Effect of 6-Benzylaminopurine on Small Intestinal Ischemia-Reperfusion Injury in Rats (Postprint)

Authors: Zhang Ziqiang, Liu Yumei, Zhu Xuemin, Wei Lan, Shi Ke, Pan Li

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Abstract

The present study was designed to investigate the protective effect of 6-benzylaminopurine (6-BA) against intestinal ischemia-reperfusion (I/R) injury in rats. Eighty male Sprague-Dawley (SD) rats were randomly divided into four groups: control group, I/R model group, and low- and high-dose 6-BA groups. The low- and high-dose 6-BA groups received continuous intragastric administration of 10 and 20 mg/kg 6-BA, respectively, for 3 weeks prior to surgery, while the control and I/R model groups received an equivalent volume of normal saline once daily. The control group underwent exposure of the superior mesenteric artery without occlusion; the I/R model group and low- and high-dose 6-BA groups underwent occlusion of the mesenteric vessels for 30 min followed by 60 min of reperfusion. Subsequently, rat jejunal tissues were harvested for determination of total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) activities and malondialdehyde (MDA) content; single-cell gel electrophoresis was employed to assess the degree of cellular DNA damage, and immunohistochemistry was used to detect cysteine-aspartic acid specific protease 3 (Caspase-3) expression. The results demonstrated that compared with the I/R model group, supplementation with 10 and 20 mg/kg 6-BA significantly increased T-SOD and GSH-Px activities ($P<0.05$) and significantly decreased MDA content ($P<0.05$); the DNA tailing phenomenon in intestinal cells was alleviated, with both tail DNA content and tail moment significantly lower than those in the I/R model group ($P<0.05$); and the number of Caspase-3-positive cells was significantly reduced ($P<0.05$). These findings indicate that 6-BA at doses of 10 and 20 mg/kg can effectively protect against I/R-induced intestinal injury, with the protective effect of 20 mg/kg 6-BA being particularly pronounced.

Full Text

Effects of 6-Benzylaminopurine on Ischemia/Reperfusion Injury of Small Intestine in Rats

ZHANG Ziqiang, LIU Yumei, ZHU Xuemin, WEI Lan, SHI Ke, PAN Li

College of Animal Science and Technology, Henan University of Science and Technology, Luoyang 471023, China

Abstract

This study investigated the protective effects of 6-benzylaminopurine (6-BA) against ischemia/reperfusion (I/R) injury in rat small intestine. Eighty male SD rats were randomly divided into four groups: control group, I/R model group, low-dose 6-BA group, and high-dose 6-BA group. The low- and high-dose 6-BA groups received daily gavage administration of 10 mg/kg and 20 mg/kg 6-BA, respectively, for three weeks before surgery, while the control and I/R model groups received equal volumes of physiological saline. In the control group, the superior mesenteric artery was exposed but not occluded, whereas in the I/R model and 6-BA groups, the mesenteric vessels were occluded for 30 minutes followed by 60 minutes of reperfusion. Jejunal tissues were then collected to measure total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) activities, as well as malondialdehyde (MDA) content. DNA damage was assessed using single-cell gel electrophoresis, and cysteinyl aspartate-specific proteinase-3 (Caspase-3) expression was evaluated by immunohistochemistry. Compared with the I/R model group, supplementation with 10 mg/kg and 20 mg/kg 6-BA significantly increased T-SOD and GSH-Px activities ($P<0.05$) and significantly decreased MDA content ($P<0.05$). DNA tailing phenomena were ameliorated, with both tail DNA content and tail moment significantly lower than in the I/R model group ($P<0.05$). The number of Caspase-3-positive cells was also significantly reduced ($P<0.05$). These results demonstrate that 6-BA at doses of 10 mg/kg and 20 mg/kg effectively protects against I/R-induced small intestine injury, with 20 mg/kg showing particularly pronounced effects.

Keywords: 6-benzylaminopurine; ischemia/reperfusion; oxidative damage; apoptosis

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Introduction

Among visceral organs, the small intestine is most susceptible to ischemia/reperfusion (I/R) injury. Research has shown that intestinal cells are highly vulnerable to local ischemia, with reperfusion further exacerbating mucosal damage. I/R commonly occurs during acute mesenteric ischemia, hemorrhagic or traumatic shock, septic shock, severe burns, and surgical procedures

such as small intestine transplantation and abdominal aortic surgery. Beyond causing direct intestinal injury, I/R damages the intestinal mucosal barrier, triggering systemic infection and multiple organ dysfunction. Consequently, small intestine I/R injury has attracted increasing research attention.

6-Benzylaminopurine (6-BA) is a cytokinin plant growth regulator widely used in plant tissue culture, fruit development, and vegetable preservation. It effectively defends plant tissues against oxidative stress, and our previous studies have confirmed its protective effects against oxidative damage in mouse brain and liver tissues. Building on this foundation, the present study further investigated the protective effects of 6-BA on rat small intestine I/R injury from multiple perspectives, including oxidative stress, DNA damage, and apoptosis-related protein expression, to provide a scientific basis for preventing intestinal I/R injury and screening antioxidants for animal feed.

