Actions of Adenosine on Nitro Blue Tetrazolium Deposition and Surface pH During Intestinal Reperfusion Injury

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Mesenteric arteries supplying an intestinal segment were occluded for 5 minutes and then released. During reperfusion, two series of measurements were made with various substances topically applied to the extraluminal surface. In the first series, reduced nitro blue tetrazolium (NBT) was extracted from tissue and measured spectrophotometrically, as an index of oxidative damage. In the second series, mucosal and serosal surface pH was measured as an index of the functional ability to maintain ion gradients. In control conditions, NBT deposition averaged 55–63 μg/g tissue. After 60 and 120 minutes of reperfusion, NBT was elevated to 446–479 μg/g, which was approximately half as large as the NBT increment (846 μg/g) produced by a 15-minute application of xanthine plus xanthine oxidase to well-perfused tissue. As expected, NBT levels were significantly lower (299 μg/g) in tissue that was continuously suffused with superoxide dismutase (SOD) plus catalase (CAT) before occlusion and during reperfusion. Similar NBT levels (274 μg/g) were observed after reperfusion in animals that were fed a diet supplemented with the antioxidant vitamin E for 4–6 weeks. These observations affirm that some, but not all, NBT deposition after reperfusion can be attributed to oxyradicals. However, with exogenous adenosine (ADO) applied for the first 30 minutes after occlusion, NBT was elevated to 174 μg/g after 60 minutes, which was only half as large as the increment with SOD plus CAT, even though those substances were continuously applied. The opposite effect was produced by an ADO receptor antagonist, 8-phenyltheophylline; NBT was increased to 516 μg/g. On the other hand, NBT after reperfusion was 294 μg/g with aminoimidazolecarboxamide riboside, an intermediate in adenine nucleotide metabolism that putatively increases endogenous ADO levels during ischemia. This value was similar to the NBT level observed in reperfused tissues treated with SOD plus CAT. With vehicle, mucosal surface pH averaged 7.1, decreased to 6.7 during occlusion, transiently increased during reperfusion, but stabilized at 6.7 after 60 minutes, which was below baseline. However, with ADO, mucosal pH did not decrease during reperfusion. With xanthine plus xanthine oxidase applied to normally perfused tissue for 15 minutes, mucosal pH was reduced to 6.7 after a 60-minute washout. Overall, these results show that 1) NBT deposition in reperfused intestinal segments is attenuated by exogenous ADO and aggravated by 8-phenyltheophylline, 2) aminoimidazolecarboxamide is approximately as effective as SOD plus CAT or a vitamin E–enriched diet for attenuating NBT deposition, but all are less effective than exogenous ADO, 3) oxygen radicals cause functional alternations that are reflected by changes in mucosal, but not serosal, surface pH, and 4) mucosal pH decreases during reperfusion are prevented by exogenous ADO. (Circulation Research 1990;66:1713–1719)

Although early reperfusion is the most effective means for salvaging ischemic tissues, several metabolic and anatomic disturbances accompany the reintroduction of blood flow, including damage of vascular integrity, tissue edema, and disturbances in cellular energy balance. Oxygen radicals have a major role in the pathogenesis of this injury in the small intestine. Exogenous adenosine (ADO) can attenuate reperfusion injury in the intestine by a mechanism linked to neutrophils. Since ADO inhibits oxygen radical generation by a receptor-mediated mechanism in stimulated neutrophils in vitro, one attractive hypothesis is that ADO reduces oxygen radical generation in the neutrophils that infiltrate reperfused intestine. Although it is difficult to detect oxygen...
radicals directly, certain indicators (e.g., nitro blue tetrazolium [NBT]) will react with oxidants and generate products that can be detected spectrophotometrically. The major purpose of this study was to compare the effect of specific oxygen radical scavengers, superoxide dismutase (SOD) and catalase (CAT), with that of ADO on the deposition of the oxidant indicator NBT in the reperfused intestine. A secondary purpose was to explore whether oxidative changes caused by reperfusion injury are accompanied by changes in tissue pH. This logic was based on the assumption that ischemia-induced oxidative membrane damage could interfere with the ability of the intestinal epithelium to maintain ion gradients, which would be reflected by changes in tissue pH.

