) control (B) and sham (C) animals. Massive intestinal resection (A) induced significant increases in total mucosal thickness (arrows), when compared to control and sham animals (B, C) (see Table 2; $p<0.05$). HE, 100x.

Discussion

The alterations in the biodistribution of $Na^{99m}TcO_4$ in organs and tissues are very well identified in several studies that used no experimental surgical models [1,2,6,7,8,13,14]. However, in the postoperative of major surgical procedures, there are no reports concerning the biodistribution of radiopharmaceuticals. The present experimental model of the short bowel syndrome in rats submitted to massive resection of the small intestine was used to determine the biodistribution profile of $Na^{99m}TcO4$ in several organs and tissues.

Intestinal failure is characterized by malnutrition and/or dehydration as a result of the inadequate digestion and absorption of nutrients. The most common cause of intestinal failure is short bowel syndrome, which occurs when the functional small bowel mass is reduced below the level necessary for adequate nutrient and water absorption. This condition frequently results from a massive resection of the small bowel. Following resection, the intestine is capable of adapt ting in response to enteral nutrients as well as other trophic stimulation. Rodents are commonly used in well-characterized models to assess the process of intestinal adaptation[16]. Following small bowel resection in the rat, the remnant intestinal mucosa undergoes compensatory alterations in an attempt to restore normal absorptive capacity. Morphologic and functional changes include increases in mucosal length, enterocyte proliferation, as well as increased electrolyte, glucose and amino acid uptake[16,17].

In humans, the alterations of intestinal absorption due to massive resection of the small intestine usually cause significant weight loss[15]. However, in rodents, there is a rapid adaptation of the intestinal mucous membrane, which minimizes weight loss[16]. These mechanisms of intestinal adaptation take place at physiologic, cellular and molecular levels and they do not correspond to what occurs in the human intestine [17]. Nutrients, electrolytes, hormones, cytokines and other elements take part in the process, which involves mainly the intestinal mucous membrane. The process begins with apoptosis and continues with an increase in epithelial cells, vilosities and mucosal crypts, and a consequent remodeling of their architecture. Functionally, this allows for increased substance transport through the intestinal mucosa[17].

In the present study a significant decrease was observed in the weights of rats submitted to massive intestinal resection, in the immediate postoperative period, and weight recovery begining at the end of the second week. These data coincide with a classic study on the subject, where morphological and functional adaptations of the jejunum were observed between the first and second postoperative weeks[18]. This phenomenon was also shown in the morphometry of the jejunum mucous membrane of the animals subjected to massive resection of the present study. Therefore, the mucous membrane hyperplasia observed in the jejunal mucosa of the SB rats of the present experiment, likely contributed to the rapid weight recovery of the animals, starting from the second postoperative week. Welters et al verified that intestinal function recovery begins with the hyperplasia of the intestinal mucosa and that absorptive function depends on the maturity of the enterocytes, a fundamental factor for nutrient metabolism[19].

The precocious postoperative recovery of the animals, represented by weight and morphology of the intestinal mucosa recovery, and the healthy behavior of the SB rats, comparable to the controls, certainly contributed to the absence of significant clinical alterations and malnutrition. Consequently, there was no negative effect on the biodistribution of $Na^{99m}TcO_4$ in the vital organs. In an experimental study using

malnutrition-inducing diets, Passos et al[20] showed that malnutrition affected the biodistribution of $Na^{99m}TcO_4$ in different organs such as the thyroid, brain, stomach and heart. In their study, the intestine was not surgically manipulated.

Studies in animals have been investigating substances that regulate the absorptive function of the intestine[21]. These mechanisms are mediated by multiple factors, including enteral or parenteral nutrition, hormones and growth factors[22]. Recently, studies on the use of the human growth hormone (GH), the epidermal growth factor (EGF) and the glucagons-like peptide-2 (GLP-2), produced in the L-cells of the small intestine, have confirmed them as agents that increase intestinal adaptation after massive resection[23]. The study suggests that, whereas GLP-2 is important in controlling adaptation, there are spatial or regional systems in place that use varying pathways. The significant increase in nutrient-stimulated GLP-2 secretion suggests that GLP-2 is involved not only in the initiation, but also in maintaining the ongoing adaptive process. The increases in mucosal proliferation that are temporally associated with a maintained GLP-2 release, suggest that GLP-2 is important in initiating and maintaining the small intestine's adaptive response to resection[24].

Curtis et al studied rats submitted to massive resection of the small intestine using marker $^{51m}Cr^{l3m}C$ and protein, and observed the animals for one week. They concluded that the rats had no alteration in absorption and digestion time when compared to the treated group and the control; this demonstrated the fast physiological adaptation of the animals[25]. A growing number of tissue factors are being investigated for having great potential in promoting intestinal adaptation in animals and humans with short bowel syndrome, in the hope of obtaining effective therapies for the syndrome in the future[23,26].

In summary, massive intestinal resection in the current study did not interfere significantly with the biodistribution of the radiopharmaceutical $Na^{99m}TcO_4$ in the organs studied. Certainly the mucosal hyperplasia of the remnant intestine was a preponderant factor for the quick weight loss reversal of the animals, and consequent preservation of their healthy metabolism.

The present study does not allow us to comment on the mechanisms by which intestinal resection results in the stimulation of trophic effects and mucosal adaptation, allowing normal biodistribution of $Na^{99m}TcO_4$ in rats. Identifying factors that may enhance the process of intestinal adaptation is an exciting area of research with important potential clinical applications. This area will require further studies.

Conclusion

In rats with experimentally-induced short bowel syndrome, an adaptive response by the intestinal mucosa reduced weight loss. The biodistribution of $Na^{99m}TcO_4$ was not affected by massive intestinal resection, suggesting that short bowel syndrome is not the cause of misleading scintigraphy interpretation when an examination with this radiopharmaceutical is indicated.

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3.3 Artigo III

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Effects of simvastatin in abdominal sepsis in rats.

Efeitos da sinvastatina na sepse abdominal em ratos.

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ABSTRACT

Purpose: Statins are widely recognized as hypolipemic drugs, but some studies have observed anti-inflammatory and immunomodulatory effects, known as pleiotropic. The aims of this work was to study possible anti-inflammatory effects of simvastatin in abdominal sepsis. Serum pro-inflammatory cytokines and leukocytes count were determined in an experimental model of abdominal sepsis, using cecal ligation and puncture (CLP) in rats. **Methods**: Twenty eigth Wistar rats weighing 285±12g were randomly divided in: CLP/Simvastatin rats (n=7), treated with 10 mg/Kg of oral simvastatin 18 and 2 hs berofe CLP; CLP/Saline group rats (n=7), treated with oral saline; group Sham/Simvastatin (n=7), treated with simvastatin, and group Sham/Saline (n=7), treated with saline. Serum TNF-α, IL-1β and IL-6 by ELISA and total leukocytes, neutrophils, lymphocytes, and eosinophils were determined 24 hs after CLP. ANOVA and Tukey test were used considering significant $p<0.05$. **Results**: It was demonstrated that serum TNF-α, IL-1β and IL-6 were respectively 364,8±42pg/mL; 46,3±18pg/mL and 28,4±13pg/mL in CLP/Simvastatin rats, significantly lower ($p<0.05$) than in group CLP/Saline (778,5±86pg/ml; 176,9±46pg/ml; 133,6±21 pg/ml, respectively). The same results were observed in total leukocytes and neutrophils counts. **Conclusion**: These results clearly demonstrate that simvastatin is an effective agent that reduces cytokines levels and leukocyte count in sepsis, independently of its well-known lipid-lowering effects. Thus, HMG-CoA reductase inhibitors like simvastatin have important anti-inflammatory effects in abdominal sepsis in rats.

Key words: Statin. Inflammation. Abdominal sepsis. Wistar rat. Cytokine. Leukocyte.

RESUMO:
Objetivo: As estatinas são agentes reconhecidamente hipolipemiantes. Vários estudos têm revelado que eles têm ações pleiotrópicas, como antiinflamatória e imunomoduladora. Tentando-se entender o papel antiinflamatório da sinvastatina na sepse, foram analisados os níveis de citocinas pró-inflamatórias e contagem de leucócitos em modelo de sepse abdominal por ligadura e punção do ceco (LPC) em ratos**. Métodos**: Foram utilizados 28 ratos *Wistar* pesando 285±12g, assim divididos: grupo sepse (n=14), submetidos a LPC e grupo *sham* (n=14), submetidos a laparotomia e manipulação suave do ceco. No grupo LPC/sinvastatina (n=7) os ratos receberam 10mg/kg de sinvastatina via oral 18 e 2 horas antes da LPC e no grupo LPC/salina (n=7) os ratos receberam injeção oral de solução salina 0,9 %. Os animais dos grupos *sham/*sinvastatina (n=7) e sham/salina (n=7) receberam o mesmo tratamento. Dosagem de TNF-α, IL-1β e IL-6 por ELISA e contagem de leucócitos totais, neutrófilos, linfócitos e eosinófilos foram realizadas em todos os animais. Análise estatística foi feita pelo ANOVA e teste de Tukey, com significância $p<0,05$. **Resultados**: Ficou demonstrado que as dosagens de TNF-α, IL-1β e IL-6 atingiram valores de 364,8±42pg/ml; 46,3±18pg/ml e 28,4±13pg/ml no grupo submetido à sepse e tratados com sinvastatina, significantemente mais baixos do que no grupo sepse não tratados (778,5±86pg/ml; 176,9±46pg/ml; 133,6±21 pg/ml, respectivamente). O mesmo ocorreu na contagem de leucócitos totais e neutrófilos. **Conclusão**: A sinvastatina mostrou ação anti-inflamatória em ratos *Wistar,* diminuiu níveis de citocinas e leucócitos, sugerindo uso potencial na prevenção ou atenuação dos efeitos da sepse abdominal.

Descritores: Estatina. Inflamação. Sepse abdominal. Rato Wistar. Citocinas. Leucócitos.

Introduction

Statins are powerful hypolipemic drugs with pleiotropic effects and have been shown to improve survival in the primary and secondary prevention of atherosclerosis in numerous large randomized clinical trials[1,2]. By inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and the mevalonate pathway to cholesterol, statins reduce not only cholesterol but also the production of several of its nonsteroidal isoprenoid precursor intermediates[3], which are necessary to membrane anchor proteins critical to the binding of signaling proteins involved in various cell functions. Several cellular and animal models demonstrate the pleiotropic activity of statins, including antiinflammatory and antioxidative properties, immunomodulatory effects, improvement in endothelial function, reduction in blood thrombogenicity, and increased nitric oxide (NO) bioavailability. Some or all of these effects may account for a substantial potential impact of statins on the complex pro and anti-inflammatory sequence of events occurring during sepsis.

Extensive research has been invested in the last 2 decades and sepsis remains the leading cause of death among patients treated in intensive care units, with mortality rates ranging between 30% and 70% [4,5]. Sepsis is generally viewed as a disease aggravated by the inappropriate and inefficient immune response encountered in the affected individual. Corticosteroids[6,7], activated protein C, [8] tumor necrosis factor (TNF) antagonists[9], interleukin-1 receptor antagonists[10], anti-endotoxin antibodies[11], and ibuprofen[12] have all been evaluated in a clinical setting, with improved outcome demonstrated recently for activated protein C. HMG-CoA reductase inhibitors (statins) such as simvastatin have been shown to exhibit important immunomodulatory effects

independent of lipid lowering[13]. These pleiotropic effects have been demonstrated to include anti-inflammatory actions[14], improvement of endothelial and microvascular function, and modulation of endothelial nitric oxide synthase (eNOS)[15]. However, statins have thus far not been used to treat severe inflammatory states such as sepsis. Knowing that infection is an important risk factor to operated people and that statins have anti-inflammatory and antioxidant properties, we hypothesized that simvastatin pretreatment would be protective against abdominal sepsis in rats.

Methods

The experimental protocol was approved by the Research Ethics Committee of the University Hospital-UFRN, Brazil. Animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals, US National Research Council, 1996.

Animals

Wistar rats weighing 285±16g were used. Rats were housed in polypropilene cages and maintained under controlled temperature conditions on a 12h light-dark cycle and allowed *ad libitum* access to commercially available rat chow (Labina, Purina®) and water.

Experimental design

A total of 28 Wistar rats were randomly distributed into the following four groups: In the sepsis group (n=14), a half of the (CLP/Simvastatin) rats (n=7) received 10 mg/Kg of simvastatin microemulsion via gavage, 18 and 2 hours before cecal ligation and puncture (CLP). The remaining (CLP/Saline group) rats (n=7) were treated with oral injection of saline 18 and 2 hs before CLP. In the group sham, 7 rats were treated with simvastatin (Sham/Simvastatin group) and 7 with saline (Sham/Saline group) as sepsis group.

Surgical models

Animals were fasted 12 hr before the experiment and anesthetized with intramuscular injection of 0.1 mL/100g weight, of a solution prepared with 1.0 mL of ketamine (50mg/mL) and 1.0 mL of xilazine (20mg/mL). They breathed spontaneously throughout the procedures. After shaving, the abdominal skin was disinfected with 70% alcohol. All procedures were performed under sterile conditions. Midline laparotomy (3 cm) and gentle manipulation of cecum was performed in the sham group. In the sepsis group the cecum was exposed, ligated with silk 2-0, one cm distally to the ileocecal valve to avoid intestinal obstruction. Four punctures were performed with a 22-gauge needle, squeezed gently to force out a small amount of feces, and then it was returned to the abdominal cavity. The abdominal incision was closed with 4-0 nylon sutures.
All animals were observed for 24 hours, weighed again and anesthetized with ketamine intramuscular (50 mg/kg). Thorax was opened, blood was collected by cardiac puncture for cytokine assay and leukocyte count.

Experimental design

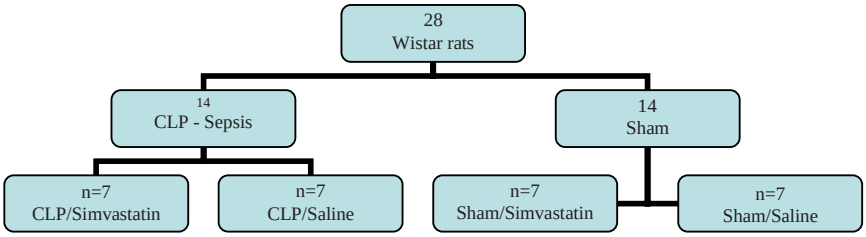

Figure 1. Experimental design: 14 rats were divided into group CLP/sepsis treated with simvastatin (CLP/Simvastatin n=7) and with saline (CLP/saline n=7). In group sham (n=14), rats were treated with simvastatin (Sham/Simvastatin n=7) and with saline (Sham/Saline n=7).

