

The effect of bacterial lipopolysaccharide on gastric emptying in rats suffering from moderate renal insufficiency

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Abstract

The objective of the present study was to evaluate the response of rats suffering from moderate renal insufficiency to bacterial lipopolysaccharide (LPS, or endotoxin). The study involved 48 eight-week-old male SPF Wistar rats (175-220 g) divided into two groups of 24 animals each. One group underwent 5/6 nephrectomy while the other was sham-operated. Two weeks after surgery, the animals were further divided into two subgroups of 12 animals each and were fasted for 20 h but with access to water *ad libitum*. One nephrectomized and one sham-treated subgroup received *E. coli* LPS (25 µg/kg, *iv*) while the other received a sterile, pyrogen-free saline solution. Gastric retention (GR) was determined 10 min after the orogastric infusion of a standard saline test meal labeled with phenol red (6 mg/dl). The gastric emptying of the saline test meal was studied after 2 h. Renal function was evaluated by measuring the plasma levels of urea and creatinine. The levels of urea and creatinine in 5/6 nephrectomized animals were two-fold higher than those observed in the sham-operated rats. Although renal insufficiency did not change gastric emptying (median %GR = 26.6 for the nephrectomized subgroup and 29.3 for the sham subgroup), LPS significantly retarded the gastric emptying of the sham and nephrectomized groups (median %GR = 42.0 and 61.0, respectively), and was significantly greater ($p < 0.01$) in the nephrectomized rats. We conclude that gastric emptying in animals suffering from moderate renal insufficiency is more sensitive to the action of LPS than in sham animals.

The most frequent symptoms of chronic renal insufficiency are anorexia, nausea and vomiting. A possible explanation for these symptoms may be a retardation of gastric emptying (1-3), although studies on gastric emptying in uremia have yielded controversial results (4). In clinical practice, the gastrointestinal symptoms of chronic renal patients are aggravated during an infection. Experimentally, a retardation of gastric emp-

tying induced by lipopolysaccharide (LPS) has been observed in rats and mice (5,6).

In an effort to clarify the relationship between infection and gastric symptoms in uremia, we have evaluated the effect of LPS on gastric emptying in rats suffering from moderate renal insufficiency.

Eight-week-old male SPF Wistar rats (175-220 g) from the University's Central Animal House were kept in collective cages

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for four days in the laboratory for adaptation to the environment before the study. The rats were housed at a temperature of 22°C to 28°C on a 12-h light/dark cycle. Labina (Purina) rodent chow and water were provided *ad libitum*. For the study, the animals were divided into two groups. Group N consisted of 24 animals submitted to 5/6 nephrectomy in two stages: initially, superior and inferior polar nephrectomy was performed on the right kidney. Hemostasis was achieved by electrocauterization and 48 h later, total nephrectomy was performed on the left kidney. Group S contained 24 animals which underwent sham nephrectomy involving a complete opening of the lumbar region but with no renal manipulation.

The tests for gastric emptying were conducted two weeks after nephrectomy. The groups were subdivided into two groups: subgroup L in which LPS was administered *iv* and subgroup V, in which the vehicle used to dissolve LPS was administered by the same route. Thus, there were four experimental groups in the study: nephrectomized + vehicle (NV), sham + vehicle (SV), nephrectomized + LPS (NL) and sham + LPS (SL).

Before the test, the animals were weighed, placed in separate cages, and deprived of food, but not of water, for 24 h. *E. coli* (strain O55:B5) LPS (Sigma Chemical Co., St. Louis, MO) was diluted to a concentration of 25 µg/ml (6) in sterile pyrogen-free saline

immediately before administration. The animals of each L subgroup (N = 12/subgroup) received 25 µg of LPS/kg, *iv* (equivalent to 1 ml of saline/kg body weight) while those in subgroup V (N = 12/subgroup) received 1 ml of 0.9% saline alone/kg, *iv*, through a tail vein. The animals were fasted for a further 2 h after these injections (6). At the end of this period, a saline test meal (2 ml/100 g body weight) containing phenol red (6 mg/dl) as a marker was given and gastric emptying determined.

Standard laboratory techniques (7,8) were used to infuse the test meal and to determine gastric retention. Before recovering the gastric residue, blood samples were collected from the abdominal vena cava of all the animals in order to determine the plasma urea and creatinine levels. The values for gastric retention were obtained 10 min after orogastric infusion of the test meal.

