Journal of Cranio-Maxillo-Facial Surgery xxx (2017) 1–8

Contents lists available at ScienceDirect

Journal of Cranio-Maxillo-Facial Surgery

journal homepage: www.jcmfs.com

Determination of VEGF, collagen type 1 and versican in the discus articularis of the temporomandibular joint in relation to dental status

Malte Michaelis [a], Ralph Lucius [a], Jörg Wiltfang [b], Falk Birkenfeld [b, *]

[a] Institute of Anatomy (Head: Prof. Dr. Ralph Lucius), Christian-Albrechts-University of Kiel, Olshausenstraße 40, 24118 Kiel, Germany
[b] Department of Oral and Maxillofacial Surgery (Head: Prof. Dr. Dr. Jörg Wiltfang), University Hospital Schleswig-Holstein, Arnold-Heller-Straße 16, 24105 Kiel, Germany

ARTICLE INFO

Article history:
Paper received 2 February 2017
Accepted 14 September 2017
Available online xxx

Keywords:
VEGF
Versican
TMJ

ABSTRACT

The aim of this study was the investigation and comparison of the presence of vascular endothelial growth factor (VEGF), collagen type 1 and the proteoglycan versican in the discus articularis in relation to dental status (full dentition [1], partial dentition [2] and edentulous [3]). The right disci articulares were removed from 17 donated bodies (6 with full dentition, 5 with partial dentition and 6 edentulous). The specimens were immunohistochemically stained for VEGF, collagen type 1 and versican. Semi-quantitative analysis of the disci was conducted within the groups based on the intensity of immunoreactivity of VEGF, collagen type 1 and versican. In addition, a pairwise comparison was carried out between the three experimental groups. The results revealed significantly higher immunoreactivity for VEGF and versican in groups 2 and 3 than in group 1. Conversely, determination of immunoreactivity was significantly higher in group 1 for collagen type 1 than in the other two groups. These results indicate an elevated presence of the proteoglycan versican and the neoangiogenesis factor VEGF when the occlusal supporting zone has been lost. By contrast, detection of collagen type 1 is reduced. The loss of collagen type 1 and rise in versican and VEGF suggest increasing degeneration when the supporting zone is lost due to the loss of teeth.

1. Introduction

The discus articularis is a cartilaginous spacer in the temporomandibular joint and serves to attenuate and disperse forces acting on it (Kubein-Meesenburg et al., 2007). Degenerative changes have been detected histologically in the cartilaginous tissue in cases of a non-physiological position, such as anterior disc displacement (Leonardi et al., 2007; Luder, 2002). Histological characteristics of degenerated cartilage are new formation and transformation of collagen fibrils, loss of proteoglycans, redistribution of glycosaminoglycans, neovascularisation, calcified regions and even perforation of the cartilage (Flygare et al., 1992; Haskin et al., 1995). Following new formation of collagen fibrils, tears appear in the surface and core of the cartilage in cases of persistent degeneration. Damage to, and loss of, collagen type 1 fibres is a result of degeneration.

The proteoglycan versican appears to play an important role in the inflammatory process in the tissue. Proteoglycans are essential to the resilience of the tissue against forces acting on it (Halper, 2014). Incorporation of proteoglycans and glycosaminoglycans, the polysaccharide side chains of the proteoglycans, are greater in the regions of the discus articularis where the highest transmission of force occurs (Blaustein and Scapino, 1986). An imbalance in the force distribution, as occurs in unilateral bite-raise, results in an increase in proteoglycans (Mao et al., 1998; Nakao et al., 2015). Unilateral loading during mastication due to the absence of teeth is sufficient to increase glycosaminoglycan synthesis in the chondrocytes (Huang et al., 2002). Research has shown that versican plays an important role in the initiation of the inflammatory response and progression of inflammation (Zhang et al., 2012).

The effect of VEGF (vascular endothelial growth factor), a multifunctional cytokine, on cartilaginous tissue has been the focus of much research. For example, VEGF has been shown to greatly influence angiogenesis in physiological tissue, but to also have effects within the scope of pathological changes (Dvorak et al., 1995). Research has demonstrated that VEGF is not solely expressed in the

* Corresponding author. Fax: +49 43150026006.
E-mail address: f.birkenfeld@mkg.uni-kiel.de (F. Birkenfeld).

https://doi.org/10.1016/j.jcms.2017.09.013
1010-5182/

endothelium, but also in other tissues, mainly in bony and cartilaginous tissue, including the discus articularis in the temporomandibular joint (Berendsen and Olsen, 2014; Lingaraj et al., 2010). Histological investigations indicate that VEGF expression increases with increasing severity of pathological tissue degeneration. Expression occurs mainly in chondrocytes and in the newly formed capillaries (Leonardi et al., 2003).

The aim of this study was the investigation of the presence of the proteoglycan versican, the neoangiogenesis factor VEGF and collagen type 1 in the discus articularis in relation to dental status. The purpose was to clarify whether there is a link between the occurrence of versican and VEGF and the severity of tissue degeneration.

2. Materials and methods

The disci articularis were sectioned and stained for microscopic examination to determine tissue degeneration. Immunohistochemical staining was carried out to detect VEGF, collagen type 1 and versican.

