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Bacterial translocation in experimental uremia

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Abstract The aim of this study was to investigate whether or not experimental uremia would induce bacterial translocation. Forty male Wistar rats were randomized into two groups: uremic (n = 20) and control (n = 20). Under anesthesia, the upper and lower left renal poles and the marginal lateral parenchyma were excised in uremic group. Seven days later, in a second operation the whole right kidney was removed. In control animals, two sham operations with the same interval were performed. After 60 days from the first operation, the liver, spleen and the mesenteric lymph nodes (MLN) were excised and cultured. Blood samples were sent for biochemical analysis (BUN, creatinine, sodium and potassium) and cultured. Specimens of the jejunum (1 cm below the Treitz angle) and ileum (1 cm above the ileocecal valve) were collected and sent for histological examination and scored for the degree of inflammation of the mucosa using a classification proposed by Chiu et al. in 1970. Uremic rats presented higher BUN, creatinine and potassium than controls. Bacterial translocation was more frequent in uremic than in control animals (8/20 (40%) vs. 1/20 (5%); $p = 0.02$). Translocation in uremic rats was observed mainly at the MLN (all eight cases). Both at the jejunum (uremic = 3 [0–5] vs. control = 2 [0–4]; $p = 0.04$) and the ileum (uremic = 2 [0–5] vs. control = 0 [0–3]; $p = 0.01$), inflammation score was higher in uremic rats than in controls. The intestinal mucosa barrier is impaired and bacterial translocation occurs in experimental uremia.

Keywords Bacterial translocation · Uremia · Small bowel · Inflammation · Renal failure

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Introduction

Bacterial translocation is currently defined as the passage of viable or not viable microorganisms as well as endotoxins through the intestinal wall [1, 2, 3]. Bacterial translocation seems to be promoted by the following three factors: disruption of the ecological balance of the intestinal indigenous flora, deficiency of the local or systemic immune defense and, mainly, loss of the integrity of mucosal barrier [4].

Experimentally, the occurrence of bacterial translocation has already been documented in hemorrhagic shock [5], intestinal obstruction [6, 7], mesenteric ischemia [8], thermal injury [9], trauma [10] and hypoxia [11] and also during the use of parenteral nutrition [12], morphine [13], corticosteroids [14] and metronidazol [15].

There is consistent evidence from the literature that the state of chronic translocation in critically ill patients may initiate a systemic inflammatory response syndrome (SIRS) even in the absence of infection [16]. The maintenance of SIRS is thought to be associated with the occurrence of multiple organ dysfunction syndrome (MODS), which is the leading cause of death in surgical patients [16, 17].

Uremia may induce intestinal mucosal injury. These damages may vary from edema and mild inflammation to ulceration with loss of the mucosal barrier [18, 19]. Moreover, the indigenous flora at the bowel lumen seems to be augmented [20] and the intestinal permeability is impaired, allowing increased passage of polyethylene glycol molecules through the intestinal wall in experimental uremia [21]. Uremia is also associated with

Table 1 Classification score of the mucosal inflammation (Chiu et al. 1970 [25])

Grade	Characteristics
0	Mucosa with normal villi
1	Developing of the sub-epithelial Gruenhagen's space, usually at the villus apex, frequently associated with capillary congestion
2	Extension of the sub-epithelial space with moderate lifting of epithelial layer from the lamina propria
3	Massive epithelial lifting down the sides of the villi
4	Denuded villi with lamina propria and dilated capillaries exposed. Increased cellularity of lamina propria may be noted
5	Digestion and disintegration of lamina propria; hemorrhage and ulceration

functional and pathological modifications of gastrointestinal mucosa including reduction of villus height and crypt depth [22].

Taking into account all of these effects related to uremia, it seems plausible to hypothesize that uremia may cause bacterial translocation. In fact, spontaneous bacterial peritonitis in chronic renal failure patients receiving hemodialysis has been reported. The authors speculated that bacterial translocation may have a role in these cases [23]. However, no previous experimental study has connected uremia to bacterial translocation and, therefore, a study focusing on this endpoint should be interesting. Thus, the aim of this study was to investigate whether or not experimental uremia would induce bacterial translocation.

Materials and methods

Forty male Wistar rats (250–300 g) entered the study. They were kept in metabolic cages at the laboratory environment in 12-h light/dark cycles at a mean temperature of 24°C for 3 days prior to the beginning of the study and had free access to tap water and standard rat chow (Ralston Purina, São Paulo, Brazil) ad libitum during the entire experiment. The Ethical Committee of the Institute of Collective Health of the Federal University of Mato Grosso approved the study.

