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Arachidonic acid causes hidden blood loss–like red blood cell damage through oxidative stress reactions

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ABSTRACT

Background: Hidden blood loss (HBL) often occurs in the prosthetic replacement for joint, but the mechanism is still not clear.

Materials and methods: This study tried to establish an animal model of HBL by injecting arachidonic acid (AA) into the Sprague-Dawley rats. Different concentrations of AA were injected into the tail veins of the rats, and blood samples were collected before and after administration at 24, 48, and 72 h. A complete blood count was obtained by to find the hemoglobin (Hb) and red blood cell (RBC) count changes. The glutathione peroxidase (GSH-PX) and total superoxide dismutase (T-SOD) activities and hydrogen peroxide (H_2O_2) levels were detected. The morphological changes of erythrocyte were observed under a polarizing microscope. The absorbance values of the blood samples were tested to determine the presence of ferryl Hb.

Results: HBL occurred in the experimental groups when the concentration of AA reached 10 mmol/L; Hb and RBC values decreased sharply at 24- and 48-h postinjection. This was followed by reduced activities of GSH-PX and T-SOD and decreased levels of H_2O_2. Moreover, the pathologic changes of red cell morphology mainly presented as pleomorphic RBC morphology, including cell rupture. The absorbance values of the blood samples were in accordance with ferryl Hb features. RBC and Hb values were relatively stable at 72 h. The GSH-PX and T-SOD activities and H_2O_2 levels gradually increased up to a balanced state.

Conclusions: The study concluded that high concentrations of AA can induce oxidative stress reactions in the body, causing acute injury of RBCs, which is closely related to HBL.

Introduction

Hidden blood loss (HBL) is one of the most common complications of arthroplasty,[1,2] which causes blood loss in spite of autologous or equivalent allogeneic blood transfusion in accordance with the dominant blood loss. Numerous hypotheses have addressed this topic[1,3-7]; however, the pathogenesis of HBL is still unknown. Pressure of the intramedullary increased rapidly, during the process of installing prosthesis in an artificial joint replacement surgery,

This work has been conducted in compliance with ethical standards.

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causing fat droplets in the bone marrow cavity enter into the blood circulation, which plays an important role in the process of pathology of some diseases and has been verified by transesophageal echocardiography.[8]

Free fatty acids (FFAs) are metabolites of fat. Studies have shown that it can stimulate neutrophils to promote the generation of reactive oxygen species (ROS).[9] ROS can attack macromolecular substances and cytoderm, damage the microstructure and membrane proteins of cells, and increase the cell membrane permeability, resulting in tissue injury and pathologic change in the body.[10-12]

In a previous research, the effect of linoleic acid on Sprague-Dawley (SD) rats was studied.[13] The hemoglobin (Hb) and red blood cell (RBC) values in that study were found to decrease. This was followed by an increase in the linoleic acid concentration and glutathione peroxidase (GSH-PX) and total superoxide dismutase (T-SOD) activities, and an obvious decrease in hydrogen peroxide (H_2O_2) levels. The study further observed the morphology of RBCs and absorbance change of the blood samples and found a large deformation of RBCs in the experimental group. The absorbance peak production reflected the existence of ferryl Hb. Thus, the study confirmed that the linoleic acid–induced oxidative stress can cause acute damage of RBCs. Arachidonic acid (AA) is an important part of the FFA and metabolites of linoleic acid.[14] In the present study, by injecting different concentrations of AA to SD rats, the changes in RBCs and the associated redox reactions were observed. The relationship between AA and HBL was also explored and the mechanism of HBL was explained, so as to obtain a useful HBL model.

Materials and methods

Animal model preparation

Adult male SD rats (weight: 225 ± 15 g) purchased from the Chinese Academy of Science, Nanjing University Animal Center, China, were used for all experiments. All animals received humane care in compliance with "The Principles of Laboratory Animal Care" formulated by the National Society of Medical Research and "The Guide for the Care and Use of Laboratory Animals" published by the U.S. National Institutes of Health (National Research Council, 1996). The animals were housed at a set temperature (24°C) in a humidity-controlled room with a 12-h light/dark photoperiod. All experimental procedures were carried out strictly in accordance with the care and use of laboratory animals, which were approved by the National Institute of Health's Guide for the Care and Use of Laboratory Animals and the Institutional Animal Care and Use Committee at Nanjing University, China. All animals were anesthetized with ether inhalation before operation.

Instruments and reagents

The following instruments were used in this study: microplate reader (Bio-Rad680, USA), hematology analyzer (Sysmex XE-5000, Japan), spectrophotometer (Hewlett Packard 8453 UV-visible diode array spectrophotometer, USA), polarizing microscope (Nikon ECLIPSE 50i, Japan), and centrifuge (Hermle Universal Centrifuge Z323, Germany).

The H_2O_2 concentration and GSH-PX and SOD activities were determined with commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). AA was obtained from Sigma-Aldrich (MO).

Experimental protocol and drug

A total of 50 SD rats were assigned randomly into five groups: one control group and four experimental groups, with 10 rats in each group. First of all, AA was dissolved in a 20% ethanol solution. Different concentrations of AA were made up by diluting AA in 20% ethanol solution. The control group was treated with 20% ethanol solution alone. The experimental groups were further divided into four groups, AA-A, AA-B, AA-C, and AA-D, with concentrations of 5, 10, 20, and 40 mmol/L, respectively. The mode of administration was intravenous injections of 0.5 m per dosage through the tail veins. The rate was slowed down, and the concentration of administration was minimized to avoid unnecessary deaths. Before formal experiments, preliminary experiments were conducted to explore the best-dosage range notation. The present study showed that half of the mortality rates occurred at a dosing concentration of 40 mmol/L, and the effective concentration ranged between 5-40 mmol/L. All the rats were monitored continuously until they recovered from anesthesia. The rats were then sacrificed by spinal dislocation method. The study found that they suffered from several symptoms, including dyspnea, cyanosis, hemoptysis labiali, and convulsions. Throughout the process, the rats had no special discomfort.

Blood samples were collected from the caudal vein before injection and at 24-, 48-, and 72-h postinjection (0.5 mL each time). Blood smears were prepared and stained with Wright's stain to observe morphologic changes. RBC and Hb values were obtained through the automatic blood analysis system. A spectrophotometer was used to measure the concentration of H_2O_2 and the activities of GSH-PX and T-SOD[15]; for each of these measurements, absorbance values were determined at 405, 412, and 550 nm, respectively, according to the manufacturer's instructions. All samples were processed within 2 h of collecting the blood samples. Spectral changes of Hb in the AA-C group were typically measured with a spectrophotometer. The Hb (10 mmol/L) was mixed with buffer (0.1 mol/L sodium phosphate) containing 100-mmol/L diethylenetriaminepentaacetic acid. All procedures were performed at 25°C.

Statistical analysis

All calculations and statistical analyses were performed using the SPSS version 19.0 software. Values were expressed as mean ± standard deviation and analyzed by one-way analysis of variance, followed by Dunnett's t-test. In all cases, $P < 0.05$ was regarded as significant.

Results

This study showed that administering AA at a concentration of 20 mmol/L caused RBC and Hb values to decrease