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Effects of early excision and skin graft on the levels of NF-κB and EPO in serum and REE in patients with deep burn wounds in hand.

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Abstract

Objective: To investigate the effects of early excision and skin graft on the expression levels of Nuclear Factor kappa B (NF-κB) and Erythrogenin (EPO) in serum and Resting Energy Expenditure (REE) in patients with deep hand burn wounds.

Methods: In this study, a total of 80 patients with deep hand burn wounds who were subjected to the Burn Department of this hospital between January 2016 and January 2017 were enrolled, and divided into two groups according to the time of initial excision and skin graft: the early group (n=42), in which patients received the surgical treatment within 5 d after being burned, and the non-early group (n=38), in which patients underwent the surgery after 5 d since being burned. After debridement, exposure treatment was carried out, in which the wound surface in Grade III and the surroundings in grade II were excised, and then covered with the autologous skin. During treatment, changes in REE were monitored using a metabolic cart (MedGraphics, USA), while the changes in expression levels of NF-κB and EPO before and after excision were detected using Enzyme-Linked Immunosorbent Assay (ELISA); variations in lipopolysaccharide were also observed before and after excision using a limulus assay, and the differences in the levels of Alanine Aminotransferase (ALT) in serum were measured using an automatic biochemical analyzer before and after excision.

Results: In the early group, REEs at 1 d and 5 d were significantly lower than those in the non-early group ($p<0.05$), while the comparisons of REEs before and after excision, as well as at 10 d and 15 d in two groups showed no statistically significant differences ($p>0.05$). Before excision, there is no statistically significant differences ($p>0.05$) in the levels of NF-κB and EPO in serum; after excision, the level of NF-κB in the early group was significantly lower than that in the non-early group ($p<0.05$); while the level of EPO in the early group was significantly higher than that in the non-early group ($p<0.05$). Before excision, no significant differences were identified in comparison of the LPS and ALT levels in serum between two groups ($p>0.05$); after excision, these indexes in the early group were significantly lower than those in the non-early group ($p<0.05$).

Conclusion: Early excision can alleviate the hypermetabolism of patients with deep burn wounds in hand, reduce the content of NF-κB in serum and increase the level of EPO in serum with less damage to liver, which might be correlated with the control of the release of major inflammatory mediators in an early stage and the accelerated recovery in surface of deep burn wounds of patients.

Keywords: Early excision and skin graft, Deep burn wounds in hand, NF-κB, EPO, REE.

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Introduction

In recent years, with an increasing trend in incidence rate of burn wound, patients usually suffer from the secondary systemic infection, or even can hardly escape from death without timely treatment [1]. In other word, burn wound has been regarded as a major factor accounting for accidental death of human beings. For patients with deep burn wounds, fluid infusion should be immediately carried out, and the maintenance of regular respiration is also necessary; escharectomy and skin graft should also be adopted as early as possible [2,3]. Nuclear Factor kappa B (NF-κB) is involved in the development and progression of many kinds of trauma and burn wounds, which could mediate the correlation between inflammation and apoptosis. According to the previous reports, Erythrogenin (EPO) can promote the recovery in surface of

burn wounds of deep Grade II in patients with moderate burn wound, thereby enhancing the recovery. Currently, there remain few studies reporting the effects of early excision and skin graft on the levels of NF-κB and EPO in serum of patients with deep burn wounds in hand [4]. Besides, with larger surface of burn wounds, those patients are usually in more severe hypermetabolism, which can decrease the resistance of the body, thereby giving rise to the infections and failure in multiple organs, directly affecting the recovery of patients, or even causing death [5-7]. It is reported that hypermetabolism may decline gradually with the recovery in wound surface. The aim of this study is to investigate the effects of early excision and skin graft on NF-κB and EPO levels in serum, as well as Resting Energy Expenditure (REE) of patients with deep burn wounds in hand, and the detailed information of this study is reported as follows.

Data and Methods

Ethical approval

Ethics approval for the present study was obtained from the local Ethics Committee of XXXXXXXXX. Written, informed agreement from each participant was obtained before surgery.

General data

In this study, a total of 80 patients with deep burn wounds in hand who were admitted to the Burn Department of this hospital between January 2016 and January 2017 were enrolled, and all of them conformed to the diagnostic criteria of deep burns, in which there were 50 males and 30 females aged between 23 and 70 y old with an average of (46.7 ± 1.2 y old). Among those patients, there were 45 in grade II and 35 in grade III; 20 were burned by gasoline or diesel, 40 by chemical burn and 20 by hot liquid. The time from burn to admission ranged between 30 min and 10 h, in which there were 60 patients between 30 min and 5 h and 20 between 5 and 10 h. They were divided according to the time of initial excision and skin graft into the early group (n=42), in which patients received the surgical treatment within 5 d after being burned, and the non-early group (n=38), in which patients underwent the surgery after 5 d since being burned. Comparisons of the gender, age, admission time and area of initial excision showed no statistically significant differences ($p>0.05$), suggesting that the data were comparable. There is no blinding in the current study.

