THE EFFECTS OF MAGGOT SECRETIONS ON THE INFLAMMATORY CYTOKINES IN SERUM OF TRAUMATIC RATS

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Abstract

The objective of this study was to analyse the changes of inflammatory cytokines level in traumatic rat serum after the maggot secretions intervention. Acute traumatic rat were randomly divided into three groups that included maggot secretions group, negative group and the control group. TNF-α, IL-6, SOD, LPO levels were measured by radioimmunoassay. The experimental results showed that TNF-α, IL-6, and SOD levels in the model group were significantly increased; LPO level was decreased and showed significant differences. Thus, the content of inflammatory cytokines in acute skin wounds could be reduced by maggot secretions, which play a role in enhancing wound healing.

Key words: Maggot secretions, serum, inflammatory cytokines, wounds

Introduction

We knew that maggot therapy had been successfully applied in many kinds of wounds, such as diabetic foot ulcer, wounds caused by vasculitis, trauma, among others (Chetan et al., 2011; Janice M.B. et al., 2005). This maggot therapy can be mostly credited to the secretions of the maggots. So far, the main fly species used in maggot therapy were Calliphora vicina, Chrysomya rufifacies, Lucilia caesar, and Lucilia cuprina. Lucilia cuprina was the species we used in this research. ES could inhibit elastase release and H_2O_2 production by fMLP-activated neutrophils, and the maximal inhibition was 5-50 mg of ES/ml. (Mariena . et al., 2007). The wound is the damage on normal skin tissue caused by the injury outside, such as external forces, heat, electric current, chemical substances and the internal factors of the body accompanied by damage to the sturdiness of the skin and to the normal function of the stress response, while producing inflammatory response. Tissue and organ damage could be caused by excessive and uncontrolled inflammation.

Material and Methods

Animals

8 week-old healthy adult rats SD 180, male and female of equal numbers, were provided by Capital Medical University. The rats we used in this research were only for our objective, not for other purpose, all the process we have been approved by the University Committee on the use and care of laboratory animals.

Reagents

TNF-α, IL-6, SOD, LPO kits were purchased from Nanjing Jiancheng Bioengineering Institute.

The preparation of maggot secretions (Gao et al., 2012)

The food for Lucilia larvae were as follows: wheat bran 500g, milk powder 60g, fish powder 60g, yeast powder 6g, peptone 10g, water 1200ml were mixed and then autoclaved in a spare refrigerator at 4□. Relative humidity was about 70%, room temperature was controlled at 25~32□, daily light by 60w incandescent for 12h, and water was added regularly to maintain a certain humidity. 100ml beaker was used for spawning. 60ml milk (15%) and bumf were used to lure flies to lay eggs regularly. The third generation egg was weighed about 0.0298g, 7.1mm length.

Maggots disinfection

The obtained Lucilia maggots needed to be sterilised, and the disinfection process was as follows: 3 aged larvae were placed in 3.5% formaldehyde saline solution for 4 min. Then, they were placed in a 2% solution of hydrogen peroxide for 2min; washed in 5% iodine form solution for 2 min; washed in 5% chlorine disinfectant for 2min, washed in 75% ethanol for 2 min. Dip and shock were included in the whole disinfection procedures, before they were washed with sterilised water at the end of every step. In a word, the maggot activity change was not big.

Model of acute wound in skin (Zhang et al., 2012; Gu et al., 2012)

SPF level SD rats were raised in sterile conditions for a week. All of the rats were anesthetised with 10% chloral hydrate; the prone position was made, and limbs were fixed, at the same, on both sides of the back spine. Hair was removed by 8% sodium sulfide removal at 24h after both sides of the spine, the same horizontal position were marked a circular area which had 1.3cm diameter.