Cytokine assays

Blood samples were used for measurement of tumor necrosis factor-alfa (TNFα), interleukin-1β (IL-1β) and interleukin-6 (IL-6) , determined using enzyme-linked immunoassay kits (all from PeproTech, Rocky Hill, NJ, USA), according to the manufacturer's recommended protocols. The fluorescence was measured by a Bio-Tec Instruments EL 808 ultra microplate reader, using KC4-V3.0 analysis software. Sensitivity of detection was 20 pg/ml for cytokines.

Leukocyte count

Whole blood was collected by cardiac puncture for leukocyte cell counts using a commercially available automated cell counter (Abbott Cell-Dyn 3500R- CD 3500 5L, USA).

Statistical analysis

Data are reported as mean± SEM. Statistical analyses were conducted with commercially available software SPSS 14.0.1 for Windows. Values of p were reported in cases in which tests were performed. A value of $p<0.05$ was considered significant. ANOVA with post hoc Tukey's test was used to compare the groups.

Results

All the animals survived to experiments. The results were tabulated and exhibited as mean±SD. Leukocyte counts obtained at 24 hours after CLP confirmed significant lowering of WBC and neutrophils in simvastatin treated (CLP/simvastatin) rats of the sepsis group, when compared with the untreated (CLP/saline) rats ($p<0.05$), as can be seen on Table 1. To address possible changes in WBC, neutrophils, limphocytes and eosinophils, secondary to the sham operation, we studied the cells count. No difference was observed comparing the simvastatin (Sham/Simvastatim) treated and saline (Sham/Saline) treated rats ($p>0.05$).

Table 1- Number of WBC and percent of neutrophils, lymphocytes and eosinophils from the studied rats.

Leukocytes / **Groups**	*WBC/µL)*(1)*	*Neutrophils (%)*(1)*	*Lymphocytes (%)*(2)*	*Eosinophils(%)*(2)*
CLP/Saline	9,46 ± 1,26ab	76,37 ± 6,57abc	16,36 ± 4,58a	0,39 ± 0,42a
CLP/Simvastatin	6,72 ± 0,39ab	56,50 ± 7,08[a]	37,03 ± 10,94a	0,57 ± 0,59a
Sham/Saline	4,68 ± 0,56a	57,17 ± 6,60b	34,14 ± 9,26b	5,41 ± 5,16b
Sham/Simvastatin	4,32 ± 0,53b	57,10 ± 8,52c	30,27 ± 8,55b	5,68 ± 5,78b

*Mean ± Standard Deviation; CLP, cecal ligation and puncture.; WBC, white blood cell.
(1) Values followed by the same letter differ among them, considering p<0.05 by Tukey test.
(2) Values followed by the same letter do not differ among them, considering p<0.05 by Tukey test.

To investigate the effects of sepsis and simvastatin treatment on cytokines, serum was isolated from all groups of rats (CLP/saline, CLP_simvastatin, sham/saline, and sham/simvastatin) and subjected to ELISA assay. The levels of TNFα, IL-1β and IL-6 from CLP/simvastatin treated rats were significantly decreased compared with that of CLP/saline rats (Table 2). Cytokines from CLP animals, treated or untreated with simvastatin, displayed an increased levels compared with the sham operated rats (p>0.05), as observed in Table 2. No difference was detected among the values of cytokines (pg/mL) from sham rats treated with simvastatin and sham-operated rats treated with saline. (p>0.05). This observation indicates that the simvastatin has interference with the expression of cytokines in septic animals, but not in the absence of sepsis.

Table 2: Values of TNFα, IL-1β e IL-6 from the studied rats.

Groups	**TNF α (pg/ml)**	**IL-1β (pg/ml)**	**IL-6 (pg/ml)**
CLP/Saline	778,5±86[a]	176,9±46[a]	133,6±21[a]
CLP/Simvastatin	364,8±42[a]	66,3±18[a]	58,4±13[a]
Sham/Saline	31,3±6,1[*]	28,1±4	29,8±2
Sham/Simvastatin	20,7±4,5[a]	27,3±5[a]	23,1±4[a]

*Mean ± Standard Deviation; CLP, cecal ligation and puncture.
(1) Values followed by the same letter differ among them, considering p<0.05 by Tukey test.
(2) Values followed by the same letter do not differ among them, considering p<0.05 by Tukey test.

Discussion

The 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitor class of drugs (statins) was introduced into clinical practice in the 1980s. They have become the most widely used drugs for lowering plasma cholesterol. Patients with coronary artery disease, highrisk elderly patients, and those having major surgery, benefit from statin therapy[16,17,18,19].

Some works have been presenting several effects (anti-inflammatory, antitrombotic, immunomodulator, etc) of statins, those denominated together as pleiotropic effects, that do not depend on the reductions in the cholesterol levels[20,21,22,23,25].

Enlarging the classic use of the statins, the challenge in subject is to evidence other actions of these molecules seen that, many pleiotropic effects have been told, as well as anti-inflammatory properties, action in the endotelial function and benefits in the

hemostasia[23,24,25]. In the best attempt to understand the anti-inflammatory effects of the simvastatin in the sepsis, rats were previously treated with this drug and submitted to the model of abdominal sepsis by CLP. The levels of pro-inflammatory cytokines and counting of total leukocytes, neutrophils, lymphocytes and eosinophils were analyzed, considering that they are factors that participate actively of the inflammatory process.

The experimental model is one of the main means to study sepsis of abdominal origin. The study of the sepsis in experimental models can be driven with administration lipopolissacárides (LPS) intravascular, bacterial peritonitis induced by introduction of feces or bacteria in the peritonial cavity, opening of an intestinal segment or cecal ligation and puncture[26,27]. The CLP model was adopted in this work by presenting some advantages, as it is easy reproducible, simple, it is not necessary the standardization of an inocule. This is the model that better approaches the human sepsis. The sepsis is polymicrobial and simulates the perforated appendicitis or diverticulitis[26]. It is believed that this experimental model is an appropriate study method to evaluate and to control the septic phenomena from its installation to the moment of failure of the organs and systems in different times in this process[28]. The experimental design of this study was elaborated in a such way that the evidences of the anti-inflammatory effect of the simvastatin in the abdominal sepse were evaluated in currently used biological models.

After the statistical treatment of the results, a discerning analysis of these data resulted in some interesting observations. Except for the groups without infection (group sham), the total leukocytes count indicated an accumulation of these cells as a consequence of the trauma and ischemia on the tissues. The use of simvastatin in the infected rats inhibited the accumulation of the neutrophils, but not in the absence of sepsis. On the other hand, it was observed that in the septic groups, the simvastatin didn't promote significant alteration in the lymphocytes and eosinophils counts.

In relation to the cytokines dosages, it was observed that the simvastatin didn't result in a significant change in the levels of TNFα, IL-1β and IL-6 in the sham rats. The abdominal sepsis served to demonstrate a significant anti-inflammatory effect of simvastatin. This fact can be corroborated by the significant reduction of the levels of these cytokines in the infected animals, when simvastatin was administered. These data suggest an important relationship between the statin and the cells of the immune system in the validity of the mechanisms of repair of the traumatic damage, as well as during the activation of the monocytes. Therefore, it was demonstrated in the present work that the serum TNFα, IL-1 β, IL-6, total leukocytes and neutrophils had statistically significant reduction ($p < 0,05$) in the groups submitted to the sepsis and treated with simvastatin, compared with those non treated rats. These data corroborate with the work of Villa et al[29], where the levels of TNF-α, IL-1 β and IL-6 became altered in the same model of CLP polymicrobial abdominal sepsis. Koo et al[30] demonstrated in CPL model that the expression of genes for these cytokines happen during the abdominal sepse, not only in the intestinal site, but also in other organs.

The results obtained in the present work are also in agreement with those visualized by Merx et al[31]. They demonstrated that the simvastatin, injected 20 hours after CLP in the same concentration used in the present study (10 mg/mL), increased the time of survival, as well as it preserved the heart and hemodinamic functions of the studied rats. In this same work it was demonstrated *in vitro* that the monocytes adhesion was increased in the group sepsis, when compared with the group sham. The adhesion decreased when these cells were incubated with simvastatin. The increasing adhesion is an important factor in the physiopathology of the sepsis.

The benefit of the anti-inflammatory action of the statins was also analyzed by Merx et al[31,32], who studied the effect of the atrovastatin, pravastatin, simvastatin and

fluvastatin in the survival in a CLP model in murines. The authors demonstrated that the treatment after 6 hours of the induction of the sepse increased the time of survival of the animals, except the fluvastatin, that didn't alter the survival. In the present study we did not find difference in survival between the groups, because no mortality occurred.

The host reaction to the peritoneal sepsis involves antibodies production, complement activation, cellular immunity and bacterial destruction by polymorphonuclear leukocytes and macrophages[33,34,35]. The mechanism of the anti-inflammatory action of simvastin is not completely elucidated. However, some hypothesis exist to explain its action. The bacterial toxins are recognized by a variety of receptors in the monocyte surface, macrophages and granulocytes. The cytokines (TNF, IL-1 and IL-6) increase the expression of adhesion molecules (selectines and iCAMs) recruiting neutrófilos for the infection site[35]. The IL-1 produces several effects similar to the exogen TNF, as fever, anorexia and hypotension. It also produces increase in the leukocyte adhesion, bone reabsorption, inhibition of the lipoprotein-lipase and the synthesis of collagen[36].

Great efforts have been used in the attempt of elucidating the action of the statins in the sepsis, because there great therapeutic potential[37,38]. Few clinical studies have been published recently to support the hypothesis of the action of therapy with simvastatin in sepse. Almog et al[39] performed a prospective observacional cohort study to determine the impact of pre-treatment with statins in the occurrence of severe sepse in infected patients. Of the 361 patients with bacterial infection, 82 (23%) had received statins at least 4 weeks before admission. The mortality tax was low and it didn't differ significantly among the 2 groups (3.7% vs 8.6%, P=0.21). Severe sepsis developed in the 2.4% and 19%, of the patients respectively, in the group with statin and without statin. In other retrospective revision of 388 patient with bacteremia, Liappis et al[40] described a significant reduction in the patients' mortality when they received statins in the period of the admission, compared with those without this therapy.

Conclusion

The data of the present study suggest that simvastatin has potential to attenuate or to prevent the effects of the abdominal sepsis in rats subjected to cecal ligation and punture, represented by the reduction of the levels of serum cytokines, total leukocytes and neutrophils.

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3.4 Artigo IV

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(Qualis Internacional C)

Prevention of bacterial translocation using b-(1-3)-D-glucan in small bowel ischemia and reperfusion in rats.

Prevenção de translocação bacteriana com b-(1-3)-D-glucana em isquemia e reperfusão intestinal em ratos.

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ABSTRACT

PURPOSE: To investigate the role of b-(1-3)-D-glucan on ^{99m}Tc labelled *Escherichia coli* translocation and cytokines secretion in rats submitted to small bowel ischemia/reperfusion injury. **METHODS:**Five groups (n=10 each) of Wistar rats were subjected to control(C), sham(S), group IR subjected to 45 min of bowel ischemia/60 min of reperfusion(I/R), and group I/R+glucan subjected to 45 min of bowel ischemia/60 min of reperfusion(I/R) and injected with 2mg/Kg intramuscular. Translocation of labelled bacteria to mesenteric lymph nodes, liver, spleen, lung and serum was determined using radioactivity/count and colony forming units/g(CFU/g). Serum TNFa, IL-1b, IL-6, IL-10 were measured by ELISA. **RESULTS:** CFU/g and radioactivity/count were higher in I/R than in I/R+glucan rats. In C, S and S+glucan groups, bacteria and radioactivity/count were rarely detected. The I/R+glucan rats had enhancement of IL-10 and suppressed production of serum TNFa, IL-1b and, IL-6,compared to I/R untreated animals.**CONCLUSION:** The b-(1-3)-D-glucan modulated the production of pro-inflammatory and anti-inflammatory cytokines during bowel ischemia/reperfusion, and attenuated translocation of labelled bacteria.

Key words: Bacterial translocation. Glucan. Intestine. Ischemia. Reperfusion. Prophylaxis.

RESUMO

OBJETIVO: Investigar o papel da b-(1-3)-D-glucana na translocação de *Escherichia coli* marcada com ^{99m}Tc e na secreção de citocinas em ratos submetidos a isquemia e reperfusão intestinal.**MÉTODOS:** Cinco grupos (n=10 cada) de ratos Wistar foram denominados controle (C), *sham* (S), grupo IR submetido a 45 minutos de isquemia do intestino delgado e 60 minutos de reperfusão(I/R), grupo I/R+glucana com 45 minutos de isquemia e 60 minutos de reperfusão(I/R) e tratados com glucana 2mg/Kg intramuscular. Translocação de *Escherichia coli* marcada com ^{99m}Tc, para Linfonodos

mesentéricos, fígado, baço, pulmão e soro foi avaliada usando contagem de radioatividade e de unidades formadoras de colônias/g (UFC/g) Dosagem sérica de TNFa, IL-1b, IL-6, IL-10 foi realizada pelo método ELISA. **RESULTADOS:** CFU/g e contagem de radioatividade foi significantemente maior nos ratos do grupo I/R do que no grupo I/R+glucana. Nos grupos C, S e S+glucana bactérias e contagem radioativa foram raramente detectadas. Os ratos do grupo I/R+glucana tiveram aumento de IL-10 sérica e significante redução da expressão de TNFa, IL-1b e IL-6, quando comparados com os animais não tratados do grupo I/R.**CONCLUSÃO:** A b-(1-3)-D-glucana modulou a produção de citocinas pró-inflamatórias e anti-inflamatórias durante a isquemia/reperfusão intestinal e contribuiu para reduzir a translocação de bactérias marcadas.

Descritores: Translocação bacteriana. Glucana. Intestino. Isquemia. Reperfusão. Profilaxia.