Inclusion criteria

The inclusion criteria included male and female burn injury patients aged between 23-70 y, admitted or followed up in the burns department, who agreed to being interviewed and were within the period of 0-10 d post burn injury.

Exclusion criteria

Patients diagnosed with psychiatric disorders (except nicotine dependence) before the burn injury were excluded from the study.

Methods

Regular systemic examinations were carried out immediately after admission after timely fluid infusion and maintenance of respiration, the condition of patients was stabilized, then surgical treatment was performed. Patients in two groups were subjected to same excision surgery: a) Anti-shock therapy; b) fluid infusion through venous channel; c) debridement immediately after vital signs of patients were stabilized; d) exposure treatment. During the course of excision, the wound surface in grade III and the surroundings in grade II were excised, and then followed by stamp skin graft or auto-microskin transplantation in combination with heterodermic graft, in which the ratio of autologous skin to the allogenic skin was 1:8. Thereafter, anti-inflammation treatment was performed.

Evaluation indexes

(1) REEs before and at 1 d, 5 d, 10 d and 15 d after excision: REEs before and at 1 d, 5 d, 10 d and 15 d after excision were detected in two groups using a metabolic cart (MedGraphics, USA) *via* indirect calorimetry. All patients underwent adaptive test, and 2 h after meal, patients were required to stay in supine position for 30 min followed by measurement of energy consumption in unit time *via* standard methods of metabolic cart or with the special hood. During measurement, patients were required to breathe only with mouth, and the exhaled air was collected for REE calculation. Each test would last for at least 10 min, and be performed in 100 kPa, 26 to 32°C and 50% to 60% humidity condition.

(2) Measurement and comparison of changes in levels of NF-κB and EPO in serum of patients before and after excision through ELISA: Corresponding ELISA kits of NF-κB and EPO were provided by Shanghai Bioleaf Biotechnology Co., Ltd., and all procedures were performed in strict accordance with the instructions.

(3) Detection of variations in lipopolysaccharide (LPS) before and after excision through limulus assay: With the kits provided by Shanghai Rongbai Biotech Co., Ltd. And Beckman AU680 Automatic Biochemical Analyzer, differences in the levels of Alanine Aminotransferase (ALT) in serum were measured before and after excision.

Statistical methods

All data were processed with SPSS 21.0 software. Chi-square test was performed for enumeration data, and t-test for measurement data. $p<0.05$ suggested that differences had statistical significance.

Results

Comparisons of REEs at different time points between two groups

In the early group, REEs at 1 d and 5 d were significantly lower than those in the non-early group ($p<0.05$), while the comparisons of REEs before and after excision, as well as at 10 d and 15 d in two groups showed no statistically significant differences ($p>0.05$; Table 1).

Table 1. *Comparisons of REEs at different time points between two groups ($kj•h^{-1}•m^{-2}$).*

Group	n	Before excision	1 d after excision	5 d after excision	10 d after excision	15 d after excision
Early group	42	278.15 ± 34.26	205.37 ± 25.84	271.48 ± 24.27	312.65 ± 28.27	321.35 ± 19.41
Non-early group	38	277.43 ± 35.14	265.28 ± 21.72	332.66 ± 27.83	345.13 ± 32.45	351.67 ± 21.39
t		0.024	-3.183	-2.925	-1.300	-1.835
p		0.982	0.033	0.043	0.2633	0.140

Comparison of NF-κB and EPO levels in serum before and after excision between two groups

Before excision, comparisons of levels of NF-κB and EPO in serum showed no statistically significant differences ($p>0.05$); after excision, the level of NF-κB in the early group was significantly lower than that in the non-early group ($p<0.05$). The level of EPO in the early group was significantly higher than that in the non-early group ($p<0.05$; Table 2).

Table 2. *Comparison of NF-κB and EPO levels in serum before and after excision between two groups.*

Group	n	NF-κB (pg/ml)		EPO (U/L)	
		Before excision	After excision	Before excision	After excision
Early group	42	77.35 ± 4.22	43.56 ± 1.67	6.45 ± 1.32	14.57 ± 2.95
Non-early group	38	77.14 ± 4.52	51.63 ± 2.81	6.59 ± 1.23	9.66 ± 0.73
t		0.168	-6.197	0.271	-7.623
p		0.224	0.003	0.144	0.000

Comparison of the levels of LPS and ALT in serum before and after excision between two groups

Before excision, no significant differences were identified in comparison of the LPS and ALT levels in serum between two groups ($p>0.05$); after excision, these indexes in the early group were significantly lower than those in the non-early group ($p<0.05$; Table 3).