Materials and Methods

Male Sprague-Dawley rats (160–220 g) were anesthetized with an aqueous mixture containing 13% urethane and 1% α-chloralose (1.0 ml/100 g body wt i.p.); supplemental doses were administered when necessary (0.1 ml/100 g body wt i.v.). The trachea, femoral artery, and femoral vein were cannulated. Respiration was spontaneous on room air supplemented with oxygen. Rectal temperature was continuously monitored (Yellow Springs Instrument, Yellow Springs, Ohio) and maintained at 36–37° C with a servo-controlled heat lamp. Femoral arterial blood pressure was continuously monitored with a transducer (P231D, Gould-Statham, Oxnard, California) connected to a polygraph (model 2600, Gould) and averaged 100±1 mm Hg (n = 79) for the 2–3-hour duration of the typical experiment.

A 2–3-cm segment of jejunum with intact innervation and vascular supply was drawn through an abdominal incision. Several sutures were attached to the antimesenteric border for traction. A rubber ligature was placed around the arteries supplying the tissue segment for later vascular occlusion. Otherwise, there was no surgical manipulation of the tissue.

The animal was positioned laterally, and the loop of tissue was immersed in a chamber containing normasol solution consisting of (mM) NaCl 90.0, KCl 5.0, CaCl₂ 2.0, MgCl₂ 1.5, sodium glutonate 23.0, and sodium acetate buffered with 0.5 mM 3-(N-morpholino)propanesulfonic acid (Sigma Chemical, St., Louis, Missouri). Solution pH averaged 7.38±0.01 (n = 54). Sutures were fixed to the bottom of a tissue chamber that was covered with a silicon elastomer (Sylgard, Dow Corning, Midland, Michigan). The position of the animal and the chamber construction prevented any tension to the mesentery or the loop of the intestine and allowed unrestricted movement of the intestinal contents through the loop. Tissue temperature was continuously monitored and maintained at 36–37° C by adjusting the flow rate (3–7 ml/min) of the heated suffusion solution.

Experimental Protocol for NBT Series

After a 30–60-minute postsurgery stabilization period, the mesenteric arteries were occluded for 5 minutes by tightening the rubber ligature. For the first 30 minutes of reperfusion, the bathing solution contained either 10⁻⁴ M ADO (Sigma Chemical) or normasol. Thereafter, that solution was removed and replaced with a normasol solution containing 1.2 mM NBT for an additional 30 minutes. The ADO concentration was based on a recent study that showed that 10⁻⁴ M ADO produced submaximal vasodilation and attenuated intestinal reperfusion injury. The concentration of NBT was based on that used in previous work. The various substances were applied only to the serosa because the mucosa presents a physical and metabolic diffusion barrier that limits the passage of ADO from the lumen to the interstitium.

The ischemia-reperfusion protocol was also performed in a group of rats fed a diet that was supplemented with the antioxidant vitamin E for at least 4–6 weeks beginning at weaning. Regular rodent laboratory chow (Purina Mills, St. Louis, Missouri) was enriched with 2.83% d-α-tocopherol (vitamin E) and 5 ppm vitamin K per 10 kg food, which provided 1,000 mg/kg body wt/day vitamin E and 10 mg/kg body wt/day vitamin K. The weight gain and activity level of these animals were similar to those of animals that were housed in identical conditions but fed a standard diet. The effectiveness of the vitamin E supplements was outwardly manifested by a smooth-textured coat.

In three other groups of animals, the basic protocol was slightly modified by continuously applying various substances to the tissue from 20–30 minutes before occlusion until the end of the reperfusion period. The purpose of this modification was to minimize the possibility of incomplete equilibration of the drug with the interstitial space. NBT was added to the solution for the last 30 minutes of reperfusion. The tissue was treated with a normasol solution containing either 10⁻⁴ M aminomidazolecarboxamide riboside (AICAR or GP-1-110-0; Genesia Pharmaceuticals, San Diego, California), 10⁻³ M 8-phenyltheophylline (Sigma Chemical), or 10 µg/ml SOD (from bovine liver; specific activity, 4,300 units/mg solid; Sigma Chemical) plus 20 µg/ml CAT (from bovine liver; specific activity, 2,800 units/mg solid, Sigma Chemical). The concentration of AICAR was based on the recommendation of the manufacturer. The concentration of 8-phenyltheophylline was based on that required to block the vasoactive effect of topically applied ADO in the intestinal microcirculation. The concentrations of SOD plus CAT (SOD+CAT) were based on those required to block the effect of oxiradicals in the microcirculation.