Introduction

Maintenance of bacteria and their products in the intestine is done by both mucin and a layer of epithelial cells, the intestinal barrier that is essential for health and survival. These gut cells are in constant division, metabolizing rapidly and forming an impermeable barrier to harmful intestinal contents. Because they are metabolically active, they are also susceptible to oxygen deprivation with subsequent ischemic damage to enterocytes and their supporting structures[1]. This insult results in epithelial cell damage, decreased absorptive function, and the loss of basement membrane integrity leading to translocation of bacteria[2]. Bacterial translocation (BT) was originally defined and described by Berg and Garlington[3] as the passage of viable bacteria through the intestinal mucosa into the mesenteric lymph nodes (MLN) and to other tissues and organs. It has been suggested that gut ischemia/reperfusion induces disruption of the intestinal mucosal barrier, allowing translocation of bacteria and endotoxin from within the bowel into the blood, an event that may initiate a systemic inflammatory response and the secretion and activation of inflammatory mediators, including cytokines[4]. Although it has been difficult to show BT in clinical cases, patients suffering from hemorrhagic shock or post-surgical syndrome are quite susceptible to endotoxemia and multiple organ failure[5]. b-(1-3)-glucan purified from fungi have been shown to have broad anti-infective activities[6]. It have been shown to bind to receptors on leukocytes and stimulate some immune responses, such as cytokine release[7], and generation of nitric oxide[8]. Soluble b-glucan has also been shown to enhance the clearance of bacteria from the blood, and reduce mortality in rat sepsis models[9]. The present experiment was designed to analyze the effect of soluble b-(1-3)-glucan in rats submitted to bowel ischemia, with and without reperfusion, on translocation of ^{99m}Tc labelled bacteria from the intestinal mucosa to MLN, liver, spleen, lung and serum. Additionally, the levels of serum cytokines were studied and correlated with BT and b-(1-3)-glucan administration.

Methods

Radiolabelling of bacteria

Escherichia coli were labelled with ^{99m}Tc, as follows. Briefly, a sample (0.1 mL) of *E. coli* ATCC-10536 culture, grown overnight in soybean casein medium, was incubated in 10mL of the same medium, under aeration, for 4 hour at 37ºC. After that, different amounts of stannous chloride were added to 2 mL of the medium to reach final concentrations of 40, 130, 290, 400 and 580 mM, respectively. The samples were then incubated at 37ºC for 10, 20, 40 and 60 min. After incubation, 37.0 MBq of ^{99m}Tc were added to each preparation and kept at 37ºC for 10 min. The tubes were then centrifuged at 3000x g for 25 min, washed and resuspended with normal saline. After three washes with saline, the ^{99m}Tc *E. coli* were incubated at 37ºC for 36h. Aliquots (100 mL) of supernatant and resuspended precipitate in saline were withdrawn for determination of radioactivity. This procedure was repeated three times. In order to evaluate the bacterial viability, aliquots were taken from the last suspension, spread into a solid culture medium and incubated at 37ºC for 24 h. The effect of the procedure on the bacterial viability was assessed by comparing the colony-forming units per mL (CFU/mL) of labelled and unlabelled *E. coli*.

Animals

Male Wistar rats weighing 285±14g were maintained under conditions with controlled temperature, on a 12h light-dark cycle and fed *ad libitum* with commercially available rat chow and water.They were randomly divided into four groups (n=10 each), and named, respectively: C group, for non-operated rats, which were the controls, S group, for sham-operated, I/R for rats submitted to 45 minutes of intestinal ischemia and 60 minutes reperfusion, and I/R+glucan for those ischemia/reperfusion group treated with glucan (2mg/Kg) intramuscular. All the animals were gavaged with ^{99m}Tc *E. coli*, two hours before the operative procedures. After fasting overnight, the animals were anesthetized with intramuscular ketamine (50mg/kg) and xilazine (7mg/kg). In the I/R and I/R+glucan groups, the superior mesenteric artery (SMA) was occluded with a microvascular clamp. The laparotomy incision was then closed, to be opened 45 minutes later for removal of the clamp. Reperfusion was confirmed by the return of pulsation to the mesenteric arcade. The incision was again closed and the animals were killed with overdose of anesthetic 60 minutes later.

Glucan administration

For each experiment, soluble b-(1-3)-D-glucan (ImunoglucanÒ) was administered intramuscularly to 10 rats of I/R+glucana group, at a dose of 2mg/Kg of body weight.

Measurement of radioactivity, bacterial counting and cytokines

At the end of the procedures, under aseptic conditions, a midline laparotomy was performed and blood was collected from the portal vein for culture, counting and cytokines assays. One mL of serum was aliquoted for radioactivity counting. One gram of MLN complex, spleen, liver and lung were removed for counting and culture, if 1g of tissue was available; otherwise, the entire organ was weighed. Tissues were homogenized and solubilized. Aliquots of 0.2mL were processed and were then counted

in a PerkinElmer - Wizard TM Gama Counter. Other portions (0,2mL) were cultured on selective MacConkey's agar and blood agar for detection of gram-negative and gram-positive bacteria, respectively. The plates were examined after 24 and 48 hours of incubation at 37ºC. Portal blood samples were used for measurement of tumor necrosis factor-alfa (TNFa), interleukin-1b (IL-1b), interleukin-6 (IL-6), and interleukin-10 (IL-10) assayed using ELISA. Sensitivity of detection was 30 pg/ml for all cytokines. Procedures involving animals and their care were conducted in conformity with the *Guide for the Care and Use of Laboratory Animals*, US National Research Council, 1996. The data analysis were performed using the BioEstat 2.0 program. The results were tabulated and compared by ANOVA using post hoc analysis with Newman-Keuls test. P<=0.05 was considered statistically significant.

Results

All animals survived the experimental protocol. The bacterial viability test showed that the number of colony forming units (CFU) of the *E. coli* under radiolabelling procedure was the same as that grown in absence of ^{99m}Tc (data not shown). When the C and S groups were compared with I/R, and I/R+glucan groups, a significant variation on the labelled bacteria migration to different organs was found. As shown in Table 1, the concentration of radio labelled *E. coli* was the greatest in the MLN, lung, and liver in ischemia/reperfusion (IR) rats. So, the MLN, spleen, liver, lung and serum from I/R rats had significantly higher levels of radioactivity than did the organs from the I/R+glucan ($p<0.01$). The level of positive cultures with CFU was significantly higher in I/R rats than in I/R+glucan group (Table 2). The C group was the only one where the organs and serum were free of any bacterial colony. In the S group the bacteria were rarely detected. As observed with the mean count of radioactivity, bacteria were less detected in the spleen than in the other organs studied (Tables 1,2). The most common bacteria cultured from the organs and serum were *E. coli* and *Enterococcus.* TNF-a, IL-1b, IL-6, and IL-10 were not detected in the serum of the C group, while there concentrations in the serum of S operated rats were 41,7±9,4pg/ml, 34±11pg/mL, 144±17pg/mL and, 94±21pg/mL respectively. Significant increase in serum level of TNF-a (753.7±91pg/ml), IL-1b (588.7±100pg/ml), IL-6 (422.1±56pg/ml) and, IL-10 (311±52pg/mL) was observed in I/R group, when compared with C and S rats ($p<0,01$). The I/R+glucan rats had the serum levels of TNF-a (98±23pg/mL), IL-1b (122±19pg/ml) and, IL-6 (110±31pg/mL) significantly lower than that observed in the I/R rats ($p<0,01$). Nevertheless, an inverse result was observed in the IL-10. There was a significant increase ($p<0,01$) in the level of IL-10 in the I/R+glucan group when compared to the I/R (Table 3).

TABLE 1 - Level of radioactivity (mean counts per minute per gram) from MLN, Spleen, Liver, Lung and Serum after ^{99m}Tc *E. coli* translocation studies.

Groups	n	MLN	Spleen	Liver	Lung	Serum
C	10	0	0	4±1.2	2±1.1	2±0.9
S	10	4±0.9	0	0	3±1.2	0
I/R	10	742±61*	334±42*	682±83*	795±132*	420±27*
I/R+Glucan	10	212±29	174±14	322±45	275±95	186±23

C, Control; S, Sham; I/R ischemia/reperfusion; I/R+glucan, ischemia/reperfusion+glucan intramuscular.
* $p < 0,01$ compared to S, C, I/R+glucan

TABLE 2 - Magnitude (CFU per gram of tissue) of bacterial translocation to several organs and serum, comparing groups with and without β-(1-3)-D-glucan intramuscular.

Groups	n	MLN	Spleen	Liver	Lung	Serum
C	10	0	0	0	0	0
S	10	2 ± 0.2	0	0	4 ± 1.3	0
I/R	10	253 ± 32*	112 ± 12*	178 ± 18*	285 ± 21*	166.5 ± 32*
I/R + Glucan	10	74 ± 18	47 ± 9	82 ± 22	77 ± 12	69 ± 14

C, Control; S, Sham; I/R ischemia/reperfusion; I/R+glucam, ischemia/reperfusion+glucan intramuscular.
*p < 0.01 compared to C, S, I/R+glucan

TABLE 3 - Portal serum levels of cytokines comparing groups with and without β-(1-3)-D-glucan intramuscular.

Groups	n	TNF-α(pg/mL)	IL-1β(pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)
C	10	0	0	0	0
S	10	41,7 ± 9,4*	34 ± 11*	144 ± 17*	94 ± 21*
I/R	10	753.7 ± 91**	588.7 ± 100**	422.1 ± 56**	311 ± 52**
I/R + Glucan	10	98 ± 23	122 ± 19	110 ± 31	430 ± 42

C, Control; S, Sham; I/R ischemia/reperfusion; I/R+glucam, ischemia/reperfusion+glucan intramuscular.
*p<0.01 compared to I/R, and I/R+glucan
** p<0.01 compared to I/R+glucan

Discussion

The gut has been suggested to be a port of entry for bacteria after intestinal mucosal injury and endotoxin challenge[10]. The translocation process involves the initial attachment of the bacteria to the gut wall, which by itself can elicit production of cytokines and initiate the subsequent inflammatory response. Once intact microbes penetrate the mucosa, they may be transported to distant organs of even the systemic circulation[11]. As shown in the present study, bowel ischemia and reperfusion promoted bacteria translocation. In addition, when compared to the control and sham, this phenomenon was significantly higher for MLN, spleen, liver, lungs and, serum in all other groups. Redan et al[12] speculate that the route of BT is through lymphatics into the right side of the heart and then to the lung. The pulmonary vascular bed would then represents the first capillary system in which the translocated bacteria encounter circulating phagocyte cells. In fact, a great amount of colony-forming units of bacteria were found in the lung. The hypoxia, followed by change in intestinal barrier function, generates a vicious cycle of increased permeability, leading to toxic mediators release, and resulting in a further increase in gut permeability, facilitating the BT[13]. However, no significant difference in radioactivity and CFU were found when they were compared the S group, where the intestines were gently manipulated, and in the C group. In this study, increased serum levels of TNF-a, IL-1b, IL-6 and IL-10 reflected the ischemia/reperfusion injury, as demonstrated by other in vivo trials[14,15]. It has been suggested that IL-6 produced by intraepithelial lymphocytes is responsible for the loss of intestinal barrier function following hemorrhage, and the extent of loss can be correlated with plasma levels of this cytokine[16]. In the rats treated with soluble b-(1-3)-D-glucan it was observed a significantly different cytokine response, which was characterized by decreased production of TNF-a, IL-1b, and IL-6, suggesting that immunomodulation with soluble glucan might act to depress the inflammatory cytokine response. The decrease in secretion of these pro-inflammatory cytokines coincided with the increase in IL-10 expression and could, at least in part, be explained by the action of

this cytokine known to have anti-inflammatory activity. In fact, IL-10 has been shown to inhibit lipopolissacharide-induced monocyte tissue factor expression in whole blood [17] and to decrease TNF-a production in human monocytes[18]. In a model of murine *E. coli* sepsis, TNF-a and IL-1 levels in soluble glucan-treated mice were significantly lower than in untreated control animals[19]. The levels of radioactivity and colony forming units of bacteria on MLN, spleen, liver, lungs and, serum were lower I/R+glucan rats than the I/R ones, meaning that the use of soluble glucan resulted in an overall decrease in bacterial translocation.

Conclusion

Based on the present data, we conclude that stimulation of the reticuloendothelial system by soluble b-(1-3)-D-glucan modulated the production of pro-inflammatory and anti-inflammatory cytokines during intestinal ischemia/reperfusion, and attenuated the translocation of ^{99m}Tc labelled bacteria.

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3.5 Artigo V

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(Qualis Internacional C)

Simvastatin improves the healing of infected skin wounds of rats.

A sinvastatina melhora a cicatrização de feridas infectadas da pele de ratos.

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ABSTRACT

Purpose: This study explores the potential of the simvastatin to ameliorate inflammation and infection in open infected skin wounds of rats. **Methods**: Fourteen *Wistar* rats weighing 285±12g were used. The study was done in a group whose open infected skin wounds were treated with topical application of sinvastatina microemulsion (SIM, n=7) and a second group with wounds treated with saline 0.9 % (SAL, n=7). A bacteriological exam of the wounds fluid for gram positive and gram negative bacteria, the tecidual expression of TNFα and IL-1β by imunohistochemical technique, and histological analysis by HE stain were performed. **Results:** The expression of TNFα could be clearly demonstrated in lower degree in skin wounds treated with simvastatin (668.6 ± 74.7 μm^2) than in saline (2120.0 ± 327.1 μm^2). In comparison, wound tissue from SIM group displayed leukocyte infiltration significantly lower than that observed in SAL group ($p<0.05$). Culture results of the samples taken from wound fluid on fourth post treatment day revealed wound infection in only one rat of group simvastatin (SIM), where *Proteus mirabilis, Escherchia coli* and *Enterobacter sp* were isolated. In the rats whose wounds were treated with saline (SAL), polymicrobial infection with more than 100.000 CFU/g was detected in all the wounds. **Conclusion:** In addition to its antiinflammatory properties, the protective effects of simvastatin in infected open skin wounds is able to reduce infection and probably has antibacterial action. The potential to treat these wounds with statins to ameliorate inflammation and infection is promising.

Key words: Statin. Inflammation. Wound Healing. Wistar rat. Skin. Cytokine.

