Inosine improves gut permeability and vascular reactivity in endotoxic shock

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Objective: To investigate the effects of inosine administration on vascular reactivity, gut permeability, neutrophil accumulation and lipid peroxidation in tissues in murine endotoxin shock.

Design: Randomized, prospective laboratory study.

Setting: Research laboratory.

Subjects: BALB/c mice 6–8 wks age.

Interventions: BALB/c mice were randomly assigned to one of five groups: a) vehicle controls, which received saline intraperitoneally; b) inosine controls, which received inosine alone (100 mg/kg, ip); c) lipopolysaccharide (LPS)-treated animals, which received LPS (40 and 100 mg/kg, ip, depending on the experimental protocol); d) inosine pretreatment group, which received inosine (100 mg/kg, ip) 30 mins before LPS; and finally, e) inosine posttreatment group, which received inosine (100 mg/kg, ip) 60 mins after LPS.

Measurements and Main Results: The passage of fluorescein isothiocyanate-conjugated dextran (4 kDa, FD4) was analyzed in everted gut ileal sacs incubated _ex vivo_ as an index of gut permeability. LPS induced a significant intestinal hyperpermeability, and inosine exerted protective effects both in pre- and posttreatment regimens. Myeloperoxidase and malondialdehyde were also measured to study neutrophil accumulation and lipid peroxidation in selected tissues. Inosine, both in pre- and posttreatment regimens ameliorated the increases in myeloperoxidase and malondialdehyde in the lung and gut. LPS-treated animals showed decreased contractile and relaxant responses, and inosine pretreatment (but not posttreatment) partially improved these responses.

Conclusions: Taken together, inosine has organ protective effects during shock. A significant portion of its protective action is maintained even in the posttreatment scenario. (Crit Care Med 2001; 29:703–708)

KEY WORDS: inosine; shock; endotoxin; gut; vascular; endothelial; permeability; reactivity; lung; liver; myeloperoxidase; malondialdehyde

It is well recognized that certain naturally occurring purines can exert powerful effects on the immune system. The nucleoside adenosine is the best characterized of these purines; both extracellular and intracellular adenosine have been shown to affect almost all aspects of an immune response (1–3). Adenosine and its analogs can alter the course of a variety of immune-mediated/inflammatory diseases, such as endotoxin shock (4, 5), rheumatoid arthritis (6, 7), pleural inflammation (8), nephritis (9), and uveitis (10). Adenosine is also recognized as one of the most important endogenous molecules able to prevent tissue injury in ischemia-reperfusion (11). Its effect is partly mediated by the inhibition of deleterious immune-mediated processes, including the release of proinflammatory cytokines and free radicals (11).

Inosine is another endogenous purine nucleoside, which is formed during the breakdown of adenosine by adenosine deaminase (12). This molecule is released into the extracellular space from cells upon metabolic stress (13–15) or from the sympathetic nervous system (16). In ischemic tissues, inosine concentration can increase to levels as high as 1–3 mM (17), and increased tissue inosine levels are present in various inflammatory states (17–20). In a recent study, we showed that—contrary to the previous, widely held belief that inosine is an inert metabolite without biological effects—inosine inhibits proinflammatory cytokine and chemokine production *in vitro* and *in vivo*, enhances the production of the anti-inflammatory interleukin-10 via a posttranscriptional mechanism, and reduces endotoxin-induced mortality in mice (21).

In the present study, we investigated whether inosine can affect the deterioration in vascular reactivity, intestinal function, neutrophil infiltration, and intraorgan oxidant production, pivotal events of the septic multiple organ dysfunction syndrome. Furthermore, we tested whether inosine maintains its protective effects in the posttreatment regimen. Our hypothesis was that inosine suppresses intraorgan oxidant production and neutrophil accumulation in endotoxemia and prevents the deterioration of vascular responsiveness. Furthermore, because of the posttranscriptional mechanism of its anti-inflammatory action, we hypothesized that some of the protective effects of inosine may be sustained, even

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Supported, in part, by grants from the National Institutes of Health (R43GM59560 and R29GM54773) to Dr. Szabó. Dr. Liaudet is on leave from the Critical Care Division, Department of Internal Medicine, University Hospital, Lausanne, Switzerland, and is supported by a grant from the ADUMED foundation, Switzerland. Dr. Soriano is on leave from the Department of Critical Care Medicine, Hospital das Clinicas da Universidade de Sao Paulo, Brasil, and is supported by a fellowship from FAPESP (Brazil).

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in the posttreatment therapeutic regimen.

MATERIALS AND METHODS

In vivo studies were performed in accordance with National Institutes of Health Guidelines and with the approval of the local Institutional Animal Care and Use Committee.

Reagents and Drugs. Lipopolysaccharide (LPS; from *Escherichia coli*, serotye 0127:B8), inosine, sodium dodecyl sulfate, 3-[*N*-morpholino]propane sulfonic acid, 2-thiobarbituric acid (4,6-dihydroxypyrimidine-2-thiol), α-phenylephrine, acetylcholine chloride, hexadecyltrimethylammonium bromide, tetramethylbenzidine, hydrogen peroxide, acetic acid, malondialdehyde bis(dimethyl acetal), and graded compound were purchased from Sigma-Aldrich (St. Louis, MO).

Mice. Male BALB/c mice (8 wks old) were purchased from Taconic Laboratories (MA).

