Xanthine Oxidase Decreases Production of Gut Wall Nitric Oxide (44190)

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Abstract. **Multiorgan failure is often the lethal outcome of intratracheal aspiration of acidic gastric juice. The pathogenesis of multiorgan failure may involve a systemic imbalance between pro-inflammatory and anti-inflammatory factors. In an anesthetized rat model, intratracheal instillation of HCl elicited intestinal inflammation which was exaggerated by xanthine oxidase (XO) and attenuated by nitric oxide (NO). We hypothesized that XO may mediate injury in part by suppression of NO formation. Therefore, we measured intestinal tissue concentrations of the stable NO oxidative metabolites (NO_2^- and NO_3^-) following intratracheal (IT) instillation of NaCl or HCl alone or in combination with interventions aimed at increasing or decreasing XO activity. Compared with IT NaCl (control treatment) jejunal tissue NO_2^- and NO_2^- + NO_3^- concentrations were increased by allopurinol pretreatment, which inhibits XO, and were decreased by systemically administered XO, as well as by IT HCl. The decreased NO_2^- and NO_2^- + NO_3^- concentrations found following IT HCl were completely reversed by either allopurinol or by systemically administered L-arginine (the precursor of NO). We conclude that manipulation of the pro-inflammatory XO system has a reciprocal effect on the intestinal anti-inflammatory NO system in either the undamaged or the endobronchially acidified lung model.** [P.S.E.B.M. 1997, Vol 216]

Multiorgan failure following intratracheal aspiration of acidic gastric juice is a common cause of death in American intensive care units (1–3). This catastrophic illness may represent a systemic imbalance between pro- and anti-inflammatory factors, although there is no consensus regarding the mechanisms underlying the transfer of injury signals to a remotely located organ (1, 3–12). In previous reports, circulating xanthine oxidase (XO) was implicated in ischemic gut- or liver-induced increases in pulmonary vascular permeability (9, 10, 13).

Recently, we found that intratracheal instillation of a 0.1 *N* HCl solution in anesthetized rats evoked significant increases in blood to lumen intestinal permeability to [^{51}Cr]-labeled EDTA and gut wall neutrophil population density compared with instillation of an isosmotic NaCl solution (14). The lung-damage–induced gut leak was attenuated by interventions which either suppressed XO-derived reactive oxidant formation (dietary allopurinol or tungstate) or increased intestinal nitric oxide (NO) content (perfusion of the gut lumen with an NO containing solution or systemic administration of L-arginine). Conversely, augmentation of oxidant production (systemic administration of XO) or suppression of NO formation (L-NAME) exaggerated the lung damage induced defect in intestinal permeability. These findings support the concept that the products of two enzyme systems play opposing roles in lung-damage–induced gut leak, with pro-inflammatory XO metabolites intensifying and anti-inflammatory NO synthase products protecting against remote organ injury.

The objective of the current investigation was to utilize high-performance liquid chromatography (HPLC) to measure stable NO oxidative metabolite concentrations in the gut wall as a reflection of tissue NO content (15). Such measurements were made in anesthetized control rats and in rats subjected to lung damage.

Materials and Methods

Sources of Reagents and Drugs. Xylazine was obtained from Miles (Bayer Corporation, West Haven, CT), Ketamine from Fort Dodge Laboratories (Fort Dodge, IA), NaCl and NaH_2PO_4 from Fluka Chemical Co. (Milwaukee, WI), the NO_2^- and NO_3^- standards for HPLC from Ricca Chemical Co. (Arlington, TX), and L-arginine and XO from Sigma Chemical Co. (St. Louis, MO). The standard diet for rats was Prolab 3000 from Agway, Inc. (St. Louis, MS). In

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two rat groups diets were enriched with allopurinol, 50 mg/kg chow, from ICN Biochemicals (Costa Mesa, CA).

