



Original Article

Effects of neuro-immuno-modulation on healing of wound combined with local radiation injury in rats

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ABSTRACT

Purpose: To investigate effects of neuro-immuno-modulation on wound healing by observing changes of cytokines and hypothalamic-pituitary-adrenal (HPA) axis hormones in acute stress reaction in rats with wound and combined local radiation injury.

Methods: Sixty female Wistar rats (weighting 200 ± 20 g) were randomly divided into normal control group, wound group and combined wound-local radiation (CWR) group (25 Gy local radiation post wound), 20 rats in each group. Contents of IL-1 β , IL-6 and IFN- γ and IL-4 in serum were measured and changes of adrenocorticotrophic hormone (ACTH) and glucocorticoid (GC) in serum were analyzed by using enzyme-linked immunosorbent assay and radioimmunologic assay, respectively at different time points post wound and radiation.

Results: (1) The level of IFN- γ , one of the Th1 cell cytokines increased significantly at 14 d post CWR, which was markedly higher than that in control group and wound group. However, the level of IL-4, IL-1 β and IL-6, one of the Th2 cell cytokines, did not show obvious change. (2) Ratio of Th1/Th2 (IFN- γ /IL-4) in wound group and CWR group increased significantly at 7 d after wound and radiation, which suggested that Th1/Th2 balance drifted to Th1 immune response. The ratio of Th1/Th2 in wound group returned to the normal level up to 14 d after the wound and radiation, while the Th1/Th2 ratio in CWR group increased persistently and was much higher than that in control and wound groups. (3) Level of serum ACTH and GC in CWR group increased at 3 d post wound and radiation, and among them, level of GC showed statistically significant increase, which was much higher than that in control and wound groups.

Conclusion: Level of serum neurohormone GC in rats increased significantly immediately after wound and radiation; while the level of IFN- γ showed significant increase only up to 14 d after wound and radiation, and the Th1/Th2 imbalance sustained till 28 d post wound and radiation. In order to reduce acute damage caused by CWR, organic immune system and nerve system showed up a marked regulate effects simultaneously and mutually. Nonetheless, the excessive stress induced by CWR causes disturbance of immunoregulation, which is one of the key reasons for delayed wound healing in CWR.

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Introduction

Combined wound and radiation injury (CWR) occurs when wound is combined simultaneously or successively with radiation exposure at a dose that is sufficient to cause injury. This kind of combined injury is common in radiotherapy during surgical

treatment of malignant tumor, nuclear accident and nuclear-attacks.¹ The feature of CWR is decreased inflammation, decreased local white blood cell infiltration, serious bleeding and tissue necrosis, easily complicated infection, bad granulation tissue formation, and delayed healing and so on. Acute stress can induce sympathetic nervous system over excited, result in immune imbalance through the nerve-endocrine-immune network, and at last cause homeostasis imbalance.² It is confirmed that the levels of adrenocorticotrophic hormone (ACTH) and glucocorticoid (GC) significantly increase in mice with combined radiation-burn injury, and severe trauma can lead to accentuation of hypothalamo-

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pituitary-adrenal (HPA) axis.³ Our previous study found that the proportions of regulatory T cells (Tregs) and Th17 cells increased, and Treg/Th17 imbalance occurred in rats with CWR, which indicate that CWR can lead to immunologic disorders.⁴ However, the effect of HPA axis accentuation on immune function and wound healing post CWR is not clear. This paper observed the changes of neurohormonal, ACTH and GC, and the changes of Th1/Th2 balance, to explore the interaction of nerve-endocrine-immune post CWR and the effects of neuro-endocrine-immune (NEI) on wound healing.

Materials and methods

Animals

Sixty female Wistar rats (weighting 200 ± 20 g) were purchased from the Laboratory Animal Center of Academy of Military Medical Sciences (AMMS) in Beijing. All rats were maintained in a specific-pathogen-free facility. The rats were randomly divided into control group, wound group and CWR group, 20 rats in each group. Enzyme-linked immunosorbent assay (ELISA) kits for IL-4 and IFN- γ from Biolegend Company (the USA), and radioimmunoassay kits for ACTH and GC from IBL Company (Germany).

Combined wound and local radiation model

After anesthetization and focal sterilization, two round wounds in rats were made at thoracic segment in the back paralleled with spine, symmetrical on both sides. The diameter of each wound was 1.5 cm, deep to the full-thickness skin, and the interval between two wounds was 1.5 cm. The animals received local irradiation immediately post trauma. The rats in CWR group were given 25 Gy γ rays using a single ⁶⁰Co source (Beijing Institute of Radiation Medicine, China) at a dose rate of 278.98 cGy/min. Meanwhile, the rats in control and wound groups received sham exposure.