Acute wound model of skin defects were made by cutting a scalpel deep fascia. Wound treatment and its packet: the rats were randomly divided into 3 groups of 60 after modelling, which included the model group, secretions group, and control group. No wound was made on rats in the control group, only the hair was removed partly. 0.8g/ml and 2ml secretions were administered on the wound in the secretions group; 0.3g Vaseline was applied on the wound in control group and model group. All rats were single caged. Debridement was accompanied with saline soaked cotton ball and dry powder or Vaseline was changed day by day. At last, the necrotic or infection tissue was removed; the wound area was covered with a net of steel, so that the secretions or Vaseline was not rubbed off by the rats themselves. 10 rats were randomly selected from each group after surgery for 1, 2, 3, 5, 7, and 14 days. The serums were got from the abdominal aorta, EP tube packaged and placed in -70 ^{o}C refrigerator.

Determination of TNF-α, IL-6 (Xia et al., 2012; Tang et al., 2012; Liang et al., 2004)

TNF-α, IL-6 levels were tested by enzyme-linked immune-absorbent assay. The detection method of SOD activity in erythrocyte was improved according to the literature. Administration concentration on rat wounds was divided into 0.2, 0.4, 0.6, 0.8g/ml. 1.5 hours after the last administration, blood was got from the abdominal aorta, heparin saline anticoagulation. The blood was centrifuged 3000r/min for 10min, and liquid erythrocytes were separated through cell separation. The quantitative haemolysis by double distilled water and SOD activity were measured using optical diffusion method.

Determination of peroxide (LPO) in rat plasma and liver tissue (Dong et al., 2005)

Peroxide (LPO) in rat plasma and liver tissue were determined by malondialdehyde TBA colorimetry. 50 male rats were randomly divided into five groups, (grouping method was as above), n=10, respectively, for five consecutive days. After the last administration within the 1.5h, plasma was prepared from abdominal aortic blood. The liver tissues were got immediately from killed rats. Residual blood was washed away using 4□ saline, filtered by paper. Part of the tissues was weighed. At last, 10% liver homogenate physiological saline was prepared to LPO test. Statistically, the experimental data are expressed as mean ± standard deviation. All data are analysed with SPSS15.0 statistical software.

Results

Contents of TNF-α, IL-6 in serum are shown in Tables 1 and 2. One day after injury, the two levels were significantly higher ($p < 0.01$), respectively. In the 2d and 3d, the contents peaked and continued; 5d, they began to decline. But in the treatment group, the value is always lower than the model group, and higher than the normal group. Until the 14d, TNF-α, IL-6 was basically decreased to normal ($p > 0.05$).

Table 1: TNF-α level in different period ($\bar{x}$ ±s, n=10, pg/ml)

Days	0d	1d	2d	3d	5d	7d	14d
Control				105.62±2.25			
Model	109.25±4.60	169.68±3.68*	216.11±4.21*	203.08±3.56*	183.68±4.21*	150.02±2.93*	113.02±4.33
Secretions	102.96±3.15	142.23±2.65*	188.35±3.64*	165.25±3.52*	149.17±4.18*	120.92±4.23	106.49±3.88

"*"Compared with control group, the value was significantly different，$p<0.01$

Table 2: IL-6 level in different period ($\bar{x}$ ±s, n=10, pg/ml)

Days	0d	1d	2d	3d	5d	7d	14d
Control				90.52±5.89			
Model	94.92±6.12	163.16±5.73*	208.63±4.97*	234.84±5.62*	198.43±6.73*	160.05±4.91*	100.93±5.66
Secretions	92.33±7.31	136.02±3.22*	179.03±4.09*	195.31±4.91*	153.42±3.87*	133.74±5.03	90.72±6.01

"*"Compared with control group, the value was significantly different，$p<0.01$

Determination of LPO and erythrocyte SOD (Zheng et al., 2012; Jin et al., 2012)

Table 3: Changes of LPO and SOD level when treated with maggot secretions($\bar{x}$ ±s)

