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Vascular Endothelial Growth Factor (VEGF) on rabbit meniscus injury in the white-white zone expressed higher blood vessels distribution and type 1 collagen bridging post meniscal suturing

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ABSTRACT

Background: Meniscus injury can occur in red – red zone with good prognostic as well as in white – white zone with bad one. General treatment of meniscus injury in white – white is menisectomy. It will destroy the articular surface of the knee joint cartilage. Administration the Vascular Endothelial Growth Factor (VEGF) will increase the proliferation of endothelial cells, fibroblast cells, collagen fibers type 1, and good prognostic for the healing of meniscus injury in white – white zone.

Aim: The study aims to explore the effect of VEGF on rabbit meniscus injury. Methods: This research implemented an experimental study with randomized post-test control group design which used 38 male New Zealand Rabbits. There were two groups (18 rabbits as a control group and 18 rabbits as treatment one). They were adapted for one week, then control group had a sharp incision and sutured in white – white zone meniscus only, and treatment group had administration VEGF after done a sharp incision and sutured in white – white zone meniscus. In the next three weeks, all rabbits have been euthanized and then examined the meniscus for the expression of blood vessels distribution and the bridging of collagen type 1 by histopathology and immunohistochemistry examination.

Results: The average median number for blood vessels distribution after administration VEGF in the sutured white-white zone was about 11.00 (interquartile range 2.00) and without VEGF was about 5.00 (interquartile range 1.00). In the treatment group with VEGF, 18 rabbits (100%) showed bridging collagen type 1, and the control one showed in 6 rabbits (33.33%). An inferential test for blood vessels distribution in white – white zone meniscus between treatment group with VEGF and control group without VEGF was significantly different (p=0.000). Analysis for bridging collagen type 1 between treatment group with VEGF and control group without VEGF showed significant differences (p=0.000).

Conclusion: Administration VEGF on rabbit meniscus injury in the post suturing white-white zone expressed higher blood vessels distribution and bridging collagen type 1 compared with suturing only without VEGF.

Keywords: VEGF, white – white zone meniscus injury, blood vessels distribution, bridging collagen type 1, suturing.

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INTRODUCTION

Meniscus injury is the most significant disease in the world. In Netherland, the incidence of the meniscus injury is about 1000 cases a year. In England and Wales are about 25.000. Then in the USA, menisectomy is the most common treatment for the meniscus injury.[1]

Meniscus is a fibrocartilage tissue, form like a couple of C letter and located between femur and tibia. The function of the meniscus is to stabilize knee joint and as a shock absorber when the femur and tibia bone are attached to each other. The biochemical content of the meniscus is 72% of water and about 28% of organic materials, especially matrix extracellular and cells. Generally, collagen forming those organic materials, such as GAG (glycosaminoglycans) 17%. Other components are chondroitin-6-sulfate, dermatan sulfate, chondroitin sulfate, and keratin sulfate, DNA (deoxyribonucleic acid) 2%, adhesive glycoprotein (fibronectin, thrombospondin, and collagen type 4) less than 1%, and elastin less than 1%. All those components are different in every human depending on the age, trauma, and other pathologic condition. Collagen is the main component of the human meniscus. Collagen is the main component of the human meniscus. In the red – red zone, collagen type 1 is the most component, about 80%, and variant collagen (type 2, 3, 4, 6, and 18 less than 1%) as remains. In white-white zone, collagen is about 70% (which are 60% collagen type 2 and 40% collagen type 1). The red – red zone consists of many fibroblast cells (its known as fibroblast-like cells) which produce an extracellular matrix of the collagen fiber type I. Other components of the meniscus are proteoglycan (aggrecan, biglycan, and decorin).[1-5]

Table 1 Characteristic of the sample

Group	Frequency (n)	Percent (%)
Intervention (with VEGF)	18	50.00
Control (without VEGF)	18	50.00
Total	36	100