Animal Preparation. Animals were injected intraperitoneally with LPS or saline. Mice for gut permeability measurements had the gut harvested after 16 hrs of 40 mg/kg LPS. For all other measurements, the tissues were harvested after 8 hrs of 100 mg/kg LPS. The two different doses of LPS for the different parts of the study were selected based on studies in the literature and preliminary dose-response and time-course work. We observed that 40 mg/kg LPS produced between 70% and 80% mortality in 24 hrs and induced significant increases in tumor necrosis factor-α, interleukin-1β, and nitrite/nitrate (peaking at 2, 4, and 8 hrs, respectively; not shown). LPS, at a dose of 100 mg/kg, produced 100% mortality at 24 hrs, i.e., initiates a very severe endotoxic shock state. We, therefore, selected a dose of 100 mg/kg for the majority of the studies to investigate whether treatment of a very severe shock state with inosine is able to improve vascular function and attenuate tissue damages. Similar doses of LPS have previously been used by other investigators (22, 23). Both doses of LPS used in the studies provided significant and reproducible changes in the respective variables studied. For inosine treatment, animals were injected intraperitoneally with saline or inosine (100 mg/kg) in a volume of 0.1 mL/10 g of body weight; inosine was injected 30 mins before LPS (INO-pre) or 60 mins after LPS (INO-post).

Treatment Protocols. Mice were divided into five experimental groups: control, inosine control, LPS, INO-pre, and INO-post: a) control—animals received physiologic saline only; b) inosine control—animals were treated with inosine and did not receive LPS; c) LPS—mice in this group received intraperitoneal LPS and physiologic saline; d) INO-pre—mice were treated with inosine (100 mg/kg, ip) 30 mins before LPS injection; e) INO-post—mice were treated with inosine (100 mg/kg, ip) 60 mins after LPS injection.

Gut Permeability Measurements. Intestinal mucosal barrier function was assessed by the mucosal-to-serosal clearance of fluorescein isothiocyanate-conjugated dextran (4 kDa, FD4) in everted gut ileal sacs incubated *ex vivo* (24). The everted gut sacs were prepared in ice-cold modified Krebs-Henseleit bicarbonate buffer (KHBB contained in mM: HEPES 10, NaCl 137, KCl 5.5, $NaHCO_3$ 4.2, Na_2HPO_4 0.3, $KHPO_4$ 0.4, $MgSO_4$ 0.4, $MgCl_2$ 0.5, $CaCl_2$ 1.3, glucose 19.5). The ileal segment was first lavaged with 3 mL of isotonic saline to remove fecal material and was then closed at one end with a 4-0 silk ligature. The gut sac was everted onto a thin plastic rod, then connected to a 3-mL syringe containing 0.4 mL of the KHBB solution, via a male luer fitting for 1/16-inch (approximately, 1.5 mm) internal diameter tubing (World Precision Instrument, Sarasota, FL), and secured with a 4-0 silk ligature. The everted gut sac was then gently distended with 0.4 mL of KHBB, then suspended in a 50-mL beaker containing FD4 (20 μg/mL) in KHBB, continuously gassed with 95% oxygen and 5% CO_2, and maintained at 37°C in a water bath.

At the beginning of the incubation, a 1-mL sample was withdrawn from the beaker to determine the initial external (i.e., mucosal) FD4 concentration ($FD4_{muc}$). After a 30-min incubation period, the gut sac was removed from the beaker, its diameter (D) and length (L) were measured, and the KHBB solution (0.4 mL) was withdrawn from within the sac to determine the internal (i.e., serosal) FD4 concentration ($FD4_{ser}$). After centrifugation (1000 × *g*, 10 mins), 300 μL of the clear supernatants were diluted with 3 mL of phosphate-buffered saline (10 mM; pH 7.4) and fluorescence was measured (λex = 492 nm, slit width = 1.5 nm; λem = 515 nm, slit width = 10 nm) in a spectrofluorometer (model RF 5301, Shimadzu, Columbia, MD). The mucosal-to-serosal clearance of FD4 was calculated using the following equations:

$$\text{Mucosal surface area (A)} = \pi LD$$

$$\text{Mass of FD4 in the gut sac after 30-min incubation (M)} = (FD4_{ser}) \times 0.4.$$

$$\text{Mucosal-to-serosal permeation rate of FD4 (PR, ng/min)} = M/30 \text{ min}$$

$$\text{Mucosal-to-serosal clearance of FD4 (C, nL/min/cm}^2) = (PR/FD4_{muc})/A$$

Vascular Reactivity in Isolated Aortic Rings. The thoracic aortae were harvested after 8 hrs of LPS injection, cleared from periadvential fat, and mounted in organ baths filled with warmed (37°C) and gas-equilibrated (95% oxygen, 5% CO_2) Krebs' solution (mM): $CaCl_2$ 1.6, $MgSO_4$ 1.17, EDTA 0.026, NaCl 130, $NaHCO_3$ 14.9, KCl 4.7, KH_2PO_4 1.18, glucose 11. Isometric tension was measured with isometric transducers (Kent Scientific, Litchfield, CT), digitized using a MacLab A/D converter and stored and displayed on a Macintosh computer. The preload was 1 g. The rings were equilibrated for 60 mins, and the solution was changed every 15 mins. Dose-response curves to phenylephrine (10^{-10} to 3×10^{-5} M) and acetylcholine (10^{-9} to 3×10^{-4} M) were obtained.