Animal Preparation, Assay Methods, and Experimental Design. Animal experiments were approved in advance by our institutional Animal Care and Use Committee. Male Sprague Dawley rats weighing 300–450 g were fasted but allowed access to water for 24 hr prior to deep anesthetization with xylazine (10 mg/kg ip) and ketamine (80 mg/kg ip). Animals were then placed on a 37°C heating pad before a tracheostomy was performed. A standard volume (1 ml/kg) of either 0.15 *N* NaCl or 0.1 *N* HCl in isosmotic saline was instilled into the trachea. A midline laparotomy was performed to expose the jejunum, and a 4-cm segment was isolated, cannulated at either end, and perfused with 37°C, isosmotic saline at 1 ml/min.

At 190 min following intratracheal instillation of NaCl or HCl, the rats were euthanized by incising and opening the thoracic cavity. A full thickness ring of jejunum was excised, weighed, and then immediately frozen in liquid nitrogen, where it was kept at −70°C until the assay (15). Tissue was homogenized (Virtishear, Virtis Co., Gardiner, NY) for 60 sec in 2 ml Na_2PO_4 (50 m*M*, pH 7.0), then sonicated at cycle 3 for 1 min (Sonifer 250, Fisher, Pittsburgh, PA). A 1.5-ml aliquot was removed and centrifuged (3000*g* for 10 min at 4°C) before 1.0 ml of the supernatant was taken for deproteinization by addition of filtered acetonitrile (250 μl) followed by vortexing and centrifugation. The supernatant was then filtered (Anopop-IC, 0.2 μm, Alltech Associates, Inc., Deerfield, IL), lyophilized, reconstituted in 100 μl of mobile phase, and analyzed by HPLC. The mobile phase (20 m*M* NaCl and 1 m*M* NaH_2PO_4 pH 7.0) was prepared with electrochemically purified water and pumped at 1.0 ml/min. NO_2^- and NO_3^- were separated using a precolumn (Prx 100, 25 × 2.3 mm) coupled to an anion exchange post column (PRP × 100, 150 × 4, 1.0 mm, Hamilton) and were detected at 210 nm and quantified using external standards.

Rats were randomly assigned to seven different treatment groups, namely (i) intratracheal (IT) instillation of NaCl only (control); (ii) IT NaCl + XO (0.5 units/ml) injected intravenously 10 min after IT instillation as described previously (9); (iii) IT NaCl + allopurinol (fed daily for 1 week prior to experimentation); (iv) IT HCl; (v) IT HCl + XO; (vi) IT HCl + allopurinol; and (vii) IT HCl + L-arginine (250 mg/kg intravenously 10 min after IT HCl instillation).

Statistical Analysis. Comparisons for parametric data were performed using one-way analysis of variance combined with the Student-Newman-Keuls multiple comparison test (16). Significant differences between rat groups were based upon a *P* value less than 0.05. All data were expressed as mean ± SEM values.

Results

Compared with the control treatment group (IT NaCl only) jejunal wall tissue NO_2^- and NO_2^- + NO_3^- concentrations were decreased significantly by systemic XO in rats treated with IT NaCl, and by IT HCl (Figs. 1 and 2). Mucosal tissue NO_2^- and NO_2^- + NO_3^- concentrations were significantly greater than control treatment values in rats treated with IT NaCl + dietary allopurinol. (Figs. 1 and 2).

In rats treated with IT HCl, addition of either allopurinol or iv L-arginine significantly increased both mucosal NO_2^- and NO_2^- + NO_3^- concentrations compared with IT HCl alone (Figs. 1 and 2).