Table 2 Distribution of blood vessels in the study

Variable	Group	
	Intervention Group (n=18) Mean ± SD	Control Group (n=18) Mean ± SD
Blood vessel distribution	10.94 ± 0.10	4.44 ± 0.70

Table 3 Distribution of type I collagen bridging in the intervention and control group

Type I collagen bridging	Group	
	Intervention Group with VEGF (n=18) n (%)	Control Group without VEGF (n=18) n (%)
Yes	18 (100)	6 (33.3)
No	0 (0)	12 (66.7)

A meniscus injury can occur in red – red zone with good prognostic, but it is in contrast to the white – white zone. One of the meniscus tear (the second most common after the longitudinal/ bucket handle) causing the traumatic cause is a radial vertical tear type (transverse).[1,2,6] Management radial vertical type of meniscus injury is still causing dangerous side effects, due to management partial or total menisectomy, which is unable to restore the function of the meniscus back to normal, and will also accelerate the adverse effects of damage to the knee joint later. This treatment is conducted because the meniscus tear occurs overall in the white-white zone and slightly widened in the red - red zone. So it can not be repaired by using a repair technique with suture, because the tear occurs in the white - white zone which is hard to achieve angiogenesis.[6-8] VEGF is the homodimeric glycoprotein and can stimulate the angiogenesis process in wound healing. Angiogenesis process involves various process, such us vasodilatation, basal membrane cell degradation, endothelial cell migration, endothelial cell proliferation, forming new capillary vessels and new basal cell membrane.[9,10]

MATERIAL AND METHOD

This is an experimental study with randomized post-test only control group design, using 38 male New Zealand Rabbits. There were two groups (18 rabbits in the control group and 18 rabbits as treatment group). They adapted for one week, then control group had a sharp incision and sutured in white – white zone meniscus only, and treatment group had administered by VEGF post sharp incision (in vertical radial) and sutured in white – white zone meniscus. In the next three weeks, all rabbits were euthanized and also examined the meniscus for the expression of blood vessels distribution and bridging collagen type 1 by both histopathological and immunohistochemical examination. Data obtained in the study were analyzed by a parametric test using independent - samples t-test or Mann-Whitney U test.

RESULT

The total number of research subjects was 36 by divided them into the treatment group and the control group (Table 1). The average of blood vessel distribution in the white - white zone post suture in the intervention group was 10.94 ± 1.10. In contrast, its average in the control group was 4.44 ± 0.70 (Table 2).

In the intervention group, 18 subjects (100%) realized the formation of type I collagen bridging in white - white zone post suture. On the other hand, six subjects (33.33%) represented the establishment of type I collagen bridging (Table 3).

The normality test used for blood vessel distribution in white - white zone and type I collagen bridging in white - white zone meniscus is <0.05. Mann - Whitney U test results showed significant differences in blood vessel distribution in white - white zone meniscus between intervention group with VEGF and control group without VEGF, and this difference was statistically significantly different with p-value <0.05. The result of the analysis also showed that the difference of type I collagen bridging in white - white zone meniscus between the intervention group and VEGF with a control group without VEGF was statistically significant with p-value <0.05.

DISCUSSION

Effect of VEGF administration on meniscus injuries in white - white zone express higher blood vessel distribution

The existence of an injury to the meniscus will cause the natural angiogenesis process, and the process will be better again in quality if there are additional VEGF. Thus, administration of VEGF to meniscus injury in white - white zone led to the expression of higher blood vessel distribution. This can be adjusted to the study by Albornoz & Forriol (2012), suggesting that when a meniscal injury