In another group, the mesenteric arteries were not occluded. Instead, the intestinal segment was exposed to a normasol solution containing 0.1 mM xanthine (grade III, sodium salt; Sigma Chemical) plus 0.1
units/ml xanthine oxidase (grade IV; Sigma Chemical) for 15 minutes. That solution was removed and replaced with normasol alone for 30 minutes followed by 30 minutes with normasol containing NBT. Those concentrations of xanthine plus xanthine oxidase (X+XO) generate relatively high levels of oxyradicals in many different biological systems.1,6,12,16–20

Assay Procedure

The oxidizing potential of tissue specimens was measured with a spectrophotometric method based on the reduction of NBT to an insoluble blue precipitate, blue formazan.11,12,21,22 In the various treatment groups, the tissue was exposed to NBT for 30 minutes, harvested, and then soaked for 10–15 minutes in a mixture of 2.5% glutaraldehyde (Fisher Scientific, Fairlawn, New Jersey) and 1% paraformaldehyde (Sigma Chemical) in 0.1 M KH₂PO₄ to rapidly stop metabolic processes. Two tissue samples were harvested in each experiment, one from the treated or injured segment and one from an adjacent untreated or uninjured segment of the jejunum, so that each animal acted as its own control.

Tissue samples were homogenized in 0.9% NaCl and centrifuged at 10,000 rpm for 10 minutes. The supernatant was removed, and the pellets were rehomogenized in KH₂PO₄ to remove the hemoglobin-containing red blood cells. This step is necessary because hemoglobin absorbs light in the same spectral region as reduced NBT.12 Samples were centrifuged again, the supernatant was removed, and the pellets were resuspended in 3 ml reagent-grade pyridine (Malinkrodt, Paris, Kentucky). Visible purple granules were extracted for 15 minutes in boiling H₂O under an exhaust hood. The residue was centrifuged again at 10,000 rpm for 10 minutes, and the extraction was repeated if purple granules were still present in the pellet. Two milliliters of extract was removed, and the optical density was determined with a spectrophotometer (model DU, Beckman Instruments, Fullerton, California) combined with a photometer (model 252, Gilford Instruments and Laboratories, Oberlin, Ohio) at 515 nm against a pyridine blank. Absorbance standards (Gilford Instruments and Laboratories) were used periodically to establish the accuracy and specificity of the readings. For given fixed points of reference, duplicate measurements were reproducible within 1–2% at 515 nm. Absorbance was converted to the amount of reduced NBT, using ascorbic acid as the reductant, as previously described.5,23 Final concentrations were expressed in micrograms of NBT per gram of tissue from a standard calibration curve.

Experimental Protocol for pH Series

For an index of the functional ability of the reperfused intestine to maintain ion gradients, an intestinal segment was surgically prepared as described above, except tissue pH was measured with a surface, flat membrane microelectrode and a flexible reference microelectrode (models MI-406 and MI-402, Microelectrodes, Londonderry, New Hampshire), connected to a pH/ion meter (Fisher Accumet, model 420 Digital, Fisher Scientific, Pittsburgh, Pennsylvania). The system was calibrated in three buffer solutions (Malinkrodt) at pH 4.0, 6.0, and 7.0, before and after each measurement.

For serosal measurements, the electrode was positioned perpendicular to the tissue with the tip gently touching the surface. For mucosal measurements, the electrode was placed through a distal incision in the intestinal wall. Spontaneous intestinal motility had no obvious effect on the readings. Mucus accumulation was periodically washed away with Ringer’s solution.

Statistical Analysis

All values are expressed as mean±SEM. All treatments were randomized, and some measurements were paired. Differences were determined with paired and unpaired t tests and analysis of variance. Significance was assessed at the 95% confidence interval.

Results

NBT Series

Figure 1 shows the amount of NBT extracted (expressed as micrograms per gram of wet tissue) from well-perfused tissue in baseline conditions and in adjacent tissue segments subjected to the ischemia-reperfusion protocol. Mean levels of reduced NBT after the 30-minute incubation in control conditions were 55–63 μg/g tissue in the various treatment groups and were not significantly different. These values can be expressed as a rate of NBT reduction by simple substitution: 56–63 μg/g=67–77 nM/g/30 min=2.2–2.6 nM/g tissue/min.

