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EGF Impact on Glycosaminoglycan Levels of the Brain

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ABSTRACT

Various growth factors such as epidermal growth factor (EGF) are effective proteins in wound healing. Glycosaminoglycans (GAG) can support the healing of wound by the growth factors as well as they are effective in recovery. Because of this reason, the changes occurring in GAG and protein levels synthesized from the hepatic tissue due to EGF applied systemically during physiologic wound healing process. 48 Wistar-Albino male rats were used in experiments. Rats to which dorsolateral excisional wound was made were divided into 2 equal groups: 1-The group left to spontaneous recovery, 2-Systemic EGF applied group. A daily dose of EGF (10 ng/mL) was given intraperitoneally (ip) to the animals in the application group. Rats were sacrificed under anesthesia in 1st, 5th, 7th and 14th days of the study. GAG and protein concentrations in brain tissues were measured spectrophotometrically. The results were compared by Mann Whitney U test. It was determined that the GAG level in brain was not effective in normal recovery process in peripheral injuries and systemically applied EGF increased GAG synthesis especially in the 7th day and it contributed to the healing by increasing the protein synthesis in the 7th day in which tissue repair is the highest.

Keywords: *Wound healing, EGF, GAG*

1. INTRODUCTION

Growth factors are effective proteins in wound healing [1]. It increases ion, glycolysis and creation of protein with DNA and RNA [2,3]. As result of the fact that EGF has effect on the development of embryo and the creation of new blood vessels, intensive studies were made on wound healing [4].

GAGs are great complexes usually consistent of small amounts of protein and negatively charged heteropolysaccharide chains [5]. The main types of GAG are hyaluronic acid, heparin, heparin sulfate, keratin sulfate, dermatan sulfate and sulfate chondroitin [5,6].

Heparin sulfate (HS), which is an important GAG, is a derivative of a polysaccharide found in all animal tissues [7] and is present on all the surfaces of cell [5,8], cell membranes [5,7-9], blood vessels and in particular in brain [7,9]. HS oligosaccharide production is associated with secondary accumulation of GM2 and GM3 ganglions in the brain, formation of large cytoplasmic content in various types of brain cells, accumulation of C subunit of synthesis of mitochondrial ATP and irregularity of growth associated protein 43 and mRNA in brain tissues [10]. Type IV is a compound of glomeruli basal membrane with collagen and laminine [8].

EGF receptors are important in proteoglycan synthesis. GAG modules the growth factors. It may be important for endothelial and smooth muscle cells such as angiogenesis, cell proliferation and inflammation. In addition, some growth factors arrange the GAG synthesis [11].

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Chondroitin sulfate-GAGs, EGF dependent maturation, cell migration and nerve roots has a brake effect on glycogenesis of the progenitor cells [12].

In accordance with all of this information, the changes occurring in GAG and protein levels synthesized from the hepatic tissue due to EGF applied systemically during physiologic wound healing process.

2. EXPERIMENTALS

2.1. Animals and treatment

In experiments, totally 48 male rats (Wistar Albino) which are 200-250 g in weigh and provided by Gazi University Laboratory Animal Rearing and Experimental Research Center (GUDAM) were used. Experimental animals were divided into two groups as Control and Systemic EGF applied group and each group was divided into 4 sub-groups as 1, 5, 7 and 14 days. Rats were fed with free feed and water during the experiment. A daily dose of EGF (10 ng/mL) was given to the animals ip in the application group. The rats were sacrificed by taking blood from their hearts under anesthesia in 1st, 5th, 7th and 14th days of the experiment.

2.2. Creation of the wound model

As general anesthesia, ketamine and xylazine were injected to the animals. Dorsolateral excisional wounds of 2 cm were made on both sides of the animal's dorsal medulla spinal. The wound lips were adapted with 2 sutures after excisional wound. The EGF serum to be applied following the injury was given ip to the animals as 1 dose in one day (10 ng/mL).

2.3 Determination of proteins in the tissue

The protein concentration in tissues was determined with Lowry method [13].

2.4. Determination of GAG in the tissue

The concentration of GAG in tissues was determined according to the modified Lowry method [14].

2.5. Statistical analyses

Mann-Whitney U test was used in evaluating the findings. All values were given with arithmetic mean ± standard deviation and $p < 0.05$ value was considered statistically significant.

3. RESULTS

The study's GAG and protein levels are given in Table 1.

