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Research Report

Modulation of FGF2 after topical application of Stichopus hermanii gel on traumatic ulcer in Wistar rats

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ABSTRACT

Background: *Stichopus hermanii (golden sea cucumber) is one of the many types of marine organisms containing glycosaminoglycans (GAGs), a polysaccharide that promote wound healing. The content of this GAGs, mainly dermatan sulfate, chondroitin sulfate and heparan sulfate has the ability to modulate FGF2. FGF2 many found in the oral mucosa to activate fibroblast proliferation.* ***Purpose:*** *The study was aimed to determine the modulation of FGF_2after topical application of Stichopus hemanii gel on traumatic ulcers in wistar rat.* ***Methods:*** *The sample was 36 male Wistar rats which were divided into 6 groups. C1 and C2 group was placebo gel, SC1 and SC2 group was Stichopus hermanii gel, HA1 and HA2 was hyaluronic acid A gel. The gel was given shortly after the traumatic ulcer (TU) formed and 24 hour later. Then all rats were sacrificed lips mucosa were taken and ELISA examination was done.* ***Results:*** *All data were analyzed by ANOVA test followed by Tukey HSD. Test results show significant differences between SC1-SC2 with C1-C2 group, while the HA1-HA2 with C1-C2 group showed no significant difference.* ***Conclusion:*** *The study showed that modulation of FGF_2 increased after topical application of Stichopus hermanii gel on traumatic ulcers in wistar rats.*

Keywords: *FGF2, Stichopus hermanii gel, traumatic ulcer*

ABSTRAK

Latar belakang: *Stichopus hermanii (teripang emas) merupakan salah satu jenis biota laut yang banyak mengandung GAG, suatu polisakarida yang sangat bermanfaat dalam proses penyembuhan luka. Kandungan GAG ini, terutama dermatan sulfat, chondroitin sulfat dan heparan sulfat memiliki kemampuan untuk memodulasi FGF2. FGF2 banyak didapatkan pada mukosa rongga mulut untuk mengaktifkan proliferasi fibroblas.* ***Tujuan:*** *Studi ini bertujuan meneliti modulasi FGF_2setelah pemberian aplikasi topikal gel Stichopus hermanii pada ulkus traumatikus pada tikus wistar.* ***Metode:*** *Sampel penelitian ini adalah 36 ekor tikus wistar jantan yang dibagi dalam 6 kelompok. Kelompok C1 dan C2 merupakan kelompok kontrol negatif (gel plasebo), Kelompok SC1 dan SC2 merupakan kelompok pemberian gel Stichopus hermanii (SC), serta kelompok asam hyaluronat HA1 dan HA2 merupakan kelompok pemberian gel asam hialuronat (AH). Pemberian gel dilakukan sesaat setelah pembuatan ulkus traumatikus dan 24 jam kemudian. Kemudian semua tikus dikorbankan untuk diambil mukosa bibirnya dan dilakukan pemeriksaan ELISA.* ***Hasil:*** *Semua data dilakukan analisa dengan uji Anova dan dilanjutkan dengan uji Tukey HSD yang hasilnya menunjukkan adanya perbedaan bermakna antara kelompok SC1-SC2 dengan kelompok C1-C2, sedangkan kelompok HA1-HA2 dengan kelompok C1-C2 tidak menunjukkan perbedaan yang bermakna.* ***Simpulan:*** *Modulasi FGF2 meningkat setelah pemberian aplikasi topikal gel Stichopus hermanii pada ulkus traumatikus tikus wistar.*

Kata kunci: *FGF2, gel Stichopus hermanii, ulkus traumatikus*

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INTRODUCTION

Aphtous ulcers have been known as oral lesions round or oval shaped, covered by white-yellowish fibrinous exudate with redness border.[1] It has been reported that one in five person in the population suffered from aphtous ulcers. These high incedence of cases used to be diagnozed as traumatic ulcer (TU) and recurrent aphtous stomatitis (RAS), which RAS prevalence is higher 83.6%.[2]

The difference between TU and RAS relied on the history of trauma in TU, while in RAS occured without any specific cause but could be triggered from trauma.[1] Mechanical trauma is the most common cause, could be from denture or accidental trauma bite of the teeth on the mucosa or physiologic trauma on patient who have bite habit on cheeck and lips. Banuarea[3] stated that RAS caused by trauma prevalence were 50,27%, while trauma of bite were the most common cause at 64,17%. The study also stated that the most common location was labial mucosa at 45,25%.