The animals were randomized into two groups: uremic (n = 20) and control (n = 20). The uremic group was submitted under ether anesthesia to two consecutive operations with an interval of 7 days to create the animal model of uremia [24]. At the first laparotomy, the upper and lower left renal poles along with the marginal lateral parenchyma were excised, leaving the pelvis and hilum intact. At the second operation, the whole right kidney was removed. This model provided a remaining functional renal tissue varying from 20 to 30% [24]. In control animals, two sham operations with the same interval of 7 days were performed. Sham operations consisted of laparotomy and touching the bowel and kidneys. No antibiotics were used during the study. It was planned to replace animals that died before the conclusion of the experiment.

After 60 days from the first operation, the rats were anesthetized again to carry on the investigative procedures. At laparotomy with aseptic technique, blood samples were obtained from the posterior cava vein and sent for biochemical analysis (serum dosages of blood urea nitrogen (BUN), creatinine, sodium and potassium) and cultured in brain-heart infusion medium. The liver, the spleen and the mesenteric lymph nodes (MLN) were excised, macerated under strictly aseptic conditions and cultured in Agar-Mac Conkey plates for 24/48 h at 37°C.

The small intestine from the ligament of Treitz to the ileocecal valve was ressected and longitudinally opened. A small specimen of the jejunum (1 cm below the Treitz angle) and ileum (1 cm above the ileocecal valve) was excised, fixed in 15% formalin solution and sent for histological examination. Under optical microscopy, hematoxilin-eosin stained slides of these regions were examined and scored for the degree of inflammation of the mucosa using a classification proposed by Chiu et al. in 1970 [25] (Table 1 and Figs. 1, 2 and 3). The final score in each slide was the mean score attributed

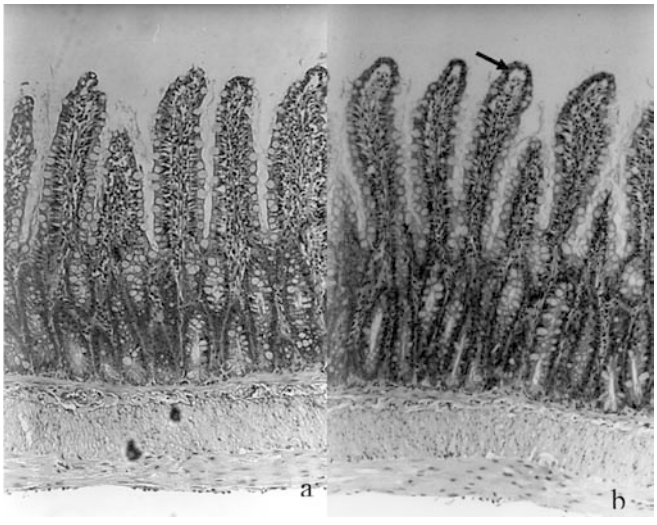

Fig. 1a, b Photomicrographs from ileal sections showing: (**a**) normal mucosa from a control animal (grade 0), and (**b**) the developing of sub-epithelial Gruenhagen's space (*arrow*) (grade 1). Original magnification: ×100

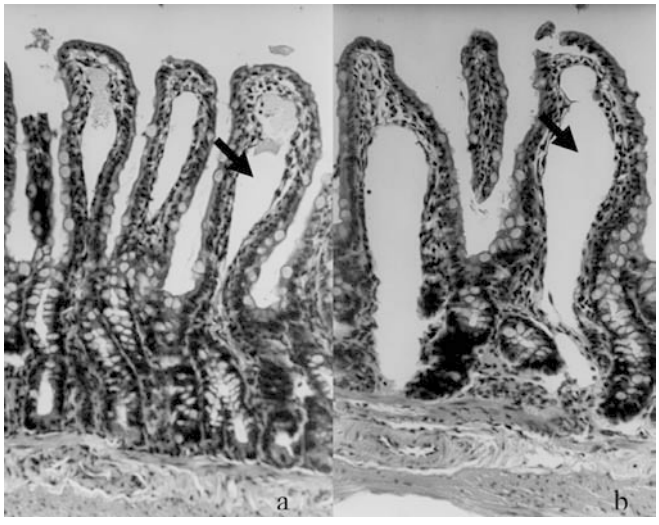

Fig. 2a, b Photomicrographs from ileal sections showing extension of the sub-epithelial space (*arrows*) with: (**a**) moderate (grade 2), and (**b**) massive epithelial lifting from the lamina propria (grade 3). Original magnification: ×200