Myeloperoxidase Assay (MPO). Samples from gut, liver, and lung were harvested, snap frozen in liquid nitrogen, and stored at −70°C for further biochemical measurements. Tissues were homogenized (50 mg/mL) in 0.5% hexadecyltrimethylammonium bromide in 10 mM 3-[*N*-morpholino]propane sulfonic acid and centrifuged at 15,000 × *g* for 40 mins. The suspension was then sonicated three times for 30 secs. An aliquot of supernatant was mixed with a solution of 1.6 mM tetramethylbenzidine and 1 mM hydrogen peroxide. Activity was measured spectrophotometrically as the change in absorbance at 650 nm at 37°C, using a Spectramax microplate reader (Molecular Devices, Sunnyvale, CA). Results are expressed as milliunits of MPO activity per mg of protein, which were determined with the Bio-Rad assay (Bio-Rad, Hercules, CA).

Malondialdehyde Assay (MDA). Malondialdehyde formation was used to quantify the lipid peroxidation in tissues and measured as thiobarbituric acid-reactive. Tissues were homogenized (100 mg/mL) in 1.15% KCl buffer. A total of 200 μL of the homogenates were then added to a reaction mixture consisting of 1.5 mL of 0.8% thiobarbituric acid, 200 μL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid (pH 3.5), and 600 μL of distilled water. The mixture was then heated at 90°C for 45 mins. After cooling to room temperature, the samples were cleared by centrifugation (10,000 × *g*, 10 mins) and their absorbance was measured at 532 nm, using 1,1,3,3-tetramethoxypropane as an external standard. The level of lipid peroxides was expressed as nmol MDA/mg of protein.

Statistical Evaluation. Values in the figures and text are expressed as mean ± SEM of n observations. Statistical analysis of the data was performed by analysis of variance, followed by the Tukey's test, as appropriate. The number of experimental determinations used was n ≥ 8 per group and is specified in the figure legends.

RESULTS

Effect of Inosine on Gut Permeability

We first determined whether inosine pretreatment or posttreatment can influence the endotoxin-induced gut hyperpermeability. The mucosal-to-serosal passage of FD4 was low in control and inosine control animals, the calcu-

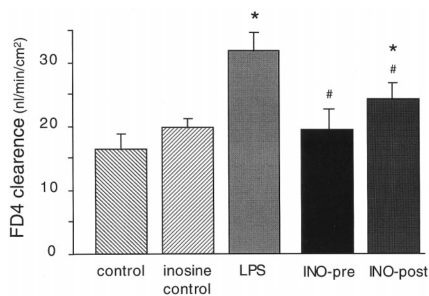

Figure 1. Gut mucosal permeability to isothiocyanate fluorescein dextran (*FD4*) in everted gut sacs incubated *ex vivo*. A segment of ileum was harvested after 16 hrs of lipopolysaccharide (*LPS*) injection to measure the mucosal-to-serosal passage of FD4. Inosine was given in the LPS-treated group as a pretreatment (*INO-pre*) or as a posttreatment (*INO-post*) (mean ± SEM of 9–12 determinations in each experimental group). $*p < .05$ control vs. LPS: significant alteration in response to LPS, compared with vehicle-treated control animals in the absence of LPS. $\#p < .05$ LPS vs. LPS plus INO-pre or LPS vs. INO-post: significant improvement by inosine (pre- or posttreatment) in the presence of LPS vs. LPS alone.

