MEDS Public Health and Preventive Medicine (2022)
Clausius Scientific Press, Canada

DOI: 10.23977/phpm.2022.020205
ISSN 2616-1915 Vol. 2 Num. 2

Effect of Xuanhuang ointment on SOD and MDA in local soft tissue of experimental acute traumatic hematoma

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Keywords: Acute soft tissue injury, Xuanhuang ointment, Superoxide dismutase, Malondialdehyde.

Abstract: Objective: To observe the effect of Xuanhuang ointment on superoxide dismutase (SOD) and malondialdehyde (MDA) in local soft tissue of experimental acute soft tissue injury model rats. Methods: The acute soft tissue injury model of rats was established and randomly divided into Xuanhuang ointment group, safflower oil group and matrix ointment control group. Another 6 rats were taken as the normal group. In addition to the normal group, each group was given Xuanhuang ointment, safflower oil and matrix ointment to apply medicine to the injured part of rats respectively. Soft tissue samples were taken from the injured part on the first, third and fifth days after treatment. Some tissues were homogenized, and the contents of SOD and MDA in the homogenization solution were measured. Results: The content of MDA in local soft tissue injury in matrix ointment group was significantly higher than that in normal group ($P < 0.01$), and the content of SOD was lower than that in normal group ($P < 0.01$); Xuanhuang ointment group could inhibit the increase of MDA content in injured tissues and effectively promote the production of SOD ($P < 0.01$), and there was no significant difference compared with safflower oil group ($P > 0.05$). Conclusion: One of the effective mechanisms of Xuanhuang ointment in the treatment of acute soft tissue injury may be related to the fact that Xuanhuang ointment can effectively reduce the content of MDA and increase the activity of SOD in local soft tissue injury.

1. Introduction

Acute soft tissue injury is a common clinical disease in orthopedics and traumatology. It belongs to the category of "tendon injury" in traditional Chinese medicine. It often leads to local tissue inflammatory reaction due to external violence factors such as accidents and strong exercise. It is often found in muscles, tendons, tendons, ligaments and other parts. It is often a kind of disease with congestion, swelling and dysfunction as the main clinical manifestations [1]. Modern medical research points out that soft tissue injury is mainly the inflammatory reaction of damaged tissue, and the key problem of inflammatory reaction is the change of microcirculation [2]. After trauma, local inflammatory mediators are secreted, released and exuded, local soft tissue ischemia and hypoxia, acid accumulation, increased vascular permeability, resulting in edema and dysfunction [3]. If the

treatment is not timely, it can cause the patient's skin cyanosis and elevated skin temperature, accompanied by severe pain. In severe cases, it can cause irreversible damage and further reduce the patient's quality of life and level. Modern medical treatment of acute soft tissue injury mainly focuses on anti-inflammatory, detumescence, analgesia and local blocking, but the curative effect is poor and recurrent, and there are some side effects [4]. Through randomized controlled animal experiments, this experiment further studied the effects on the contents of SOD and MDA in local soft tissue of experimental acute soft tissue injury model rats, so as to provide further scientific basis for the clinical application and promotion of xuanhuang ointment.

2. Materials and methods

2.1 Experimental animal selection

Sixty six Wistar rats, half male and half female, weighing 160 ~ 190g, were purchased from the experimental animal center of the Fourth Military Medical University with animal license No.: scxk (Shaanxi 2007-002).

2.2 Experimental drugs and reagents

Xuanhuang ointment (self-made), the positive control drug safflower oil is provided by Changchun Jingkai Pharmaceutical Co., Ltd. (gyzz z22024033, batch No. 20040103), and SOD and MDA kits are purchased from Nanjing Jiancheng Institute of biology.

2.3 Experimental animal modeling [5]

The rats were fixed on the self-made rat operating table, and a hollow tube with a diameter of 1.5cm and a height of 50cm was placed with a smooth wall. Place it on the soft tissue of the right lower limb of rats (remove the hair of the thigh of the right lower limb with 8% sodium sulfide hair remover one day before the experiment), and then hit the soft tissue of the right lower limb of rats with a weight of 100g free fall from top to bottom for three times, resulting in a non open soft tissue injury model with an area of about 4cm2 and obvious subcutaneous bleeding and swelling. There is an obvious swelling feeling after hand touch.