After 120 minutes in the tissue bath with no occlusion and continuous vehicle suffusion, NBT levels were only slightly elevated (70±2 μg/g tissue, n=5). However, after a 5-minute occlusion and 60 and 120 minutes of reperfusion, NBT significantly increased to 446±18 μg/g tissue (n=10) and 479±46 μg/g tissue (n=6), respectively, which represented increases to 790±70% of control and 746±36% of control. There was no significant difference between NBT deposition after 60 and 120 minutes of reperfusion with vehicle, which suggested that most of the damage occurred early during reperfusion. The lack of difference between 60 and 120 minutes of reperfusion could not be attributed to a supramaximal stimulus. In a separate series, the intestinal segment was exposed to X+XO for 15 minutes and then normasol for 60 minutes. In this condition, NBT was 824±32 μg/g tissue (or 1,408±83% of control, n=8). When SOD or SOD+CAT was in the suffusate, NBT deposition was 258±7 μg/g tissue and 199±9 μg/g, respectively (or 437±18% of control, n=5, and 336±16% of control, n=5). These values were significantly reduced compared with vehicle and significantly different from each other.

With SOD+CAT, NBT deposition averaged 57±4 μg/g tissue (n=7) in control conditions before occlu-
sion. After 60 minutes of reperfusion, the value increased to 299±16 μg/g tissue (537±55% of control, n=7). This value was reduced by one half compared with vehicle, and the difference was significant.

In a separate group of animals fed a diet supplemented with vitamin E for 4–6 weeks, control NBT was 55±4 μg/g tissue (n=6). The levels after reperfusion (274±14 μg/g tissue, n=6) were similar to those with SOD+CAT.

With ADO in the suffusate for the first 30 minutes of reperfusion, NBT was elevated to 174±9 μg/g tissue (297±16% of control, n=12) after 60 minutes. This value was reduced by one half compared with SOD+CAT or vitamin E, and the difference was significant.

In two animals fed the vitamin E–enriched diet, baseline NBT measured was 52 and 64 μg/g. During the first 30 minutes of reperfusion, ADO was topically applied, and NBT deposition was 156 and 167 μg/g after 60 minutes of reperfusion, which was similar to that in ADO-treated but normally fed animals.

With AICAR in the solution in control conditions before occlusion, NBT was 61±3 μg/g tissue (n=5). After 60 minutes of reperfusion, NBT levels were 294±12 μg/g tissue (487±32% of control, n=5). This value was similar to that with SOD+CAT, significantly lower than that with vehicle, and higher than that with exogenous ADO. In contrast, with the ADO receptor antagonist 8-phenylethoplyline in the suffusate, NBT was 62±3 μg/g tissue initially and increased to 516±16 μg/g tissue (857±58% of control, n=8) with reperfusion, which was significantly higher than the value with vehicle.

**pH Series**

Figure 2 shows serosal and mucosal surface pH as a function of time during 60 minutes of reperfusion in the groups treated with vehicle only or ADO. The mean preocclusion pH values with vehicle were 7.36±0.02 for the serosa (n=7) and 7.12±0.02 for the mucosa (n=8). In the ADO group, the mean preocclusion pH values for the serosa and mucosa were similar (7.34±0.01, n=8, and 7.10±0.03, n=5, respectively). In both groups, ischemic insult caused a rapid and significant decrease in pH to 6.69±0.04 in the serosa and 6.70±0.05 in the mucosa with vehicle and 6.69±0.02 and 6.71±0.02, respectively, with ADO suffusion. During the first 15 minutes of reperfusion, pH gradually increased to near preocclusion values in both groups, but thereafter there were regional differences related to the treatment. After 60 minutes of reperfusion, serosal pH was 7.30±0.02 and 7.38±0.02 in the vehicle and ADO groups, respectively, and not different from preocclusion controls. However, after 60 minutes of reperfusion, mucosal pH was 6.73±0.05 with vehicle and 7.12±0.01 with ADO. The vehicle value was significantly below its preocclusion baseline, but the ADO value was similar to its preocclusion baseline. Thus, 1) mucosal pH was lower than serosal pH, 2) both values decreased during ischemia and rebounded during reperfusion, and 3) posts ischemic mucosal pH did not fully recover to its preocclusion baseline, but ADO prevented this change.

Figure 2 also shows mucosal pH values (n=7) in a group treated with X+XO, but with no occlusion. Baseline pH was similar to that in the other groups (7.11±0.02). After 15 minutes of X+XO treatment, followed by 60 minutes of perfusion with normosal,
pH was 6.70±0.04, which was significantly lower than the pretreatment baseline. Thus, oxidative tissue damage is accompanied by decreases in mucosal pH.