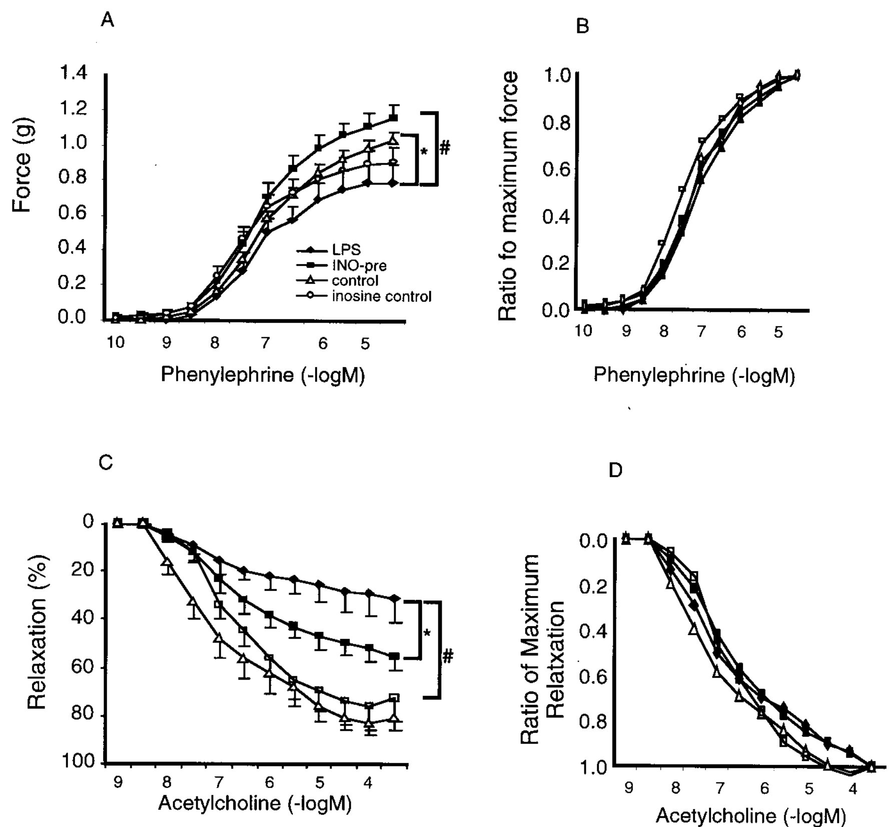

Figure 2. Vascular rings experiments. Thoracic aortae were harvested after 8 hrs of lipopolysaccharide (*LPS*) injection to measure their contraction (*A*) to phenylephrine or relaxation (*C*) to acetylcholine. In *panels B* and *D,* the values are percentage of corresponding maximum effect. Mean ± SEM of 10–12 determinations in each experimental group. $\#p < .05$ control vs. LPS: significant alteration in response to LPS compared with vehicle-treated control animals in the absence of LPS. $*p < .05$ LPS vs. pretreatment (*INO-pre*): significant improvement by inosine pretreatment in the presence of LPS vs. LPS alone.

lated clearance being 16.4 ± 2.4 and 19.7 ± 1.4 nL/min/cm^2, respectively (Fig. 1). LPS (40 mg/kg, ip)-treated animals demonstrated an increase in gut permeability, the calculated clearance reaching 31.9 ± 2.8 nL/min/cm^2. In inosine-treated animals, there was a significantly reduction of permeability. The calculated clearance was 19.4 ± 3.0 and 24.3 ± 2.6 nL/min/cm^2, INO-pre and INO-post respectively (Fig. 1).

Effect of Inosine on Vascular Reactivity

The aortic rings taken from LPS-treated animals (100 mg/kg, ip) showed a significantly reduced peak response to phenylephrine (0.792 ± 0.109 g) compared with controls. INO-pre animals showed improved contractility (1.162 ± 0.079 g) (Fig. 2). However, inosine 60 mins after LPS failed to protect against the endotoxin-induced loss of vascular contractility (data not shown).

The endothelial function was analyzed by relaxant responsiveness of precontracted vascular rings to the endothelium-dependent vasodilator, nitric oxide-liberating hormone, acetylcholine. LPS animals developed a greater endothelial dysfunction with a reduced relaxant response (30.9% ± 10.1%) than controls, and inosine (applied as a pretreatment) improved endothelial function (55.0% ± 5.6%) (Fig. 2). The data were also plotted as a percentage of the maximum developed tension or relaxation to determine the sensitivity of rings to phenylephrine or acetylcholine. The percentage maximum developed tension and relaxation curves were nearly identical for LPS, INO-pre, control, and inosine control groups (Fig. 2, *B* and *D*).

Inosine Reduces the LPS-Induced Intraorgan Inflammatory Processes

Tissue Myeloperoxidase. The activity of MPO in selected tissues is demonstrated in Figure 3. In the lung, there is an increase in MPO levels in LPS (100 mg/kg, ip)-treated animals and both pre- and posttreatment with inosine protected against this alteration. Also, in the gut, MPO activity was reduced in the presence of inosine, both in pre- and posttreatment regimens (for example, respective MPO values were 5.7 ± 1.1, 3.5 ± 0.6, and 3.8 ± 0.7 mU/mg for LPS, INO-pre, and INO-post) (Fig. 3*A*). In the liver, there was a slight increase in MPO levels in response to LPS, and there were no significant differences among the groups studied (Fig. 3*C*).

Tissue Levels of MDA. In gut and lung, there were significant increases in the levels of MDA in response to LPS (gut, 1.45 ± 0.09 nmol/mg of protein; lung, 14.41 ± 1.36 nmol/mg of protein). Inosine pretreatment (gut, 1.13 ± 0.07 nmol/mg of protein; lung, 3.66 ± 0.32 nmol/mg of protein) and inosine posttreatment (gut, 0.88 ± 0.21 nmol/mg of protein; lung, 4.79 ± 0.46 nmol/mg of protein) both reduced the tissue MDA levels, as shown in Figure 4. In the liver, LPS significantly increased MDA generation, and inosine did not have any significant effects (Fig. 4, *bottom*).