Trauma of TU also caused by chemical trauma as missused of drug as aspirin attach on the mucosa, teeth and on mouth rinse. [1,4] Other cause of TU is thermal trauma occured when having hot meals.[5] Pain resulted on ulcers is annoying and cause trouble on mastication, speaking and swallowing, even bother patient's emotional stability so that TU and RAS affect not only oral function but also systemic in general.[6] Treatment on TU need to be done by eliminating the cause factors, reducing the pain and accelerate the wound healing process. Antibiotic sometimes applied to prevent secondary infection, usually on the case of chronic TU. Severe TU could be applied topical therapy, for example corticosteroid.[1]

Therapeutical agents to accelerate wound healing have been developed recently, one of it was hyaluronic acid (HA). Hyaluronic acid is one of major glycosaminoglycan (GAG) secreted on tissue repair which is the integral part of extracellular matix. HA produced by fibroblast during proliferative phase on wound healing to induce migration an mitosis of fibroblast and epithelial cells.[7] It increases the activity of *growth factor* (GF) to induce migration and fibroblast proliferation and collagen deposition on the ulcer.[8]

GF is a key component in wound healing which give chemical signalling to regulate biological response and tissue differentiation. One of representative potential growth factor which have the role in repair and tissue regeneration is fibroblast growth factor (FGF). FGF bind and then activate *fibroblast growth factor receptor* (FGFRs) which dominant in RAS/ MAP (mitogen-activated protein) kinase pathway with high affinity. Regarding to its potential function, FGFs have been used as indicator in detecting regenerative process of damaged tissue, including skin, blood vessel, muscle, adipose, tendon/ ligament, cartilage, bone, teeth, nerves.

Some studies reported that FGF have many potential functions in proliferation, migration, diferentiation and angiogenesis in many cells and tissue. FGF2 is a family of FGF that have been found in all that functions. FGF2 has the target on set up the proliferations of preadipose, endothelial cell, epithelial cells, fibroblast and neural stem cells. In arranging migration cell process, the target of FGF is on myogenic cells, in differentiation process its target is on neuroepithelial cells while in angiogenesis function, its role is on the development of endothelial cells.[9]

Sea cucumber is a valuable natural source that has not been explored, specially in dentistry. Among 1.250 types of sea cucumbers, the Stichopus hermanii is the species that contains GAG components the most.It contains less saponin compare to *Holothuriae scabra* species, and also contain more *arachidonic acid* (AA), *eicosapentaenoic acid* (EPA) dan *docosahexaenoic acid* (DHA).[10]

In addition to HA, Stichopus hermanii also contain chondroitin sulphate, heparin sulphate and dermatan sulphate which have the ability to activate and bind to GF specially FGF2.[11,12] Unsaturated fatty acids also found as content in Stichopus hermanii are key mediators to control inflammation process in wound healing, arranging fibroblast proliferation and collagen synthesis to produce healthy collagen. Another advantage of the presence of this unsaturated fatty acid is to minimalize the scar formation and increase the strength of connective tissue.[13,14] The study was aimed to determine the effect of topical application of *Stichopus hermanii* gel in modulating FGF2 on traumatic ulcer of wistar rats.

MATERIALS AND METHODS

The study was true experimental study with post test only control group design. FGF2 were examined in the early phase of traumatic ulcer. Samples were healthy male 36 wistar rats, weight 200-300 grams, age 3 months[15,16] and were divided randomly into 6 groups each consisted of 6 rats, i.e : negative control group given placebo gel right after TU formed for one day (C_1), negative control group given placebo gel 24 hour after TU formed for one day (C_2), treatment group given 60%Stichopus hermanii extract (SC) gel right after TU formed for one day (SC_1), treatment group given 60% Stichopus hermanii extract gel 24 hours after TU induction for one day (SC_2), treatment group given hyaluronic acid (HA) gel right after TU induction for one day (HA_1), treatment group given given hyaluronic acid (HA) gel 24 hours after TU formed or one day(HA_2). Wistar rats were being adapted for one week, feed and drink daily. Research were performed in biochemistry laboratory Faculty of Dentistry Hang Tuah University

Fresh Stichopus hermanii were cleaned from dirt, put under running water in a mesh sized 30-40 mesh, squeezed to remove saponin in foam form, washed then put on blender, performed freeze drying resulted in rough extract particle.[17] Extract were performed by sohlet method then added mucoadhesive agent.[18]

Wistar rats were given ketamine-xylazin then traumatic ulcer were induced on labial mucosa. Sterile amalgam stopper was flammed in 45 second, touched in labial mucosa for 1 second.[16,19,20] After the ulcer formed, gels were applied as its groups of treatment for 24 and 48 hours, then being terminated to obtain the labial mucosa samples on ELISA test.

Samples were rinsed in cold PBS (0,01 mol/L, pH 7,0-7,2) to remove excessive bleeding and weighed. Tissue were chopped in small cut then being homogenized in 5-10 mL PBS above the ice. Suspension were sonicated with ultrasonic to disrupt cells or performed freeze-thaw cycle twice to disrupt cell membrane, sentrifuged for 5 minute on 5000g. Supernatan were released then being examined immediately or stored in -20°C.

ELISA test were done using enzyme linked immunosorbent assay (ELISA) kit for untuk FGF_2 mouse (Uscn Life Science Inc, Wuhan, China). Reagent and 96 well strip plate were prepared as manufacturer standard and incubated for 25 minutes. Absorbance were measured in microplate reader infinite® M200 PRO (Tecan Deutschland GmbH, Crailsheim, Germany) on wave length 450 nm. All samples were replicated on measure.