Discussion

Severe insult delivered to a single organ can lead to widespread inflammatory involvement of other organs with catastrophic results. We recently studied two instructive examples of this adverse sequence, namely acute lung injury due to mesenteric ischemia-reperfusion injury, and gut mucosal inflammation secondary to pulmonary acid aspiration (9, 10, 14). A common theme in these antiparallel models was that secondary inflammatory injury was promoted by the pro-oxidant XO and diminished by local production of NO. For example, lung-injury–induced gut leak was worsened by infusion of XO or inhibition of endogenous NO synthase and was lessened by inhibition of XO or local delivery of NO to the intestinal mucosa. The present study extends these observations and suggests that the divergent effects of XO and NO may reflect interrelated processes. Specifically, augmenting XO from exogenous or endogenous sources appears to suppress dramatically formation of protective NO by the gut.

We found that pulmonary acid aspiration abolished gut NO_2^- levels, suggesting that lung injury imposed an oxidant stress upon the intestines. In situations where NO is formed

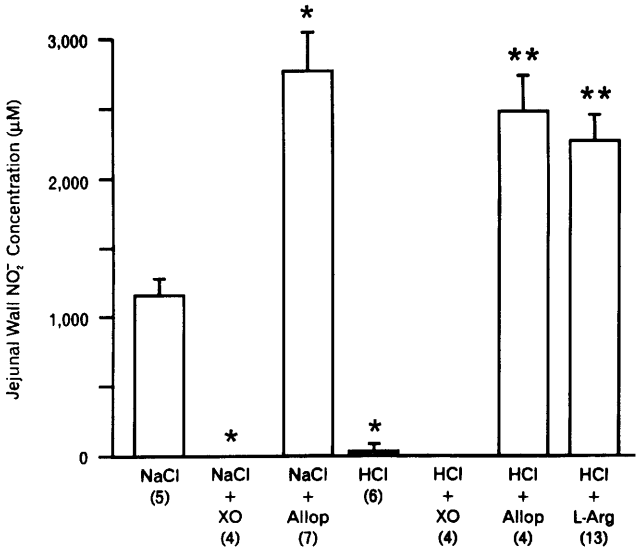

Figure 1. IT HCl or IV XO treatment decreased and allopurinol increased jejunal wall NO_2^- levels compared with the IT NaCl control (*$P < 0.01$). Allopurinol increased jejunal wall NO_2^- levels in rats treated with IT NaCl or IT HCl, compared with their respective controls (**$P < 0.001$ compared with IT HCl). L-Arginine increased NO_2^- levels in rats treated with IT HCl. Values are means ± SEM, and numbers in parentheses indicate group size.

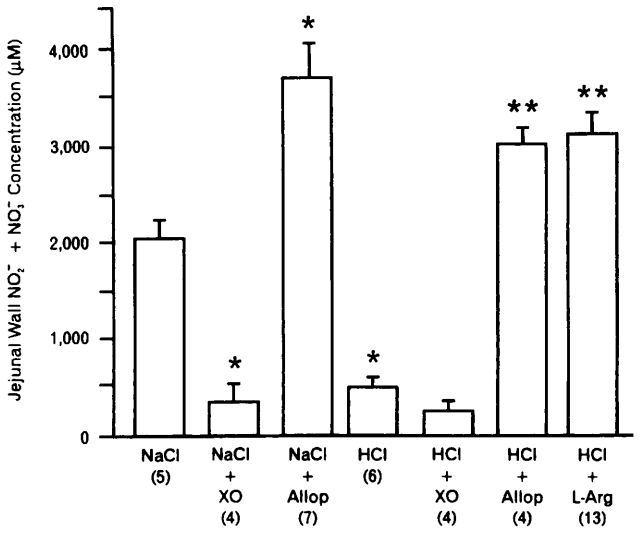

Figure 2. IT HCl or IV XO treatment decreased and allopurinol increased jejunal wall NO_2^- + NO_3^- levels compared with IT saline controls (*$P < 0.001$). Allopurinol increased jejunal wall NO_2^- + NO_3^- levels in rats treated with IT saline or IT HCl, compared with their respective controls (**$P < 0.001$ compared with IT HCl). L-Arginine increased NO_2^- + NO_3^- levels in rats treated with IT HCl. Values are means ± SEM, and numbers in parentheses indicate group size.