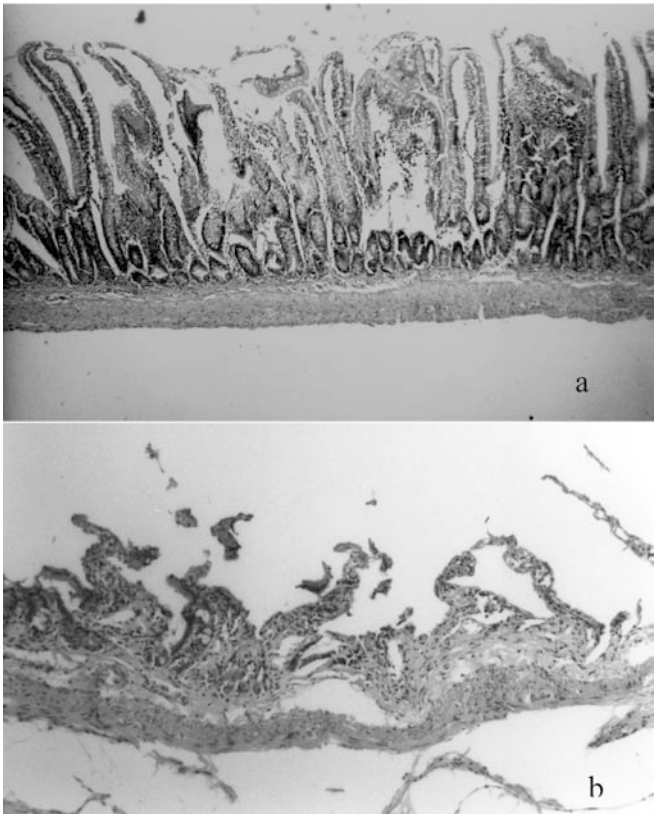

Fig. 3a, b Photomicrographs from jejunal sections showing: (**a**) denuded villi with lamina propria exposed (grade 4), and (**b**) digestion and disintegration of lamina propria (grade 5). Original magnification: ×40

by two observers blind to group assignment and to the objective of the study. Each observer considered the mean score of ten consecutive microscopic fields (100× magnification) in each slide.

Translocation rates were compared between groups by the Fisher's exact test. Continuous data were compared using the Student's T test or Mann-Whitney test according to the distribution and homogeneity of the data. Correlations between the level of both seric creatinine and BUN and the inflammation score were done using the Spearman's test. Data were presented as mean ± sd or median (range) accordingly. A 5% level ($p < 0.05$) was adopted for significance.

Results

Twenty-one animals (19 in uremic group and two in control group; $P < 0.05$) died during the experiment and according to the design of the study, they were replaced. Autopsies performed in those animals showed no case of peritonitis or abdominal abscesses. Most of these animals (90%) died between the 30th and 60th day of the experiment and the cause of death was not known. The replaced animals underwent the same protocol of the study design. In both groups, rats significantly increased in weight (control group, from 263 ± 16 g to 323 ± 34 g, $P < 0.01$; and uremic group, from 253 ± 14 g to 286 ± 37 g, $P < 0.05$). However, the gain in controls was significantly higher than that observed in uremic group ($P < 0.01$).

Table 2 Levels of serum BUN, creatinine, potassium and sodium in the two groups

Biochemistry	Group		
	Uremic	Control	P
BUN (mmol/L)*	55.7 (23.5–134.9)	23.7 (17.5–27.8)	< 0.001
Creatinine (μmol/L)*	95.5 (60.1–450.8)	47.7 (10.6–79.6)	< 0.001
Potassium (mEq/l)**	5.3 ± 1.5	4.1 ± 1.0	< 0.001
Sodium (mEq/l)**	142.4 ± 9.6	140.9 ± 2.0	0.51

*median (range), **mean ± SD

Table 3 Bacterial translocation in the two groups

Group	Bacterial Translocation			
	Yes		No	
	N	%	N	%
Uremic (n = 20)	8*	40	12	60
Control (n = 20)	1	5	19	95

*$p = 0.02$, Fisher's exact test

Uremic rats presented higher BUN, creatinine and potassium seric levels than controls, whereas seric sodium did not differ between groups (Table 2). At necropsy, uremic rats showed variable degrees of edema at both the intestines and the peritoneal membrane. The bowel serosa in uremic animals was dull.

Bacterial translocation occurred in eight animals (40%) of uremic group and in one animal (5%) of the control group ($p = 0.02$). Translocation in uremic rats was observed at the MLN complex (all eight cases) and blood (two cases). All animals with blood positive cultures presented bacterial translocation at the MLN complex. The single event in controls was verified at the MLN complex (Table 3). No positive cultures were seen at either the liver or spleen. The median colony-forming units (CFU) in uremic group was 500 [100–4500] CFU/g of MLN tissue, 1025 [850–1200] CFU/mL of blood, and in the single event of controls, 350 CFU/g of MLN tissue. Escherichia coli (64%) followed by Proteus mirabilis (29%) and Proteus vulgaris (7%) were the most frequent bacteria found in cultures.