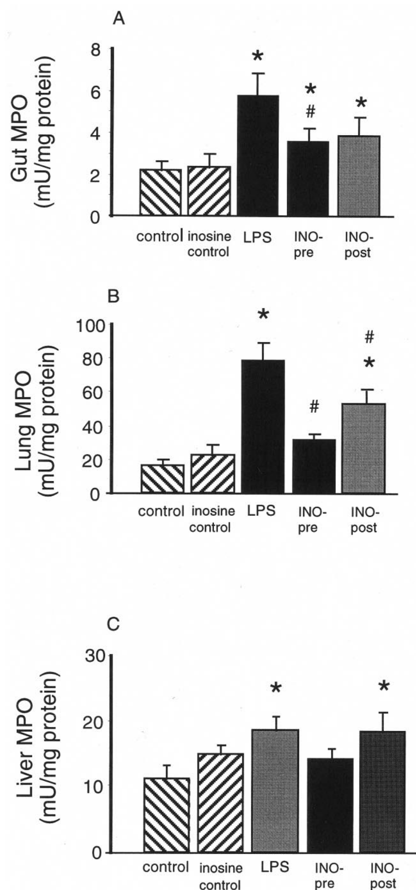

Figure 3. Myeloperoxidase (*MPO*) activity in the gut (*A*), lung (*B*), and liver (*C*) obtained from mice after lipopolysaccharide (*LPS*) injection. The tissues were harvested after 8 hrs. Mean ± SEM of 8 determinations in each experimental group. *$p < .05$ control vs. LPS: significant alteration in response to LPS compared with vehicle-treated control animals in the absence of LPS. #$p < .05$ LPS vs. LPS plus pretreatment (*INO-pre*) or LPS vs. posttreatment (*INO-post*): significant improvement by inosine (pre- or posttreatment) in the presence of LPS vs. LPS alone.

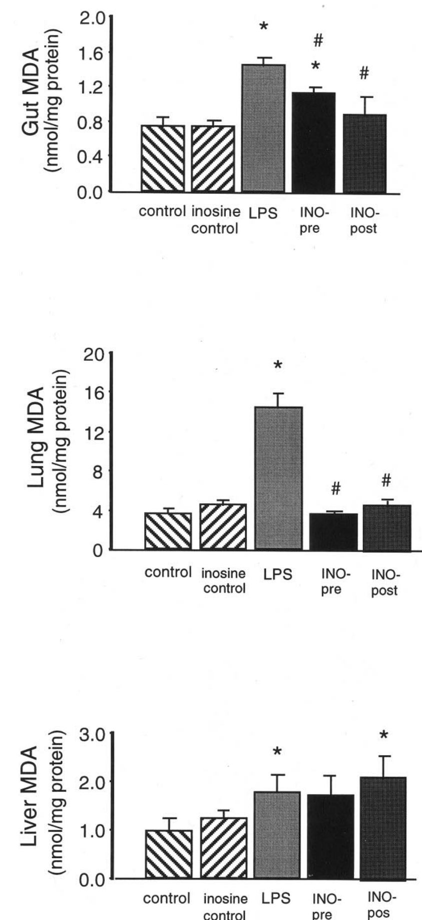

Figure 4. Concentrations of malondialdehyde (*MDA*) measured in the gut (*top*) lung (*middle*), and liver (*bottom*) obtained from mice after lipopolysaccharide (*LPS*) injection. The tissues were harvested after 8 hrs. Mean ± SEM of 8 determinations in each experimental group. *$p < .05$ control vs. LPS: significant alteration in response to LPS compared with vehicle-treated control animals in the absence of LPS. #$p < .05$ LPS vs. LPS plus pretreatment (*INO-pre*) or LPS vs. posttreatment (*INO-post*): significant improvement by inosine (pre- or posttreatment) in the presence of LPS vs. LPS alone.

DISCUSSION

Until recently, inosine was considered an inactive purine metabolite in most biological systems. However, evidence from our laboratory, as well as other groups of investigators, indicates that extracellular inosine has powerful cellular protective effects. In addition to the already mentioned modulation of pro- and anti-inflammatory chemokine and cytokine production (see Introduction and Reference 21), it prevents glial cell death during glucose deprivation (25), decreases the release of intracellular enzymes from hypoxic lymphocytes (26), improves renal function during ischemia (27, 28), and removes the harmful effects of total hepatic ischemia (29). Inosine administration has also been shown to improve myocardial function during acute left ventricular failure (28, 29) and decrease infarct size after coronary occlusion (30–32). Most of these actions were previously ascribed to the potential vasodilatory effects of this purine.

In the current study, we present evidence that inosine exerts potent organ protective effects *in vivo*. Part of these actions is related to inosine's ability to decrease polymorphonuclear accumulation and lipid peroxidation in lung and gut. Gut and lung are pivotal organs affected in multiple organ dysfunction. A number of studies have highlighted the central role of the gut in the pathophysiology of shock of various origins. The gut is particularly prone to develop both ischemic and reperfusion injuries because of the countercurrent shunting of blood in the villi (33), as well as the abundant content of xanthine dehydrogenase/xanthine oxidase in the gut mucosa (34). In line with these concepts, we found that animals subjected to endotoxin shock developed a significant gut mucosal barrier failure, as evidenced by a marked increase of gut permeability to FD4 (Fig. 1). Our current results demonstrate that inosine reduces the inflammatory response in the gut and preserves the intestinal permeability function. Recent data have indicated that gut adenosine triphosphate content is directly correlated to the severity of mucosal barrier failure (35). Reactive oxidant species are known to activate futile cellular adenosine triphosphate consuming pathways, such as the one triggered by poly(ADP-ribose) synthetase (36–38). The reduction by inosine of malondialdehyde formation (an index of lipid peroxidation and reactive oxidant production) indicates that the site of inosine's action is proximal to free radical generation and the above-mentioned energy-consuming processes.

