

Effect of 6-Benzylaminopurine on Small Intestinal Ischemia-Reperfusion Injury in Rats: Post-print

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Abstract

This study aimed to investigate the protective effect of 6-benzylaminopurine (6-BA) against small 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, low-dose 6-BA group, and high-dose 6-BA group. The low- and high-dose 6-BA groups received continuous intragastric administration of 10 mg/kg and 20 mg/kg 6-BA, respectively, for 3 weeks before surgery, while the control and I/R model groups received the same 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 mesenteric vessels for 30 min followed by 60 min of reperfusion. Subsequently, rat jejunal tissues were collected for measurement 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 protease-3 (Caspase-3) expression. The results demonstrated that 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 ($P < 0.05$); the tailing phenomenon of small intestinal cells was ameliorated, with both tail DNA content and tail moment significantly lower than those in the I/R model group ($P < 0.05$); the number of Caspase-3 positively expressed cells was significantly reduced ($P < 0.05$). These findings indicate that 10 mg/kg and 20 mg/kg 6-BA can effectively protect against I/R-induced small intestinal injury, with the effect of 20 mg/kg 6-BA being particularly pronounced.

Full Text

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

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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 3 weeks before surgery, while the control and I/R model groups received equivalent volumes of physiological saline. In the control group, the superior mesenteric artery was exposed but not occluded; in the I/R model and 6-BA groups, the mesenteric vessels were occluded for 30 minutes followed by 60 minutes of reperfusion.

Jejunal tissue samples were collected for analysis of 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.

The results demonstrated that 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 ($P < 0.05$) while significantly decreasing MDA content ($P < 0.05$). DNA damage, as evidenced by comet tail formation, was substantially ameliorated, with both tail DNA content and comet tail percentage significantly lower than in the I/R model group ($P < 0.05$). Additionally, the number of Caspase-3-positive cells was significantly reduced ($P < 0.05$). These findings indicate that 6-BA at doses of 10 mg/kg and 20 mg/kg effectively protects against small intestinal I/R injury, with the 20 mg/kg dose showing particularly pronounced effects.

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

Introduction

Among visceral organs, the small intestine is particularly 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 in conditions such as acute mesenteric ischemia, hemorrhagic or traumatic shock, septic shock, severe burns, and during surgical procedures including small intestine transplantation and abdominal aortic surgery. Beyond causing direct intestinal damage, I/R-induced destruction of the intestinal mucosal barrier can trigger systemic infection and multiple organ dysfunction, leading researchers to increasingly recognize the small intestine as a “motor” driving systemic organ failure.

Although the precise mechanisms of small intestinal I/R injury remain incompletely understood, it is well-established that I/R promotes free radical formation, DNA damage, mitochondrial membrane depolarization, and ultimately apoptosis and necrosis of intestinal cells. Caspases, also known as death proteases, represent the most important proteases in apoptosis, directly hydrolyzing and activating proteins associated with characteristic apoptotic changes including DNA fragmentation. Caspase-3 serves as the final effector in the caspase cascade and is essential for the apoptotic proteolytic cascade.

6-Benzylaminopurine (6-BA) is a cytokinin plant growth regulator widely used in plant tissue culture, fruit development, and vegetable preservation. Beyond its agricultural applications, 6-BA has demonstrated efficacy in defending against oxidative stress in plant tissues. Our previous research confirmed that 6-BA exerts protective effects against oxidative damage in mouse brain and liver tissues. Building upon these findings, the present study further investigated the protective effects of 6-BA on rat small intestinal 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 using 0.06 mol/L hydrochloric acid. MDA content assay kit, GSH-Px activity assay kit, T-SOD activity assay kit, and Coomassie brilliant blue reagent 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 obtained from the Experimental Animal Center of Zhengzhou University. After 7 days of acclimation, the animals 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

3 weeks before surgery, while the control and I/R model groups received equivalent 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. 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 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 Instruments Biological tissue slicer (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); electrophoresis tank (DYCP-33A, Beijing Liuyi Instrument Factory).

1.4 Determination of T-SOD, GSH-Px Activities and MDA Content Ten percent small intestinal tissue homogenate was prepared and diluted with physiological saline to 1% for T-SOD activity measurement. Absorbance 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 absorbance was measured at 412 nm. For MDA content, 10% tissue homogenate was diluted to 5% and analyzed by the thiobarbituric acid (TBA) method with absorbance measured at 532 nm.

1.5 Detection of DNA Damage by Single-Cell Gel Electrophoresis Small intestinal single-cell suspensions were prepared and stored for analysis. A base layer was prepared by applying 100 μ L 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 created by adding 110 μ L of the same agarose solution onto the base layer and solidifying at 4°C for 20 minutes. The third layer was prepared by mixing 70 μ L of single-cell suspension with 140 μ L of 0.65% low-melting-point agarose maintained at 37°C, then applying 110 μ L of this mixture onto the second layer and solidifying at 4°C for 20 minutes.