ELISA assay

On the 3rd, 7th, 14th and 28th d after injury and radiation (simple injury), 5 rats from each group were sacrificed and serums were taken for ELISA. ELISA assay for IL-1 β , IL-4, IL-6 and IFN- γ were performed according to the manufacturer's instructions. Serum was diluted 1:2 in 2% bovine serum albumin of phosphate buffer saline (PBS) containing 0.05% Tween 20 (Sigma-Aldrich, PBST) and was added 100 μ l to the wells. After 90 min incubation at 37 °C with shaking, plates were washed (4 \times PBST) and biotin-conjugated detection antibody was added. Following 1 h incubation at 37 °C, microwells were washed four times and streptavidin-HRP was added. Following 30 min incubation at 37 °C, microwells were washed four times and tetramethylbenzidine (TMB, Thermo Fisher Scientific, the USA) added for 10–15 min at 37 °C. Reactions were stopped by addition of 0.9 M H₂SO₄ (50 μ l) and product absorbance was determined at 450 nm. Non-specific background (measured at 630 nm) was subtracted.

Radioimmunologic assay

On the 3rd, 7th, 14th, and 28th d after injury, 5 rats from each group were sacrificed and serums were taken for radioimmunologic assay. According to the manufacturer's instructions, the samples were incubated and centrifugated, and then the radioactivity of precipitation was measured by a gamma counter. The concentration of ACTH and GC in serum was calculated according to the standard curve.

Statistical analysis

All data are presented as means \pm SD. Student's unpaired *t*-test was used to compare the data among groups. *P* < 0.05 was considered statistically significant.

Results

Changes of IFN- γ and IL-4 in serum post injury

There was no significant difference in IFN- γ among three groups 3–7 d post injury. However, on the 14th d post injury, the level of serum IFN- γ in CWR group increased significantly, and was markedly higher than that in the other two groups. The level of serum IL-4 in wound and CWR groups decreased in some extent post injury; but the change did not show statistical significance. Meanwhile, there were no statistically significant changes in the level of serum IL-4, IL-1 β and IL-6 between the wound and CWR groups (Fig. 1).

Changes of ratio of IFN- γ /IL-4 (Th1/Th2) post injury

IFN- γ and IL-4 represented the characterized cytokines of Th1 cells and Th2 cells respectively, so the ratio of IFN- γ /IL-4 could objectively stand for the balance of Th1/Th2.⁵ As shown in Fig. 2, the ratio of IFN- γ /IL-4 in wound and CWR groups was significantly higher than that in control group at 7 d post injury. At 14 d post injury, the level IFN- γ in the wound group recovered to the controlled level. However, because of durative high level of IFN- γ , the ratio of IFN- γ /IL-4 in CWR group was significantly higher than that in the control and the wound groups, that is, the Th1/Th2 balance showed a shift to the Th1 immune response. Until 28 d post injury, the ratio of IFN- γ /IL-4 in CWR group was still significantly higher than that in control group.

Changes of ACTH and GC in serum post injury

Analysis of serous ACTH and GC contents showed that the level of ACTH and GC in CWR group increased at 3 d post injury, and the level of GC significantly increased. At 7 d post injury, the level of serous ACTH and GC in CWR group was lower than that in wound group; however there was no significant difference between two groups (Fig. 3).

Discussion

Stress is a non-specific response of the body to the external or internal stimuli. When the body receives stimuli and shows stress reaction, the stress signal can cause the secretion of corticotropin releasing hormone (CRH) in hypothalamus, and then lead to the secretion of ACTH in pituitary gland, afterwards, ACTH can induce secretion of GC in adrenal cortex, and thereby results in excitation of HPA axis. The neurotransmitters, neuropeptides and hormones secreted from HPA axis play an important role in the regulation of immune system by binding to the receptor. And the immune system achieves the feedback regulation of neuroendocrine system through various biological active molecules generated in immune response,^{6,7} and thus forms the NEI network, which also plays an important role in the maintenance of homeostasis. Stress has a dual role; appropriate stress can improve the body's adaptability, while excessive stress can lead to dysfunction of the body. In the study of single radiation on neuroimmuno-modulation, Marquette et al.⁸ found that IL-1 β levels increased in the hypothalamus, thalamus and hippocampus, and TNF- α and IL-6 levels increased significantly in the hypothalamus post irradiation, indicating that radiation has an important influence on the neuroimmunomodulation. In CWR,

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