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Study on the Bacteriostatic Effect of Baitouweng on Pseudomonas Aeruginosa Infection of Wounds in Rats

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Abstract: **Objective:** To analyze the bacteriostatic effect of Baitouweng on Pseudomonas aeruginosa infection of wounds in rats. **Methods:** Forty Wistar rats were enrolled in the study, among which excisions were made on 30 rats on their upper layer of dorsal skin with an area of 1 cm x 1 cm, the other 10 rats as the control group of sterile wound. Prepared Pseudomonas aeruginosa was applied on the wounds of rats to create infection models. Forty rats were divided into three groups (control group, mafenide group and Baitouweng group) according to different infection methods, and were treated with normal saline, 100g/L mafenide, and 1g/L Baitouweng respectively after 3 hours of injury. The changes in the number of white blood cells in both the wound surface and body of the three groups were observed within one to four days after injury. After that, the changes on the number of both white blood cells and body weight were continuously observed. The survival of the rats in each group was observed on the 14th day after injury. **Results:** From the observation after injury, compared with the other two groups, rats in control group had more exudation and moist wounds, and the activities of rats decreased while the death rate increased. On the 3rd day after injury, the number of white blood cells in each group decreased, and the number of Pseudomonas aeruginosa in the control group was significantly higher than that in the other two groups ($P < 0.01$). The rats in the sterile wound control group did not die and continued to gain weight. After 14 days, the survival number of rats in control group was significantly less than that in mafenide group and Baitouweng group ($P<0.05$). **Conclusion:** Baitouweng has obvious bacteriostatic and virus-killing effects on Pseudomonas aeruginosa infection of wounds in Wistar rats, reducing mortality rate effectively, and has practical value as well as development and application prospects.

Keywords: Baitouweng; Pseudomonas Aeruginosa; Wound Infection

1. Foreword

Pseudomonas aeruginosa, also known as pseudomonas, often occurs in nature, such as soil, and wet parts of the body, such as human intestinal tract. Pseudomonas aeruginosa has low nutritional requirements but with strong drug resistance and has great harm, especially to the middle-aged, the elderly and infants who has low immunity. This kind of bacterium is easy to cause disease, such as septicemia, and acute gastroenteritis. Therefore, early prevention, early detection and early treatment are the main treatment principle, and the inhibition of the bacteria has become a crucial issue to overcome. Baitouweng was first recorded in Shennong's Herbal Classic of Materia Medica and had been widely used in ancient Chinese medicine. With the development of modern medical science and technology, it has been found that Baitouweng has clear bacteriostatic effect on pathogens such as Escherichia coli and Paratyphoid Bacillus, and the extracted effective components are protoanemonin and anemonin. Safe drug usage has become a broad consensus in the medical field nowadays. In this case, exploring antibacterial drugs from traditional Chinese herbal medicines has become a hot research topic. This study deeply explores the antibacterial effect of Baitouweng on Pseudomonas aeruginosa. Details are reported as the following contents[1].

2. Materials and methods

2.1 Animal origin, strains tested and experimental drugs

Wistar rats were all male and introduced from animal experiment center of a medical university. Their body weight ranged from 200 g to 250 g. Pseudomonas aeruginosa was provided by microbiology laboratory. Baitouweng is purchased from the Chinese medicine pharmacy in our hospital.

2.2 Preparation and purification of protoanemonin

Baitouweng weighing 150g was put into a round bottom flask, and was mixed with distilled water at a ratio of 1: 10. After soaking for one hour and heating for one hour, the dregs were filtered with gauze. Then, eight times and six times of distilled water were poured into the dregs to repeat soaking and heating operation. The solution obtained from the above three treatments was concentrated and purified, so that the concentration of the liquid was stabilized at 1g/L and stored in the freezer.

2.3 Preparation of Pseudomonas aeruginosa suspension

Pseudomonas aeruginosa in culture medium was separated through centrifugal separation, with 2000r/min centrifugal rate and 15min duration. It was washed repeatedly with normal saline and then re-suspended to a concentration of 1×10^8CFU/ml. After incubation at 37℃ for 24 hours, the number of colonies was identified by blood cell counting plate.

2.4 Detection of antibacterial activity

Pseudomonas aeruginosa was cultivated on the culture medium, and solution and distilled water of Baitouweng were injected respectively as control group. After being cultivated at 37℃ for 24 hours, the results showed that Baitouweng had a strong inhibitory effect on Pseudomonas aeruginosa.

2.5 Establish infection models

One day before injury, 30 rats were selected, and the other 10 used as control group of sterile wound. After anesthesia, fixation and hair removal, the upper layer of the skin was cut with a wound area of 1cm×1cm by a scalpel. Prepared Pseudomonas aeruginosa bacterial solution was smeared on the wound of rats to establish infection models.

2.6 Groups

Randomized sampling and different nursing methods were used to divide rats into three groups, which are control group, mafenide group and Baitouweng group. A total of 40 rats in the three groups were kept in the same environment, where was clean and dry with 25℃ of constant temperature. The changes of the number of white blood cells in the wound and body of the three groups were observed within one to four days after injury. Then the changes of white blood cells and body weight were continuously observed, and the survival of the rats in each group was observed on the 14th day after injury[2].