The prepared slides were immersed in cell lysis solution for 1 hour at 4°C, then placed in an electrophoresis tank with electrophoresis buffer for 25 minutes at 4°C to unwind DNA. Electrophoresis was performed for 20 minutes (20 V, 200 mA). After electrophoresis, slides were neutralized for 15 minutes, then stained with 40 μ L of 10 mg/L ethidium bromide (EB) solution for 10 minutes. Images were captured using a fluorescence microscope in a dark room. For each group, three slides were randomly selected and eight fields of view were examined at 100 \times magnification. Comet tails longer than 35 μ m were considered positive. CASP software was used to measure tail DNA content, tail length, and calculate the percentage of comet tails.

1.6 Detection of Caspase-3 Expression by Immunohistochemistry Caspase-3 expression in small intestinal tissue sections was detected by immunohistochemistry according to the kit protocol. Primary antibody was applied overnight at 4°C, with phosphate-buffered saline used as negative control. Diaminobenzidine (DAB) staining was performed, followed by hematoxylin counterstaining, routine dehydration, clearing, and mounting. Yellow-brown granules in cells observed under light microscopy indicated positive expression.

1.7 Statistical Analysis All data are expressed as mean \pm standard deviation. One-way ANOVA was performed using SPSS 18.0 software. Inter-group differences were analyzed by Duncan's test, with $P < 0.05$ considered statistically significant.

Results

2.1 Effects of 6-BA on T-SOD, GSH-Px Activities and MDA Content after Small Intestinal 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 and significantly lower MDA content compared with the I/R model group ($P < 0.05$). MDA content in the high-dose 6-BA group did not differ significantly from the control group ($P > 0.05$).

2.2 Effects of 6-BA on Cell DNA Damage after Small Intestinal I/R Injury DNA damage in small intestinal cells after I/R injury is shown in Figure 1 [Figure 1: see original paper]. In the control group, cell nuclei were uniformly sized, round fluorescent masses with uniform fluorescence intensity and smooth edges, without comet tail formation. The I/R model group exhibited severe DNA damage with smaller comet heads and prominent tail formation. Both low- and high-dose 6-BA groups showed reduced comet tails and decreased tail lengths.

Tail DNA content and comet tail percentage are presented in Table 2. Compared with the control group, the I/R model group showed significantly increased tail DNA content and comet tail percentage ($P < 0.05$). Both 6-BA treatment 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 Intestinal I/R Injury Caspase-3 expression after small intestinal I/R injury is shown in Figure 2 [Figure 2: see original paper]. The I/R model group exhibited markedly

increased numbers of Caspase-3-positive cells compared with the control group, while both low- and high-dose 6-BA groups showed fewer Caspase-3-positive cells than the I/R model group, confirming that 6-BA ameliorated I/R-induced injury.

Discussion

Ischemia/reperfusion injury occurs when restoration of blood flow exacerbates tissue damage following a period of insufficient blood supply. As the largest reservoir of bacteria and endotoxins in the body, intestinal I/R injury can trigger systemic sepsis, systemic inflammatory response syndrome, and multiple organ dysfunction (affecting liver, lungs, kidneys, etc.), leading to the concept of the gut as a “motor” driving systemic organ failure. While the detailed mechanisms of small intestinal I/R injury remain to be fully elucidated, I/R-induced free radical formation, DNA damage, and mitochondrial membrane depolarization culminating in apoptosis and necrosis are well-established pathological features.

Caspases, also known as death proteases, are the most important proteases in apoptosis, directly hydrolyzing and activating proteins associated with characteristic apoptotic changes including DNA fragmentation. Caspase-3 serves as the final effector in the caspase cascade and is essential for the apoptotic proteolytic pathway. Therefore, this study evaluated the protective effects of 6-BA against small intestinal I/R injury by examining antioxidant enzyme activities, lipid peroxidation products, DNA damage, and Caspase-3 protein expression.

By occluding mesenteric vessels for 30 minutes followed by 60 minutes of reperfusion, we established a small intestinal I/R model that exhibited severe DNA damage and significantly increased numbers of Caspase-3-positive cells, confirming the high susceptibility of the small intestine to I/R injury. Assessment of antioxidant enzyme activities and lipid peroxidation products demonstrated the involvement of free radicals in small intestinal I/R injury.

6-BA inhibits the degradation of chlorophyll, nucleic acids, and proteins in plant leaves, preserving green color and preventing senescence. It can transport amino acids, auxins, and inorganic salts to treated areas, offering stability, low cost, and ease of use as a plant growth regulator safe for humans and livestock, with widespread application throughout agricultural, fruit, and horticultural crop production from germination to harvest. Previous studies have shown that many plant growth regulators not only promote plant growth but also protect against oxidative damage and resist aging in animal tissues. For example, kinetin (6-furfurylaminopurine) inhibits oxidative stress in liver and ovary tissues and can improve immune function in aging rats. Given the structural similarity between 6-BA and kinetin, and the significant antioxidant and anti-aging effects of 6-BA in plants, we hypothesized that 6-BA might also possess antioxidant capacity in animal tissues.