Materials and Methods

1.1 Drugs and Reagents

6-BA was purchased from Sanland Company (USA) and prepared as 1,000 mg/L and 2,000 mg/L stock solutions with 0.06 mol/L hydrochloric acid. MDA content assay kit, GSH-Px activity assay kit, T-SOD activity assay kit, and Coomassie brilliant blue assay kit were obtained from Nanjing Jiancheng Bioengineering Institute. Low-melting-point agarose was purchased from Sigma (USA). Rabbit anti-rat Caspase-3 monoclonal antibody was purchased from Abcam (USA). Immunohistochemistry kit was purchased from Wuhan Boster Biological Engineering Co., Ltd.

1.2 Experimental Animals

Eighty male SD rats were purchased from the Experimental Animal Center of Zhengzhou University. After seven days of acclimation, they were randomly divided into four groups (n=20 each): control group, I/R model group, low-dose 6-BA group, and high-dose 6-BA group. The 6-BA groups received daily gavage administration of 10 mg/kg or 20 mg/kg 6-BA for three weeks, while the control and I/R model groups received equal volumes of physiological saline. Animals were fasted for 12 hours before surgery with free access to water. Anesthesia was induced by intraperitoneal injection of 3% pentobarbital sodium (1 mL/kg). After fixation, a midline abdominal incision was made. The control group had the superior mesenteric artery exposed without occlusion, while the I/R model and 6-BA groups had their mesenteric vessels occluded with atraumatic arterial clamps for 30 minutes followed by 60 minutes of reperfusion. Immediately after the procedure, jejunal tissue samples were collected for analysis.

1.3 Main Equipment

Biological tissue microtome (RM-2235, Leica, Germany), spectrophotometer (UV-6300, Shanghai Mapada Instrument Co., Ltd.), fluorescence microscope (BX41TF, Olympus, Japan), electrophoresis apparatus (DYY-11, Beijing Liuyi Instrument Factory), and electrophoresis tank (DYCP-33A, Beijing Liuyi Instrument Factory).

1.4 Determination of T-SOD, GSH-Px Activities and MDA Content

Ten percent small intestine tissue homogenate was prepared and diluted to 1% with physiological saline. T-SOD activity was measured at 550 nm using a UV/visible spectrophotometer according to the kit instructions. For GSH-Px activity, 10% tissue homogenate was diluted to 0.8% and measured at 412 nm. MDA content was determined by the thiobarbituric acid (TBA) method at 532 nm using 5% tissue homogenate.

1.5 Detection of DNA Damage by Single-Cell Gel Electrophoresis

Small intestine single-cell suspensions were prepared and stored for use. A base layer was prepared by spreading 100 µL of 0.75% normal-melting-point agarose solution at 50°C onto fully frosted slides, which were then dried overnight at 37°C. A second layer was added by applying 110 µL of the same agarose solution, leveled and solidified at 4°C for 20 minutes. The third layer was prepared by mixing 70 µL of single-cell suspension with 140 µL of 0.65% low-melting-point agarose maintained at 37°C, then applying 110 µL onto the second layer and solidifying at 4°C for 20 minutes. The prepared slides were immersed in cell lysis solution at 4°C for 1 hour, then placed in an electrophoresis tank with electrophoresis buffer for 25 minutes of unwinding at 4°C, followed by electrophoresis for 20 minutes (20 V, 200 mA). After electrophoresis, slides were neutralized for 15 minutes, stained with 40 µL of 10 mg/L ethidium bromide (EB) solution for 10 minutes, and observed/photographed under a fluorescence microscope in a dark room. For each group, three slides were randomly selected, and eight fields of view were examined at 100× magnification. Comets with tail length >35 µm were considered positive. CASP software was used to measure tail DNA content, tail length, and tail moment.

1.6 Detection of Caspase-3 Expression by Immunohistochemistry

Caspase-3 expression in rat small intestine tissue sections was detected by immunohistochemistry according to the kit protocol. Primary antibody was incubated overnight at 4°C, with phosphate-buffered saline as negative control. Diaminobenzidine (DAB) staining was performed, followed by hematoxylin counterstaining, routine dehydration, clearing, and mounting. Yellow granules observed under optical microscope indicated positive expression.

1.7 Statistical Analysis

Data are expressed as mean ± standard deviation. SPSS 18.0 software was used for one-way ANOVA, with Duncan' s test for inter-group comparisons. $P<0.05$ was considered statistically significant.

Results

2.1 Effects of 6-BA on T-SOD, GSH-Px Activities and MDA Content After Small Intestine I/R Injury

The effects of 6-BA on T-SOD, GSH-Px activities and MDA content are shown in Table 1 . Compared with the control group, T-SOD and GSH-Px activities in the I/R model group were significantly decreased ($P<0.05$), while MDA content was significantly increased ($P<0.05$), confirming successful establishment of the I/R model. Both low- and high-dose 6-BA groups showed significantly higher T-SOD and GSH-Px activities ($P<0.05$) and significantly lower MDA content ($P<0.05$) compared with the I/R model group. MDA content in the high-dose 6-BA group showed no significant difference from the control group ($P>0.05$).

