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Juvenile porcine temporomandibular joint: Three different cartilaginous structures?

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ABSTRACT

Objective: The temporomandibular joint (TMJ) consists of three cartilaginous structures: the fossa, disc, and condyle. In juvenile idiopathic arthritis (JIA), inflammation of the TMJ leads to destruction of the condyle, but not of the fossa or the disc. Such a different effect of inflammation might be related to differences in matrix composition of the cartilaginous structures.
Methods: The matrix composition of the three TMJ structures was analyzed in juvenile porcine samples and in an *in vitro* system of cells isolated from each anatomical structure embedded in 3% agarose gels.
Results: The matrix of all three anatomical structures of the TMJ contained collagen type I and its gene expression was maintained after isolation. The condyle and the fossa stained positive for collagen type II and proteoglycans, but the condyle contained considerably more collagen type II and proteoglycans than the fossa. The disc contained neither collagen type II protein nor expression of its gene, and the disc did not stain positive for proteoglycans. Aggrecan gene expression was lower in the disc compared to condyle and fossa cell-isolates. In general, the cell-isolates *in vitro* closely mimicked the characteristic features found in the tissue.
Conclusion: The collagen type II content of the condyle clearly distinguished this cartilaginous structure from the disc and fossa. Since autoimmunity against collagen type II is associated with JIA, the relatively abundant presence of this type of collagen in the condyle might provide an explanation why primarily this cartilaginous structure of the TMJ is affected in JIA patients.

1. Introduction

The temporomandibular joint (TMJ) contains two synovial compartments. The upper compartment is delineated by the fossa in the temporal bone at the base of the skull. The lower compartment is formed by the mandibular condyle. The intra-articular disc divides the upper and the lower compartment. Although the condyle and the disc have been subject of investigation in several *in vitro* studies (Detamore & Athanasiou, 2005; Tanimoto et al., 2011), the fossa is generally disregarded, even though investigating the fossa might provide valuable insights in the underlying causes of TMJ destruction during inflammation. For example, in children suffering from juvenile idiopathic arthritis (JIA) Svensson et al. found that the condylar cartilage of some JIA patients was completely destroyed, whereas the fossa seemed unaffected (Svensson, Larsson, & Adell, 2001). Therefore, studies in which the composition of all three cartilage parts are compared, preferably from children or young animals whose TMJ closely resembles that of humans, may contribute to our understanding of why the condyle is affected in JIA and the fossa is not. Ultimately, this could help to develop novel strategies to prevent joint damage at these sites.

JIA is a chronic rheumatic disease that is initiated before the age of 16. JIA has an unknown etiology and affects ~1 of 1000 children worldwide (Olsen-Bergem, Kristoffersen, Bjørnland, Reseland, & Aas, 2016). It is characterized by persistent inflammation that can cause destruction of the matrix of joints, including the TMJ

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(Abramowicz, Kim, Prahalad, Chouinard, & Kaban, 2016). With 75% of the JIA subjects suffering from acute TMJ arthritis at the time of diagnosis, it has been suggested that the TMJ is one of the most frequently affected joints in children with JIA (Weiss et al., 2008). These children are prone to jaw pain and jaw dysfunction in adulthood. Furthermore, persistent inflammation of the TMJ can result in asymmetric growth of the mandible (Kjellberg, 1998), an undersized jaw (Larheim & Haanaes, 1981), an abnormal positioning of the maxilla (Twilt, Schulten, Nicolaas, Dülger, & van Suijlekom-Smit, 2006), malocclusion (Hu, Billiau, Verdonck, Wouters, & Carels, 2009), and a reduced maximum mouth opening (Ringold, Torgerson, Egbert, & Wallace, 2008).

Ethical issues limit the number and the nature of clinical studies and biological sampling from TMJ in children with JIA. This may explain the lack of knowledge of the underlying cause of the changes occurring in the TMJ in JIA. Furthermore, there is an absence of *in vitro* and *in situ* models that take into account the three different articular structures of the TMJ.

Unlike other synovial joints, which are covered by hyaline cartilage, the three different articular structures of the TMJ are considered to be fibrocartilage (Benjamin & Ralphs, 2004). Fibrocartilage is characterized by the presence of not only collagen type II (COL2) and proteoglycans, but also collagen type I (COL1) (Benjamin & Ralphs, 2004). It is not known whether the matrix composition of the three cartilaginous structures is the same. It is possible that the preferential destruction of the condyle in JIA could be related to a matrix and/or cellular composition that differs from the fossa and disc.

Our aim was to analyze the composition of the matrix of the fossa, the disc, and the condyle by assessing the presence and/or absence of three main matrix components of fibrocartilage: proteoglycans and collagen type I and type II. We tested the hypothesis that the matrix composition of the condyle differs from that of the other cartilaginous structures (*i.e.* fossa and disc). Porcine TMJs were used because the TMJ of this species is comparable with that of human; (Bermejo, González, & González, 1993; Detamore et al., 2006; Springer, Fleiner, Jepsen, & Açil, 2001). In order to analyze the cellular characteristics of the three cartilaginous structures, we isolated cells together with their surrounding pericellular matrix (chondrons) using a chondron-preserving enzyme digestion.