Our laboratory previously demonstrated that 6-BA effectively inhibited CCl₄-induced decreases in antioxidant enzyme activity and accumulation of lipid peroxidation products in a mouse model of hepatic oxidative damage. Considering the relationship between intestinal I/R injury and oxidative stress, we further investigated the protective effects of 6-BA against small intestinal I/R injury. The results confirmed that 6-BA effectively inhibited the I/R-induced decrease in antioxidant enzyme activity, accumulation of lipid peroxidation products, DNA damage, and morphological changes in small intestinal cells. These findings suggest that appropriate doses of 6-BA exert protective effects against small intestinal I/R injury in rats, providing new insights for developing active substances against intestinal I/R injury, though the detailed mechanisms require further investigation.

Conclusions

1. 6-BA at doses of 10 mg/kg and 20 mg/kg effectively reduces I/R-induced oxidative stress injury in small intestine.
2. 6-BA at doses of 10 mg/kg and 20 mg/kg effectively reduces I/R-induced cell DNA damage in small intestine.
3. 6-BA at doses of 10 mg/kg and 20 mg/kg effectively reduces I/R-induced increase in apoptosis-related protein Caspase-3-positive cell numbers.
4. 6-BA at doses of 10 mg/kg and 20 mg/kg effectively protects against small intestinal I/R injury, with the 20 mg/kg dose demonstrating particularly prominent effects.

References

- [1] HE X H, YAN X T, WANG Y L, et al. Transduced PEP-1-heme oxygenase-1 fusion protein protects against intestinal ischemia/reperfusion injury[J]. *Journal Surgical Research*, 2014, 187(1): 77-84.
- [2] YANG X K, BAI H, CAI W X, et al. Lycium barbarum polysaccharides reduce intestinal ischemia/reperfusion injuries in rats[J]. *Chemico-Biological Interactions*, 2013, 204(3): 166-173.
- [3] INAN M, UZ Y H, KIZILAY G, et al. Protective effect of sildenafil on liver injury induced by intestinal ischemia/reperfusion[J]. *Journal of Pediatric Surgery*, 2013, 48(8): 1707-1715.
- [4] LI Y S, WANG Z X, LI C, et al. Proteomics of ischemia/reperfusion injury in rat intestine with and without ischemic postconditioning[J]. *Journal of Surgical Research*, 2010, 164(1): e173-e180.
- [5] 陈建雷, 孙庆林. 白藜芦醇对大鼠肠缺血再灌注损伤的保护作用 [J]. *中华小儿外科杂志*, 2011, 32(2): 139-142.

- [6] 李杰, 况胜利, 房晓敏, 等. 6-苄氨基嘌呤的合成研究 [J]. 安徽农业科学, 2009, 37(15): 6841-6842.
- [7] 张自强, 刘玉梅, 位兰, 等. 6-苄氨基嘌呤对 D-半乳糖致小鼠脑组织氧化损伤的保护作用 [J]. 中国临床药理学杂志, 2015, 31(14): 1422-1425.
- [8] 张自强, 刘玉梅, 朱雪敏, 等. 6-苄氨基嘌呤对四氯化碳致小鼠肝脏氧化损伤的保护作用 [J]. 中国临床药理学杂志, 2015, 31(11): 928-931.
- [9] ZHANG R, HE G Z, WANG Y K, et al. Omega-3 polyunsaturated fatty acids inhibit the increase in cytokines and chemotactic factors induced in vitro by lymph fluid from an intestinal ischemia-reperfusion injury model[J]. Nutrition, 2015, 31(3): 508-514.
- [10] CHEN H, SUN Y P, HU P F, et al. The effects of hydrogen-rich saline on the contractile and structural changes of intestine induced by ischemia-reperfusion in rats[J]. Journal of Surgical Research, 2011, 167(2): 316-322.
- [11] 樊永梅, 王如蜜, 张长杰, 等. 高频电疗对大鼠脑缺血再灌注损伤后 caspase-3 和海马神经细胞超微结构的影响 [J]. 中南大学学报: 医学版, 2017, 42(1): 21-25.
- [12] 茹琴, 欧阳五庆, 张黎. 激动素对小鼠急性四氯化碳肝损伤的保护作用 [J]. 中国新药与临床杂志, 2007, 26(4): 263-267.
- [13] 孙江宏, 刘玉梅, 曹统, 等. 激动素对 D-半乳糖致衰雌性小鼠卵巢和子宫的影响 [J]. 生理学报, 2013, 65(4): 389-394.
- [14] 李梦云, 欧阳五庆, 吴小利, 等. 激动素对衰老大鼠免疫功能及其脾淋巴细胞体外增殖的影响 [J]. 生理学报, 2014, 66(5): 605-611.

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