We also analyzed certain indicators of vascular failure in the current model of endotoxin shock. Inosine treatment improved vascular reactivity, as evidenced by a higher developed tension of the thoracic aorta to phenylephrine (Fig. 2). The endothelial function was also improved by inosine, with better endothelium-dependent relaxant responses elicited by acetylcholine. A normal vascular reactivity is a crucial factor in maintaining adequate blood perfusion regulation, and its impairment is associated with end-organ damage (39–42). The endothelial dysfunction is initiated, at least in part, by leukocyte adherence to the blood vessels (39). In hemorrhagic shock, an early microvascular dysfunction has been shown to contribute to the late sequelae of intestinal ischemia and can alter microvascular responses to subsequent systemic insults (40). It is conceivable that also in our current model, an early endothelial dysfunction is followed by leukocyte accumulation and eventual increases in gut permeability. In this sequence of events, intestinal damage will subsequently trigger additional, late multiple organ dysfunction. The vascular dysfunction is, at

We propose the potential use of inosine for the experimental therapy of circulatory shock.

least in part, dependent on the production of proinflammatory cytokines, such as tumor necrosis factor-α: its neutralization can partially protect against the development of vascular hyporeactivity (43). Because inosine can suppress proinflammatory cytokine production (21), it is possible that its protective effects are, at least in part, related to reduced proinflammatory cytokine levels. Taken together, we propose that the protective effects of inosine on the vascular responsiveness may, at least partly, be attributable to its effects on inflammatory mediator production.

Although we have now provided evidence that inosine improves multiple constituents of multiple organ failure induced by endotoxin, we have not presented data in the current manuscript for endotoxin-induced alterations in hemodynamic variables or tissue acidosis. Nevertheless, previous studies, using LPS doses similar to the ones used in the current manuscript, have indeed reported significant hemodynamic and metabolic alterations. Based on the current data and our recent work (21) demonstrating that inosine reduced LPS-induced mortality in mice, it is possible that inosine also improves additional (hemodynamic or metabolic) aspects of the shock syndrome. However, the effects of inosine on the hemodynamic and metabolic alterations in various forms of circulatory shock remain to be further characterized.

We have previously demonstrated that the *in vitro* inhibitory effect of inosine on proinflammatory mediator production is maintained when the purine is given later than the stimulus of the inflammatory mediator production (e.g., LPS). We have also demonstrated that the mode of inosine's action is not related to activation of the p38 and p42/p44 mitogen-activated protein kinases, phosphorylation of the c-Jun terminal kinase, degradation of inhibitory factor κB, or elevation of intracellular cAMP, and it was found to be posttranscriptional or pretranslational (21). This delayed mode of action encouraged us to perform the comparison of pre- and posttreatment effects in the current series of experiments. In line with the *in vitro* studies, in fact, the *in vivo* results also confirmed that a significant portion of inosine's beneficial effects is maintained, even in the posttreatment regimen. Although the current experimental model cannot be directly compared with a clinical situation, the findings are nevertheless encouraging and may indicate a significant window of opportunity for future therapy of shock with inosine.

There was a significant interorgan variability in the degree of neutrophil infiltration and lipid peroxidation in our study. This interorgan variability has previously been shown in the literature: in rodents, LPS was found to induce significantly higher levels of MPO in lung than liver, and these changes are associated with different degree of the expression of the transcription factor nuclear factor κB (44, 45). This difference between the severity of inflammation between various organs may be related to the fact that the expression of adhesion molecules and the infiltration of inflamed organs by mononuclear cells exhibit an organ-specific pattern after LPS challenge (46, 47). Although the exact reasons why the degree of neutrophil infiltration and the degree of oxidant generation show marked organ-specific differences are not completely understood, our work clearly shows that inosine is able to downregulate these processes in all affected organs tested.

Inosine has been, in fact, sporadically used in clinical practice for various forms of cardiovascular disorders, including certain ischemic events (17). The daily tolerated doses of inosine in humans are extremely high, up to levels of 5–6 g/day (48–50) (which is comparable with the 100-mg/kg dose used in our current rodent studies). Based on our data, coupled with the excellent safety profile of inosine, the concept of testing inosine in patients with circulatory shock may be not at all far fetched.

In vivo, inosine is metabolized to urate. In fact, humans taking high doses of inosine orally have significant increases in plasma urate levels (48). Urate is a potent scavenger of peroxynitrite or peroxynitrite-derived reactive nitrogen species (51, 52). Therefore, the possibility exists that urate-mediated antioxidant effects also contribute to the protection seen with inosine. It is noteworthy, in this context, that a recent study demonstrated that urate treatment exerts potent hepatoprotective effects in a rodent model of hemorrhagic shock (53).

In summary, our study demonstrated that inosine reduces a variety of features of multiple organ dysfunction associated with LPS, as demonstrated in the lung, gut, endothelium, and vascular smooth muscle. A significant proportion of the protective effects of inosine is maintained in the posttreatment scenario. We propose the potential use of inosine for the experimental therapy of circulatory shock.