Statistical analysis was performed using the Kruskal-Wallis test (9) and the test for multiple comparisons of differences between pairs (10) with alpha values set at 0.05 and 0.01, respectively.

The levels of urea and creatinine (Table 1) in 5/6 nephrectomized animals were two-fold higher than those observed in the sham-treated rats ($P < 0.01$). The gastric retention (Figure 1) of sham rats given LPS was greater than that of sham rats receiving saline alone ($P < 0.01$). A similar relationship was observed for the nephrectomized rats. There was no difference in the gastric retention of the nephrectomized and sham-treated rats given vehicle alone. In contrast, the gastric retention of nephrectomized animals given LPS was significantly greater than in the corresponding sham rats. These results confirm data in the literature regarding the ability of LPS to retard gastric emptying (5,6).

Although the gastric retention values for the sham and nephrectomized rats receiving saline alone were the same, there was a significant delay in gastric emptying in LPS-treated nephrectomized rats when compared

Table 1 - Plasma creatinine and urea levels two weeks after 5/6 nephrectomy.

The results are reported as mg/dl and represent the mean \pm SEM of 12 rats in each subgroup. Subgroups: sham + vehicle (SV); sham + LPS (SL); nephrectomized + vehicle (NV); nephrectomized + LPS (NL). The urea and creatinine levels in 5/6 nephrectomized rats (subgroups NV and NL) were approximately two-fold greater than those in the sham groups (subgroups SV and SL). a = $P < 0.01$: NV vs SV and NV vs SL; b = $P < 0.01$: NL vs SL and NL vs SV (multiple comparisons test).

	SV	SL	NV	NL
Urea (mg/dl)	33.4 \pm 1.25	40.1 \pm 1.44	75.9 ^a \pm 3.26	94.1 ^b \pm 7.27
Creatinine (mg/dl)	0.43 \pm 0.01	0.46 \pm 0.01	0.73 ^a \pm 0.02	0.80 ^b \pm 0.05

to the corresponding sham animals. This finding suggests that animals suffering from renal insufficiency are more sensitive to LPS.

No studies have investigated the effect of LPS in uremia, although the greater morbidity and mortality observed in uremic infections (11,12) may involve the presence of this toxin. The mechanisms involved in the effect of LPS on gastric emptying are still unclear. Anorexia with LPS involves the release of prostaglandins. However, pretreatment with indomethacin did not eliminate the effect of the toxin on gastric emptying (5). Raybould et al. (13) reported delayed emptying of a solid meal while studying gastric emptying in animals with chronic renal insufficiency. This response was not modified by a specific inhibitor of nitric oxide synthesis. Nitric oxide may act as an inhibitory neurotransmitter in the gastrointestinal tract (13,14) and possibly as a modulator of gastric tonus (15). Thus, while having no effect on the gastric antrum which is an important segment for the emptying of solids, it may act on the gastric fundus which is involved in the emptying of gastric liquids (16,17). We are currently investigating

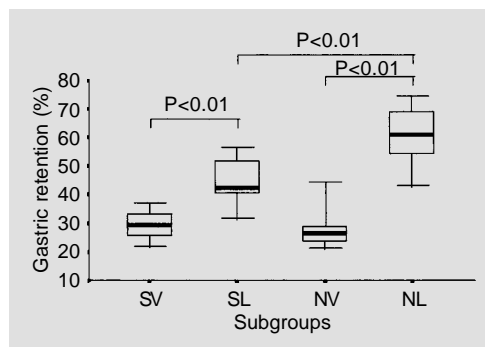


Figure 1 - Gastric retention in rats in the absence and presence of LPS. The gastric retention (%) obtained 10 min after an orogastric saline meal was determined 2 h after the *iv* administration of LPS (25 µg/kg) to the appropriate subgroups: sham + vehicle (SV); sham + LPS (SL); nephrectomized + vehicle (NV), and nephrectomized + LPS (NL). The results are shown as box-plots for 12 rats/subgroup. The thick horizontal bars dividing the rectangles represent the medians. LPS retarded gastric emptying in the sham and 5/6 nephrectomized groups that received saline alone. The gastric retention in 5/6 nephrectomized rats given LPS was significantly greater than that observed in the corresponding sham group. Statistical comparisons were performed using the multiple comparisons test.

the mechanisms by which LPS can delay gastric emptying.

Studies on gastric emptying in uremic patients generally do not state whether the patient had an infectious process. The latter could conceivably compromise the results in view of the influence of renal insufficiency on emptying observed in the present investigation.

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