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Expression of VEGF-receptors in TMJ synovium of rabbits with experimentally induced internal derangement☆

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Abstract

Our aim was to evaluate the expression of vascular endothelial growth factor receptors (VEGFRs) in the synovium of the temporomandibular joints (TMJ) of rabbits with experimentally induced internal derangement. Internal derangement was experimentally induced in 52 rabbit TMJ, and established on the right side of TMJ while the left side was used as the control. Each joint and its control was evaluated by magnetic resonance imaging (MRI) and endoscopy. The synovial tissues on both sides were harvested after one, two, three, and four weeks. The expression of VEGFRs mRNA was investigated in the experimental joint and its control using real-time polymerase chain reaction (PCR). Internal derangement was successfully confirmed in 45 of the 52 of the experimental joints (87%) on the right side by MRI and endoscopy. In the first and fourth week, the VEGFR-2 mRNA expression was higher in the experimental joints than in the controls ($P = 0.008$ and $P = 0.02$). Meanwhile, the VEGFR-1 mRNA expression was up-regulated in the experimental group compared with the controls during the fourth week ($P = 0.02$). However, we found no significant differences in VEGFR-3 mRNA expression in the two groups during the first and fourth weeks. During the second and third weeks, the mRNA expression of the three receptors did not differ significantly among the groups. Our data have shown increased expression of VEGFR-1 and VEGFR-2 mRNA in the synovium of rabbit TMJ with internal derangement, which indicates that VEGFR-1 and VEGFR-2 may have important roles in the processes of internal derangement and formation of adhesions.

Keywords: Temporomandibular joint internal derangement; Rabbit model; Synovium; VEGF receptors; Real time-PCR

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Introduction

Intra-articular adhesion is the most common internal derangement of the temporomandibular joint (TMJ). It accounts for 55–93% of all dysfunction of the TMJ, particularly in stages III–V of internal derangement.[1–8] It has been reported that the formation of the intra-articular adhesions and synovitis are highly correlated.[8] Clinically, when magnetic resonance imaging (MRI) was used to detect an abnormal amount of fluid in patients' joints, synovitis and synovial hyperaemia were commonly detected on arthroscopy.[3] It has been reported that the formation of intra-articular adhesions follows several steps: displacement of the articular disc; the condyle covered by a bilaminar zone; extrusion of the bilaminar zone and anterior recess into the synovium; hyperaemia and exudation of the synovium; an increase in, and accumulation of, inflammatory substance into the upper anterior recess; accumulation of cellulose to form the primary adhesion; the growth of capillary vessels, synovium, and fibroblasts; and, finally, formation of different types of adhesion.[1]

However, until now the underlying mechanism of the formation of intra-articular adhesions in the TMJ has been poorly understood. Candidate biomarkers as preventative targets have not been identified. To better understand the process, therefore, and to search for the candidate targets involved in the process, we constructed an experimental model in rabbits in which internal derangement was induced that partly mimics the formation of internal derangement in human TMJ.

Vascular endothelial growth factor (VEGF) and its receptor play an important part in increasing the permeability of the capillary vessel, and Sato et al.[9] found that expression of VEGF in the synovium was highly correlated with the amount of joint fluid.

Over-expression of VEGF was also noted in synovial tissue of TMJ with synovitis.[6] Until now three types of VEGF receptors (VEGFRs) had been identified: VEGFR-1, VEGFR-2, and VEGFR-3. It had been reported that the expression of the VEGF/VEGFR-1 system may be involved in angiogenesis in inflamed synovial tissue in the TMJ,[10] which suggests that aberrant alteration of the VEGF/VEGFR-1 pathway might contribute to inflammation of the synovial tissue of the TMJ and the formation of intra-articular adhesions. However, whether other subtypes of VEGFR may also be involved in angiogenesis in inflamed synovial tissue in the TMJ and the formation of intra-articular adhesions in the TMJ do not need to be addressed.

To test our hypothesis, we have successfully induced internal derangement experimentally into rabbit TMJ and evaluated the mRNA expression of three VEGFRs in them and compared it with a matched control group using real time PCR at indicated time points. We have found that VEGFR-2 mRNA expression was higher in the experimental than in the control group ($P = 0.008$) during the first and fourth weeks. The VEGFR-1 mRNA expression was increased in the experimental compared with the control group during the fourth week ($P = 0.02$), indicating that VEGFR-1 and VEGFR-2 might have important roles in the processes of internal derangement and the formation of adhesions.

Materials and methods

Experimental animals

Fifty-two adult, New Zealand, big-eared rabbits 6 months old and weighing 2.5–3 kg were provided by the Animal Experiment Laboratory of Shanghai Jiao Tong University School of Medicine. The rabbits were randomly divided into 4 groups, with 13 in each group. The selection standards of animals were as follows: no teeth missing, no malocclusion, good nutrition, no systemic disease, and the ability to move freely. The right craniomandibular joint was chosen as the experimental side, and the left side used as control. MRI and arthroscopic examinations were made 1, 2, 3, and 4 weeks after the operation to evaluate the severity of disc displacement and the formation of internal adhesions. The experimental animals were then killed and samples were taken for histological examination. This study was approved by the Animal Care and Use Ethics Committee of Shanghai Jiao Tong University School of Medicine.

Experimental induction of internal derangement of the TMJ

The experimental animals were given general anaesthetics in the form of ketamine 0.2 ml and sulphamethoxazole 0.8 ml by intramuscular injection. The fur over the right preauricular region was shaved and the area sterilised. An injection of 2% lignocaine (1.5 ml) was given locally. The skin was incised forwards and downwards from the root of the zygomatic arch to its surface for about 2.5–3.0 cm to expose the zygomatic arch and the orbital floor. Two small processes can be seen on the upper edge of the orbital floor, the anterior one of which was about 40 mm from the root of the zygomatic arch. A hole was drilled using a titanium pulling nail with a 2/0 suture (Johnson & Johnson) tied to it. Part of the zygomatic branch was removed and the articular capsule exposed. The anterior part of the articular disc was penetrated by a 2/0 suture, and the disc pulled forward with an elastic band that deforms from 9.5 mm before, to 40 mm after, creating a pulling force of 1.20 N. The elastic band was fixed on to the orbital bone with a suture, and located in the internal part of the orbital bone to simulate the effort of the pterygoid muscle.

After those procedures we washed the wound with iodine, and then sutured it in layers. Penicillin 200 000 iu was injected intramuscularly every day for 3 days after the operation, and the animals were fed with a soft diet for one week, followed by a normal diet.