RESUMO:

Objetivo: O presente estudo avaliou o potencial da sinvastatina para atenuar a inflamação e a infecção em feridas abertas infectadas de pele de ratos. **Métodos**: Foram utilizados 14 ratos *Wistar* pesando 285±12g. O estudo foi realizado com um grupo de animais cujas feridas abertas infectadas foram tratadas com aplicação tópica de sinvastatina microemulsão (SIM, n=7) e um segundo grupo com feridas tratadas com solução salina 0,9% (SAL n=7). Foi realizado exame bacteriológico do fluido das feridas para detecção de bactérias gram positivas e negativas, a expressão tecidual de TNFα e IL-1β por imunohistoquímica e análise histológica pela coloração H-E. **Resultados:** A expressão do TNFα pode ser claramente demonstrada em menor grau nas feridas de pele tratadas com sinvastatina (668.6 ± 74.7 μm^2) do que no grupo salina (2120.0 ± 327.1 μm^2). Em comparação, os tecidos das feridas do grupo SIM mostrou infiltração leucocitária significantemente menor do que a observada no grupo SAL ($p<0,05$). O resultado das culturas realizadas no fluido das feridas no 4º dia de tratamento revelou infecção em apenas um rato do grupo sinvastatina (SIM), onde *Proteus mirabilis, Escherchia coli* e *Enterobacter sp* foram isolados. Nos ratos cujas feridas foram tratadas com solução salina (SAL), infecção polimicrobiana com mais de 100.000 UFC/g foi detectada em todas as feridas. **Conclusão:** Além de suas propriedades antiinflamatórias, o efeito protetor da sinvastatina em feridas abertas e infectadas de pele é capaz de reduzir a infecção e provavelmente tem ação antibacteriana. O potencial da droga para atenuar inflamação e infecção de feridas é promissor.

Descritores: Estatinas. Inflamação. Cicatrização de Feridas. Rato Wistar. Pele. Citocinas.

Introduction

The healing of open skin wounds involves the epithelium and underlying stroma. Processes such as angiogenesis, activation, migration, and proliferation of fibroblasts, myofibroblasts and endothelial cells; formation of granulation tissue; and wound contraction are needed to close these defects[1,2]. Some wounds are also frequently inflamed and, in general, stromal involvement and inflammation greatly increase the risk of subsequent complications[3,4] The repair process begins immediately after injury by the release of various growth factors, cytokines, and low-molecular weight compounds. Disruption of blood vessels also leads to the formation of the blood clot, which is composed of cross-linked fibrin, and of extracellular matrix proteins such as fibronectin, tenascin, and thrombospondin[5] .

Wound infection develops in 2% to 5% of patients undergoing surgical procedures each year in most hospitals world-wide and continues being considered one of the most important problems in surgical wards nowadays. It is one of the main factors that alter the physiologic evolution of the wound healing[6,7] The bacteria inhibit the angiogenesis, secrete plasminogen activators, and proteolitic enzymes that may affect the extracelular matrix, blocking the wound contraction[8]

Several substances have been used to treat infected skin wounds, like honey, sugar, antibiotics, phytotherapics[9,10,11,12,13]

Statins are a class of compounds that competitively inhibit the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the first committed step in

cholesterol biosynthesis. Increasingly, the pleiotropic properties of statins are being described. In endothelial cells, all of these effects seem to result from the inhibition of cholesterol's precursor mevalonic acid, which is critical to the isoprenylation of a diverse family of proteins[14,15]. Simvastatin, a HMG-CoA reductase inhibitor, have been shown to exhibit important immunomodulatory effects independent of lipid lowering[16]. These pleiotropic effects have been demonstrated to include anti-inflammatory actions[17]. improvement of endothelial and microvascular function, modulation of endothelial nitric oxide synthase (eNOS)[18], ischemia/reperfusion[19] and sepsis[20] . However, statins have not been used to treat skin infected wounds. We thus approached the question of whether topical treatment with simvastatin might improve the healing of skin infected wounds in a rat model.

Methods

The experimental protocol was approved by the Research Ethics Committee (Animal Research Ethics Division) of the University Hospital-UFRN, Brazil. Animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals, Brazilian College of Animal Experimentation.

Animals

Wistar rats (*Rattus norvegicus albinus, Rodentia mammalia*) weighing 282±14g were used. They were housed in polypropilene cages and maintained under controlled temperature conditions on a 12h light-dark cycle and allowed *ad libitum* access to commercially available rat chow (Labina, Purina®) and water.

Experimental design and procedures

The animals were randomly separated in two groups of seven rats each. After 12 hours of fasting, the rats were anesthetized by intramuscular injection of 30mg/Kg of ketamine and thiopental 20mg/Kg intraperitoneal. After dorsal skin depilation and anti-sepsis with 1% povidona, the surgical procedures were performed under aseptic conditions. An open skin squared wound ($1cm^2$), was done in the dorsal skin of all the rats. Immediately after the surgical procedure, the wounds were contaminated with topical application of 0.5mL of multibacterial solution prepared with 1g of rats fresh feces and 10mL of saline. In the following day, the infected wounds of the simvastatin group (SIM) (n=7) were topically treated with 0.2 ml of simvastatin microemulsion (10mg/mL) once a day. The wounds of group saline (SAL) (n=7) rats were treated with 0.2 ml of saline solution daily.

When the wounds were totally epithelialized, the healing time was recorded and the resection of the scar was performed under anesthesia. The healed tissues were used for histopathological study and immunohistochemical dosage of tumor necrosis factor-α (TNF-α) and interleukine-1 (IL-1β).

Immunohistochemical staining of TNF-α and IL-1β.

Immunohistochemical staining for TNF-α and IL-1β was performed on tissue samples obtained from the healing skin. These samples were fixed in 4% paraformaldehyde, processed in routine technique, cut into 5μm-thick frozen sections,

and dried at room temperature. Absolute methanol containing 3% hydrogen peroxide was added to block endogenous peroxidase. After three washings with a phosphate-buffered solution (PBS) for 5 min each, these sections were treated with 1% polyoxyethylene-10-octylphenyl ether in PBS for 20 min at room temperature. After washing in the same way, these were reacted with 100 μl of biotinylated anti-rat TNF-α monoclonal antibody (Pharmingen, San Diego, CA) or biotinylated anti-rat IL-1β monoclonal antibody (Pharmingen), diluted in 9 mL of PBS and 1 ml of whole goat serum at room temperature in a moist chamber for 2 h. After washing, the preparations were incubated, with two drops each of avidin solution and biotinylated peroxidase solution in 4.5 ml of PBS and 0.5 ml of 5% skim milk for 2 h at 37°C. After PBS rinsing, diaminobenzidine and nickel were applied for 8 min to achieve permanent color change. Six views were selected randomly for each section and observed under a light microscope (x100). The mean number of reactive cells in the six views was regarded as the data for each sample.

Sequential images of microscopic sections were photographed within 72 hours after immunostaining, by a digital camera (Sony, Tokyo, Japan) mounted on a light microscope (Olympus B-50, Tokyo, Japan) at a magnification of 100x, and saved in jpg file format. Images were then analyzed in ImagePro-Plus software (Media Cybernetics, LP, USA). Briefly, the entire area colored by cytokines was marked, and the total marked area was calculated. The area stained by the antibody of interest was identified and calculated by using the software color algorithm. The integrated optical areas stained by anti-bodys were then recorded. The score index was calculated for each of the antibodies and it was averaged.

Histopathology

The biopsies of skin wounds were processed following the routine and stained with hematoxilin and eosin (HE) for histological analysis of the inflammatory reaction, using the optical microscope Olympus B-50, Japan, Tokyo. The quantification of cells, fibers and elements of the inflammatory reaction was performed by the Image Pro-Plus Média Cybernetics software, LP, USA.

Bacteriological examination

At the 4^{th} postoperative day, exsudato was collected from the wounds for microbiology and for quantitation of bacterial population. The materials were processed and cultured on selective MacConkey's agar, blood agar and salt manitol agar. The agar plates were incubated at 37 ^{o}C and examined for growth after 24, 48 and 72 hours. Any growth in the plates of bacteria of the same biotype as cultured in the wounds was considered positive and expressed as colony-forming units per gram of tissue (CFU/g). All procedures were performed under laminar air flux.

Statistical analysis

Data are presented as the mean±standard deviation. Results were analyzed with ANOVA and Student t test. Statistical significance was assumed at $p< 0.05$.

Results

Tumor necrosis factor alpha (TNFα)

The expression of TNFα could be clearly demonstrated in lower degree in skin wounds treated with simvastatin (668.6 ± 74.7 μm^2) than in saline (2120.0 ± 327.1 μm^2) treated wounds, as can be shown in figure 1 and table 1. So, a distinct decrease of tissue reactivity occurred when the simvastatin microemulsion was applied to the infected wounds.

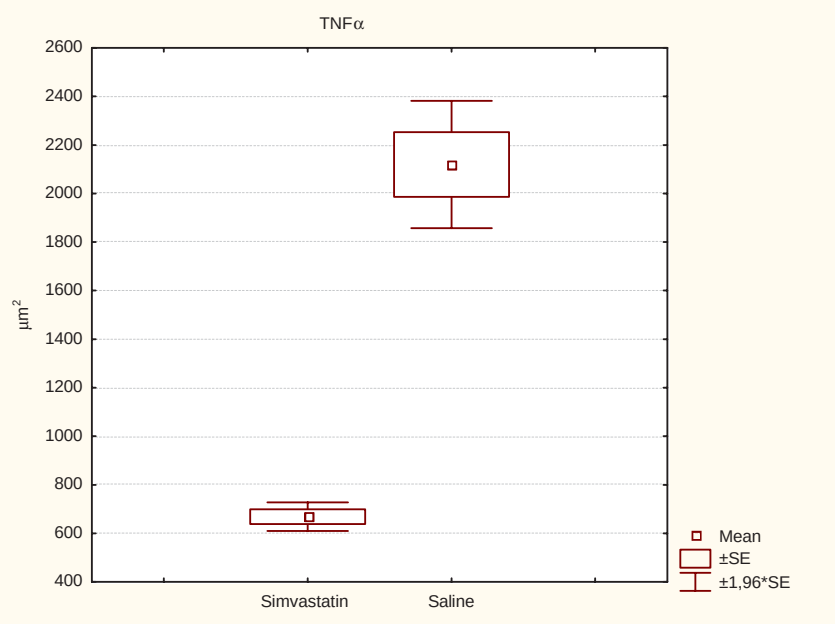

FIGURE 1 – Mean areas corresponding to the expression of TNFα. Significant difference comparing the groups simvastatin and saline ($p<0.05$).

Interleukin-1β

IL-1β expression was significantly more enhanced in the saline (SAL) group than in the Simvastatin (SIM) group after total epithelialization of the skin wounds. A clearly decreased immunohistochemical stainability could be noticed in the SIM group, whose data are expressed in figure 2 and table 1.

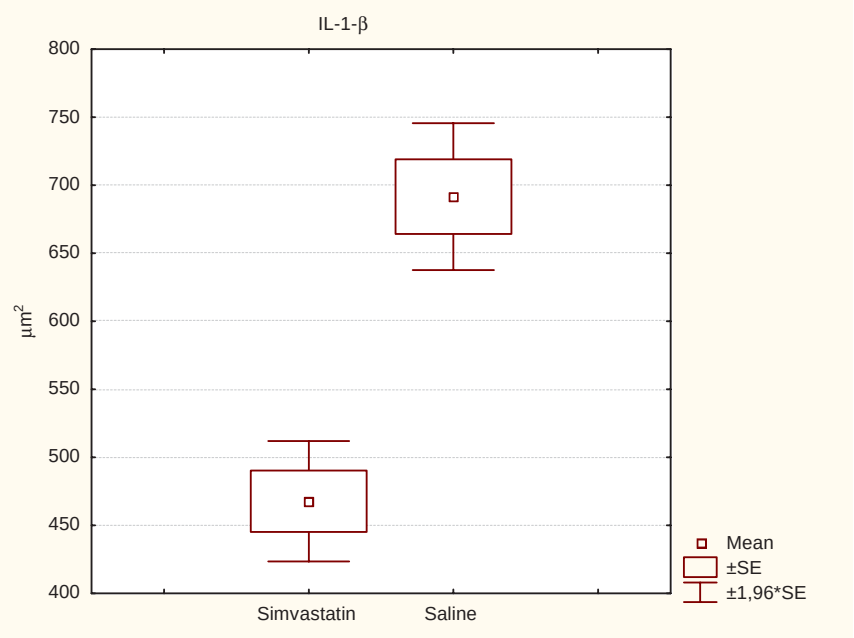

FIGURE 2 – Mean areas corresponding to the expression of IL-1β. Significant difference comparing the groups simvastatin and saline ($p<0.05$).

TABLE 1. Mean and statistical analysis of optical density related to the expression of cytokines in tissues of skin infected wounds treated with simvastatin and saline (μm^2)

Groups / Cytokines	Simvastatin (SIM)*	Saline (SAL)*	p-value
TNFα	668.6 ± 74.7	2120.0 ± 327.1	0.0000[1]
IL-1 β	467.6 ± 55.2	691.6 ± 67.4	0.0000[1]

* Mean ± Standard deviation
1 – Difference statistically significant comparing the groups SIM/SAL (Student t test).
Image Pro-plus software Media Cybernetics was used.

Histopathology

Contamination of skin wounds with multibacterial fecal solution caused intense inflammatory reaction in tissues of group SAL rats, with edema and marked leukocyte infiltration consistent with acute injury (Figure 3). In comparison, wound tissue from SIM group displayed leukocyte infiltration significantly lower than that observed in SAL group. ($p<0.05$). These pathologic changes were reduced by the administration of simvastatin topically in the infected wounds (Table 2) The histological slides (figures 3 and 4) suggest differences in neutrophil accumulation between the SAL and SIM groups.

TABLE 2. Histological grading based in the optical density related related to the inflammatory reaction in tissues of skin infected wounds treated with simvastatin and saline (μm^2)

Groups / Inflammation	Simvastatin (SIM)*	Saline (SAL)*	p-value
Inflammatory reaction	844.7 ± 65.2	3416.1 ± 233.4	0.0000[1]

* Mean ± Standard deviation

1 – Difference statistically significant comparing the groups SIM/SAL (Student t test).
Image Pro-plus software Media Cybernetics was used.

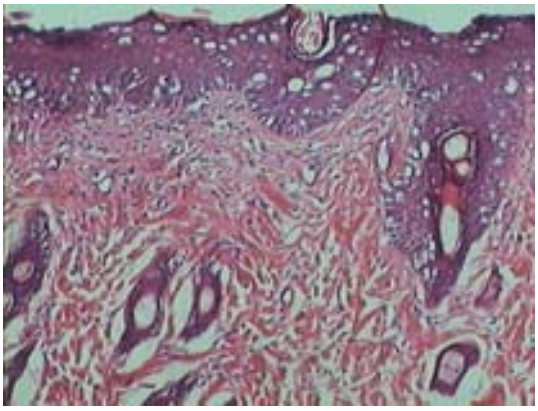

FIGURE 3 – Histological section of wound tissue taken from a SAL group rat, demonstrating significant neutrophil infiltration, giant cells and folicules. HE, 100x.