RESULT

Topical application of placebo gel, *Stichopus hermanii* extract gel (SC) and hyaluronic acid gel (HA) were evaluated on first and second day after the formation of ulcer (Figure 1). Anova result showed significant difference on mean of FGF2 protein level (p=0,000).Result on SC group was better than placebo group but not significant compare to HA group (p>0.05) (Table 1).

DISCUSSION

FGF2 is the key factor on promoting fibroblast proliferation. The FGF main pathway is RAS/ MAP kinase which contain many protein signaling. An important event of FGF signal pathway is tyrosine residue phosphorilation on protein docking. FGF substrat 2α (FRS2α) receptor prepare the new binding site to take direct or indirectly protein responsible for signalling.[21,22] FRS2α rectruit complex of adaptor protein, guanine nucleotide exchange factor 2 (GRB2), son of sevenless (SOS), the tyrosine phosphatase (SHP2) and protein docking GRB2-associated

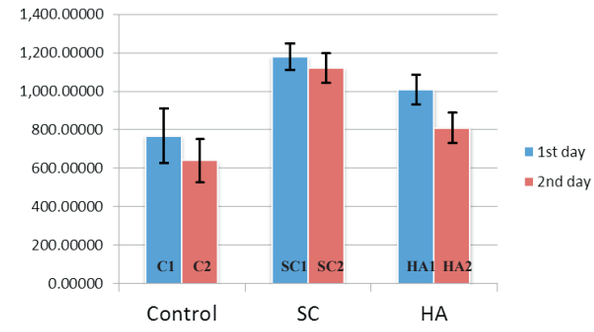

Figure 1. Mean of FGF_2protein level on control and treatment groups.

binding protein 1 (GAB1). Signalling complex FRS2 resulted in activation of RAS/ MAP kinase and PI3 kinase/ Akt pathway.[23] Many studies stated that RAS/ MAP kinase pathway involved in cell growth and differentiation. One of target molecule for FGFR is phospholipase C gamma (PLCγ), which bind to phosphorilated Tyr-766 result on its receptor and then become phosphorilated tyrosin PLCγ and activate PLCγ. Activation of PLCγ hidrolyze phosphatidylinosito and produce inocyol triphosphate (IP3) dan diacylglycerol (DAG).[24] IP3 is second massenger which facilitate calcium release from endoplasmic reticulum. Increasing level of calcium in cytosol and DAG together activate protein kinase C (PKC).Physiologic relevance of this pathway has not been described in details on mitogenesis process or cell differentiation.

Result of FGF2 expression showed the protein level produced on the first day after TU formed generally higher compared to those on second day (Figure 1), although LSD result showed no significant difference for FGF_2 protein level on each group (K1 and K2; SC1 and SC2; HA1 and HA2) as shown on Table 1.

Level of FGF2 in control group were higher on the first day application right after injury performed and tend to decrease when given after the ulcer formed. This result was related to Numata *et al.*[24] stated that one day after injury the level of FGF2 increased and re increased on the third day but the later increasement was less than on the earlier on the beginning of the injury. Treatment with SC resulted in increasing of FGF2 compare to other groups and slightly reduced on the second day (p>0.05). SC contain more type of GAGs compare to HA which have role in increasing the level of FGF2.

Table 1. LSD test result

Dependent variable	Treatment group	K2	SC1	SC2	HA1	HA2
FGF2	K 1	0,931	0,004*	0,024*	0,302	1,000
	K 2	-	0,000*	0,001*	0,016	0,742
	SC1	-	-	0,999	0,726	0,015*
	SC2		-	-	0,968	0,071
	HA1					0,556

Note: *significant different

Chondroitin sulphate is needed in organizing granulation tissue formation during wound healing. Chondroitin sulphate is able to bind to FGF2 and maintaining the level of FGF2 in the tissue and implicated in triggering fibroblast proliferation.[11]Heparan sulphate and dermatan sulphate are great component in wound liquid and become dissolved. The dissolved dermatan sulphate have the ability to activate growth factors like FGF2 and FGF7 which activate MAP kinase pathway and increase the level of Ca+ and then trigger differentiation process and cell mitogenesis.[9,12]

HA have its role on proliferation, migration and cytokine synthesis mediated by CD44 on the cell surface. Along with the increasing of inflammatory cell on HA treatment, the inflammation process accelerated so FGF2 production was not inhibited dan ready to perform the signalling in MAP kinase pathway. But the treatment given on the second day was resulted on delayed of FGF2 increasement. The FGF2 level on HA2 was lower than in SC group, even not significant different.

Stichopus hermanii is marine biota that contain GAG which have the important role in increasing and binding of FGF2.By that active component, the level of FGF2 after topical appication of SC were increased compared to control group and HA group in first day, and compared to control group in second day. It is concluded that FGF2 modulation was increased after topical application of Stichopus extract gel on traumatic ulcers of wistar rats.

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