PROTECTION OF SALVIA MILTIORRHIZA AGAINST ACUTE SODIUM NITRITE POISONING IN MICE

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ABSTRACT

The present study evaluated the protection of Salvia miltiorrhiza injection against acute sodium nitrite poisoning in mice. Forty male C57mice were randomly divided into control group, sodium nitrite poisoning group (SN), Methylene blue (MB) and Salvia miltiorrhizae (SM) group. In SN, MB and SM group, 200 mg/kg sodium nitrite was injected intraperitoneal. Mice in MB and SM group were given 1.5mg/kg Methylene blue and 0.2ml Salvia miltiorrhiza injection immediately after sodium nitrite injection respectively. Mice in SN and SM group demonstrated higher blood methemoglobin and Serum TNFα and IL-6 but lower IL-10 levels than control group (P< 0.05). HE and TUNEL staining showed developed histological damage accompanied with increased apoptosis in heart, lung, liver and kidney. But the values were much less severe in MB and SM group than those in SN group(P< 0.05). The results of this study showed that Salvia miltiorrhiza injection reduced tissue injury induced by sodium nitrite effectively.

Key words: Mice, Salvia miltiorrhiza, Sodium nitrite.

INTRODUCTION

Sodium nitrite is a white to slight yellowish crystalline powder that is very soluble in water and is hygroscopic( Hassan et al., 2010). It is a useful precursor to a variety of organic compounds, such as pharmaceuticals, dyes, and pesticides, but it is probably best known as a food additive to prevent botulism( Luca et al.,1987). While this chemical will prevent the growth of bacteria, it can be toxic in high amounts for animals, including humans. If one is eating much sodium nitrite, methemoglobinemia will happen( Gautami et al.,1995). Methemoglobinemia is a disorder characterized by the presence of a higher than normal level of methemoglobin in the blood. Methemoglobin is a form of hemoglobin that contains ferric (Fe3+) iron and has a decreased ability to bind oxygen. When methemoglobin concentration is elevated in red blood cells, tissue hypoxia can occur ( Brown et al., 2013). Signs and symptoms of methemoglobinemia include shortness of breath, cyanosis is the classic symptom, mental status changes, headache, fatigue, exercise intolerance, dizziness and loss of consciousness. Arterial blood with elevated methemoglobin levels has a characteristic chocolate-brown color as compared to normal bright red oxygen-containing arterial blood( Hall et al., 2013). Methemoglobinemia can be treated with supplemental oxygen and methylene blue. Methylene blue restores the iron in hemoglobin to its normal (reduced) oxygen-carrying state(Singh et al., 2012). But at high doses, however, methylene blue actually induces methemoglobinemia.

Traditional Chinese medicine has evolved into a well-developed, coherent system of medicine to treat illness( Ni et al., 2012). Herbal medicine has been used for more than 2000 years ( Zheng et al., 2011). Salvia miltiorrhiza is a traditional Chinese drug commonly used for activating circulation( Han et al., 2008), the research on the effect of it in the treatment of sodium nitrite poisoning has not been reported. Salvia miltiorrhiza injection is the extraction of wild Salvia roots, the main activeing redients include danshensu, salvianolic acid, as well as tanshinone, dihydrotanshinone, and cryptotanshinone(Yu et al., 2012), which are able to protect endothelial cells, fight against inûammation, and prevent lipid peroxidation and

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calcium overload (Zhao et al., 2004; Xia et al., 2003; Chen et al., 2006). Some studies have shown that Salvia miltiorrhiza is used to treat some kind of poisoning such as mercury poisoning (Jing et al., 2010) and carbon tetrachloride poisoning (Wang et al., 2012) through protecting multi organ functions. Although salvia miltiorrhiza has been used to treat some kind of poisoning, the exact mechanism is not very clear. For this reason, the purpose of this study is to evaluate the protection of salvia miltiorrhiza injection against acute sodium nitrite poisoning in mice through observing the pathological alterations in the heart, lung, liver and kidney tissues.

