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Effect of exogenous nitrite in postoperative ileus.

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Exogenous administration of nitrite has been shown to protect heart, liver, kidney and brain from ischemia/reperfusion (I/R) injury. The protective effect of exogenous nitrite is not completely understood, but evidence suggests that exogenous nitrite might be a source of NO under hypoxic conditions. A possible mechanism of action is activation of soluble guanylate cyclase (sGC) by NO, with reduction of platelet aggregation and opening of mitochondrial inner membrane K_{ATP} channels. Impairment of gastrointestinal motility after abdominal surgery (postoperative ileus, POI) is mainly due to intestinal inflammation with leukocyte infiltration, triggered by surgical handling. As the latter will also lead to repetitive momentary I/R of the bowel, the aim of this study was to investigate the effect of nitrite in a model of postoperative ileus.

C57Bl6J mice were anesthetized (ketamine 100 mg/kg, xylazine 10 mg/kg; intraperitoneally) and after laparotomy, POI was induced by compressing the small intestine by cotton applicators (intestinal manipulation; IM) for 5 min. Sodium nitrite (48 nmol) or its solvent (PBS) was administered in the inferior vena cava just before IM. Intestinal transit was assessed 24h postoperatively using fluorescent imaging, 90 min after fluorescein gavaging (geometric centre, GC of intestinal fluorescein progression). Contractile activity to bethanechol (cumulative 0.3 µM - 300 µM) was evaluated in circular mucosa-free mid-jejunal muscle strips, obtained 24h after IM. Additional mucosa-free muscularis segments were stored at -80°C for later analysis of myeloperoxidase (MPO) activity as an index of leukocyte infiltration and of cGMP by enzyme immunoassay. A hepatic I/R model, where nitrite was already shown to be efficient, was studied in parallel. Hepatic left lateral and median lobes were rendered ischemic by clamping the hepatic artery and portal vein for 45 min. Halfway through the ischemia, sodium nitrite (48 nmol) or its solvent was administered in the inferior vena cava. After 5h of reperfusion, serum aspartate and alanine transaminase (AST/ALT) levels were analysed as a marker for liver injury.

Pre-treatment with nitrite partially prevented the delayed transit seen after IM, and tended to increase the reduced contractile response of the circular muscle strips to bethanechol. However, nitrite did not have an influence on elevated leukocyte infiltration (MPO) or on decreased cGMP levels in the muscularis of operated mice. The protective effect of nitrite was confirmed in the hepatic I/R model (Table 1).

	Control	IM	IM+nitrite
GC	8.5 ± 0.5	4.1 ± 0.5***	6.0 ± 0.5^{+}
E_{max} bethanechol (g.s/mg wet weight)	14.8 ± 3.1	5.7 ± 2.8	9.9 ± 2.0
MPO (U/mg protein)	2.7 ± 0.4	9.3 ± 1.1**	11.9 ± 1.8
cGMP (pmol/g tissue)	25.1 ± 3.5	13.6 ± 1.9*	11.3 ± 1.8

Table 1: Influence of nitrite on intestinal changes induced by IM, and on increased serum AST/ALT levels induced by hepatic I/R

	Control	I/R	I/R+nitrite
ALT (U/L)	92.0 ± 13.8	628.8 ± 88.9***	193.9 ± 17.5^{+++}
AST (U/L)	162.1 ± 19.5	458.8 ± 44.1***	257.1 ± 24.6^{+++}

*P<0.05, **P<0.01,***P<0.001 vs. control; $^{+}$P<0.05, $^{+++}$P<0.001 vs. IM or I/R; n = 5-8

The present study indicates that pre-treatment with exogenous nitrite partially prevents delayed intestinal transit upon IM; this is not related to reduction of leukocyte infiltration. Activation of sGC in the muscular layer does not seem to play a role in the effect of nitrite in this model.