Recombinant Human Growth Hormone Promotes Burn Wound Repair by Regulating Vascular Endothelial Growth Factor and Micro Vessel Density

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To explore the effect of recombinant human growth hormone on burn wound healing by regulating vascular endothelial growth factor and micro vessel density. Sixty rats were randomly divided into control group (n=20) (group A), low dose recombinant human growth hormone intervention group (n=20) (group B) and high dose recombinant human growth hormone intervention group (n=20) (group C). After the deep II burn wounds were formed in all rats, 1.0 ml saline was injected into the back burn wounds in the group A, 1.0 ml of 0.2 IU/ml recombinant human growth hormone saline solution was injected into the back burn wounds in the low dose recombinant human growth hormone intervention group, and 0.4 IU/ml recombinant human growth hormone saline solution was injected into the back burn wounds in the group C every day. The time of scab removal and hair growth, vascular endothelial growth factor expression, micro vessel density, oxidative stress index and inflammation index were compared in each group. Rats in group B showed shorter decrustation and hair growth times than those in group A, while rats in group C showed shorter decrustation and hair growth times. In group A, vascular endothelial growth factor expression was higher than in group B, and in group C, vascular endothelial growth factor expression was higher than in group B. In group B, the level of micro vessel density was higher than in group A, and in group C, it was higher than in group A. Superoxide dismutase levels in the group B were higher than those in the group A, and malondialdehyde levels in the group C were higher than those in the group B, while malondialdehyde levels in the group C were lower than those in the group A. As compared to group A, the levels of interleukin-6, tumor necrosis factor-alpha, and interleukin-1 alpha were reduced in group B, while in group C they were decreased. Recombinant human growth hormone can promote burn wound repair by regulating vascular endothelial growth factor and micro vessel density.

Key words: Recombinant human growth hormone, vascular endothelial growth factor, micro vessel density, burn, wound repair

Skin and subcutaneous tissue can be damaged by hot gases, hot fluids, hot solids, and strongly corrosive substances, resulting in burns. Burn pain is excessive inflammation caused by burns can damage other organs. Although personal awareness of protection has been gradually enhanced, burns can still be seen everywhere in daily life and workplaces[1]. Burn wound healing is an overlapping stage, including hemostasis, inflammation and so on[2]. Each stage crosses and overlaps each other, involving a variety of inflammatory cells, repair cells, growth factors, inflammatory mediators and extracellular matrix, including Vascular Endothelial Growth Factor (VEGF), Micro Vessel Density (MVD) and other burn-related growth factors[3]. Growth hormone is a synthetic metabolic polypeptide hormone, which has shown its application prospect in burn treatment by promoting protein synthesis and weakening global protein catabolism[4]. After major surgery, trauma, septicemia, or heat injury, recombinant human Growth Hormone (r-hGH) has been shown to enhance immune function and wound healing[5]. After injury, r-hGH stimulates protein synthesis and reduces nitrogen loss. In addition, it has been shown to improve clinical