MATERIALS AND METHODS

Animals: Eight to ten weeks old male C57BL/6 mice weighing 20–25 g were obtained from the Experimental Animal Center of China Medical University. All were fed a standard laboratory chow and allowed tap water ad libitum. All the experimental procedures were performed according to the guidelines for the care and use of animals established by China Medical University. Forty age-matched male C57 mice were randomly divided into control group, sodium nitrite poisoning group (SN), Methylene blue (MB) and Salvia miltiorrhiza-treated (SM) group. In SN, MB and SM group, 200 mg/kg sodium nitrite was injected intraperitoneal. Mice in MB and SM group, 1.5 mg/kg Methylene blue and 0.2 ml Salvia miltiorrhiza injection (each 10 mL vial contains active components equivalent to 15 g of the original medicine, Shenghe Pharmaceutical Co., Ltd. China.) immediately after sodium nitrite injection respectively. In control group, an equal volume of sterile saline was administered. All the mice were sacrificed after 24 h of sodium nitrite injection. Blood samples and heart, lung, liver and kidney tissues were collected while they were under anesthesia.

Methemoglobin analysis: After 24h of sodium nitrite injection, the blood was collected for the measurement of methemoglobin analysis. First hemoglobin content was determined by hemoglobin determination reagent, then methemoglobin was determined by methemoglobin determination reagent. And then methemoglobin percentage was calculated according to the manufacturer’s instructions. (Nanjing Jiancheng, China).

Observation of mortality rate and pathological changes: After 24h of Sodium nitrite injection, the mortality rates of mice in various groups were recorded and the animals were killed by cervical dislocation, followed by immediate organ collection for histologic analysis. Fresh heart, lung, liver and kidney tissues sections were fixed in 10% buffered formalin and embedded in paraffin; 4 µm sections were stained with hematoxylin and eosin. A pathologist blind to group assignment analyzed the samples and determined the levels of injury.

Determination of apoptosis: Detection of apoptotic cells was carried out using the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay kit. Fresh heart, lung, liver and kidney sections were fixed in 10% buffered formalin and embedded in paraffin; 4 µm thick of slices were stained using the in situ Cell Death Detection kit (Promega technology. USA) according to the manufacturer’s instructions. Three sections from each sample were randomly selected and ten microscopic fields per section were evaluated by two independent observers. In each field, nuclei were counted and the percentage of TUNEL-positive nuclei was calculated.

Measurement of concentrations of cytokines in serum: The concentrations of TNF-a, IL-6 and IL-10 in serum were measured by using commercial available ELISA kits (Ray Biotech, USA) following the manufacturer’s instructions. They were carefully checked for specify, sensitivity and reliability. Serum concentration of TNF-a, IL-6 and IL-10 were measured using mouse ELISA kits: ELM-TNFalpha-001, ELM-IL6-001 and ELM-IL10-001 respectively. The absorbance was measured using a spectrophotometer (Biotek, USA); concentration of each cytokine was calculated by a comparison curve established in the same measurement using prism 5 Graphpad. Each cytokine assay was performed in duplicate each time.

Statistical analysis: All values presented as means ± SE. Differences were compared by ANOVA followed by Bonferroni correction for post hoc t-test, where appropriate. Probabilities of < 0.05 were considered to be statistically significant. All of the statistical tests were performed with the SPSS software 13.0.
RESULTS AND DISCUSSION

Comparison of mortality rates: Four mice died in the sodium nitrite group; 2 mice died in SM group and no mice died in the MB group and control group.

Methemoglobin analysis: Sodium Nitrite induced increase of methemoglobin levels compared with control group (P < 0.05), however, in MB and SM group was significant lower than in SN group (P < 0.05). As illustrated in Table 1.

Histological changes of heart, lung, liver and kidney tissues: Tissues sections were stained with hematoxylin and eosin. The results were shown in Figure 1. From this figure it can be seen that in control group, there is no obvious abnormality under the light microscope. While in the SN group, swelling and necrosis of tissues and inflammatory cell infiltration and edema of connective tissue were seen. In MB and SM Group, which was lighter than that in SN group.

Figure 1. Heart, lung, liver and kidney sections were stained with hematoxylin and eosin. A-D means heart tissue, E-H means lung tissue, I-L means liver tissue and M-P means kidney tissue. CON means control group, SN means sodium nitrite poisoning group, MB means methylene blue and SM means Salvia miltiorrhizae-treated group. There is no obvious abnormality in control group. While in the SN group, swelling and necrosis and inflammatory cell infiltration and edema of connective tissue were seen. In SM group and MB group, which was lighter than that in SN group.

Apoptosis in renal tissue: Sodium nitrite induced increase of TUNEL staining compared with control group (P < 0.05), however, these increase in MB and SM group was significant lower than in SN group (P < 0.05). As illustrated in Figure 2.