The degree of mucosal inflammation was significantly higher in uremic rats than in controls in both the jejunum (uremic = 3 [0–5] vs. control = 2 [0–4]; $p = 0.04$) and the ileum sites (uremic = 2 [0–5] vs. control = 0 [0–3]; $p = 0.01$). The two uremic animals with positive blood cultures presented high inflammation scores at both the jejunum (5 and 4) and the ileum (4 and 3).

None of the rats with normal values of either creatinine or BUN presented bacterial translocation. Animals with bacterial translocation presented significantly ($p < 0.01$) higher creatinine (150.3 [10.6–450.8] μmol/L) than animals with negative cultures (70.7 [10.6–150.2] μmol/L). There was no statistical difference ($p = 0.09$) between the level of BUN and the occurrence of

bacterial translocation when animals with positive cultures (39.6 [23.5–131.4] mmol/L) were compared with animals with negative cultures (25.3 [17.5–91.4] mmol/L). Both at the jejunum (4[1–5] vs. 2[0–5]; $p = 0.047$) and the ileum (2[0–5] vs. 1[0–4]; $p = 0.043$) sites, the inflammation score was higher in rats that presented bacterial translocation. The level of creatinine correlated with the inflammation score at both the jejunum ($p = 0.02$) and the ileum ($p < 0.01$). The seric BUN level correlated with the inflammation score at the jejunum ($p = 0.01$) but not with the ileum ($p = 0.15$).

Discussion

The animal model of uremia used in this study provided a reasonable percentage of living animals along with elevated levels of seric BUN, creatinine and potassium, thus offering an appropriate model to attain the aims of the experiment. According to those parameters, the animals in uremic group were in renal failure. At necropsy, the bowel serosa in uremic rats seems dull in almost all animals. It was thought that this could be due to edema, though this finding was not reported in previous papers. Similarly to the findings reported by other authors [24, 26, 27], the mortality in uremic animals was 10-fold greater than the controls. As in the quoted papers, the cause of death in those animals was not found. Some models described to study uremia in rats provide acute instead of chronic renal failure. The model chosen and used in this experiment has the following advantages: a) it is associated with a progressive placement of toxic effects with a progressive installation of renal failure evidenced by gradual increase of BUN and creatinine; and b) it provides animals with absence of uncompensated metabolic acidosis, anemia and hypertensive state [24].

The gastrointestinal tube has been associated with mucosal lesions in renal failure patients. The most often reported alterations are esophagitis, gastrointestinal ulcers, enteritis and colitis [18, 19]. Apparently, the inner aspect of the intestinal mucosa in this experiment showed no gross ulceration at unarmed vision. However, at optical microscopy, a substantial degree of inflammation was documented in uremic animals. Both the jejunum and the ileum presented significantly more lesions than the controls. The severity of these lesions had a tendency to be greater at the jejunum when compared with the ileum specimens. Furthermore, there was a significant correlation between the level of creatinine and the degree of inflammation of the intestinal mucosa, and in rats with bacterial translocation the inflammation score was higher. These findings were relevant and imply that the mucosal barrier is impaired in uremia and, therefore, may explain the occurrence of bacterial translocation in the uremia group.

The results of this study demonstrated that bacterial translocation occurs in uremia. The MLN complex, after the intestinal wall, is the next barrier of defense to avoid the spread of translocated bacteria from the intestinal lumen [1, 2, 3]. Thus, it was not a surprise that in all positive cases of translocation there was the involvement of the MLN. In a small number of cases the blood culture was positive in uremic animals, implying that the event is almost limited to the MLN complex. However, the two cases with positive blood cultures also had bacterial translocation at the MLN complex. This finding is in agreement with the line of defense constituted by the MLN. A physiological bacterial translocation may occur in up to 10% of normal rats [2, 4]. Therefore, the 5% rate of translocation observed in control rats in this experiment was considered as normal values.

Infection is frequently a serious problem during the follow-up of patients suffering from chronic renal failure. In fact, sepsis and MODS are important causes of mortality in this group of patients and enterobacterias are commonly found in cultures [28]. Although the gateway in this process is the intravenous or intraperitoneal devices and also the urinary and pulmonary tract, in many cases the source of the infection is not found [29]. In this context, primary peritonitis may occur in both acute and chronic renal failure [30]. Thus, the hypothesis of bacterial translocation may explain these cases.

Although caution is necessary to transpose the findings of an experimental study to the clinical setting, the main conclusions in this study are: a) the intestinal mucosa barrier is impaired during uremia and b) bacterial translocation occurs in experimental chronic renal failure.

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