Table1: Brain tissue GAG and protein levels

		GAG (mg/g tissue)	Protein (mg/g tissue)
1 day wound	Control group	0.098 ± 0.014	0.510 ± 0.160
	Systemic EGF applied group	0.203 ± 0.53 a	1.086 ± 0.325
5 day wound	Control group	0.086 ± 0.015	0.490 ± 0.190
	Systemic EGF applied group	0.086 ± 0.01 b	2.513 ± 0.937
7 day wound	Control group	0.058 ± 0.009 a	2.300 ± 0.883 a,c
	Systemic EGF applied group	0.100 ± 0.022 b	1.050 ± 0.449
14day wound	Control group	0.073 ± 0.018	1.321 ± 0.150 a,c
	Systemic EGF applied group	0.120 ± 0.009 d,g,a	1.880 ± 0.204 b,f,g

a= P < 0,05 when compared with the same values of 1-day incision wound group
b= P < 0,05 when compared with the same values of 1-day incision + EGF applied group
c= P < 0,05 when compared with the same values of 5-day incision wound group
d= P < 0,05 when compared with the same values of 5-day incision + EGF applied group
e= P < 0,05 when compared with the same values of 7-day incision wound group
f= P < 0,05 when compared with the same values of 7-day incision + EGF applied group
g= P < 0,05 when compared with the same values of 14-day incision wound group

It was seen that the GAG release level significantly increased in the brain tissue belonging to the 1st day of EGF application group. It was seen that the GAG release level significantly decreased in the brain tissue belonging to the 5th day of EGF application group. It was seen that

the GAG level significantly decreased in the brain tissue belonging to the 7th day of EGF application group and its own control group. It was seen that the GAG level significantly decreased in the brain tissue belonging to the 14th day of EGF application group and significant change in GAG level was seen in its own control group. No change in the protein levels were seen in the brain tissue belonging to the 1st day of EGF application group and its own control group. Increase in the protein levels were seen in the brain tissue belonging to the 5th day of EGF application group. No change in the protein levels were seen in the brain tissue belonging to the 7th day of EGF application group and increase was seen in its own control group. Increase in the protein levels were seen in the brain tissue belonging to the 14th day of EGF application group and its own control group.

4. DISCUSSION

In our study, the role of brain tissue in this process was revealed and it was concluded that GAG release do not get impacted from peripheral injury generally, although it seems like there is decrease in brain's GAG level only in the 7th day.

Yeo and colleagues determined that GAG synthesis increase more than normal skin in the granulation tissues. But their studies made them think that the roles of GAG in wound healing are insufficiently understood [15]. Savage and his colleagues compared hypertrophic scar and normal scar, fibroblasts isolated from normal skin tissues, their growth curves, protein content and GAG synthesis and determined that there were changes in in vivo scar and GAG content in skin [16]. Olczyk and his colleagues observed that GAG has a key role in extracellular matrix's organization and metabolism and cell migration stimulation, differentiation and proliferation and all stages of wound healing [17]. In this sense, a tight relation between the growth factors in GAG and wound healing is considered.

Kosir and colleagues mentioned that the early wound recovery contains the release of GAGs by the fibroblasts in hydrophilic matrix form appropriate for remodeling [18]. Fibroblasts are the main elements of healing process and they are responsible of the production of structural proteins used in reproduction of the tissues. They are seemed in the 3rd and 4th days after injury [19,20,21]. However, they reach to the peak level in the 7th day [19,22,23]. The wound does not get a significant resistance in the first 5 days.

In our study, GAG release of brain began to increase due to increasing fibroblasts in the 5th day in which the fibroplasia phase is intensive in rats applies with EGF. So the GAG release level decreased until the 7th day in which vascular proliferation and fibroblast growth started. But in this fibroplasia phase in which the fibroblasts reached to the peak level, GAG level began to increase again in the 7th day. Biological system started over and the release of GAG in brain increased due to the fact that wound starts to heal itself after the 14th day.

Savage and colleagues determined that GAG may have impact on the scar formation by fibroblasts rising in wound tissue, that Platelet derived growth factor stimulates the cell bunches more effectively than the normal skin fibroblasts and that GAG synthesis increased in skin and scar fibroblasts [24]. Fan and colleagues observed that GAG may be a wound healing moderator as well as it can help on collagen formation by the growth factors and the creation of dermal fibroblasts and stimulate the fibroblasts in wound and normal skins with its effects on in vivo wound healing [25]. Webber and his colleagues showed that TGF β-1 is an important source for diverge-based myofibroblast and HS is an important modulator of this process [26]. Chondroitin sulfate-GAGs, EGF dependent maturation, cell migration and nerve roots has a brake effect on glycogenesis of the progenitor cells [27].

In our study, on the other hand, the protein levels released by the brain tissue were examined in order to reveal the biological answer given by brain to the peripheral injuries and the protein level increased rapidly to increase the protein synthesis that is the main factor of wound healing in the 7th day in which the wound resistance was on the top level. In addition, as a contribution to the wound healing to the brains of the rat applied with EGF as ip, the protein level in brain increased in the 14th day due to EGF. It is thought that this increase in brain tissue's protein levels may be associated with the hormones in protein level released from the brain, growth factors and/or mediators and the brain may have important role in wound healing with this way.

In the study of Caglikulekci and colleagues which support our thought, it was found that the protein origin growth factor released from the brain may have accelerating effect in wound healing [28].

In the light of all these information, it was determined that the GAG level in brain was not effective in normal recovery process in peripheral injuries and systemically applied EGF increased GAG synthesis especially in the 7th day and it contributed to the healing by increasing the protein synthesis in the 7th day in which tissue repair is the highest. Further studies are needed to find out the role of brain in this process.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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