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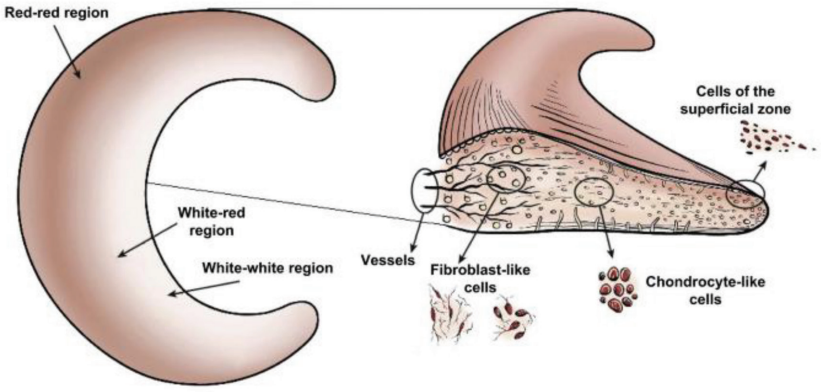

Figure 1 Zone of the meniscus cells[2]

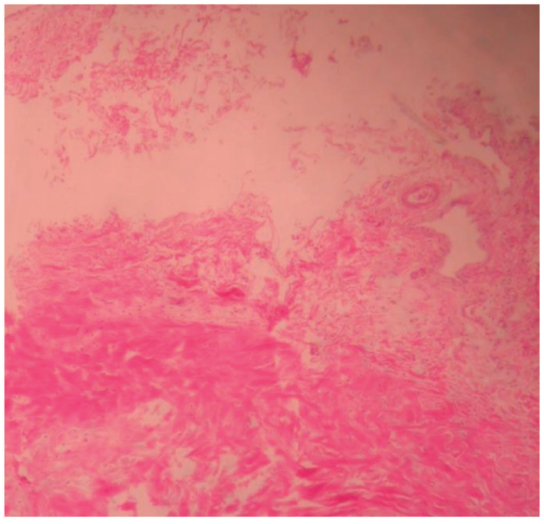

Figure 2 Histopathological results of blood vessels distribution in control group without VEGF

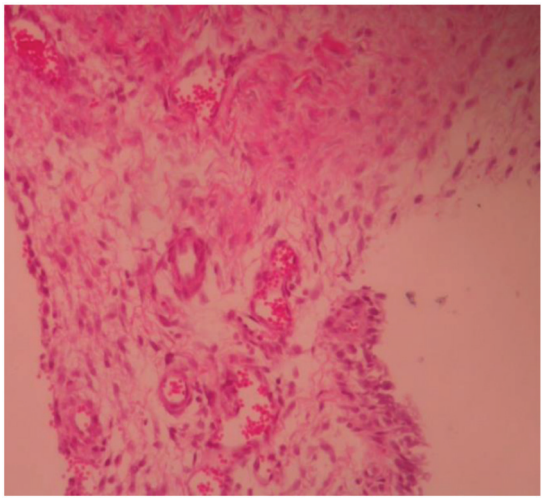

Figure 3 Histopathological results of blood vessels distribution in the treatment group with VEGF

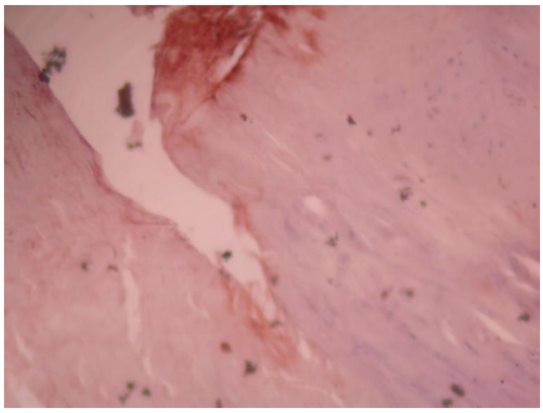

Figure 4 Immunohistochemical findings of type 1 collagen bridging in control group without VEGF