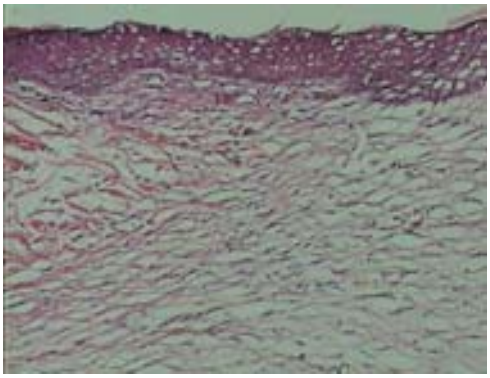

FIGURE 4 – Histological section of wound tissue taken from SIM group rat, demonstrating low neutrophil infiltration and good epithelial regeneration. HE, 100x.

Bacteriological examination

Culture results of the samples taken from wound fluid on fourth post treatment day revealed wound infection in only the rat number 4 of group simvastatin (SIM), where *Proteus mirabilis, Escherchia coli* and *Enterobacter sp* were isolated. In the rats whose wounds were treated with saline (SAL), polymicrobial infection with more than

100,000 CFU/g was detected in all the wounds (Table 3). The most frequently isolated microorganisms can be observed in table 3.

TABLE 3 - Detection of bacteria in the wound fluid of animals in groups treated with simvastatin (SIM) and treated with saline (SAL).

Rat number	SIM Group / bacteria	CFU/g	SAL Group/ bacteria	CFU/g
1	NBG	0	Klebsiella sp; Proteus Mirabilis; Proteus vulgaris; Staphylococcus coagulase negative	> 100,000
2	NBG	0	Proteus mirabilis; Escherichia coli; Staphylococcus coagulase negative	90,000
3	NBG	0	Proteus mirabilis; Escherichia coli; Staphylococcus coagulase negative	> 100,000
4	Proteus mirabilis; Escherichia coli; Enterobacter sp	50,000	Klebsiella sp; Proteus mirabilis	>100,000
5	NBG	0	Escherichia coli; Enterobacter sp; Proteus mirabilis	> 100,000
6	NBG	0	Proteus mirabilis; Escherichia coli	> 100,000

NBG, No bacterial growth; CFU/g, Colony forming unit per gram.

Discussion

Cutaneous wound healing is a complex process involving blood clotting, inflammation, new tissue formation, and finally tissue remodeling[21]. It is well described at the histological level and many experimental and clinical studies have demonstrated varied, but in most cases beneficial, effects of exogenous substances[11,12,13] on the healing process. However, the roles played by these exogenous treatments have remained largely unclear.

HMG-CoA reductase inhibitors (statins) are used clinically for lowering hypercholesterolemia because of their inhibitory effect on hepatic biosynthesis of cholesterol at the mevalonate step[22]. These statins such as simvastatin have been shown to exhibit important immunomodulatory effects independent of lipid lowering[23] These pleiotropic effects have been demonstrated to include anti-inflammatory actions[24].

The present study is one of the first to demonstrate that HMG-CoA reductase inhibitors significantly improve healing of infected skin wounds in an experimental model in rats. The improvement in inflammatory reaction and in cytokines expression corroborates some results in the literature that clearly demonstrate that simvastatin is a potent and effective endothelium-protective agent that reduces leukocyte–endothelial cell interactions independently of its well-known lipid-lowering effects. This effect has been found to be at least partially mediated via downregulation of P-selectin expression on the microvascular endothelium. Thus, HMG-CoA reductase inhibitors like simvastatin have important anti-inflammatory effects besides their well-known lipid-lowering action[25,26].

In the present study we demonstrated that the topical application of simvastatin microemulsion attenuated the inflammatory reaction in wound healing of infected tissues, but to date the mechanism is not clear. Prueffer et al[27].demonstrated a protective effect of simvastatin under conditions of acute inflammation induced by an exotoxin

within the microcirculation. In particular, they provide strong evidence that simvastatin is able to attenuate enhanced leukocyte-endothelium interaction after *S aureus* toxin administration. Pore-forming *S aureus* toxin is known to provoke inflammatory acctivation[28,29,30]. These evidences may explain hour results with the topical use of simvastatin in the healing of infected wounds.
Statins affect the production of many acute phase reactants, including CRP, which is produced in the liver under stimulation by cytokines (IL-1 and IL-6). In nonatherosclerotic huCRP transgenic mice, statins decreased basal and IL-1*b*-induced plasma huCRP levels independently of cholesterol lowering and of an effect on IL-6 production[31] . In fact, in this work simvastatin was able to induce a marked decrease in TNFα anf IL-1β in healing tissues, as demonstrated by immunohystochemical analysis. A probable antibacterial effect was also observed, and the exact mechanism to explain this action is to be described. In an other study the pretreatment was found to decrease cytokine-stimulated transcription factor activation and iNOS expression in the endothelium, stating that simvastatin affect cytokines with several ways[32].

Conclusion

In addition to its antiinflammatory properties, the protective effects of simvastatin in infected open skin wounds is able to reduce infection and probably has antibacterial action. The potential to treat these wounds with statins to ameliorate inflammation and infection is promising.

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3.6 Artigo VI

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(Qualis internacional B)

Comparing Reconstruction with Ileocecal Graft to Jejunal Interposition Pouch after Total Gastrectomy in rats.

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ABSTRACT After total gastrectomy, the ileocecal graft could act as a reservoir, protect against reflux but gives rise to transposition of the ileum and causes possible changes in bile acid metabolism and nutrition. This study compared the ileocecal graft and jejunal pouch. Male Wistar rats weighing 265±22g were submitted to sham operation (S), Ileocecal interposition graft (IIG) and jejunal pouch interposition graft (JP) after total gastrectomy. Eight weeks later the esophagus was examined for evidence of esophagitis. Nutritional biochemistry and weight profile were documented preoperative and 8 weeks after surgery. Oral glucose tolerance test was investigated. Thirty three rats were operated on and thirty survived for eight weeks. Esophagitis occurred in 7 JP rats. Body weight was significantly higher in IIG than JP rats ($p<0.05$). Normal glucose tolerance to intragastric glucose load in sham and operated rats was observed, meaning a normal emptying from stomach and pouches. JP rats had a significant decrease in serum albumin, glucose, transferrin, hemoglobin, iron, folate and calcium, compared to sham ($p<0.05$). Cobalamine was significantly lower in IIG rats than in JP ($p<0.05$). In the IIG and JP groups, serum/hepatic total bile acid did not differ significantly from preoperative and sham values. In conclusion, the IIG interposition graft in rats prevented esophagitis, preserved nutrition and did not interfere with enterohepatic total bile acid circulation.

KEYWORDS total gastrectomy, ileocecal graft, jejunal pouch, interposition, nutrition, bile acid, esophagitis, reflux.

Total gastrectomy is one of the most used operative procedures for gastric cancer treatment.[1] It is followed by a variety of symptoms and impairments, commonly referred to as postgastrectomy syndromes. [2] One important feature of the postgastrectomy syndromes is considerable body weight loss.[3,4] The loss averages around 25% of preoperative body weight [4,5] and leaves 60% to 70% of patients

permanently below ideal body weight. [3,6] Total gastric resection and the selection of the appropriate operation for the reconstruction of the passage between the esophagus and the intestine is controversial. Many different types of reconstruction after total gastrectomy have been proposed, but a great deal of research is still being done to validate the potential advantages of various procedures. The main focus of such reconstruction should be to retain the nutritional status and quality of life of the patient, and also to achieve similar function to that of a normal gut.

The preservation of the duodenal passage is considered important. It has been hypothesized that passage of food across the duodenum, resulting in the mixture of chyme with biliary and pancreatic secretions, aids in digestion, absorption, and stimulation of the remaining intestinal tract.[7,8] These processes should result in better calcium and iron absorption with improved lipid and protein digestion. [9] The formation of an appropriate replacement gastric reservoir to simulate pre-operative gastric volume is considered important.

In the present study a comparison was done between the ileocecal segment and the jejunal pouch as esophagus-duodenum interpositional grafts for replacing the stomach. Because experience with these pouches is limited and the ileocecal graft could act as a reservoir and protect against reflux and lead to transposition of the ileum and cause possible changes in bile acid metabolism and nutrition, we investigated the effect of the two procedures on esophagus histology, gastric emptying, nutritional state and serum bile acid in rats.

MATERIALS AND METHODS

Male Wistar rats weighing 265 to 315 g, were randomly divided in three groups and used for experiments. Rats were housed under controlled conditions of illumination (12/12 hours light/dark cycle), humidity (60–70%), and temperature (21°C). The International guidelines for the care and use of laboratory animals were followed throughout the study. All the surgical procedures were performed by the same investigator, a well trained and experienced surgeon in animal surgery.

Surgical Procedures

a)Sham: (S) Sham operation was performed by 3 cm midline laparotomy under ketamine hydrochloride (100 mg/kg) and xylazine (15 mg/kg) anesthesia. The stomach and the intestine were covered with saline-moistened gauze for 40 minutes, which corresponds to the time period required for the other surgical procedures.

b) Ileocecal interposition graft: (I IG) The total gastrectomy was done and the ileocolon was isolated (Ileum 5 cm; cecum and colon 10 cm). After transection of the proximal colon and ileum, the ileocecal vascularized segment was rotated 180º clockwise upward. Intestinal continuity was restored with an ileocolic anastomosis. The colonic end of the ileocecal graft was sewn to the end of the duodenal stump, (Figure 1) and the ileum was anastomosed with the transected esophagus using single stitches (6-0 polipropilene). Anastomosis were performed by using a (9x) binocular microscope (DF Vasconcelos®, Brazil).

c) Jejunal pouch interposition graft: (JPIG) After total gastrectomy, the jejunum was divided 5 cm distal to the ligament of Treitz. The digestive reconstruction method included interposition of a jejunal pouch reservoir between the esophagus and duodenum, preserving the duodenal passage. The gut was divided again 10 cm more distally and a jejunojejunostomy was performed. A pouch was created at the proximal end of the jejunal graft and an end-to-side anastomosis to the esophagus was done

(Figure 2). The aborad end of the jejunal graft was anastomosed with the duodenum by single stitches, (6-0 polypropilene).

After the operations, the rats had an infusion of Ringer 10mL/Kg intraperitoneal and free access to oral glucose 10% was permitted. Rats resumed normal diet on the third postoperative day. They were weighed on the same scale every two weeks. A recovery period of eight weeks was allowed for all operated animals before the following experiments were commenced.

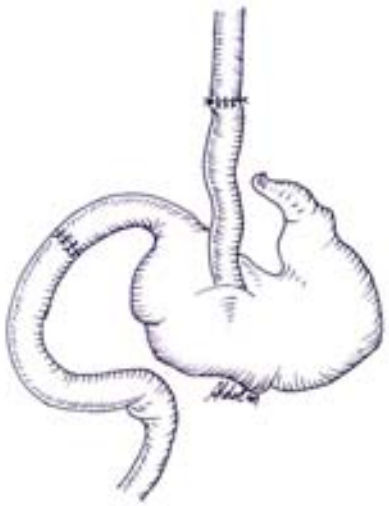

Figure 1. Ileocecal pouch. Esophagus is anastomosed to terminal ileum; Proximal colon is anastomosed to duodenum.

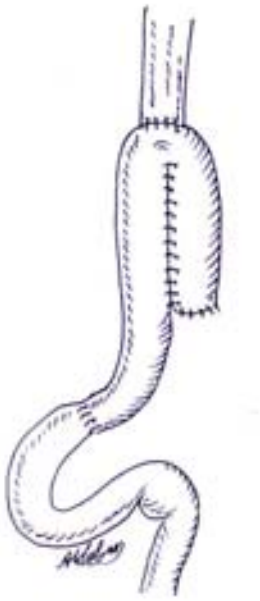

Figure 2. Jejunal interposition pouch. Esophagus is anastomosed to pouch and jejunum to duodenum.

Histological procedures

After eight weeks the rats were killed with an overdose of anesthetic and tissue samples were taken from the distal esophagus (1 cm from the anastomosis) to examine for the presence of esophagitis. They were fixed in 10% formalin, embedded in paraffin, cut at 4μm, and stained with hematoxylin and eosin. The following parameters were considered: 1- loss of surface epithelium; 2- neutrophil infiltration; 3 - increased height of the basal cell layer of the squamous epithelium; 4 - increased depth of the papillae. Diagnosis of esophagitis was positive when 2 or more of these parameters were present.

Oral glucose tolerance test

After overnight fasting, on the day of animal sacrifice, blood sample was taken from tip of the tail vein from all the rats in S, IIG, and JP groups. After the fasting blood glucose was determined, the animals were loaded by gavage with a solution composed of 3.5 g glucose/kg body wt dissolved in distilled water. Blood samples were taken from tail vein at 30th, 60th, 90th and 120th minute after glucose administration and blood glucose levels were assessed by single touch glucometer (Accu-chek, Roche Diagnostics, Mannheim, Germany).

Laboratory tests

Laboratory measurements were performed before and eight weeks after the operations. Serum levels of total proteins, albumin, cholesterol, triglyceride,

hemoglobin, iron, transferrin, folate, cobalamine, and calcium were determined with an autoanalyzer (Weiner Lab BT Plus 3000).

Determination of serum and liver bile acid concentration

Two hundred milligrams of liver obtained from biopsies at day zero and 60th postoperative were hydrolyzed with 10% KOH in 90% ethanol at 80ºC for 2 hours. Bile acids were extracted on Bond Elut Cartridge C^{14} columns and total bile acid concentration in the liver tissue was determined by the 3α-hydroxysteroid dehydrogenase method [10]. The serum total bile acid was analyzed by gas-liquid chromatography. A Hewlett-Packard gas chromatograph equipped with a 30 m, 0.25-mm inner diameter fused silica column coated with a 0.25-µm layer of cross-linked methyl silicone was used.

Statistics

For statistical analysis the chi-square test with Yates' correction and one way ANOVA with Newman-Keuls and Tukey tests were used. Differences were considered significant at $p<0.05$ in the two-tailed tests.

RESULTS

Thirty three rats were operated on. Thirty survived for eight weeks. One died in the early postoperative period in the IIG group, and 2 of the JP group died before the fourth week (Table 1). The difference in mortality was not significant ($p>0.05$).