2.4 Experimental animal grouping

Sixty rats were numbered in the order of weight from large to small, and randomly divided into Xuanhuang ointment group, safflower oil group and matrix ointment group. There were 20 rats in each group, and the other 6 rats were taken as the normal group.

2.5 Experimental methods

After successful modeling, all rats were fed with standard rat feed and drank freely under the same conditions (in the biochemical laboratory of Shaanxi University of traditional Chinese medicine, room temperature 19 ~ 24℃, relative humidity about 40%). Administration was started 1h after modeling. After modeling, the animals in each group were killed at three hours on day 1, day 3 and day 5, with 6 rats in each hour, and 2 rats in the blank group. The skin of the hit part of the rats was cut with a scalpel, the subcutaneous tissue was separated, and the muscle tissue in the center of the injury was cut. The tissue of the same part was taken in the normal group.

2.6 SOD, MDA detection

2.6.1 Detection of SOD

① Tissue sampling: cut a 1cm with a scalpel × 1cm × Tissue slices with the size of 0.5cm were homogenized with a tissue homogenizer;

② The instruments and self prepared reagents required in the experiment are: 550nm spectrophotometer; 37℃ constant temperature water bath pot; Bench Centrifuge; Micro pipette; Distilled water; Glacial acetic acid (analytical purity);

③ Composition and preparation of reagents

Reagent 1: 10ml of stock solution × 1 bottle (there will be some crystallization in cold weather or in the refrigerator, which needs to be dissolved in hot water before reuse); Preparation of reagent I application solution: dilute each bottle with distilled water to 100ml and store it at 4 °C;

Reagent 2: liquid 10ml × 1 bottle, stored at 4 °C ~ 10 °C;

Reagent 3: liquid 10ml × 1 bottle, stored at 4 °C ~ 10 °C;

Reagent 4: liquid 350ul × 2 sticks, stored at 4 °C, non freezing; No. 4 diluent 5ml × 1 bottle, Store at 4 °C. Do not freeze. It can be stored for 2 ~ 3 months after preparation. Note: all washing nozzles should be special and disinfected;

Reagent 5: Powder × 1. Add 75ml of hot double distilled water at 70 °C ~ 80 °C to dissolve it and then use it for standby. If the evaporation of water decreases during heating, it must be supplemented to 75ml with distilled water at this time, and the prepared reagent shall be refrigerated at 4 °C away from light;

Reagent 6: Powder × 1. Add 75ml distilled water to dissolve it when it is used, and then use it for standby. The prepared reagent shall be kept away from light and refrigerated at 4 °C; Preparation of developer: prepare the developer according to the volume ratio of reagent 5: reagent 6: glacial acetic acid = 3:3:2, and store it at 4 °C.

④ Operation steps of SOD activity determination:

Fully mix it with a vortex mixer, place it in a constant temperature water bath at 37 °C for 40 minutes, mix it, place it at room temperature for 10 minutes, place it at the wavelength of 550nm, 1cm light path cuvette, adjust the distilled water to zero and compare the color. Determination of SOD (See Table 1.)

Table 1: Determination of SOD

Reagents	Measuring tube	Contrasting tube
Reagent 1(ml)	1.0	1.0
Specimen(ml)	0.3	0.3
Distilled water(ml)	0.1	0.1
Reagent 2(ml)	0.1	0.1
Reagent 3(ml)	0.1	0.1
Reagent 4(ml)	0.1	0.1
Chromogenic agent(ml)	2	2

⑤ Definition: when the inhibition rate of SOD per milligram of tissue protein in 1ml reaction solution reaches 50%, the corresponding SOD amount is a SOD activity unit.