2.1. Samples

The right disci articulares were removed from 17 donated bodies (11 × female, 6 × male, mean age: 77.5 years; Institute of Anatomy, Christian-Albrechts-University of Kiel, Kiel, Germany) for the immunohistochemical investigations carried out here. The donors were allocated to three experimental groups in relation to their dental status: full dentition (1), $n = 6$; partial dentition (2), $n = 5$ and edentulous (3), $n = 6$. Allocation to these groups was carried out based on Eichner's (1955) classification of supporting zones. This stipulates that full dentition is composed of four supporting zones that are characterised by tooth contact between the antagonists of the premolars and molars. The number of supporting zones that were still present formed the basis for the three main groups. The jaw is edentulous if all supporting zones are missing (3). If one to three supporting zones are present, the jaw has partial dentition (2) and the presence of all four supporting zones means the jaw has full dentition (1). Subgroups are not considered here for reasons of clarity.

In a first step, the mandibles were disarticulated from the donor bodies so the disci articulares could be harvested. Due care was taken to ensure the spacer was not damaged or distorted when the discus articularis was detached from the condyle. The discus was always removed whole. The right disci were quartered in an axial direction. They were then marked in the corners with suture material (3/0 Seralon, SERAG Wiessner KG, Naila, Germany) to aid in orientation. This ensured that the specimens were correctly embedded later on and the desired side of the cartilage was sectioned. The specimens were stored in 4% paraformaldehyde (Merck KGaA, Darmstadt, Germany). A total of 68 specimens were produced and investigated.

2.2. Embedding and sectioning

The paraformaldehyde served the purpose of post-fixing the specimens. The pieces were cleaned for 1 h under running water and then treated in a series of alcoholic solutions of ascending concentrations (2 × 50%, 2 × 70%, 2 × 90%, 1 × 96% and 1 × 100%). The alcohol was undenatured ethanol (Walter-CMP GmbH & Co. KG, Kiel, Germany). The specimens were stored overnight in the interim medium of ethyl benzoate or benzoic acid methyl ester (Carl Roth GmbH & Co. KG, Karlsruhe, Germany). On the following day, the tissue was embedded in paraffin for 3 × 1 h (Histo Comp, VOGEL med. Technik & Elektronik GmbH & Co. KG, Fernwald, Germany) and then poured into moulds. The sections were made using a microtome (Leica RM2155, Leica Microsystems GmbH, Wetzlar, Germany) after the block had hardened. The block was first cut into at a thickness of 30 μm, the histological sections were 7 μm in thickness.

2.3. Immunohistochemical staining of the specimens

The antibodies used for immunohistochemical staining were the vascular endothelial growth factor (VEGF), the proteoglycan versican and collagen type 1. Table 1 lists the primary antibodies that were used, their clonality, the species from which the antibodies were obtained and what species these antibodies target, and the manufacturer. Table 2 lists the secondary antibody that was used for VEGF, versican and collagen type 1.

2.4. Preparation of the collagen type 1 specimens

The specimens for the immunohistochemical determination of collagen type 1 were pre-treated with proteolytic enzymes to unmask the antigens. After rinsing with distilled water for 5 min, the specimens were incubated with 0.1% pepsin (in 0.5 M acetic acid) for 30 min at 37 °C. Following this, the specimens were subjected to threefold interim rinsing with phosphate buffered saline (PBS, Merck KGaA, Darmstadt, Germany) and then treated with hyaluronidase, also for 30 min at 37 °C. The buffer solution prevents desiccation of the specimens. Threefold interim rinsing with PBS was then repeated. Finally, the specimens were left to stand for 20 min in 0.6% H_2O_2 (1 ml 30% H_2O_2 in 50 ml 100% methanol). These steps blocked the endogenous peroxidase in the chondrocytes and erythrocytes and are particularly important for the prevention of false-positive colour changes.

2.5. Preparation of the VEGF specimens

The endogenous peroxidase was blocked with 3% H_2O_2 for 30 min in the specimens used for the immunohistochemical determination of VEGF. This was followed by threefold rinsing in PBS. The specimens were then heated in citrate buffer in a microwave for 2 min at 800 W and for 14 min at 140 W. The citrate buffer is a solution made from 1.05 g citric acid monohydrate (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) in 500 ml distilled water.

2.6. Preparation of the versican specimens

The endogenous peroxidase was blocked for the immunohistochemical determination of versican by incubating the specimens for 30 min in 3% H_2O_2 and then rinsing them three times in PBS.

Table 1
List of the primary antibodies used for immunohistochemical staining.

Antibody	Host	Reactivity	Manufacturer
VEGF monoclonal	Mouse	Human	R&D Systems, Minneapolis, USA
Versican monoclonal	Mouse	Human	US Biological, Massachusetts, USA
Collagen type 1 monoclonal IgG1	Mouse	Human	Biotrend Chemikalien GmbH, Köln, Germany

Table 2
The secondary antibody used for immunohistochemical staining.

Antibody	Host	Reactivity	Manufacturer
Biotinylated IgG	Goat	Mouse	Vector Laboratories Inc., California, USA

Please cite this article in press as: Michaelis M, et al., Determination of VEGF, collagen type 1 and versican in the discus articularis of the temporomandibular joint in relation to dental status, Journal of Cranio-Maxillo-Facial Surgery (2017), https://doi.org/10.1016/j.jcms.2017.09.013