TABLE 1 Operative mortality

Groups	Operated rats (n)	Mortality n(%)	Survived after 8 weeks
IIG	11	1 (9%)	10
JP	12	2(16.6%)*	10
Sham	10	0 (0.0%)	10
Total	33	3 (9%)	30

* $p>0.05$ compared to IIG group. Values are expressed as mean±SEM; IIG, Ileocecal interposition graft; JP, jejunal pouch interposition graft.

TABLE 2 Body weight of rats before and after operation (g)

	Sham	IIG	JP
Preoperative	268.3±10.6	277.5±12.4	278.6±11.2
2nd week	284.7±13.0	253.7±10.3	265.3±10.6
4th week	313.2±12.3	268.3±10.8	262.7±12.3
6th week	370.8±17.4	287.0±14.5	261.2±12.0
8th week	388.2±17.8	317.5±13.1*	255.4±14.7

* $p<0.05$ compared to JP group. Values are expressed as mean±SEM; IIG, Ileocecal interposition graft; JP, jejunal pouch interposition graft.

TABLE 3 Laboratory findings at preoperative and after eight weeks (60 days)

Laboratory parameter	Group	Preoperative	60 days
Albumin (g/dL)	Sham	3.5±0.5	3.8±0.3
	IIG	3.2±0.2	3.0±0.2
	JP	3.6±0.4**	2.8±0.3*
Glucose (mg/dL)	Sham	102±14.2	98±7.3
	IIG	90±12.1	86±10.7*
	JP	104±12.3**	75±14.1*
Transferrin (mg/dL)	Sham	233±22.4	230±28.1
	IIG	224±26.8§	183±18**
	JP	230±23**	151±26.3*
Hemoglobin (g/dL)	Sham	14±2.5	13±1.6
	IIG	13±3.2	11±1.7
	JP	13.8±3.0**	10±1.1*
Iron (µg/dL)	Sham	280±18	276±25
	IIG	264±28.4	262±17.7
	JP	269±12**	247±32.1*
Folate (ng/mL)	Sham	8.6±3.3	8.2±1.9
	IIG	8.4±2.2	7.5±2.5
	JP	8.0±3.4**	5.9±0.9*
Cobalamine (pmol/L)	Sham	223±21	214±27.8
	IIG	234±32§	161±18*
	JP	218±27.2	183±16.7
Calcium (mg/dL)	Sham	8.5±0.8	9.7±1.8
	IIG	9.3±0.9	7.4±3.1
	JP	8.8±1.4**	6.4±2.2*
Cholesterol (mg/dL)	Sham	166±21.4	170±14.2
	IIG	173±23.3§	156±12.7
	JP	170±15.6	167±22.3
Triglycerides (mg/dL)	Sham	147±15.5	133±18.4
	IIG	140±19.4§	126±11.6
	JP	153±21.2	144±15.7

Values are expressed as means±SEM; IIG, Ileocecal interposition graft; JP, jejunal pouch interposition graft.

* $p<0.05$ compared to sham 60 days; § $p<0.05$ compared to IIG 60 days; ** $p<0.05$ compared to JP 60 days.

TABLE 4 Total serum and hepatic bile acid concentration (nmol/ml)

Laboratory bile acid	Group	Preoperative	60 days
Serum bile acid nmol/ml	Sham	30.4±8.2	33.2±8.5
	IIG	35.3±4.0	29.7±6.4*
	JP	34.1±7.3	37.5±10.2**
Hepatic bile acid	Sham	51.6±12.0	48.7±9.3
	IIG	47.4±10.6	34.2±12.1*
	JP	40.2±17.3	36.6±15.0**

Values are expressed as means±SEM; IIG, Ileocecal interposition graft; JP, jejunal pouch interposition graft.
*p>0.05 compared to preoperative; p>0.05 compared to sham and IIG.

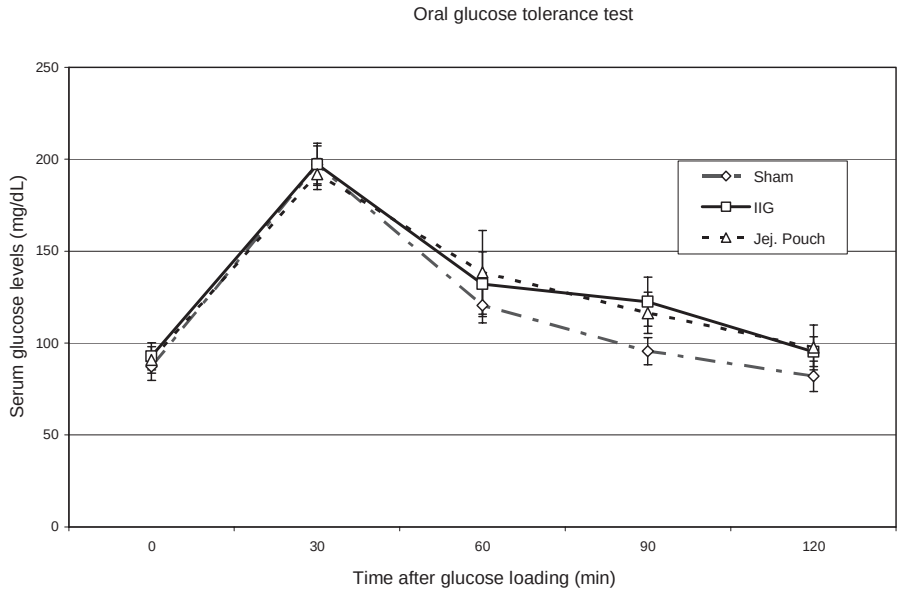

Figure 3. The oral glucose tolerance test in sham, IIG and JP groups: serum glucose had a significant elevation after oral administration of glucose, and peaked in 30 min. In all the groups, serum glucose level of 185 mg/dl or more decreased significantly at 60, 90 and 120 min. Comparing the sham, IIG and JP groups at each point in time, the difference was not significant (p>0.05).

Postoperative esophagitis occurred in 7 rats of the JP group. Loss of surface epithelium, neutrophil infiltration and, increased depth of the papillae were found in 3 rats of this group. Increased height of the basal cell layer of the squamous epithelium and neutrophil infiltration were found in 4 rats. The IIG group had 2 rats with low height increase of the basal cell layer, and the sham rats had no signs of histological esophagitis.

The oral glucose tolerance test in sham, IIG and JP groups showed that the level of serum glucose had a significant elevation after intragastric administration of glucose. In all the groups, serum glucose level of 185 mg/dl or more at 30 minutes decreased significantly and from 90th minutes, this parameter was near the initial level (Figure 3). Differences between the sham, IIG and JP groups at each point in time were shown to

be not significant ($p>0.05$). These results suggest that a normal glucose tolerance to intragastric glucose load in sham and operated rats may primarily be due to normal gastric emptying, and that glucose absorption from small intestine was not significantly changed in rats.

No significant difference in the body weight of rats before surgery was detected among sham, IIG and JP groups. At Two, four, six and eight weeks post-surgery, the body weight in S rats was significantly higher than in IIG and JP rats ($p<0.05$). In S rats an increasing body weight was observed from preoperative to eighth week, while in the JP group, the body weight constantly decreased (Table 2). At second and fourth week postoperatively, the weights of IIG and JP rats were similar, while at eighth week the body weight was significantly higher in IIG than in JP rats ($p<0.05$). Although the weight of the IIG rats was not as high as that of the sham group, it still increased significantly compared to preoperative weight (317.5 ± 13.1 vs 277.5 ± 12.4, $p<0.05$). Nevertheless, the rats in the JP group lost weight compared to preoperative weight (278.6 ± 11.2g vs 255.4 ± 14.7g, $p<0.05$) and they were significantly lower at eighth week than those of the S and IIG groups ($p<0.05$).

The JP interposition graft was associated with a significant decrease in serum albumin, glucose, transferrin, hemoglobin, iron, folate and calcium concentration in the 60^{th} postoperative day, when compared to sham-operated rats ($p<0.05$). Parameters concerning cholesterol and triglycerides serum levels were unaffected when comparing JP to S and IIG rats (Table 3). JP rats in the preoperative period, as compared to the 60^{th} day, displayed a significant decrease in serum albumin, glucose, transferrin, iron, folate, cobalamine and calcium ($p<0.05$). Glucose and cobalamine serum concentrations were significantly reduced in IIG rats when compared to S rats on the 60^{th} postoperative day ($p<0.05$). The cobalamine serum level was significantly lower in IIG than in JP rats. ($p<0.05$). In IIG rats, transferrin, cobalamine, cholesterol and triglycerides showed a significant decrease when comparing the preoperative and 60^{th} postoperative levels ($p<0.05$).

The total serum bile acids for each group is presented in table 4. Although the bile acids showed a trend toward a decrease 60 days following surgery in the IIG group, this did not represent a significant decrease compared with that determined prior to the operation ($p=0.12$). There was a trend toward an increase in the serum total bile acids in the JP group postoperatively, but the difference was not significant ($p=0.062$). In both IIG and JP groups, the total serum bile acid concentration behaved in a fashion similar to that of the hepatic bile acids. In sham rats, total serum and hepatic bile acid concentration did not differ significantly from the preoperative values.

DISCUSSION

Currently, there is no agreement with regard to the ideal reconstruction type after total gastrectomy and it have long been a controversial issue. The importance of the duodenal passage [7] and the need for pouch reconstruction are matters of controversy [18,19]. Further randomized and standardized studies are necessary to fully elucidate the optimum approach in restoring intestinal continuity after total gastrectomy. In 1951, Lee [11] began his experiments in dogs using the ileocecal segment as a gastric replacement, and later used it on the first human. Hunnicutt [12] performed 4 operations on humans with minimal risks. The ileocecal segment has rarely been used with this finality, but some studies have reported good results in children[13] and in adults [14, 15,16,17]. In this study we tested two interpositional pouches with reestablishment of the duodenal passage, whose advantages have been frequently described [7,20]. After

total gastrectomy the primary problems are loss of reservoir function, nutrition deficit, exclusion of the duodenal route, and intestinal alkaline reflux. The postgastrectomy reconstruction procedures differ primarily in the origin of the graft (small or large intestine), configuration and size of the reservoir, and the preservation or exclusion of duodenal passage. The optimal stomach replacement should sufficiently preserve the digestive function and nutrition.

Our study showed that the mortality of construction of a jejunal pouch after total gastrectomy did not differ of ileocecal graft, both interpositioned between the esophagus and the duodenum. The fatal complications were apparently minimized because all the surgical procedures were performed by a trained investigator, experienced in animal surgery. The benefits of certain anatomic advantages of the IIG over the JP procedure included similar caliper of the terminal ileum and terminal esophagus, as well as of the proximal duodenum and proximal colon, making tension-free anastomosis feasible.

The oral glucose tolerance test is considered to be an indirect measure of emptying of liquids, and this test results showed no significant differences between the values in rats which underwent IIG, JP and sham operation. These findings are consistent with those previously reported by Sakamoto et al [15], who showed no significant differences between the 50g oral glucose tolerance test values in patients subjected to ileocecal graft after total and partial gastrectomy.

Histologic signs of esophagitis were rarely found in IIG rats, probably because the ileocecal valve effectively functioned, preventing the regurgitation of bile and pancreatic juice into the lower esophagus. Using (the) IIG in two patients, von Flüe [14] reported that reflux was not provoked even in the anti-Trendelenburg position. Pediatric surgeons have reported excellent results in replacing the total esophagus with the retrosternal ileocolonic conduit. None of the 8 operated children had aspiration pneumonia or gastroesophageal reflux, despite suture location at the cervical esophagus [13]. No patient reported reflux symptoms or showed endoscopic findings of reflux esophagitis in 23 patients subjected to total gastrectomy and subdiaphagmatic ileocecal interpositional graft [17]. Similar results were reported with 47 patients, supported by manometry and acid loading pH-metry [15].

In the present study the rats constantly lost weight in the JP group from preoperative to 8^{th} week, but rats in the IIG group gained weight from the 4^{th} to 8^{th} postoperative week. These results revealed that the jejunal pouch interposition procedure may affect the nutritional status of rats, when compared to sham and IIG rats. As the ileocecal pouch in rats has higher reservoir quality than the jejunal pouch, it seems that IIG is superior to JP to maintain nutritional status after total gastrectomy. Schwarz et al[7] concluded that the reconstruction method with the installation of a pouch preserving the duodenum passage resulted in a statistically significant increased quality of life after gastrectomy but, pouch volume itself had no influence on the results. Serum iron levels depend on the duodenum passage because of iron absorption in the duodenum and proximal jejunum [21].

In our study the serum iron levels did not alter after total gastrectomy in the IIG rats, compared to the sham rats. Some studies have showed that serum iron is initially low and returns to normal after total gastrectomy [4, 22]. Schwarz et al [7] showed that serum iron levels fell markedly after gastrectomy without preservation of the duodenal passage, being about 45% lower than in the reconstruction groups with preservation of the duodenal passage. We observed that, since IIG preserves the duodenal passage, and gives rise to a high quality reservoir, the serum levels of iron, albumin, glucose, folate and calcium were unaffected, when comparing the preoperative and 60^{th} postoperative days. The cobalamine serum level was significantly lower in IIG rats on the 60^{th}

postoperative day than JP and S rats, possibly because of ileum transposition and total gastrectomy. Cobalamine deficiency may develop early after total gastrectomy, before the onset of anemia [23]. In a study of 31 patients with cobalamine deficiency after total gastrectomy, the authors concluded that prophylactic administration should be initiated immediately to prevent anemia and symptoms associated with cobalamine deficiency [24].

Iron absorption is elevated in mice with hypotransferrinaemia [25], possibly caused by an effect on progenitor enterocytes in the crypts or in the mechanism of its absorption in villus enterocytes. Iron uptake from plasma transferrin is directly correlated with plasma iron concentration that, in turn, is affected by storage iron levels [26]. This is in keeping with the hypothesis that the regulation of iron absorption by storage iron levels is determined by the amount of iron acquired by precursor absorptive cells while they are in the crypts. These facts may explain the unaffected iron serum levels associated with hipotransferrinaemia after IIG in the present study.