Table 3. *Comparison of the levels of LPS and ALT in serum before and after excision between two groups.*

Group	n	LPS (EU/ml)		ALT (U/L)	
		Before excision	After excision	Before excision	After excision
Early group	42	0.71 ± 0.1	0.29 ± 0.12	83.42 ± 5.03	43.53 ± 2.9
Non-early group	38	0.68 ± 0.3	0.65 ± 0.17	83.11 ± 4.78	56.74 ± 3.85
t		0.193	7.502	0.185	7.414
p		0.163	0.031	0.206	0.002

Discussion

For patients with deep burn wound in degree higher than grade II, epidermis and dermis in wound surface are usually severely damaged with necrosis, but the residue of reticular layer beneath the dermis can repair the wound. According to relevant report [8-11], the positive rate in bacterial culture on surface of deep burn wound can be as high as 100%. This situation makes patients more susceptible to infection, thereby severely affecting the survival rate of patients and giving rise to the complications. For patients undergoing early excision therapy, only a few bacteria or no bacteria are spread on the surface of

wound, which can significantly elevate the survival rate of skin, and maximally remove the necrotic tissues, thus reducing the origins of inflammatory mediators, controlling the infections and decreasing the incidence rate of sepsis caused by wound surface. As for patients with burn wounds in grade II or above, surgical duration should be limited within 7 d after burn, because after 7 d, angiogenesis or granulation may take place in wound surface, while excision at that time will increase the chance of infection [10]. NF-κB, as one of the major nuclear transcription factors, can activate multiple inflammatory cytokines. Then inflammatory cytokines such as TNF-α can further enhance the activation of NF-κB, thus forming a vicious cycle to produce the cascade amplification effect, further aggravating the condition of patients [11]. EPO is a kind of multifunctional cytokines, and not only is involved in the generation of red blood cells and angiogenesis, but also inhibits cell apoptosis and promotes the angiogenesis under the hypoxia. All of these effects could protect the tissues from further damage. A previous study [12] has indicated that EPO can increase the curative rate of the burn wound surface, shorten the recovery time and facilitate the healing of wound in rats with deep grade II burn wounds. Results in this study showed that before excision, comparisons of levels of NF-κB and EPO in serum showed no statistically significant differences ($p>0.05$); however, after excision, the level of NF-κB in the early group was significantly lower than that in the non-early group ($p<0.05$). The level of EPO in the early group was significantly higher than that in the non-early group ($p<0.05$). These data suggested that early excision and skin graft can reduce the level of NF-κB and increase the level of EPO in serum, thus declining the release of inflammatory cytokines and promoting the healing of wound. The recovery effect of EPO on surface of wound in patients with burn wound might be explained as follows [13]: a) EPO in serum can ameliorate the migration of cells in traumatic tissues, thus increasing the migration rate of cells, especially the fibroblasts and keratinocytes, thus accelerating the recovery of wound; b) EPO in serum may promote the angiogenesis and improve the microcirculations in traumatic tissues, thus accelerating the recovery of wounds through increasing the oxygen and nutrient supply to traumatic tissues; c) EPO in serum can ameliorate the inflammatory responses, thus shortening the secretion period, and reducing the quantity of cells surrounding the wounds, finally accelerating the reabsorption of granulation tissues.

Infection has been regarded as the No. 1 cause of death in patients with severe burn wounds, but it is usually generated in wound surface. Inflammatory responses in necrotic tissues in wound surface results in hypermetabolism to induce the disorders in immunological functions, and myelosuppression. Hence, the aim of removing the surface tissue of deep burn wound in early stage was to convert the composite injury into simple injury. And transplantation of autologous or allogenic skin can accelerate the early recovery, which has been considered as a key step. Besides, it cannot only relieve the hypertension and toxicity, but also reduce the incidence rate of infection and increase the curative rate. In clinical practice, measurement of REE for patients aims to provide guidance for treatment, and evidence for stipulation of individualized therapeutic strategy [14,15]. Results in current study showed that in the early group, REEs at 1 d and 5 d were significantly lower than those in the non-early group ($p<0.05$), while there is no statistically significant differences ($p>0.05$) between before and after excision group, as well as at 10 d and 15 d in two groups. Taken together, the above results indicated that before excision, patients are already in hyper-metabolism situation, and regardless the time of excision, early excision can reverse this situation.

LPS might be one of the major pathogens leading to damage to liver, or even hepatic failure after burn injury [15]. ALT, mainly located in cytoplasm of hepatocytes, can be immediately released into blood once the hepatocytes are damaged. AST mainly exists in mitochondria of hepatocytes, and when liver injury and mitochondria damage were occurred, the level of ALT in serum also elevated [16]. Thus, level of ALT in serum can accurately reflect the damage to hepatocytes. Results in the present study showed that before excision, no significant differences were identified in comparison of the LPS and ALT levels in serum between two groups ($p>0.05$); however, after excision, these indexes in the early group were significantly lower than those in the non-early group ($p<0.05$). These findings demonstrated that early excision can significantly reduce the levels of LPS and ALT in serum, thereby relieving the damage to liver. Thus, early excision plays a protective effect on liver.

In conclusion, early excision can alleviate the hyper-metabolism of patients with deep burn wounds in hand, reduce the content of NF-κB in serum and increase the level of EPO in serum with less damage to liver, which might be correlated with the control of the release of major inflammatory mediators in an early stage and the accelerated recovery in surface of deep burn wounds of patients.

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