Effect of Huoxuequyu Recipe on Erythrocyte Ultrastructure and Membrane ATPase Activity in Rats with Passive Smoking*

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Summary: The effect of “Huoxuequyu” recipe on erythrocyte membrane Ca$^{2+}$-ATPase activity, Na$^+-$K$^+$-ATPase activity and erythrocyte ultrastructure in rats exposed to passive smoking were observed. The erythrocyte membrane Ca$^{2+}$-ATPase activity and Na$^+-$K$^+$-ATPase activity were found to be decreased in the model group as compared with the control group ($P<0.01, P<0.01$), while no significant difference existed between the treated group and the control group ($P>0.05$). Our results also showed that there were more deformed red blood cells in model group than in control group under TCM and SEM ($P<0.0001$). No significant difference was found between control group and treated group ($P>0.05$). Furthermore, these findings indicated that “Huoxuequyu” recipe, a prepared natural herbal medicine, may be involved in modulation of the activity of erythrocyte membrane enzymes, thereby protecting erythrocyte membrane, so as to improve the erythrocyte function.

Key words: smoking; erythrocyte membrane; ultrastruture; Na$^+-$K$^+$-ATPase; Ca$^{2+}$-ATPase; Huoxuequyu natural herbs

In recent years, the changes of erythrocyte shape following its functional impairment in some diseases or in aging process have been reported$^{[1-11]}$. Effort has been directed to finding out an effective medicine capable of recovering membrane damage. This study observed the effect of “Huoxuequyu” recipe, a prepared natural medicinal herb on erythrocyte ultrastructure and membrane ATPase activity of erythrocyte in rats with exposure to passive smoking.

1 MATERIALS AND METHODS

1.1 Animals
Male Sprague-Dawley rats weighing 200 -250 g, which were provided by the Experimental Animal Center of Tongji Medical University were used.

1.2 Drugs and Reagents
Cigarettes of trade mark Hong Jing Long were purchased from market, which, according to the manufacturer, contain approximately 15 mg of nicotine in each cigarette. Huoxuequyu recipe, decoction made up of Radix angelicae sinensis, Rhizoma Ligustici Chuanxiong and Radix salviae miltiorrhizae, was provided by the Pharmaceutical Factory of Tongji Hospital, ATP-Na$_2$, and Ouabain, were obtained from Sigma Chemical Co., USA. All other reagents such as Tris, EDTA, EGTA and TCA etc. were of analytical grade. Solution was prepared in deionized double-distilled water and pH was controlled with PHs-10C Digital pH meter.

1.3 Experimental Animals
Rats were randomly divided into three groups: control group, model group and treated group. The model group and treated group rats were housed in the instrument for 20 days. They were exposed to smoke 4 times daily (15 min each time) in an'cage, with dosage CO$_2$1.2 %, CO 900 ppm, O$_2$ 19.8 % and Nox 8 ppm. The rats in treated group were given Huoyuequyu decoction by artificial feeding (2.0 ml per animal each
day), while the model group and control group rats were given the normal saline of same volume. The rats in the control group were treated in the same way but without exposure to cigarette smoke. All the animals were killed with pentobarbital saline on twenty-first day after exposure.

1.4 Assay for Ca²⁺-ATPase, Na⁺-K⁺-ATPase Activity

Blood samples were drawn and immediately mixed with heparin. Erythrocytes were separated and washed by centrifugation in ice-cold saline containing 50 mmol/L Tris-HCl and 1 mmol/L EDTA buffer (pH=7.40). The membrane were then obtained by lysing and centrifugation[41]. All the processes were controlled at the temperature between 0 °C - 4 °C. Membrane protein was determined with folin phenol reagent. Human serum albumin was used as standard. The technique used for measurement of erythrocyte membrane ATPase activity were similar to those described in the literature[5,6].

1.5 Erythrocyte Ultrastructure

Four blood samples were obtained with 2.5 % pentoanel randomly in each group to make photos to be observed under scanning electronic microscope (SEM). Three randomly obtained blood samples with heparin from each group were centrifuged at 4 °C (3000 g, for 10 min), with plasma and buffy coats removed. Then the erythrocyte were fixed by 2.5 % pentoanel for making transmission electronic microscope photos.

The data were analyzed by SAS software. A P value less than 0.05 was regarded as significant.

2 RESULTS

The erythrocyte membrane Ca²⁺-ATPase activity, Na⁺-K⁺-ATPase activity were found to be decreased in the model group. There was a significant difference between the model and the control group (P <0.01, P <0.01). However, no significant difference was found between the treated group and the control group (P >0.05). In model group, some spinous processes on the surface of erythrocytes, such as spherocytes, rigid cells, spike cells, were found under the SEM. The transmission electron microscopy revealed that some parts of the red cells membrane became thicker, the density were changed. All ultrastructural abnormalities were significantly serious as compared with the control group (P <0.0001), while these changes were significantly mild in the treated group, which were similar to those of the control group (P >0.05).

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<th>Table 1 ATPase activity (x±s)</th>
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P <0.01 compared with the control group

3 DISCUSSION

Normal red cell can undergo distortion undamaged when passing through small capillaries. The membrane permeability and transportation and enzymatic activity within the membrane of the red blood cell are paramount in the passage through narrow capillaries. Abnormal red cells, such as spherocytes, spike cells, rigid cells, sick cells etc. in some disease, or in aging process, accompanying membrane damage have been reported[1-3]. The role of the erythrocyte membrane in the regulation of the microcirculation and cell biofunction has draw more attention of researchers. Effort has been made to find out effective medicine capable of recovering membrane damage. In order to further understand the effect of Huoxuequyu recipe on erythrocyte membrane, the erythrocyte damage model,