Tsuchiya et al [27] studied whether ileal transposition affects the absorption and transport of lipids and bile salts in rats and demonstrated that transposition attenuates cholesterol absorption and transport, possibly by promoting premature absorption of bile salts. It is known that active transport of bile acids is limited to the ileum and passive diffusion is higher in the ileum than in the jejunum [28]. Despite the advantages of the IIG reconstruction method, there has been no evaluation in the literature studying the enterohepatic metabolism. In the present study a transposition of terminal ileum was performed in the IIG rats and the serum and hepatic total bile acids were not significantly affected, apparently suggesting that the enterohepatic metabolism was not altered. This may be explained by the fact that in rats, the proximal small intestine is as important in the absorption of bile acids as the ileum [29]. It was demonstrated in Rhesus monkeys that, while ileal resection appreciably reduced bile salt secretion, enterohepatic circulation was by no means abolished. Bile salt reabsorption from the residual intestine was greater after one-third than after two-third small bowel resection. These observations suggest that jejunal reabsorption of bile salts occurs and may well contribute to normal enterohepatic circulation [30]. After proctocolectomy, it was shown that the transposed terminal ileum possesses a high capability for absorption of bile acids and sodium following the terminal ileal transposition procedure [31]. Moreover, the mid-jejunum also showed a high rate of absorption for bile acids, glucose, and chloride after this procedure. Another study has suggested that the mucosal weight and DNA content of the terminal ileum, duodenum, and pancreas are increased following ileo–jejunal transposition. [32].

In conclusion, the IIG interposition graft in rats had low mortality, prevented esophagitis, resulted in good pouch emptying, preserved nutrition and did not interfere with enterohepatic total bile acid circulation.

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3.7 Artigo VII

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(Qualis internacional B)

Prevalence of *Helicobacter pylori* infection in advanced gastric carcinoma

Associação do carcinoma gástrico avançado com infecção por *Helicobacter pylori*

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ABSTRACT

BACKGROUD: There is substantial evidence that infection with *Helicobacter pylori* plays a role in the development of gastric cancer and that it is rarely found in gastric biopsy of atrophic gastritis and gastric cancer. On advanced gastric tumors, the bacteria can be lost from the stomach. **AIMS:** To analyze the hypothesis that the prevalence of *H.pylori* in operated advanced gastric carcinomas and adjacent non-tumor tissues is high, comparing intestinal and diffuse tumors according to Lauren's classification **METHODS:** A prospective controlled study enrolled 56 patients from "Hospital Universitário", Federal University of Rio Grande do Norte, Natal, RN, Brazil, with advanced gastric cancer, treated from February 2000 to March 2003. Immediately after partial gastrectomy, the resected stomach was opened and several mucosal biopsy samples were taken from the gastric tumor and from the adjacent mucosa within 4 cm distance from the tumor margin. Tissue sections were stained with hematoxylin and eosin. Lauren's classification for gastric cancer was used, to analyse the prevalence of *H. pylori* in intestinal or diffuse carcinomas assessed by the urease rapid test, IgG by ELISA and Giemsa staining. *H. pylori* infected patients were treated with omeprazole, clarithromycin and amoxicillin for 7 days. Follow-up endoscopy and serology were performed 6 months after treatment to determine successful eradication of *H. pylori* in non-tumor tissue. Thereafter, follow-up endoscopies were scheduled annually. Chi-square and MacNemar tests with 0.05 significance were used. **RESULTS:** Thirty-four tumors (60.7%) were intestinal-type and 22 (39.3%) diffuse type carcinomas. In adjacent non-tumor gastric mucosa, chronic gastritis were found in 53 cases (94.6%) and atrophic mucosa in 36 patients (64.3%). All the patients with atrophic mucosa were *H. pylori* positive. When examined by Giemsa and urease test, *H. pylori* positive rate in tumor tissue of intestinal type carcinomas was higher than that in diffuse carcinomas. In tumor tissues, 34 (60.7%) *H. pylori*-positive in gastric carcinomas were detected by Giemsa method. *H. pylori* was observed in 30 of 56 cases (53.5%) in tissues 4 cm adjacent to tumors. This difference was not significant. Eradication of *H. pylori* in non-tumor tissue of gastric remnant led to a complete negativity on the 12th postoperative

month **CONCLUSIONS:** The data confirmed the hypothesis of a high prevalence of *H. pylori* in tumor tissue of gastric advanced carcinomas and in adjacent non-tumor mucosa of operated stomachs. The presence of *H. pylori* was predominant in the intestinal-type carcinoma.

Headings: Stomach neoplasms. Carcinoma. Helicobacter infections.

INTRODUCTION

Gastric carcinoma is one of the most common human malignant cancers in the world. There is substantial evidence that infection with the gastric bacterium *Helicobacter pylori* plays a role in the etiology of gastric cancer[2, 48]. The International Agency for Research on Cancer, sponsored by the World Health Organization, has categorized *H. pylori* infection as a definite human carcinogen since 1994[19]. Some years after that decision, it is well established that persistent infection with *H. pylori* is associated with an increased risk for gastric malignancies[11, 17].

The magnitude of the risk of gastric cancer associated with infection remains unclear and there have been suggestions that this risk varies with sex[14, 32], age[30], and the histological subtype of the cancer[33]. There is evidence that *H. pylori* is frequently found in gastric biopsy specimens from individuals with atrophic gastritis, intestinal metaplasia and gastric cancer, and that with the development of advanced gastric tumors, the bacteria can be lost from the stomach[20, 21]. With loss of infection, the level of circulating anti-*H. pylori* antibodies will fall, so that patients with gastric cancer may be *H. pylori* seronegative even though they have been infected in the past[10]. Most researchers believe that the pathogenesis of human gastric cancer is a multifactorial and multistage process[13, 19, 22]. Recent studies linked cytokine gene polymorphisms to *H. pylori*-related gastric cancer development[23, 37]. RAD et al.[37] observed that pro-inflammatory IL-1 polymorphisms (IL-1RN2[+]/IL-1B-511T/-31C[+]) were associated with increased IL-1b expression, more severe degrees of inflammation, and an increased prevalence of intestinal metaplasia and atrophic gastritis. LU et al.[24] observed that the risk of gastric cancer was significantly elevated in subjects with the IL-8-251 AA or IL-10-1082 G or TNFa-308 AG genotypes. These findings suggest that genetic polymorphisms in IL-8, IL-10, and TNF-a may play important roles in developing gastric cancer in the Chinese population. YANG et al.[51] reported that, in Chinese population, the risks associated with the IL-1b variant genotypes were 1.64 for -31TT and 1.52 for -511CC, respectively, compared with their wildtype homozygotes. The risks were significantly more evident in individuals with *H. pylori* infection, which was consistent with the biological effects of IL-1b.

H. pylori cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) proteins interact with multiple host proteins, although downstream signaling events need further characterization. It does appear, however, that CagA may participate in a negative feedback loop on Src family kinases to prevent further phosphorylation of CagA. ARGENT et al.[1] reported that *H. pylori* strains that deliver CagA with more phosphorylation motifs induce higher levels of CagA phosphorylation in epithelial cells, induce more cytoskeletal changes, and are more likely to be associated with gastric cancer. Functional variability of *cagA* gene has been reported in Japanese isolates of *H. pylori*[15]. The BagA2 and CagA genes were investigated in 208 Brazilian *H. pylori* strains. A strong association between BabA2 and duodenal ulcer or gastric

carcinoma was observed, even after adjusting for confounding factors, such as age, gender, and CagA status. CagA-positive strains were also independently associated with *H. pylori*-related diseases[31].

Undoubtedly, the most significant association of *H. pylori* is with gastric cancer, both intestinal and diffuse types, and meta-analysis has shown that infection confers a 2–3-fold increased chance of developing gastric cancer[18]. Epidemiological and histopathological studies have shown that *H. pylori* infection is closely associated with gastric carcinogenesis[13, 46].

Beginning from the evidence that the *H. pylori* infection predominantly occurs in initial gastric carcinomas, the aim of this study was to analyze the prevalence of *H. pylori* infection in advanced gastric carcinomas and adjacent mucosa from operated patients, comparing intestinal and diffuse tumors according to LAUREN'S classification[22]. Localization of *H. pylori* in gastric carcinomas and adjacent non-tumor tissues were demonstrated.

METHODS

The prospective controlled study enrolled 56 patients from "Hospital Universitário", Federal University of Rio Grande do Norte, Natal, RN, Brazil. with advanced gastric cancer according to the TNM classification, treated from February 2000 to March 2003. Patients with chronic diseases, immunossupressed, using non-steroid anti-inflammatory, previous radiotherapy/chemotherapy and H2 blockers were excluded. All patients were subjected to partial gastrectomy. After resection, the greater curvature of the stomach was opened and several (usually 12) mucosal biopsy samples were taken from the gastric tumor, and the adjacent macroscopically non-tumorous mucosa within 4 cm distance from the tumor margin. For morphological analysis, tissue sections were routinely stained with hematoxylin and eosin. Adjacent non-tumor tissue was examined for diagnosis of atrophy of mucosa and chronic gastritis. The histological typing of gastric cancer was assessed according to Lauren's classification[18]. The *H. pylori* infection status was assessed by the urease rapid test, observed during 30 min (Gastroteste kit). *H. pylori* IgG antibody in plasma was measured by an enzyme-linked immunosorbent assay (ELISA), using commercially available kit Cobas Core II (Roche). A cut off value of >7.5 U was taken to categorize positive samples, as recommended by the manufacturer. For histopathological evaluation of the *H. pylori* colonization, the specimens from tumor tissue and adjacent mucosa were loaded into 1% formalin and routinely screened with microscope (Giemsa staining).

For those patients infected with *H. pylori* in non tumor tissue, treatment was performed after the 30^{th} postoperative day. Patients received omeprazole 2 × 20 mg, clarithromycin 2 × 500 mg, and amoxicillin 2 × 1000 mg given before breakfast and before dinner for 7 days. The first follow-up endoscopy was performed 6 months after treatment to determine successful eradication of *H. pylori* and tumor recurrence. Thereafter, follow-up endoscopies were scheduled annually.

All patients gave an informed consent before the surgical procedures. The study was conducted in accordance with the Declaration of Helsinki and the 196/96 Resolution from National Council of Health, Brazil and was approved by the Ethics on Research Committee of the Federal University of Rio Grande do Norte, Brazil (Protocol 261.01).

The statistical analysis was performed using the chi-square test and Yates correction, to compare the association between proportions for independent groups. The McNemar test was used for dependent paired groups. $P<0.05$ was considered statistically significant.

RESULTS

Demographic data are expressed in Table 1. Thirty-four tumors (60.7%) were classified as intestinal-type, and the remaining 22 (39.3%), as diffuse type carcinomas. When the cancer was separated according to the histological type, the prevalence of *H. pylori* infection was higher in intestinal than in diffuse-type carcinoma (Table 2). Statistically significant differences were found between these groups, when the diagnosis was performed by Giemsa staining and urease rapid test ($P<0.05$). Histopathological changes of adjacent non-tumor gastric mucosa were observed; chronic gastritis was found in 53 cases (94.6%) and atrophic mucosa in 36 patients (64.3%). All the patients with accentuated reduction in the epithelial thickness were *H. pylori* positive when assessed by Giemsa and urease test.

TABLE 1 – Demographic data of patients operated with gastric cancer

Number	56
Male/female	38/18
Mean age (year)	62
Age range (year)	21–78
H. pylori infection rate (%)	60.7

TABLE 2 – H. pylori infection detected by ELISA, Giemsa and urease test. Comparison with histological types of gastric carcinoma (tumor tissue)

Histological types	Total cases	ELISA		Giemsa		Urease test	
		p	n	p	n	p	n
Intestinal	34	20	14	25*	9	26*	8
Diffuse	22	16	6	9	13	8	14
Total	56	36	20	34	22	34	22

p = positive;
n = negative
* $P<0.05$ compared to diffuse GC

Helicobacter pylori in tumor tissue

Giemsa staining showed 34 positive cases (60.7%) of tissue sections carrying bacterial bodies of *H. pylori* from 56 gastric carcinomas, and the positive rate was lower than that detected by ELISA method in serum of 36 patients (64.3%). The urease rapid test detected *H. pylori* in tumor tissue of 34 patients (60,7%). The differences among these proportions were not significant ($P>0.05$). When detected by Giemsa, *H. pylori* positive rate in intestinal type carcinomas was higher than that in diffuse carcinomas ($P<0.05$) (Table 2). The difference was also significant comparing *H. pylori* positive in intestinal and diffuse carcinomas by rapid urease test ($P<0.05$). So, in diffuse carcinomas, *H.*

pylori was predominantly negative when Giemsa staining and urease rapid test were used (Table 2).

Helicobacter pylori in non-tumor tissue

H. pylori was detected by Giemsa and urease test in 30 of 56 cases (53.5%) in the glands and mucous pool of normal tissues 4 cm adjacent to tumors. *H. pylori* microscopic positive rate in non-tumor sites was lower than that in tumor (60,7%) sites, but this difference was not significant ($P>0.05$).

ELISA antibody and urease test after eradication therapy

To examine the effect of *H. pylori* treatment on antibody expression and urease rapid test, a total of 40 patients were followed endoscopically (16 were lost of follow-up). We analyzed the gastric biopsies obtained from gastric remnant of patients who had infection of adjacent non-tumor tissue, before and after *H. pylori* eradication therapy. Six months later, only two patients had IgG/ELISA and urease positive tests 2/36 (5%) and the treatment was repeated. The second treatment of *H. pylori* led to a complete negativity of IgG/ELISA and urease test on the 12th postoperative month. On the second follow-up year, tumor recurrence occurred in five patients who had diffuse carcinomas, whose *H. pylori* tests had been negative after 6 and 12 postoperative months. These patients died 4 months later.

DISCUSSION

The epidemiology of *H. pylori* infection has been studied in the Brazilian population. ROCHA et al.[38], using indirect immunofluorescence, detected a prevalence of 62.1% *H. pylori* infection in asymptomatic Brazilian blood donors in an urban area. A prevalence of 84.7% *H. pylori* infection in adults in a rural area of a central region of Brazil was also reported[42]. Thus, the prevalence is highest in developing regions, including all the countries of Latin America. Around the world, the prevalence of *H. pylori* infection ranges from 20% to over 90% in adult populations[36]. It has been postulated that transmission decreases as sanitation improves[1]. Within countries, *H. pylori* infection is linked to low socioeconomic status, residential conditions and migration from high prevalence regions[4, 41, 48].

Histological studies have reported the association between *H. pylori* infection and gastric cancer[5, 16, 28, 33]. However, the results have not been always consistent; higher rates of serologically and histologically detected *H. pylori* positivity have been reported for early stage cancer than for advanced gastric cancer[5]. TANG et al.[43] demonstrated positive rates of *H. pylori* 75.0% and 49.5% in early gastric carcinomas and advanced gastric carcinomas, respectively. Different from what could be expected, in the present study all the patients had advanced cancer and the prevalence of positive *H. pylori* in tumor tissue was 60.7%, as detected by Giemsa staining. As *H. pylori* infection had a high frequency in gastric cancer tissue, one possible interpretation of the results includes the possibility that it could be one of the carcinogenic factors in our patients. The prevalence of *H. pylori* in non-tumor tissue, 4 cm adjacent to gastric cancer, was not different from that detected in tumor tissue. In a Brazilian study of 40 patients receiving gastrectomy for gastric carcinoma, *H. pylori* was detected in 82% of the

cases. Of the cases evaluated by histologic and microbiologic methods, 94% had positive results by at least one method[29].