⑥ Calculation formula

SOD activity in tissue homogenate = (absorbance of control tube - absorbance of measuring tube)/ 2(absorbance of control tube) × Total volume of reaction solution / sampling volume (ML)/protein content in tissue.

2.6.2 Detection of MDA

① Tissue sampling: cut a 1cm with a scalpel × 1cm × 0.5cm tissue slices were homogenized with tissue homogenizer.

② Instruments and self prepared reagents required in the experiment: 550nm spectrophotometer; 37℃ constant temperature water bath pot; Bench Centrifuge; Distilled water; Glacial acetic acid (analytical purity); Absolute ethanol.

③ Composition and preparation of reagent

Reagent 1: 20ml liquid × 1 bottle, stored at room temperature, will solidify in cold weather, and heated appropriately before each test to accelerate dissolution until transparent before application.

Reagent 2: 12ml solution × 1 bottle, add double distilled water to 340ml and mix well.

Reagent 3: Powder × Add the powder into 60ml of hot double distilled water at 90 ℃ ~ 100 ℃, heat appropriately, fully dissolve, make up to 60ml with double distilled water, and then add 60ml of glacial acetic acid,mix well, and refrigerate the prepared reagent away from light.

Standard: 10nmol / ml Tetraethoxypropane 5ml × 1 bottle.

④ The steps of MDA determination:

Mix well with a vortex mixer, tie the mouth of the test tube tightly with fresh-keeping film, prick a small hole with a needle, take a 95 ℃ water bath (or boil with a pot cover) for 40 minutes, take it out and cool it with running water, then 3500 rpm, centrifuge for 10 minutes, take the supernatant, adjust the distilled water to zero, and measure the absorbance value of each tube. Determination of MDA (see Table 2.)

Table 2: Determination of MDA

Reagents	standard tube	Standard blank tube	Measuring tube	Measuring blank tube
10nmol / ml standard(ml)	0.1	0.1	0.1	0.1
Anhydrous alcohol(ml)	0.1	0.1	0.1	0.1
Test sample(ml)	0.1	0.1	0.1	0.1
Mixed reagent(ml)	0.4	0.4	0.4	0.4

⑤ Calculation formula

MDA content in tissue homogenate (nmol / mgprot) = (absorbance of control tube - absorbance of measuring tube) / (absorbance of standard tube - absorbance of standard blank tube) × Standard concentration (10nmol / ml) / protein content (mgprot / ml) / protein content in tissue.

3. Statistical Analysis

The data of each group are expressed in ($\bar{x} \pm s$), and the difference between groups is judged by two-sided t-test of the mean of two samples. SPSS 22.0 genuine statistical software was used for analysis, and LSD method was used for significance test.

4. Results and analysis

4.1 Observation of SOD content

The content of SOD in the injured local soft tissue of model rats in each group was significantly lower than that in the normal group ($P < 0.01$). Although the content of SOD in xuanhuang ointment group was higher than that in safflower oil group, it was not statistically significant ($P > 0.05$). (See Table 3 and Figure 1).

Table 3: Comparison of SOD content in local tissue of rats in each group after injury (nu/mg) (± s)

Groups	n	1 day SOD	3 day SOD	5 day SOD
Normal group	6	215.70±1.1271	215.25±1.1247	214.37±1.1064
Matrix paste control group	18	141.03±1.1034	160.32±1.2011	180.21±1.1941
Safflower oil control group	18	160.24±1.3108	179.24±1.3219	203.03±1.1557
Xuanhuang ointment group	18	162.56±1.1314	182.92±1.1642	203.34±1.1247

4.2 Observation of MDA content

The content of MDA in the injured local soft tissue of model rats in each group was significantly higher than that of normal at all stages after injury ($P < 0.01$). Xuanhuang ointment can inhibit the increase of MDA content in the injured tissue of model rats. Although the content of MDA in xuanhuang ointment group was lower than that in safflower oil group, it was not statistically significant ($P > 0.05$). (See Table 4 and Figure 2).