2. Materials and methods

2.1. Dissection of the porcine TMJ

Heads Dutch Landrace pigs (*Sus scrofa*), with a body weight in the range of 70–80 kg, aged 6–8 months old, were obtained from a local abattoir (Westford, Gorinchem, The Netherlands). In total 9 pig heads were used in this study. In each experiment 6 porcine TMJs from 3 pigs were pooled together and the experiments were repeated two times.

2.2. Histological and immunohistochemical analysis of porcine TMJ tissue

The fossa, the disc, and the condyle were dissected within 4 h after sacrifice, embedded in Tissue-Tek® (Sakura Finetek Europe BV.; Zoeterwoude, The Netherlands), and snap-frozen in liquid nitrogen. Sagittal cryosections of 10 μm thickness of the medial part of the fossa, the disc, and the condyle were cut and placed on silane-coated glass slides. Proteoglycan staining was performed by using Safranin-O (C.I. 50240)/fast green (C.I. 42053) (Gurr, BDH, Poole, UK).

Immunolocalization of the collagens was performed by using rabbit polyclonal anti-COL1 (1:1000) (ab292; Abcam, Cambridge, MA) and mouse monoclonal IgG2a anti-COL2 (1:1000 dilution) (ab3092; Abcam). The secondary antibodies alexa-555 goat anti-rabbit (1:2000 dilution) (A31630; Invitrogen, Carlsbad, CA) was used against COL1 primary antibody, and alexa-555 goat anti-mouse (1:2000 dilution) (A31622; Invitrogen) was used against COL2 primary antibody. Negative controls were performed with mouse IgG2a (Dako, Glostrup, Denmark) and rabbit immunoglobulin fraction (Dako). These antibodies replaced the first antibody. The slides were analyzed with a Leica DMRx400F microscope (Leica, Wetzlar, Germany).

2.3. Cell isolation and culturing

The articular cartilage of the fossa and condyle and the whole disc were dissected in a sterile operation room under aseptic conditions, within 4 h after sacrifice. The cell-isolates were isolated as previously described (Vonk et al., 2010) by enzymatic digestion with bacterial collagenase type II 0.3% (w/v) (Worthington Biochemical; Lakewood, NJ) and dispase 0.2% w/v (Gibco) for 5 h at 37 °C. The released cells and chondrons were passed through a 100 μm pore size cell sieve (Invitrogen). The cells were centrifuged and resuspended in 2× Dulbecco's modified Eagle's medium (DMEM) (Gibco). To create three dimensional microenvironment for the cells, the medium containing the cells was mixed 1:1 with 6% ultra pure low melting point agarose (Invitrogen) to a final concentration of 1×10^6 cells/ml, 3% agarose, 1× DMEM supplemented with 50 μg/ml ascorbic acid (Merck, Darmstadt, Germany), 10% fetal bovine serum (FBS) (Gibco), and 2% penicillin/streptomycine/fungizone (Invitrogen). As shown in supplemental Table 1, the cell-gel constructs were cultured for a maximum of 6 days, after which the FBS in the medium was substituted by ITS (Insulin–Transferrin–Selenium) (Sigma-Aldrich) (Supplemental Table 1; (Bougault, Paumier, Aubert-Foucher, & Mallein-Gerin, 2009)).

2.4. Histological and immunohistochemical analysis of cell-isolates embedded in agarose gel

The agarose gels containing cells that were isolated from the fossa, disc, and condyle were embedded in Tissue-Tek® and snap-frozen in liquid nitrogen prior to cryosectioning. Proteoglycan staining was performed by using toluidine blue (C.I. 52040) (Gurr, BDH) and analyzed with a Leica DMRx400F (Leica). The Toluidine blue positive cells were counted as follows: Of each TMJ structure (fossa, disc, and condyle) 10 randomly chosen sections were used, and the number of blue cells, as well as the total number of cells were counted.

For the immunohistological staining of COL1 and COL2 of the isolated cells from the fossa, disc, and condyle the sections were stained with the same primary and secondary antibodies used for the staining of the tissue sections. The cell-gel constructs were fixed with formaldehyde and analyzed with Axio Zoom V16 microscope (Zeiss, Munich, Germany).

The Axio ZoomV16 microscope enabled the analysis of ±1 mm thick sections of the cell-gel construct. 200 μm of the cell-gel construct was scanned, and from this scan 6 μm optical sections were made for further analysis. The micrographs were then superimposed and the positive cells were counted. With this technique, we were able to count the COL1 and COL2 positive cells manually, with high accuracy.

2.5. RNA extraction and real-time quantitative PCR

The entire articular cartilage of the fossa and condyle, and the whole disc were snap-frozen in liquid nitrogen, and pulverized using a small cryogenic mill (freezer/mill 6700; SpexIndustries,