**Discussion**

There are five major new findings from this study: 1) The deposition of NBT after 60 minutes of reperfusion was reduced by exogenous ADO, relative to SOD+CAT. Within the framework of the limitations considered below, this observation suggests that postocclusion treatment with ADO is at least as effective (and possibly more effective) for reducing oxidative damage than SOD+CAT, even though those substances were continuously applied before, during, and after occlusion. 2) The deposition of NBT after reperfusion in animals treated with AICAR (which putatively increases endogenous ADO levels during ischemia and the rate of ATP resynthesis) was similar to that observed in animals treated with SOD+CAT, whereas NBT levels with 8-phenyltheophylline (a nonselective antagonist of ADO receptors) changed in the opposite direction. These observations support the hypothesis that endogenous ADO may reduce oxidative damage in reperfused intestine and that methylxanthines can aggravate ischemic injury. 3) In animals fed a diet supplemented with vitamin E for 4–6 weeks, the baseline level of NBT deposition was similar to that of uninjured controls, and the value after 60 minutes of reperfusion was similar to that with SOD+CAT or AICAR but less than that with ADO. However, in two animals fed vitamin E, ADO during reperfusion produced a decrement in NBT to values that were similar to those with ADO alone. These observations support the idea that antioxidant nutrients can protect against some forms of oxidative tissue damage and that vitamin E can attenuate ischemia-reperfusion damage in the intestine, as well as the heart. However, a vitamin E–enriched diet was not as effective as exogenous ADO. 4) Topical application of X+XO to the well-perfused serosa for 15 minutes followed by a 60-minute washout period produced a mucosal surface pH decrease showing that oxidative injury interferes with the ability of the intestinal mucosa to maintain ion gradients. A corollary is that tissue pH may be a useful index for estimating oxidative damage. 5) Reperfusion of intestinal segments was accompanied by a reduction in mucosal, but not serosal, pH, and the reduction was prevented by ADO. This observation suggests that ADO can preserve the ability of the mucosal epithelium to maintain ion gradients after ischemic injury.

**Critique**

The major limitation of this study is that the deposition of NBT is not a specific test for oxygen radicals or oxidative damage. Indeed, virtually any oxidant can convert the yellow water-soluble indicator to the insoluble blue precipitate. The deposition of NBT was considered an index of oxidative damage that accompanied reperfusion injury because the baseline levels in well-perfused tissue were relatively low (55–63 μg/g tissue) and remained relatively low (70±2 μg/g) for the 2-hour duration of the experiment unless the tissue was subjected to an ischemia-reperfusion protocol or was exposed to X+XO. After these experimental stimuli, the NBT levels increased sevenfold to 14-fold, unless various drugs were added to the sulfosate solution.

NBT has been used to estimate oxygen radical production in the extracellular space of cerebral tissue by comparing the amount of precipitate in the presence and absence of SOD+CAT. However, the validity of this approach can be challenged because of potential diffusion limitations caused by electrostatic effects and size differences between the large enzyme and the small indicator molecule. Whereas NBT is a small (molecular weight, 818), positively charged molecule, SOD is a large (molecular weight, 34,000), negatively charged molecule, which could lead to a differential equilibration of these two substances at the site of oxygen radical generation at the cell surface, which is both ruffled and negatively charged. In other words, exogenous
SOD probably cannot scavenge all the endogenously generated oxiradicals that have access to NBT. Therefore, even though NBT deposition may reflect damage caused by oxiradicals and might even be used to measure radicals in some conditions, it may be difficult to equate the SOD-inhibitable fraction of NBT deposition in reperfused intestine to oxygen radicals, per se.

It was assumed that a continuous topical application of 10 μg/ml (43 units/ml) SOD plus 20 μg/ml (56 units/ml) CAT would have scavenged endogenously generated oxygen radicals that entered the extracellular space. Figure 1 shows that SOD+CAT after X+XO prevented most of the increment in NBT deposition. These enzyme concentrations are similar to those used by previous investigators for blocking the effect of oxygen radicals in the microcirculation and were based on the maximum theoretical rates of radical generation, enzyme diffusion coefficients, and equilibration times. Furthermore, at the concentration of SOD used in this present study, the reduction of NBT by bradykinin, arachidonate, and X+XO should have been more than 80% inhibited. Nevertheless, it is possible that SOD+CAT is less effective when topically applied to the intestine because the peptidases in this tissue may have partially inactivated the enzymes. The efficacy of enzymatic scavengers in the reperfused intestine has been most thoroughly studied by Granger’s group, but those investigators typically use a bolus application by an intravascular route, rather than a continuous topical application to the serosal surface, so it is difficult to make direct comparisons with this present study.