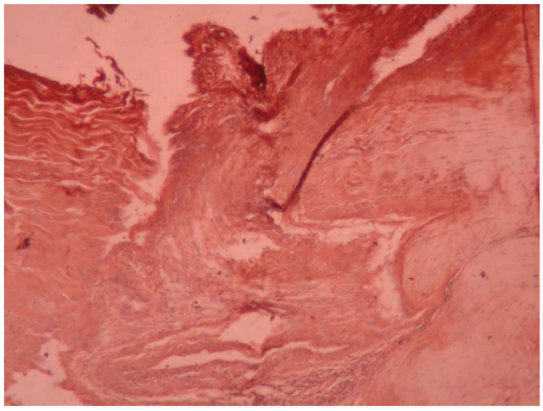

Figure 5 Immunohistochemical results of bridging collagen type 1 in the treatment group with VEGF

occurs within the vascular (red-red zone) and avascular (white-white zone) areas will cause release the growth factor by cells in the injured region and accompanied by the release of inflammatory mediators. Thus, it causes the meniscus cells to undergo a process of proliferation, migration, differentiation, and the synthesis of the meniscal cell matrix. Giving recombinant protein growth factor (VEGF) in high doses and repeatedly will lead to meniscus stimulation for better angiogenesis process and of course will stimulate the healing process of meniscus.[9-11]

The theory proposed by Bao et al. (2009), in which VEGF binds to 2 receptors Tyrosinkinase, the Flt-1 receptor, and KDR on the surface of endothelial cells and blood vessels that have matured in a healing process of injury and will induce phosphorylation and trigger the migration of endothelial cells and angiogenesis processes. VEGF will induce the movement of endothelial cell membranes,

chemotaxis, the proliferation of endothelial cells, vasodilation, basal cell membrane degradation, and endothelial cell migration. Then there will be the formation of blood capillary pipes and followed by the formation of parallel anastomosis of the capillaries and eventually the formation of new basal cell membrane.[9,10,12]

In addition, the results of a study conducted by Wolf Petersen et al. (2004), suggest that studies conducted by administering VEGF post suturing in the avascular area of Merino sheep's meniscus injury indicate an increase in VEGF expression, the stimulus process of proliferating endothelial cells of blood vessels and angiogenesis processes. But the study denied the healing process in the avascular (white - white zone) area of the meniscus sheep (Petersen et al., 2004). This can be concluded the tear of the meniscus in the sheep is undergone longitudinally (bucket handle), so there is no stimulus in the formation of angiogenesis process that crosses from the vascular area (red - red zone) to the avascular area (white - white zone).[13]

The effect of VEGF administration on rabbit meniscus injury in the white-white zone in expressing type 1 collagen bridging

The administration of VEGF to meniscus injury in white - white zone causes bridging collagen type 1 higher post suturing. It can be assumed that the initial process of a meniscus injury in the vascular (red - red zone) and avascular (white - white zone) areas are the occurrences of an angiogenesis process involving the role of VEGF naturally. It will be better if the injury we give recombinant additional VEGF proteins, which of course will lead to better angiogenesis process, and stimulate the formation of new blood vessels more so that the meniscus will contain many fibroblast cells. As well as research conducted by Makris et al. (2011) and Fox et al. (2012), the histologic content of the meniscus in general, especially in red-red zones contains many fibroblast cells. These cells will multiply if there is a good role of VEGF in forming an angiogenesis process. Thus the fibroblast cells will produce an extracellular matrix of type 1 collagen fibers that are plentiful.[2,3] Finally, in the presence of more blood vessels due to VEGF, it induces the movement of endothelial cell membranes, chemotaxis, the proliferation of endothelial cells, vasodilation, endothelial cell migration, and better formation of new basal cell membranes. It results in a large number of fibroblast cells forming, which in turn leads to increased production of extracellular fibers of type collagen fibers.[2] This leads to the bridging of collagen type 1 from red-red zone to the white-white zone meniscus.

In addition, studies conducted by Kim et al. (2016) conclude that the effect of VEGF is to promote blood vessel formation and will form a framework for the proliferation of tissue regeneration cells after injury, as well as to increase the proliferation of fibroblasts and deposition or collagen fiber deposits in a meniscal injury.[9,10,14]

CONCLUSION

The conclusion from this researched are the blood vessels distribution and type I collagen bridging are higher in white – white zone of rabbit meniscus post suturing with VEGF than without VEGF.

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