Inflammatory cytokines release in serum: Sodium nitrite induced increase of TNF-α and IL-6 release and decrease of IL-10 compared with control.
group (P < 0.05), however, TNF-± and IL-6 in MB and SM group was significant lower and IL-10 significantly higher than in SN group (P < 0.05). As illustrated in Table 2.

Sodium nitrite is widely used in industry, construction and meat products. Nitrite is highly toxic substances, adult intake of 0.2 to 0.5 g can cause poisoning, 3 grams can be lethal (Luca et al., 1987). Due to the consumption of high levels of nitrate or nitrite cured meat, pickles, and the spoiled vegetables can cause poisoning (Lukin-Butenko et al., 1983). Nitrite will trigger oxidation of low iron in the normal oxygen-carrying hemoglobin into methemoglobin, thus losing the oxygen-carrying capacity and cause tissue hypoxia (Gautami et al., 1995). Nitrite is also a carcinogen, according to the previous studies, esophageal cancer patients was positively correlated with nitrite salt intake (Xie et al., 2011). Nitrite poisoning incidence rapid incubation period is generally one to three hours, the main features of the poisoning is due to tissue hypoxia caused cyanosis phenomenon, such as the lips, tongue and body skin bruising. Sleepiness or irritability, difficulty breathing, dizziness, headache, fatigue, respiratory failure and death (Gautami et al., 1995). In our study, 4 mice died in the sodium nitrite group, and after sodium nitrite treatment, all the extremities and the tip of the nose, skin and mucous membrane of mice cyanosis, the mice showed incontinence and decreased activities. Which means we successfully established nitrite poisoning animal model and presented methemoglobinemia obviously. Methemoglobinemia can be treated with supplemental oxygen and methylene blue. Methylene blue restores the iron in hemoglobin to its normal (reduced) oxygen-carrying state. This is achieved by providing an artificial electron acceptor for NADPH methemoglobin reductase. At high doses, however, methylene blue actually induces methemoglobinemia, Salvia miltiorrhiza in the experiment, as an old Chinese herb, was shown to be valuable for many kinds of disease previously. All these research reveal that Salvia miltiorrhiza play a role in the treatment of some kind of disease by its properties such as protect endothelial cells, light against inflammation, and prevent lipid peroxidation. In our study, we used the animal model to mimic the sodium nitrite poisoning in humans both biochemically and morphologically. The characteristic histological features of our models were findings of swelling and necrosis of tissues, inflammatory cell infiltration and edema of connective tissue in interstitium. And the model also shown an increase of methemoglobin accompanied with increase of inflammatory cytokines and apoptosis in tissues. In the study, we observed that Salvia miltiorrhiza attenuated the histologic severity of tissues. So it can be speculated on the mechanism of Salvia miltiorrhiza on experimental sodium nitrite poisoning. It can prevent the inflammation and apoptosis in tissue. However, there is still a great need for more and better research on the efficacy and safety of Salvia miltiorrhiza. Although it is difficult to extrapolate animal data to humans, best Salvia miltiorrhiza is recommended as a beneficial treatment for sodium nitrite poisoning.

**CONCLUSIONS**

Salvia miltiorrhizae injection reduced tissue injury induced by Sodium nitrite effectively, which may served as a beneficial treatment accompanied with methylene blue to rescue acute sodium nitrite poisoning patients.

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### TABLE 2: TNF-α, IL-6 and IL-10 concentration in serum (x±s, n=8).

<table>
<thead>
<tr>
<th></th>
<th>TNF-± (pg/µg protein)</th>
<th>IL-6 (pg/µg protein)</th>
<th>IL-10 (pg/µg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL group</td>
<td>13.8±0.9</td>
<td>11.3±0.8</td>
<td>10.4±0.7</td>
</tr>
<tr>
<td>Sodium nitrite group</td>
<td>19.5±1.9*</td>
<td>18.8±1.5†</td>
<td>3.7±0.4*</td>
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<tr>
<td>Methylene blue-treated group</td>
<td>14.3±1.2*#</td>
<td>13.6±1.4†</td>
<td>9.4±0.9*#</td>
</tr>
<tr>
<td>Salvia miltiorrhizae-treated group</td>
<td>15.4±0.7*</td>
<td>15.6±1.3†</td>
<td>8.3±0.6*</td>
</tr>
</tbody>
</table>

Note: *P<0.05 vs Control group, †P<0.05 vs Sodium nitrite group
REFERENCES