REFERENCES

1. Cronstein BN: Adenosine, an endogenous anti-inflammatory agent. *J Appl Physiol* 1994; 76:5–13
2. Hasko G, Szabo C: Regulation of cytokine and chemokine production by transmitters and co-transmitters of the autonomic nervous system. *Biochem Pharmacol* 1998; 56: 1079–1087
3. Apasov S, Koshiba M, Redegeld F, et al: Role of extracellular ATP and P1 and P2 classes of purinergic receptors in T-cell development and cytotoxic T lymphocyte effector functions. *Immunol Rev* 1995; 146:5–19
4. Hasko G, Nemeth ZH, Vizi ES, et al: An agonist of adenosine A3 receptors decreases interleukin-12 and interferon-gamma production and prevents lethality in endotoxemic mice. *Eur J Pharmacol* 1998; 358: 261–268
5. Parmely MJ, Zhou WW, Edwards CK, et al: Adenosine and a related carbocyclic nucleoside analogue selectively inhibit tumor necrosis factor-alpha production and protect mice against endotoxin challenge. *J Immunol* 1993; 151:389–396
6. Green PG, Basbaum AI, Helms C, et al: Purinergic regulation of bradykinin-induced plasma extravasation and adjuvant-induced arthritis in the rat. *Proc Natl Acad Sci U S A* 1991; 88:4162–4165
7. Szabo C, Scott GS, Virag L, et al: Suppression of macrophage inflammatory protein (MIP)-1alpha production and collagen-induced arthritis by adenosine receptor agonists. *Br J Pharmacol* 1998; 125:379–387
8. Schrier DJ, Lesch ME, Wright CD, et al: The antiinflammatory effects of adenosine receptor agonists on the carrageenan-induced pleural inflammatory response in rats. *J Immunol* 1990; 145:1874–1879
9. Poelstra K, Heynen ER, Baller JF, et al: Modulation of anti-Thy1 nephritis in the rat by adenine nucleotides: Evidence for an anti-inflammatory role for nucleotidases. *Lab Invest* 1992; 66:555–563