Groups	Dose g/ml	Plasma LPO (nmol/ml)	Liver tissue LPO (nmol/g Wet weight)	Erythrocyte SOD (u/gHb)
Vaseline		3.125±0.875	184.2±21.3	58.27±8.16
Secretions	0.2	3.167±0.734	176.2±30.2	51.34±9.87
	0.4	4.694±1.150	215.8±26.2	97.8±11.53*
	0.6	4.138±1.047	200.7±18.5	96.12±10.51**
	0.8	3.716±0.897	174.9±32.7*	102.89±10.63**
Erythromycin	0.1	2.838±0.734**	165.9±25.3**	110.31±8.41**

Compared with vaseline group, "*" and "**" exhibits significant difference, *, $p<0.05$; **, $p<0.01$.

We can read from Table 3 that LPO level in the liver tissues and the SOD level in serum could be decreased by maggot secretions. However, the LPO level in the plasma could not be decreased. And we found that when the concentration of the maggot secretion was 0.8g/ml, the susceptibility of SOD to secretions was higher than that of LPO in liver tissues. As for erythromycin group, when the concentration was 0.1g/ml, the LPO level in both of the liver tissues and plasma could be decreased significantly.

Discussion

In this research, TNF-α, IL-6, SOD, and LPO levels were measured by radioimmunoassay. The experimental results showed that TNF-α, IL-6, and SOD levels in the model group were significantly increased; LPO level was decreased to a certain extent. Thus, the content of inflammatory cytokines in acute skin wounds could be reduced by maggot secretions, which enhance wound healing. Maggot secretion may be integrated into the materials which belonged to treatment of wounds (Michael et al., 2007; Rhonda et al., 2010). Detection of SOD activity and LPO activity in experimental rats will give us suggestion on multiple trauma patients with injury severity score. There is a certain relationship in the generation of oxygen free radicals in the body with a clear imbalance of free radical damage caused by the body after multiple trauma patients with trauma and lipid peroxidation injury, injury severity and patient's injury or death.. Thus, this experiment could contribute well to this work.

Inflammatory cells such as neutrophils were activated after severe trauma; then, cytokines and a large number of inflammatory factors were released. There was a close connection between the release process and systemic inflammatory response. Recent studies have shown that the systemic inflammatory response actually was the imbalance in the unbalance between the body's inflammatory response and anti-inflammatory response, because the release of a variety of cytokines and other inflammatory mediators such as TNF-α, IL-6 was runaway. ES could dose-dependently reduce the fMLP-stimulated expression of CD11b/CD18 by neutrophils, which means ES could modulate neutrophil adhesion to endothelial cells (M. J. A. van der Plas et al., 2009). The pro-inflammatory responses of human monocytes could also be inhibited through a cyclic AMP-dependent mechanism. This kind of process could contribute to their beneficial effects on chronic wounds. Now, we have a mature, standardised and efficient method of culturing maggots. Therefore, the quality and consistency of the maggot secretions could be assured; there will be no obvious individual differences in future. Current inspection standards were to evaluate its quality standards by testing the total protein content in each batch of the secretions.

TNF-α, IL-6 are important pro-inflammatory cytokines in body, and they play a key role in the cytokine network as well as development of systemic inflammatory. The release of other cytokines, the promotion of adhesion between neutrophils and endothelial cells as well as neutrophil migration were all done by TNF-α, IL-6. Further damage on tissue and organ would be made by the increase in inflammatory mediators. Currently, in addition to the farming methods mentioned in this article, there are also other, for example, Lucillia sericata (Green-bottle flies) which could be caught from the environment. They live on water, dry sugar, and occasional meat, and their eggs were collected from the underside of meat. Clusters were separated in 0.5% sodium hypochlorite, sterilised in 1% Lysol for 5 minutes, and hatched on chicken liver. The hatching maggots (larvae) were then transferred to sterile vials. The collected sterile maggots were kept for 24 hours on Columbia agar until they reached the third larval stage before being used for the experiments (Al-Saeed WM et al., 2012).

It is noted that Xiaorong Li and Ning Liu contribute same work to this research.

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