Table 4: Comparison of MDA content in injured local tissue of rats in each group (nu / mg) (± s)

Groups	n	1Day MDA	3Day MDA	5Day MDA
Normal group	6	2.69±0.1019	2.67±0.1113	2.71±0.1120
Matrix paste control group	18	4.95±0.1123	4.66±0.1219	3.30±0.1214
Safflower oil control group	18	4.49±0.1227	4.16±0.1429	2.95±0.1029
Xuanhuang ointment group	18	4.43±0.1318	4.01±0.1642	2.81±0.1034

5. Conclusions and Discussion

Under normal circumstances, all tissues of the body contain certain active antioxidant enzyme systems, which can remove oxygen free radicals in time and effectively. MDA is one of the most important products of lipid peroxidation, which can directly damage biological macromolecules and cell membranes [6], and the content of MDA in vivo can indirectly reflect the degree of damage caused by free radicals to the body. Sod is an important scavenger of oxidative free radicals in the body. It has important antioxidant effect and is one of the most important antioxidants [7-8]. It is a natural antioxidant enzyme in the human body. It can effectively remove free radicals and maintain the dynamic balance of free radicals in the body by catalyzing the self redox reaction of superoxide anion free radicals. Lipid peroxidation not only converts reactive oxygen species into active chemicals (i.e. non free radical lipid decomposition products), but also amplifies the role of reactive oxygen species through chain or chain branched chain reaction. Therefore, the initial reactive oxygen species can lead to the formation of many lipid decomposition products. Some of these decomposition products are harmless, while others can cause cell metabolism, dysfunction and even death. Oxygen free radicals can not only cause cell damage through the peroxidation of polyunsaturated fatty acids (PUFA) in biofilm, but also cause cell damage through the decomposition products of lipid hydroperoxide. Therefore, the amount of MDA often reflects the degree of lipid peroxidation in the body and indirectly reflects the degree of cell damage.

The determination of MDA often cooperates with the determination of SOD. The activity of SOD indirectly reflects the body's ability to eliminate oxygen free radicals [9], and the level of MDA indirectly reflects the severity of free radical attack on body cells. The analysis of the results of SOD and MDA is helpful to explore the preliminary mechanism of Xuanhuang ointment in the treatment of acute soft tissue injury.

In the pathological state of acute soft tissue injury, the generation of free radicals increases, the

activity of free radical scavenging enzymes decreases, the dynamic balance is destroyed, the free radical chain reaction is started, the lipid peroxidation of membrane is triggered, and the tissue injury is further worsened. MDA is the metabolic end product of lipid peroxidation caused by oxygen free radical attack, which reflects the generation level of oxygen free radical and the degree of tissue damage. Sod can catalyze the disproportionation of superoxide free radicals to produce H2O and O2. After the leg muscles of model rats are damaged, the generation of free radicals and MDA in the body increase. At the same time, the protective mechanism in the body starts, and SOD increases accordingly. The decrease of MDA in Xuanhuang ointment group was significantly different from that in matrix ointment control group ($P < 0.01$), indicating that Xuanhuang ointment can effectively reduce lipid peroxidation, and the increase of SOD was significantly different from that in matrix ointment control group ($P < 0.01$), indicating that Xuanhuang ointment can effectively stimulate the body to produce a large amount of SOD, so as to effectively prevent and control the damage of oxygen free radicals.

Through the detection of MDA and SOD contents in local tissues of model rats, this experiment confirmed that Xuanhuang ointment can significantly improve the activity of SOD and reduce the production of MDA, improve the body's antioxidant and protect the function of vascular endothelium. It is speculated that Xuanhuang ointment may achieve the purpose of treating acute soft tissue injury by enhancing the activity of SOD, inhibiting the production of MDA, protecting the function of vascular endothelium and preventing lipid peroxidation.

Acknowledgements

The author sincerely thanks Xi'an Science and Technology Bureau for the support of the experimental study on the effect of Xuanhuang ointment on immune function in rats with acute soft tissue injury [201805093yx1sf27 (16)].

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