10. Marak GE Jr, de Kozak Y, Faure JP, et al: Pharmacologic modulation of acute ocular inflammation: I. Adenosine. *Ophthalmic Res* 1988; 20:220–226
11. Cain BS, Harken AH, Meldrum DR: Therapeutic strategies to reduce TNF-alpha mediated cardiac contractile depression following ischemia and reperfusion. *J Mol Cell Cardiol* 1999; 31:931–947
12. Barankiewicz J, Cohen A: Purine nucleotide metabolism in resident and activated rat macrophages in vitro. *Eur J Immunol* 1985; 15:627–631
13. Bell MJ, Kochanek PM, Carcillo JA, et al: Interstitial adenosine, inosine, and hypoxanthine are increased after experimental traumatic brain injury in the rat. *J Neurotrauma* 1998; 15:163–170
14. Wang T, Sodhi J, Mentzer RM Jr, et al: Changes in interstitial adenosine during hypoxia: Relationship to oxygen supply:demand imbalance, and effects of adenosine deaminase. *Cardiovasc Res* 1994; 28:1320–1325
15. Rego AC, Santos MS, Oliveira CR: Adenosine triphosphate degradation products after oxidative stress and metabolic dysfunction in cultured retinal cells. *J Neurochem* 1997; 69:1228–1235
16. Fredholm BB, Sollevi A: The release of adenosine and inosine from canine subcutaneous adipose tissue by nerve stimulation and noradrenaline. *J Physiol* 1981; 313:351–367
17. Juhasz-Nagy A, Aviado DM: Inosine as a cardiotonic agent that reverses adrenergic beta blockade. *J Pharmacol Exp Ther* 1977; 202: 683–695
18. Jabs CM, Neglen P, Eklof B: Breakdown of adenine nucleotides, formation of oxygen free radicals, and early markers of cellular injury in endotoxic shock. *Eur J Surg* 1995; 161:147–155
19. Grum CM, Simon RH, Dantzker DR, et al: Evidence for adenosine triphosphate degradation in critically-ill patients. *Chest* 1985; 88:763–767
20. Schmidt H, Siems WG, Grune T, et al: Concentration of purine compounds in the cerebrospinal fluid of infants suffering from sepsis, convulsions and hydrocephalus. *J Perinat Med* 1995; 23:167–174
21. Hasko G, Kuhel DG, Nemeth ZH, et al: Inosine inhibits inflammatory cytokine production by a posttranscriptional mechanism and protects against endotoxin-induced shock. *J Immunol* 2000; 164:1013–1019
22. Wattanasirichaigoon S, Menconi MJ, Delude RL, et al: Effect of mesenteric ischemia and reperfusion or hemorrhagic shock on intestinal mucosal permeability and ATP content in rats. *Shock* 1999; 12:127–133
23. Lowell CA, Berto G: Resistance to endotoxic shock and reduced neutrophil migration in mice deficient for the Src-family kinases Hck and Fgr. *Proc Natl Acad Sci U S A* 1998; 95:7580–7584
24. Hanasaki K, Yokota Y, Ishizaki J, et al: Resistance to endotoxic shock in phospholipase A_2 receptor-deficient mice. *J Biol Chem* 1997; 272:32792–32797
25. Haun SE, Segeleon JE, Trapp VL, et al: Inosine mediates the protective effect of adenosine in rat astrocyte cultures subjected to combined glucose-oxygen deprivation. *J Neurochem* 1996; 67:2051–2059
26. Cole AW, Palmer TN: Action of purine nucleosides on the release of intracellular enzymes from rat lymphocytes. *Clin Chim Acta* 1979; 92:93–100
27. Fernando AR, Armstrong DM, Griffiths JR, et al: Enhanced preservation of the ischaemic kidney with inosine. *Lancet* 1976; 1:555–557
28. de Rougemont D, Brunner FP, Torhorst J, et al: Superficial nephron obstruction and medullary congestion after ischemic injury: Effect of protective treatments. *Nephron* 1982; 31:310–320
29. Tilser I, Martinkova J, Chladek J: The effect of metipranolol and inosine on total hepatic ischemia of rats in vivo. *Sb Ved Pr Lek Fak Karlovy Univerzity Hradci Kralove* 1993; 36: 25–29
30. Woollard KV, Kingaby RO, Lab MJ, et al: Inosine as a selective inotropic agent on ischaemic myocardium? *Cardiovasc Res* 1981; 15:659–667
31. Devous MD Sr, Jones CE: Effect of inosine on ventricular regional perfusion and infarct size after coronary occlusion. *Cardiology* 1979; 64:149–161
32. Czarnecki W, Herbaczynska-Cedro K: The influence of inosine on the size of myocardial ischaemia and myocardial metabolism in the pig. *Clin Physiol* 1982; 2:189–197
33. Revelly JP, Ayuse T, Brienza N, et al: Endotoxic shock alters distribution of blood flow within the intestinal wall. *Crit Care Med* 1996; 24:1345–1351
34. Qu XW, Rozenfeld RA, Huang W, et al: The role of xanthine oxidase in platelet activating factor induced intestinal injury in the rat. *Gut* 1999; 44:203–211
35. Wattanasirichaigoon S, Menconi MJ, Delude RL, et al: Effect of mesenteric ischemia and reperfusion or hemorrhagic shock on intestinal mucosal permeability and ATP content in rats. *Shock* 1999; 12:127–133
36. Ha HC, Snyder SH: Poly(ADP-ribose) polymerase is a mediator of necrotic cell death by ATP depletion. *Proc Natl Acad Sci U S A* 1999; 96:13978–13982
37. Kennedy M, Denenberg AG, Szabo C, et al: Poly(ADP-ribose) synthetase activation mediates increased permeability induced by peroxynitrite in Caco-2BBe cells [see comments]. *Gastroenterology* 1998; 114: 510–518
38. Szabo C, Dawson VL: Role of poly(ADP-ribose) synthetase in inflammation and ischaemia- reperfusion. *Trends Pharmacol Sci* 1998; 19:287–298
39. Scalia R, Pearlman S, Campbell B, et al: Time course of endothelial dysfunction and neutrophil adherence and infiltration during murine traumatic shock. *Shock* 1996; 6:177–182
40. Fruchterman TM, Spain DA, Wilson MA, et al: Selective microvascular endothelial cell dysfunction in the small intestine following resuscitated hemorrhagic shock. *Shock* 1998; 10:417–422
41. Hayward R, Lefer AM: L-arginine attenuates endothelial dysfunction and prolongs survival in rats subjected to traumatic shock. *Endothelium* 1998; 6:71–79
42. Szabo C, Cuzzocrea S, Zingarelli B, et al: Endothelial dysfunction in a rat model of endotoxic shock: Importance of the activation of poly (ADP-ribose) synthetase by peroxynitrite. *J Clin Invest* 1997; 100:723–735
43. Thiemermann C, Wu CC, Szabo C, et al: Role of tumor necrosis factor in the induction of nitric oxide synthase in a rat model of endotoxin shock. *Br J Pharmacol* 1993; 110: 177–182
44. Kamochi M, Kamochi F, Kim YB, et al: P-selectin and ICAM-1 mediate endotoxin-induced neutrophil recruitment and injury to the lung and liver. *Am J Physiol* 1999; 2777:L310–L319
45. Liu SF, Ye X, Malik AB: Pyrrolidine dithiocarbamate prevents I-kappa B degradation and reduces microvascular injury induced by lipopolysaccharide in multiple organs. *Mol Pharmacol* 1999; 55:658–667
46. Gabellec MM, Jafarian-Tehrani M, Griffais R, et al: Interleukin-1 receptor accessory protein transcripts in the brain and spleen: kinetics after peripheral administration of bacterial lipopolysaccharide in mice. *Neuroimmunomodulation* 1996; 3:304–309
47. Henninger DD, Panes J, Eppihimer M, et al: Cytokine-induced VCAM-1 and ICAM-1 expression in different organs of the mouse. *J Immunol* 1997; 158:1825–1832
48. McNaughton L, Dalton B, Tarr J: Inosine supplementation has no effect on aerobic or anaerobic cycling performance. *Int J Sport Nutr* 1999; 9:333–344
49. Reid PG, Watt AH, Routledge PA, et al: Intravenous infusion of adenosine but not inosine stimulates respiration in man. *Br J Clin Pharmacol* 1987; 23:331–338
50. Williams MH, Kreider RB, Hunter DW, et al: Effect of inosine supplementation on 3-mile treadmill run performance and VO2 peak. *Med Sci Sports Exerc* 1990; 22:517–522
51. Szabo C, Salzman AL: Endogenous peroxynitrite is involved in the inhibition of mitochondrial respiration in immuno-stimulated J774.2 macrophages. *Biochem Biophys Res Commun* 1995; 209:739–743
52. Squadrito GL, Cueto R, Splenser AE, et al: Reaction of uric acid with peroxynitrite and implications for the mechanism of neuroprotection by uric acid. *Arch Biochem Biophys* 2000; 376:333–7
53. Tsukada K, Hasegawa T, Tsutsumi S, et al: Effect of uric acid on liver injury during hemorrhagic shock. *Surgery* 2000; 127: 439–46