2.2 Effects of 6-BA on Cell DNA Damage After Small Intestine I/R Injury

DNA damage in small intestine cells after I/R injury is shown in Figure 1 [Figure 1: see original paper]. Control group cells exhibited uniform nuclear size with round fluorescent masses, uniform fluorescence intensity, and smooth edges without comet tails. The I/R model group showed severe DNA damage with smaller comet heads and prominent comet tails. Both 6-BA groups exhibited reduced DNA tailing and shorter tail lengths. Tail DNA content and tail moment are presented in Table 2 . Compared with the control group, the I/R model group showed significantly increased tail DNA content and tail moment ($P<0.05$). Both low- and high-dose 6-BA groups significantly reduced these parameters compared with the I/R model group ($P<0.05$).

2.3 Effects of 6-BA on Caspase-3 Expression After Small Intestine I/R Injury

Caspase-3 expression after small intestine I/R injury is shown in Figure 2 [Figure 2: see original paper]. Compared with the control group, the I/R model group showed markedly increased numbers of Caspase-3-positive cells, while both 6-BA groups showed fewer positive cells, confirming that 6-BA ameliorated I/R-induced injury.

Discussion

When blood supply to tissues and organs is insufficient, cellular dysfunction and even necrosis can occur; however, rapid restoration of blood flow can further aggravate organ damage, a phenomenon known as I/R injury. The intestine

represents the largest reservoir of bacteria and endotoxins in the body. When I/R injury destroys intestinal barrier function, it can trigger a series of diseases including sepsis, systemic inflammatory response syndrome, and multiple organ dysfunction (affecting liver, lungs, kidneys, etc.). Consequently, the intestine is considered the "motor" that drives systemic organ dysfunction. Although the detailed mechanisms of small intestine I/R injury remain unclear, it is well established that I/R can induce free radical formation, DNA damage, mitochondrial membrane potential depolarization, and ultimately lead to apoptosis and necrosis of intestinal cells. Caspases, also known as death proteases, are the most important proteases in apoptosis, directly hydrolyzing and activating proteins associated with apoptotic features such as DNA fragmentation. Among them, Caspase-3 is the final effector in the caspase cascade and an essential pathway in the apoptotic protein cascade reaction. Therefore, this study evaluated the protective effects of 6-BA against small intestine I/R injury by examining antioxidant enzyme activities, lipid peroxidation products, DNA damage, and Caspase-3 protein expression.

In this study, a small intestine I/R model was established by occluding mesenteric vessels for 30 minutes followed by 60 minutes of reperfusion. The results demonstrated severe DNA damage and significantly increased numbers of apoptotic protein Caspase-3-positive cells, confirming the high susceptibility of small intestine to I/R injury. Detection of antioxidant enzyme activities and lipid peroxidation products revealed that small intestine I/R injury is associated with free radical generation. 6-BA can inhibit the degradation of chlorophyll, nucleic acids, and proteins in plant leaves, preserve green color, and prevent senescence. It can also transport amino acids, auxins, and inorganic salts to treated areas. As a stable, inexpensive, and easy-to-use plant growth regulator that is safe for humans and animals, 6-BA is widely applied at various stages from germination to harvest in agriculture, fruit trees, and horticultural crops. Literature indicates that many plant growth regulators not only promote plant growth but also prevent oxidative damage and resist aging in animal tissues. For example, kinetin can inhibit oxidative stress in animal liver and ovary tissues and even improve immune function in aging rats. 6-BA has a chemical structure similar to kinetin and exhibits significant antioxidant and anti-aging effects in plants. Whether 6-BA also possesses antioxidant capacity in animal tissues has not been reported. Our laboratory previously established a mouse liver oxidative damage model by intraperitoneal injection of carbon tetrachloride (CCl4) and demonstrated that 6-BA effectively inhibited CCl4-induced decreases in antioxidant enzyme activities and accumulation of lipid peroxidation products. Given the relationship between intestinal I/R injury and oxidative stress, this study further examined the protective effects of 6-BA on small intestine I/R injury. The results confirmed that 6-BA effectively inhibited the decrease in antioxidant enzyme activities, accumulation of lipid peroxidation products, DNA damage, and morphological lesions in small intestine cells during I/R injury in rats. These findings suggest that 6-BA at appropriate doses exerts protective effects against rat small intestine I/R injury, providing new insights for develop-

ing active substances against intestinal I/R injury, though detailed mechanisms require further investigation.

Conclusion

1. 6-BA at 10 mg/kg and 20 mg/kg effectively reduces I/R-induced oxidative stress injury in small intestine.

2. 6-BA at 10 mg/kg and 20 mg/kg effectively reduces I/R-induced cell DNA damage in small intestine.

3. 6-BA at 10 mg/kg and 20 mg/kg effectively reduces I/R-induced elevation of apoptotic protein Caspase-3-positive cell numbers in small intestine.

4. 6-BA at 10 mg/kg and 20 mg/kg effectively protects against I/R injury in small intestine, with 20 mg/kg showing particularly prominent effects.

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Note: Figure translations are in progress. See original paper for figures.