2.7 Statistical analysis

All the collected prescription-related data in this paper are processed and analyzed through SPSS20.0 software, saving the cost of manual calculation and improving the accuracy and efficiency of calculation. The unit of measurement is expressed by ($\bar{x}\pm s$) with t test. If $P<0.05$, it is statistically significant.

3. Results

3.1 Changes of wound surface

From the observation after injury, compared with the other two groups, the wounds in the control group had more secretions and moist wounds. In addition, the activities of rats in the control group decreased and the death toll continued to

increase.

3.2 Quantification of Pseudomonas aeruginosa

After data collection and statistics, it was found that Pseudomonas aeruginosa in the control group was significantly higher than that in the other two groups. See **Table 1** for detailed data.

Table 1. Quantification of Pseudomonas aeruginosa in the scab of rats in each group on the third post-injury day

groups	Before injury	Different quantitative levels of bacteria		
		$<10^3$	10^3~10^5	$>10^5$
Control group	10	0	0	10
Mafenide group	10	5	5	0
Baitouweng group	10	10	0	0

3.3 Survival rate and weight test

After data collection and analyzing, it was found that on the 3rd post-injury day, the death number of rats reached five in control group, followed by mafenide group, two death rats, and Baitouweng group, one rat. By the 14th day, the survival rate of rats in control group was only 30%, and the other two groups remained unchanged. On the 6th post-injury day, the weight of rats decreased significantly in the control group compared with other two groups. Besides, there was little difference between the mafenide group and Baitouweng group. See **Table 2** for details.

Table 2. White blood cells on the third post-injury day and weight of rats on the sixth post-injury day

Rats number	White blood cell count ($\times 10^9$/l)			Weight (g)		
	control group	Mafenide group	Baitouweng group	control group	Mafenide group	Baitouweng group
1	—	3.95	3.9	—	19.1	20.9
2	—	5.65	4.25		18.3	19.5
3	2.95	4.10	3.1	16.9	20.3	18.4
4	—	4.3	4.25	—	20.8	19.0
5	3.65	—	4.2	16.7	—	18.8
6	3.35	3.75	4.9	16	19.7	19.8
7	—	3.95	3.9	—	19.4	19.1
8	3.5	4.05	—	—	19.8	—
9	3.45	4.25	3.6	—	20.6	20.4
10	—	—	5.95	—	—	19.6
$\bar{x}\pm s$	3.38±0.26	4.44±0.79	4.01±0.53	16.53±0.47	19.73±0.77	19.49±0.84

Notes: "-" suggests that the rat died.

4. Discussion

As one of the representatives of pathogenic bacteria in current clinical treatment, Pseudomonas aeruginosa, widely distributing in nature, its drug resistance is increasing year by year, while its morbidity and mortality are also high, being a

tricky issue in medical treatment. Even in hospitals, patients can be infected with Pseudomonas aeruginosa, because the pathogen mostly grows in humid environment. It is surveyed that the infection rate of Pseudomonas aeruginosa in hospitals is about 10% to 15% around the world. The isolation rate of Pseudomonas aeruginosa in the diagnosis of acquired pneumonia is as high as 20.9% in China, which is a very serious situation. Under such situation that there are many drug-resistant pathogenic bacteria strains at present, how to realize symptomatic prevention and treatment and maximize the effectiveness of antibacterial drugs in the treatment process has become an urgent issue for each clinician[3].

Baitouweng belongs to a kind of Chinese herbal medicine, which has been used to kill bacteria and insects since ancient times. In recent years, with the continuous advancement of the medical research and analysis on the chemical constituents and pharmacological effects, Baitouweng has drawn people's attention with a brand-new understanding. In modern research, Baitouweng has clear inhibitory effect on known strains such as Shigella dysenteriae and Bacillus subtilis. Compared with western medicine, Baitouweng will not produce too many toxic and side effects, especially in the case of antibiotic abuse.

Mafenide is recognized as an effective antibacterial drug against Pseudomonas aeruginosa in present clinical treatment. However, with the long-term use of the drug, it is inevitable that the therapeutic effect will be reduced due to the increase of bacterial drug resistance. Therefore, it is necessary to find new effective inhibitory components to alleviate this phenomenon. In this study, mafenide group and Baitouweng group were compared to on the one hand, verify the antibacterial effect, on the other hand, to verify whether the extract from Baitouweng can further replace mafenide as an antibacterial medicine for wound. From the wound infection, the wounds of the two groups of rats are basically the same, both of which are scabbed, dry and have little secretion. From the quantitative point of view of Pseudomonas aeruginosa, the antibacterial effect of Baitouweng group is better. It is because the protoanemonin and anemonin in Baitouweng can destroy the cell membrane and cell wall of Pseudomonas aeruginosa to achieve the bactericidal effect. At the same time, it will enhance the antibacterial effect with the extension of time. From the other indicators, there is little difference between the weight of the two groups and the number of white blood cells. The mortality rate of rats in the Baitouweng group will be lower, which indicates that it is feasible to use Baitouweng as an anti-Pseudomonas aeruginosa drug. However, whether it is functional in human body remains to be confirmed.

In summary, Baitouweng has obvious bacteriostatic and virus-killing effects on Pseudomonas aeruginosa infected wounds on Wistar rats. It effectively reduces rats' mortality rate, and has practical value and development and application prospects.

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