Study of L-Arginine in Intestinal Lesions Caused by Ischemia-Reperfusion in Rats


ABSTRACT

To examine whether treatment with L-arginine (ARG), a substrate of nitric oxide biosynthesis, attenuated intestinal dysfunction caused by ischemia (I) and reperfusion (R), we treated rats with ARG (100 mg/kg intravenously) or saline solution (SS) before 60 minutes of I produced by occlusion of the superior mesenteric artery and/or during 120 minutes of R. After I or I/R, we isolated 2-cm jejunal segments for mounting in an organ bath to study neurogenic contractions stimulated by electrical pulses or KCl with the use of a digital recording system. Thin jejunal slices were stained with hematoxylin and eosin for optical microscopy. Jejunal contractions were similar in the sham and I+ARG, but reduced in I+SS, I/R+SS, and I/R+ARG groups. Jejunal enteric nerves were damaged in I+SS, IR+SS, and IR+ARG, but not in the I+ARG group, suggesting that ARG attenuate intestinal dysfunctions due to I but not to R.

It is well known that ischemia (I) and reperfusion (R) are dangerous processes for mammalian tissues, including the intestine. This significant problem may result from hypovolemia, hypotension, hypoxia, sepsis, or mechanical vascular obstruction, some of which are involved in transplant surgery. They can dramatically compromise motor and secretory intestinal functions owing to cellular lesions and death caused by deprivation of oxygen and nutrients. In addition, R after I can produce cellular lesions and death due mainly to lipid peroxidation of cell membrane caused by accumulation of oxygen free radicals and other cytotoxic substances.

Several excitatory and inhibitory transmitters released from enteric nerves regulate intestinal motility, including acetylcholine, 5’-adenosine triphosphate (ATP), nitric oxide (NO), and neuropeptides. The regulatory actions of these transmitters highly depend on the integrity of enteric nerves. However, intestinal motor activity is severely reduced by I and R (IR) owing to loss of structural and functional integrity of the enteric nerves.

Many drugs have been proposed to attenuate or prevent these motor and neural dysfunctions caused by IR, including agents that increase NO production. Several types of cells, including those of endothelial, immune and neural origin, produce NO through oxidation of L-arginine (ARG) by NO synthase. Some studies have suggested that NO inhibits lipid peroxidation via lipophilic free radicals, reducing the lesions caused by I and R.

In our previous study, ARG was shown to improve the jejunal function in rabbits submitted to I but not to R. The morphologic study showed that treatment with ARG after IR was similar to the sham group for jejunal strips from rabbits suggesting cytoprotection by ARG. Seeking to understand these effects, we investigated whether ARG attenuated or prevented intestinal I/R injury by analyzing its effects on the motility and histology of jejunal segments from rats undergoing IR.

MATERIALS AND METHODS

Male Wistar EPM-1 rats (270–300 g) anesthetized with ketamine (60 mg/kg) and xylazine (40 mg/kg) intravenously underwent 60 minutes’ occlusion of the superior mesenteric artery with the use of...
a metallic clip (I) followed by 120 minutes of blood recirculation (R) following clip removal. Twelve rats underwent only I (I group), 12 I plus R (IR group), and 6 constituted a sham group.

Among the I group, 6 rats were treated with 0.9% saline solution (SS) and 6 with ARG (100 mg/kg) injected into the femoral vein 5 minutes before I. In the IR group, 6 rats were treated with SS and 6 with ARG (100 mg/kg) injected into the femoral vein 5 minutes before I, 5 minutes before R, and 55 minutes after R.

After I or IR, the rats were killed and isolated jejunal segments (2 cm) were washed, cleared of surrounding tissues, and mounted under 1 g tension at 37°C in an organ bath containing 10 mL aerated nutrient solution (pH 7.4): NaCl 138, KCl 5.7, CaCl₂ 1.8, NaH₂PO₄ 0.36, NaHCO₃ 15, and dextrose 5.5 mmol/L. We studied neurogenic contractions induced by electrical field stimulation (EFS) or by the depolarizing agent KCl (70 mmol/L) with the use of a digital recording system.⁶⁻⁸ EFS (5 and 30 Hz, 1 ms duration, 60 V) was performed by means of platinum electrodes connected to an electrical stimulator S88 (Grass, USA).⁶⁻⁸

**Table 1. Values of Amplitude of Neurogenic Contractions (Expressed in Grams of Tension) Induced by EFS (5 and 30 Hz) or KCl (70 mmol/L) in Jejunal Segments of Rats Treated with ARG or SS and Submitted to Intestinal I or IR**