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results after heat injury[6]. The purpose of this study is to explore that r-hGH promotes burn wound repair by regulating VEGF and MVD, in order to provide reference for the choice of clinical treatment. 60 male Sprague-Dawley (SD) rats were purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd., r-hGH (Changchun Jinsai Pharmaceutical Co., Ltd.,); ether (Sigma Co., Ltd.,); VEGF, Superoxide Dismutase (SOD), Malondialdehyde (MDA) kits were purchased from Nanjing Jiancheng Institute of Biological function, China; Interleukin (IL)-6, Tumor Necrosis Factor-Alpha (TNF-α), IL-1 Beta (β) Enzyme-Linked Immunosorbent Assay (ELISA) kit (Shanghai Biyuntian Co., Ltd.,). Sixty rats were divided into control group (n=20) (group A), low dose r-hGH intervention group (n=20) (group B) and high dose r-hGH intervention group (n=20) (group C). The rats were anesthetized in a covered sealed container with ether gauze. After anesthesia, the long hairs on the back of the rats were first cut off with scissors, and then the remaining short hairs were shaved with a special electric razor to expose the skin on the back. Pour the water into a beaker and gently touch the exposed skin on the back of the rats for 15 s to form a deep II burn wound. The group A received intradermal injection of 1.0 ml saline into the back burn wound every day, the low-dose r-hGH intervention group injected 1.0 ml r-hGH saline solution of 0.2 IU/ml concentration into the back burn wound, and the high-dose r-hGH intervention group injected 1.0 ml r-hGH saline solution of 0.4 IU/ml concentration into the back burn wound every day for 2 w. Calculate the scab removal time and hair growth time of each group. 1 w after the end of the administration time in each group, the blood was collected from the tail vein about 0.5 ml, and the supernatant was taken and stored in the refrigerator for examination. The 1 cm sized full-layer skin samples were removed from the back wounds of the rats and stored in the refrigerator at -80° for examination. A 4 % paraformaldehyde solution was used to fix the lung overnight and Hematoxylin and Eosin (H&E) was used to stain it. After slicing, dehydration, embedding and slicing, the paraffin sections were stained with H&E, and the MVD was measured by immunohistochemical SP method. The venous blood was separated by 3000 r/min for 20 min, and the supernatant was reserved for detection. The levels of IL-6, TNF-α, IL-1 β, SOD, MDA and VEGF were detected strictly according to the instructions of ELISA detection kit. Statistical Package for the Social Sciences (SPSS) 20.0 was used for statistical analysis, and the measurement data were expressed by ($\bar{x}\pm s$). Analysis of variance was used for comparison among groups, and Least Significant Difference (LSD) test or Tamhane test was used for pairwise comparison between groups. Compared with the group A, $^{a}p<0.05$ and compared with the low-dose r-hGH intervention group, $^{b}p<0.05$ The decrustation time and hair growth time of rats in the group B were reduced than those in the group A, while those in the group C were decreased than those in the group B as shown in Table 1. A higher level of VEGF expression was seen in group B than in group A; while it was raised in group C than in group B as shown in Table 2. MVD levels were higher in group B than in group A; and it was raised in group C than in group B as shown in Table 3. In group B, SOD levels were higher, and MDA levels were lower than in group A; SOD levels in group C were higher than those in group B, while MDA levels in group C were lower than those in group B as shown in Table 4. It was found that the levels of IL-6 and TNF-α were reduced in group B and that they were decreased in group C as shown in Table 5. Burns are injuries to the skin, respiratory system or body organs caused by accidental exposure to flames, hot liquids, chemicals, electricity or radiation[7]. When the human body experiences burns, the integrity of the skin tissue will be destroyed, and its barrier defense function will also be affected. At the same time, a large number of cells and blood vessels in the deep tissue of the human body will also be damaged to varying degrees[8]. Severe pain can lead to stress response and shock caused by a large amount of body fluid loss, changing the homeostasis of the body's internal environment, resulting in immune dysfunction after burn. In many chronic wounds, burns can easily cause skin damage, resulting in shock, water-electrolyte imbalance, abnormal pH balance, wound infection and malnutrition. This will not only lead to other complications, but also lead to longer wound healing time[9]. Therefore, timely treatment after burn, especially in the early stage of burn, is very important to promote wound healing. Currently, prescription drugs such as sulfadiazine and zinc silver are used for burns, which can relieve