Atrophy of the gastric mucosa adjacent to tumor tissue was observed in all patients with *H. pylori* positive gastric carcinomas, with intestinal or diffuse types. This finding, associated with the high prevalence of chronic gastritis in adjacent tumor tissue (94.6%), may be a predetermining condition in the carcinogenesis of the gastric tumor of our patients. CRAANEN et al.[9] showed that atrophic mucosal changes were present in 90.3% of patients with intestinal-type early gastric cancer. UEMURA et al.[45] reported that subjects with severe gastric atrophy, corpus predominant gastritis, or intestinal metaplasia had an increased risk for gastric cancer. Another study confirmed that gastric atrophy status was essential for cancer development[50].

According to CORREA's[8] model of gastric carcinogenesis, continuous exposure to irritants of the gastric mucosa produces repeated episodes of superficial gastritis**.** When this occurs in patients with nutritional deficits, a degenerative sequential process causes atrophic gastritis, intestinal metaplasia, dysplasia and, ultimately, carcinoma. *H. pylori* may be considered an agent that causes chronic inflammation of the gastric mucosa. Histological studies have described a corpus-dominant pattern of mucosal inflammation, which is found not only in most *H. pylori* infected gastric cancer patients irrespective of the clinical stage[24, 43], but also in healthy relatives of gastric cancer patients[25]. Based on histological studies, patients with a corpus-dominant *H. pylori* gastritis have about 9-fold increased risk for gastric cancer[26, 27, 28].

Although cancer development is a multifactorial process[7], *H. pylori* infection increases the risk of gastric cancer[40]. Mongolian gerbils were orally inoculated and infected with *H pylori*, which induced gastric carcinomas located in the pyloric region. After the 26th week, severe active chronic gastritis, ulcers, and intestinal metaplasia could be observed in the infected animals. After the 62nd week, adenocarcinoma had been developed in the pyloric region of 37% (10/27) of the infected animals. It was found that adenocarcinoma development seemed to be closely related to intestinal metaplasia[47]. In our study the presence of *H. pylori* in tumor tissue with intestinal-type gastric adenocarcinoma was more prevalent than in the diffuse-type, and the difference was significant. In the diffuse-type carcinoma the *H. pylori* was predominantly negative when the Giemsa and urease test were used. Our results are in contradiction to the works published by other authors[6, 35]. *H. pylori* infection was found in 63.6% of patients with intestinal type early gastric cancer and in 54.5% of patients with diffuse-type early gastric cancer[9].

"The Maastricht Consensus Report"[12] recommends *H. pylori* eradication therapy following early resection for gastric cancer. There are some data showing that *H. pylori* eradication is associated with a decrease in the recurrence rate in patients with early gastric cancer that is resected endoscopically[39, 44] Some reports emphasize the importance of *H. pylori* treatment at a young age. These studies conclude that *H. pylori* eradication is also useful for the prevention of new cancer development from high-risk mucosa for gastric cancer[3, 21, 40]. WONG et al.[49] reported a 7.5-year follow-up study in a high-risk region in China and showed that treatment of *H. pylori* reduced the overall incidence of gastric cancer by 37%, although there was no statistical significance. The above recommendations are in agreement with the management adopted and the results of the present study. All the patients operated with gastric

cancer were treated for *H. pylori* infection, when it was present. All of them, previously with infection in non-tumor tissue, were *H. pylori* negative after 1 year of follow-up and no recurrence of cancer was observed. After 2 years, endoscopy showed tumor recurrence in five patients, with *H. pylori* negative tests. These recurrences may be explained by the fact that all the patients were operated with advanced gastric carcinomas, with poor prognosis. These results suggest that *H. pylori* eradication, even in advanced tumors, may reduce gastric cancer recurrence.

As suggested by an economical analysis based on the United States data, a screen-and-treat strategy for *H. pylori* infection, even under conservative assumptions, may be a cost-effective strategy for gastric cancer prevention comparable to the costs of breast mammography screening programs[34].

CONCLUSIONS

The data of the present study suggest a high prevalence of *H. pylori* in tumor tissue of gastric advanced carcinomas and in adjacent non-tumor mucosa of operated stomachs. A significant difference was detected in the presence of *H. pylori* between intestinal and diffuse histological types of gastric carcinoma.

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4. COMENTÁRIOS, CRÍTICAS E CONCLUSÕES

As vantagens da cirurgia bariátrica no tratamento da obesidade têm sido muito difundidas nos últimos anos[2]. Estudos enfatizam que o tratamento cirúrgico permite um controle de peso a médio e longo prazos, reduzindo as comorbidades. Isso possibilitou um maior conhecimento da eficácia como também das alterações orgânicas provocadas pelos diversos procedimentos, em particular, as atribuídas ao *switch* duodenal[2,42]. Dado o crescente volume de procedimentos bariátricos realizados nos tempos atuais, sobretudo após o advento da videolaparoscopia, é mister salientar a necessidade de acompanhamento desses pacientes. Para tanto, a realização de exames cintilográficos pós-operatórios não está descartada.

Partindo desses princípios, julgamos importante utilizar a técnica do *switch* duodenal e observar, essencialmente, se havia ou não alterações na biodistribuição do pertecnetato no pós-operatório. Nesse estudo inicial, da linha de pesquisa biodistribuição de radionuclídeos após intervenções cirúrgicas de grande porte, o primeiro publicado na literatura a respeito, não foram utilizados ratos obesos. Dando continuidade aos estudos nessa linha, passamos a utilizar modelos experimentais de animais com obesidade.

Baseados no projeto pré-estabelecido, durante um período máximo de dois anos, foram publicados 07 artigos científicos, em periódicos internacionais, o que trouxe como contribuição o enriquecimento do ponto vista crítico e científico do autor, correspondendo as expectativas e possibilitando o cumprimento de um conograma prévio.

A presente tese teve como mérito a originalidade na criação de um modelo experimental de cirurgia bariátrica que contribuirá para futuras pesquisas e publicações nesta área, possibilitando ao autor, o seu ingresso em base de pesquisa em cirurgia experimental assim como a orientação de alunos da graduação na pesquisa com animais.

Dentre as dificuldades encontradas, há o fato de se tratar de uma intervenção cirúrgica de grande porte, realizada em pequenos animais, o que necessitou do uso de microscópio cirúrgico e instrumental cirúrgico especial para realização dos procedimentos.

Um projeto piloto com pelo menos três séries de experimentos foi necessário, até que se conseguiu sistematizar a técnica com baixa mortalidade e observação dos animais pelo período pré-estabelecido, pois houve perdas no projeto piloto antes da sistematização do procedimento.

Além disso, trabalhando com material radioativo, utilizamos equipamentos de radioproteção como aventais de chumbo, que dificultavam as manobras operatórias. O caráter expoliante da técnica empregada, resultando em rápida perda de peso dos animais, nos fez limitar o tempo de observação pós-operatório para 10 dias.

No presente estudo foi possível determinar alterações na biodistribuição do pertecnetato de sódio em órgãos do aparelho digestivo e à distância. A redução na captação do radiofármaco na tireóide pode ter relação com a deficiência energético-protéica pós-operatória, uma vez que metade do intestino delgado estava disfuncionalizado e o estômago reduzido a um quarto de sua capacidade, no modelo experimental utilizado. Passos et al. (2000) atribuíram esse fenômeno a uma redução do transporte de pertecnetato para o

interior da glândula, assim como ocorre com o iodo na presença de desnutrição[73,74]. A maior atividade radioativa observada no pâncreas e baço parece ser devido ao processo inflamatório no sítio cirúrgico, decorrente da migração de polimorfonucleares marcados**.** Montero et al. (1998) observaram uma maior captação do Gálio (Ga^{67}) no osso esterno, em pacientes submetidos à toracotomia mediana, como preditor de osteomielite pós-operatória[75]. Barreto et al. (2005) utilizaram antibiótico marcado com ^{99m}tecnécio no diagnóstico de infecção osteo-articular[76].

A polineuropatia e a miopatia são resultantes da deficiência de vitamina B12, tiamina e vitamina D respectivamente[55,77,78]. A maior captação muscular do pertecnetato no presente estudo pode ser conseqüência de uma miosite por déficit de vitaminas D e E nos operados pelo switch duodenal[79,80]. Para Khurana et al. (2004) a miopatia é uma das causas não traumáticas de rabdomiólise pós-operatória na cirurgia bariátrica[81].

Tratando-se de uma cirurgia de grande porte, uma provável síndrome de angústia respiratória pós-operatória, que não foi avaliada no presente trabalho, pode ter influenciado a maior captação radioativa no pulmão no grupo experimental. Lesão pulmonar aguda ocorre frequentemente após trauma de grande vulto com elevada mortalidade[82]. Os mediadores intimamente implicados são as endotoxinas, citocinas, metabólitos do ácido araquidônico, enzimas proteolíticas derivadas de leucócitos e produtos tóxicos do oxigênio. Estima-se que após o trauma cirúrgico os neutrófilos produzam anions superóxidos, proteases e citocinas inflamatórias[83]. Em adição aos neutrófilos, macrófagos circulantes e alveolares assim como células endoteliais agravam a lesão pulmonar[84]. Em resposta a vários estímulos inflamatórios, células

endoteliais pulmonares, alveolares, do trato respiratório e macrófagos teciduais produzem ambos oxido nítrico e superóxido[85]. Estudos relatam que uma vez na circulação, o ^{99m}tecnécio marca não só hemácias, como também leucócitos presentes no plasma, que migram aos locais de inflamação, neste caso os pulmões[86-90].

As justificativas acima descritas ficam na fase de hipóteses, aliadas ao fato de que a explicação para as alterações na biodistribuição do pertecnetato de sódio não foi o objetivo principal deste estudo. Muitos outros aspectos pós-operatórios necessitam ser analisados a respeito da cirurgia bariátrica, com o objetivo de explicar as mudanças decorrentes do catabolismo excessivo, alterações imunológicas, moleculares, entre outras. Estudos subseqüentes serão realizados nesta linha de pesquisa, na busca do esclarecimento de repercussões da cirurgia bariátrica, que possam explicar as alterações observadas no presente trabalho.

O primeiro trabalho anexado a esta tese teve como contribuição adicional alertar aqueles que venham utilizar exames cintilográficos para controle pós-operatório em casos de cirurgia bariátrica. Com isso, evitando possíveis resultados falso-positivos em exames de baço, pâncreas, tireóide, pulmão e músculo, reduzindo custos e protegendo os pacientes da exposição repetida a radiação.

4.1 Perspectivas de trabalhos na mesma linha de pesquisa

Como perspectivas de estudos posteriores, algumas perguntas podem ser respondidas em trabalhos futuros relacionados com a cirurgia bariátrica, como: outras técnicas de cirurgia bariátrica podem ter o mesmo

comportamento do *switch* duodenal? Técnicas cirúrgicas outras, não bariátricas, podem provocar alteração na biodistribuição do pertecnetato e outros radiofármacos e radiotraçadores? Até que ponto tais alterações seriam significativas, a ponto de ser possível a inferência para cirurgia em humanos, ocorrendo repercussão em resultados falso-positivos e falso-negativos em exames de imagem na medicina nuclear?

A linha de pesquisa terá continuidade, a procurar explicações, em trabalhos futuros, para as alterações de biodistribuição nos órgãos onde ela ocorreu, através de parâmetros laboratoriais.

4.2 Conclusão

A partir do modelo experimental utilizado no presente trabalho, concluíu-se que a cirurgia do desvio bioliopancreático com *switch* duodenal contribuiu para alterar a biodistribuição do pertecnetato de sódio na tireóide, pulmão, pâncreas, baço e músculo de ratos operados, devido a prováveis alterações metabólicas e estruturais causadas por técnica cirúrgica mutilante

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Abstract

The biliopancretic diversion with duodenal switch is one of the mixing techniques used in the treatment of morbid obesity. The duodenal switch reduces the stomach capacity and leaves only 50-100 cm of small intestine for nutrition and absorption. The surgery produces hormonal, structural and biochemical changes, which can influence on the result of scintigraphic examinations in operated patients. With the objective of evaluate the postoperative biodistribution of sodium pertechnetate ($Na^{99m}TcO_4$) in brain, thyroid, heart, lung, liver, spleen, kidney, stomach, duodenum, pancreas, small intestine, bladder, muscle and bone of Wistar rats. The rats were randomly allocated into 3 groups of 7 rats each: the duodenal switch group (DS), the control group (C) and the sham group (S). They were operated under anesthesia and aseptic technique. In the tenth postoperative day, 0.1mL of sodium pertechnetate was injected via orbital plexus. After 30 min the animals were killed with overdose of anesthetic and samples of liver, spleen, pancreas, stomach, duodenum, small intestine, thyroid, lung, heart, kidney, bladder, muscle, bone and brain were harvested, washed with saline and weighed. The detention of radioactivity was made using the automatic Gamma Counter Wizard, PerkinElmer and the percentage of activity per gram of tissue (%ATI/g) was calculated. There was no early or late mortality in either rats groups. The values of percent radioactivity per gram of tissue (%ATI/g), showed no significant difference in liver, stomach, small bowel, duodenum, kidney, heart, bladder, bone and brain, when compared the DS rats with sham and controls rats. A postoperative significant increase in mean %ATI/g levels was observed

in spleen, pancreas and muscle in group DS rats, as compared to group S and C rats ($p<0.05$). In the lung there was an increase and in thyroid a decrease in mean %ATI/g of DS rats, when compared to sham rats ($p>0.05$). In conclusion, the biliopancreatic diversion with duodenal switch in rats modified the biodistribution of sodium pertechnetate in thyroid, lung, pancreas, spleen and muscle. The study had the participation of the departments and laboratories researches, as Nucleus of Experimental Surgery, Department of Surgery, Laboratory of Radiobiology, Department of Pathology and Service of Nuclear Medicine, certifying the character of a multidisciplinary research.

Key words: Biliopancreatic diversion, Duodenal switch, Bariatric surgery, Sodium pertechnetate, Technetium, Biodistribution, Postoperative, Rats.