The rat has an extremely high level of mucosal xanthine oxidase activity (twofold to fourfold higher than that in dogs and fivefold to sixfold higher than that in the cats), and xanthine oxidase is a major source of oxygen radicals in intestinal reperfusion injury, as well as a potential contaminant in the NBT assay. Nevertheless, the rate of NBT reduction in baseline conditions in these experiments averaged 2.2–2.6 nM/g/min, which is within the range of NBT reduction in cerebral tissue. Therefore, there was no obvious interference of intestinal xanthine oxidase with NBT deposition. The purpose of the pH measurements was to determine whether reperfusion injury might alter the ability of the intestine to maintain ion gradients and whether ADO had any effect on this change. It should be emphasized that the pH measurements were obtained with a surface, rather than an interstitial, electrode. This method reflects leakage across damaged epithelium but ignores effects of glandular secretion and ion changes in the villus core.

Finally, in these present experiments, the total period of occlusion was 5 minutes, and the duration of reperfusion was 1 hour. It is not known whether ADO has similar effects in other species, after longer periods of occlusion, or whether the effects persist during longer periods of reperfusion. These technical details are not trivial, because alterations in purine metabolism and the generation of oxygen radicals depend on the nature of the ischemic stimulus as well as the duration of reperfusion.

**Interpretation**

Since exogenous ADO was more effective at reducing the deposition of NBT in reperfused tissue than SOD+CAT (Figure 1) and since ADO alone was as effective as ADO applied to animals fed a diet supplemented with vitamin E, it is reasonable to conclude that ADO has beneficial properties beyond its inhibitory effect on the generation of oxygen radicals. Based on earlier studies, those properties could include an inhibitory effect on neutrophil migration and adherence, which is relevant because neutrophils are important in the pathogenesis of reperfusion injury and the no-reflow phenomenon. In addition, ADO is a powerful vasodilator in the intestinal microcirculation, which could partially offset the no-reflow phenomenon either by a direct action on vascular smooth muscle or by inhibition of neurally mediated vasoconstriction. And, finally, one of the oldest ideas is that ADO could accelerate the postischemic regeneration of depleted purines.

An especially interesting observation is that manipulating the action of endogenous ADO with drugs had corresponding effects on NBT in reperfused tissue; that is, the precipitation was more extensive with 8-phenyltheophylline than with vehicle. Methylxanthines, such as caffeine and theophylline, are present in therapeutic concentrations in coffee, tea, soft drinks, and many nonprescription medicines and are among the most widely consumed drugs in the world. The results from this present study are consistent with the idea that methylxanthines can aggravate ischemic injury by antagonizing the receptor-mediated effect of ADO.

Another interesting observation was that AICAR, an intermediate in the pathway of adenine metabolism, attenuated NBT deposition in reperfused tissue, compared with vehicle. In the canine heart during reperfusion, AICAR accelerated the rate of adenine nucleotide de novo synthesis by ninefold, whereas ADO accelerated the rate by 90-fold. Maintenance of ion gradients is one of the most energy-intensive functions for cells, and it is reasonable to speculate that oxidative membrane damage accompanying reperfusion, or depletion of high-energy phosphates after ischemia, would interfere with this fundamental process. The values of mucosal surface pH in the rat jejunum observed in this study in baseline conditions (7.12±0.02 [vehicle control] and 7.10±0.03 [experimental, ADO]) were virtually identical to those reported earlier. The results of this present study show that intestinal reperfusion injury is accompanied by decreases in mucosal surface pH and that ADO prevents those changes (Figure 2). To our knowledge, this observation has not been previously reported.
In summary, it has been reported that ADO attenuates the no-reflow phenomenon, neutrophil infiltration, and histological evidence of reperfusion injury in the partially isolated intestine. The results of this present study extend those findings: ADO reduces evidence of oxidative damage and prevents pH decreases that accompany reperfusion and the effect is apparently more potent than that of SOD+CAT or nutritional supplements with the antioxidant vitamin E.

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References


Key Words • jejunum • superoxide dismutase • ischemia • theophylline • catalase • vitamin E • xanthine oxidase
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