in an oxidant-rich environment, for instance, the primary oxidative metabolite of NO is NO_3^- (17), formed by rearrangement and further oxidation of peroxynitrite (18). This proposed increase in intestinal oxidant burden is consistent with our previous findings which implicated XO as a participant in secondary gut leak, and also with the appearance of neutrophils in the gut wall (14). Indeed, in a similar model, interventions aimed at decreasing gut neutrophil retention resulted in diminished secondary intestinal injury (3). Likewise, in the present study introduction of an oxidant stress by infusion of XO in sham rats also completely abolished gut NO_2^- levels.

We also observed that pulmonary acid aspiration reduced total intestinal NO_2^- + NO_3^- levels, suggesting not only that NO was being oxidatively consumed (which alone would manifest as a shift from NO_2^- to NO_3^-) but also that the production of NO itself was probably diminished in response to lung injury (Fig. 3). Although the mechanism of this decrease is not clear, it is interesting that infusion of the NO substrate L-arginine restored NO_2^- + NO_3^- levels in the jejunal wall of acid aspiration–treated rats. This apparent decrease in NO production appeared to be linked to XO since (i) allopurinol restored gut NO_2^- + NO_3^- levels in rats subjected to acid aspiration; (ii) infused XO decreased gut NO_2^- + NO_3^- levels in sham rats; and (iii) acid aspiration, which injures the intestines in part through an XO pathway (44), decreased gut NO_2^- + NO_3^- levels. Inasmuch as endogenous and exogenously delivered NO protect the gut from oxidative damage (14, 19–22), our findings raise the possibility that XO may promote secondary intestinal injury by decreasing gut NO production as well as increasing the oxidative consumption of NO (Fig. 3). Of further relevance to neutrophil-dependent, secondary gut injury are the findings that XO promotes whereas NO diminishes shear-dependent neutrophil-endothelial cell interactions *in vitro* (20).

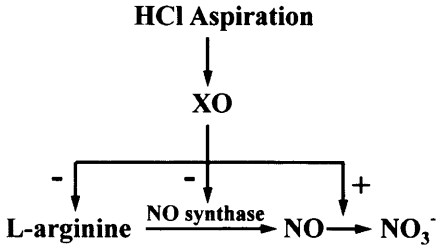

Figure 3. Schematic diagram of potential modulatory roles of XO on the presence of NO in the gut wall following HCl aspiration. Although XO may reduce the presence by inhibiting NO synthase, it is also possible that XO decreases the availability of L-arginine. XO may also accelerate the oxidation of NO → → → NO_3^- and may augment NO conversion into peroxynitrite.

XO may modulate gut NO production under basal conditions, since allopurinol increased gut NO_2^- + NO_3^- levels, even in sham rats. NO has been shown to promote mucosal integrity in the uninjured gut (21), suggesting that a balance between the disparate effects of XO and NO may also determine homeostasis in the resting bowel wall.

In addition, XO may reduce the availability of the NO substrate, L-arginine. This mechanism is supported by our findings that exogenously administered L-arginine abrogated the effects of acid aspiration on gut wall NO presence in the present study and on intestinal hyperpermeability in a previous report (14).

Intestinal ischemia also causes many of the gut responses that occur following endobronchial acidification (10, 14, 19, 22). It is therefore possible that intratracheal HCl releases a cytokine whose initial effect is intestinal ischemia, although our findings do not address this possibility.

We conclude that, following pulmonary injury, XO decreases intestinal formation of NO and increases oxidative consumption of NO, potentially worsening gut mucosal injury and inflammation. Secondary injury mechanisms appear to be complex and manifold, however, and the suppressive effect of XO on NO is likely to provide only a partial explanation for the secondary spread of inflammation characteristic of multiorgan failure. Finally, it should be noted that NO has a similar counterregulatory effect on XO, because NO inactivates XO and thereby diminishes its production of oxidant metabolites (23).

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