symptoms and promote recovery[10]. However, taking into account the side effects of these drugs, affect the long-term efficacy of patients. In order to treat burns and promote wound healing, it is imperative and necessary to find local topical drugs with definite curative effects. This study aimed to explore that r-hGH promotes burn wound repair by regulating VEGF and MVD, in order to provide reference for the choice of clinical treatment. Wound therapy, such as growth factors, that play a role at the molecular and cellular level plays a major role in wound healing[11]. As a synthetic metabolite, human growth hormone stimulates the growth and mitosis of many cell types through the direct or indirect action of insulin-like growth factor[12]. The effects of this drug have been confirmed in a variety of tissues, including the skin, nerves, muscles, bones, corneas, etc. It could shorten healing times in animal models[13]. Rats in group B showed a reduced decrustation time and hair growth time than rats in group A, while rats in group C showed a reduced decrustation time and hair growth time. Compared with group A, group B had higher levels of SOD and significantly lower levels of MDA; SOD levels in group C were higher than those in group B, and MDA levels were lower. Both group B and C had reduced IL-6, TNF-α, and IL-1 levels, while group A had increased levels of IL-6, TNF-α, and IL-1. It is suggested that r-hGH can promote burn wound healing by regulating oxidative inflammation. Burn wound healing is a complex process, including inflammatory process and the release of a variety of growth factors. Previous studies have shown that VEGF is a highly specific VEGF, which can promote vascular endothelial cell migration, extracellular matrix degeneration and other physiological effects[8]. As we all know, growth hormone is one of the molecules with multiple effects on skin cells. It participates in the inflammation, proliferation and maturation stage of wound healing[14]. During the inflammatory phase, macrophages transmit growth factors, attract fibroblasts, and stimulate the next phase. It stimulates the release of VEGF, which stimulates fibroblasts; VEGF, which stimulates wound angiogenesis; and VEGF[15]. Angiogenesis plays a key role in granulation and tissue remodeling because the progress of wound healing requires new blood vessels[16]. Endothelial cells express VEGF receptors and produce VEGF to participate in the latter process[17]. After a large number of experiments and studies, it has been proved that r-hGH plays critical role in the establishment of neovascularization and inhibition of inflammation[18]. The results also showed that the expression of VEGF in the group B was increased than that in the group A, and the VEGF expression level in the group C group was raised than that in the group B. The level of MVD in the group B was raised than that in the group A, and the MVD level in the group C was increased than that in the group B. It is suggested that r-hGH can promote burn wound repair by regulating VEGF and MVD. It has been found that the expression of VEGF in rat wound can be enhanced by injection of r-hGH.

TABLE 1: SCAB REMOVAL AND HAIR GROWTH TIME OF RATS (D)

Group	n	Scab removal time	Hair growing time
A	20	22.68±3.20	30.83±4.35
B	20	20.40±3.18[a]	28.59±4.17[a]
C	20	17.62±3.24[ab]	26.06±3.23[ab]
F		12.49	10.737
p		0.000	0.000

Note: Compared with the group B, [a]p<0.05 and compared with the group C, [b]p<0.05

TABLE 2: VEGF EXPRESSION AMONG DIFFERENT GROUPS

Group	n	VEGF (ng/ml)
A	20	12.93±1.38
B	20	15.53±2.26[a]
C		20.19±3.06[ab]
F		49.576
p		0.000

Note: Compared with the group B, [a]p<0.05 and compared with the group C, [b]p<0.05

TABLE 3: MVD COUNT IN WOUND SURFACE OF RATS

Group	n	MVD
A	20	35.38±11.12
B	20	44.62±12.19[a]
C	20	55.74±13.23[ab]
F		46.545
p		0.000

Note: Compared with the group B, [a]p<0.05 and compared with the group C, [b]p<0.05

TABLE 4: OXIDATIVE STRESS INDEXES IN RATS

Group	n	SOD (kU/g)	MDA (μmol/g)
A	20	51.52±12.49[a]	8.23±2.69[a]
B	20	64.28±14.39[b]	6.34±1.47[b]
C	20	88.38±14.32	4.14±1.13
F		37.033	23.553
p		0.000	0.000

Note: Compared with the group A, [a]p<0.05 and compared with the group B, [b]p<0.05

TABLE 5: SERUM INFLAMMATORY INDEXES IN RATS

Group	n	IL-6 (μg/l)	TNF-α (ng/l)	IL-1β (pg/l)
A	20	180.28±18.04	170.33±17.11	30.28±4.84
B	20	150.37±16.09[a]	121.41±13.14[a]	16.36±2.13[a]
C	20	97.86±11.28[ab]	50.71±8.22[ab]	9.34±1.76[ab]
F		146.788	407.156	219.423
p		0.000	0.000	0.000

Note: Compared with the group B, [a]p<0.05 and compared with the group C, [b]p<0.05

By increasing the number of MVD in the wound and accelerating the blood circulation in the wound, it is more conducive to burn wound healing. To sum up, r-hGH can promote burn wound repair by regulating VEGF and MVD.

Conflict of interests:

The authors declared no conflict of interests.

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