<table>
<thead>
<tr>
<th>Group</th>
<th>5 Hz</th>
<th>30 Hz</th>
<th>KCl</th>
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<tr>
<td>Sham</td>
<td>1.95 ± 0.19</td>
<td>2.15 ± 0.19</td>
<td>2.25 ± 0.18</td>
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<tr>
<td>I+SS</td>
<td>0.19 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.29 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.41 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>I+ARG</td>
<td>0.27 ± 0.02&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.55 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.79 ± 0.07&lt;sup&gt;ns&lt;/sup&gt;</td>
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<tr>
<td>IR+SS</td>
<td>0.18 ± 0.06</td>
<td>0.45 ± 0.10</td>
<td>0.61 ± 0.17</td>
</tr>
<tr>
<td>IR+ARG</td>
<td>0.10 ± 0.08&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.15 ± 0.02&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.30 ± 0.05&lt;sup&gt;ns&lt;/sup&gt;</td>
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Data represented as mean ± SEM (n = 6).
<sup>a</sup>Statistically different from Sham (P < .05).
<sup>b</sup>Statistically different from I+SS (P < .05).
<sup>ns</sup>Not statistically different.
Responses to EFS and KCl were recorded by force-displacement transducers connected via a bridge amplifier to an analog/digital recording system (AD Instruments, USA). Data on contractile responses were subjected to statistical evaluation using 1-way analysis of variance and Student t test.7,8 We also performed histologic analyses of jejunal pieces embedded in paraffin, cut into thin slices, and stained with hematoxylin and eosin, using optical microscopy.

RESULTS

Figure 1 shows that EFS (5 and 30 Hz) and KCl (70 mmol/L) produced contractile responses in all jejunal segments: sham (Fig 1A), I+/H11001 SS (Fig 1C), I+/H11001 ARG (Fig 1E), IR+/H11001 SS (Fig 1D), and IR+/H11001 ARG (Fig 1F). However, the amplitude of these contractions was similar in I+/H11001 ARG, but reduced in I+/H11001 SS, I/R+/H11001 SS, and IR+/H11001 ARG groups compared with the sham group (Fig 1B; Table 1).

Histologic analysis showed a loss of structural integrity of enteric nerves in the jejunal segments of I+/H11001 SS (Fig 2B), but not I+/H11001 ARG group (Fig 2C).

DISCUSSION

The present work demonstrated that the motility stimulated by transmitters released by and the amount of enteric nerves were significantly reduced in jejunal segments of rats undergoing intestinal I and IR. However, the intestinal dysfunctions caused by I, but not by R, were attenuated in rats treated with ARG. These results suggested that ARG attenuated or prevented the motor and neural dysfunctions caused by I but not by R.

The molecular mechanisms involved in the cellular injuries caused by I are not fully understood. The formation of oxygen free radicals with oxidative stress generated thereby, the activation of phospholipase A2, and the alterations of calcium flux are involved in ischemic lesions.17 Mason et al (2000) reported increased oxygen free radicals associated with decreased tissue NO levels.18 In addition, endogenous NO can inhibit or delay the injury caused by free oxygen radicals in the early periods of IR.15 Studies using NO donors have suggested that increased NO levels may cause vasodilatation and scavenge oxygen free radicals, thereby reducing cellular injuries caused by IR.13 However, NO functions are still not fully understood, because NO reacts with superoxide anions to form peroxynitrite anions (ONOO−).13 These molecular mechanisms appear to be involved in the protective effects of ARG against cellular lesions caused by intestinal I, but not by R, as observed in the present work.

Our previous study showed ARG to improve the functional response of rabbit jejunae after I, but not after R.18 It suggested that lesions caused by R were more pronounced than those caused by I, perhaps because of the long period that blood flow ceased during this process.13 We suggested that the NO levels during I after ARG treatment were important to protect the tissue.18,19

In contrast, Luo et al19 reported that intro-L-arginine methyl ester (L-NAME) reduced the IR injury by inhibiting endogenous NO, suggesting that high levels of NO during R were deleterious to tissues. Taha et al20 showed that the dysfunctions caused by IR were reduced after treatment with L-NAME, with improved jejunal motility during R but not I. The authors suggested that L-NAME, an inhibitor of NO biosynthesis, attenuated or prevented motor and neural dysfunctions produced by IR because it controls excessively high NO levels during R.20 The authors suggested that high NO levels could contribute to lesions produced during R. Decreasing NO levels with L-NAME narrowed the injury.11,19,20

In conclusion, treatment with ARG attenuated the motor and neural dysfunctions of small bowel caused by I, but not by R, in rats.

REFERENCES