Tissue and Cell 45 (2013) 145–152

Contents lists available at SciVerse ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

# Aquaporin 1 (AQP1) expression in experimentally induced osteoarthritic knee menisci: An *in vivo* and *in vitro* study

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ARTICLE INFO

*Article history:*
Received 18 June 2012
Received in revised form 8 October 2012
Accepted 11 October 2012
Available online 17 November 2012

*Keywords:*
AQP1
Knee menisci
Inflammation
Joint
Osteoarthritis
Tissue degeneration

ABSTRACT

Osteoarthritis (OA) of the knee is a major problem in our society. The development of new treatment options for OA is limited, because the pathophysiological mechanisms are not clearly understood, especially on the molecular level. Aquaporin 1 (AQP1) is a specific protein channels for water transport; it is expressed in articular chondrocytes, human synovitis, in chondrocytes of patients with rheumatoid arthritis or OA and in chondrocyte-like cells of human intervertebral disc. The aim of this study was to investigate the expression of AQP1, through immunohistochemistry, immunocytochemistry and Western blot, in experimentally induced OA knee menisci. AQP1 was studied *in vivo* in knee OA menisci from 36 rats that underwent medial or lateral meniscectomy, and *in vitro* on fibrochondrocytes derived from knee OA menisci rats. OA in rats was experimentally induced and tested by histomorphometric analysis. Histological results demonstrated structural alterations in OA menisci accompanied by a very strong AQP1 immunohistochemical and immunocytochemical staining. The Western blot analysis confirmed a strong expression of AQP1 in OA fibrochondrocytes cells. The results of the present research suggest that an activation of AQP1, induced by the OA process, may represent an endogenous mechanism, which can be used to control the tissue degeneration within OA articular joints.



## 1. Introduction

Osteoarthritis (OA) is a common cause of pain and disability in the general population and its socioeconomic significance is well known (Badley and Tennant, 1993; Musumeci et al., 2011a, 2012a). OA results from an imbalance between chondrocyte-controlled anabolic and catabolic processes that is characterized by a progressive degradation of extracellular matrix components within articular cartilage (Musumeci et al., 2011b,c). Many joints can be affected, although OA is more evident in the knees. At this level, one of the protection mechanisms against cartilage wear comes from the presence of the two menisci. They play a vital role in load transmission, shock absorption, stability and jointly with the synovial fluid, nourish and lubricate the articular cartilage of the knee (Musumeci et al., 2012b).

The meniscal body is composed of three parts: the inner rim, the central core and the outer rim. The meniscus is typically an avascular structure with the primary blood supply limited to the periphery. Studies have demonstrated that the meniscus is vascularized only in the outer rim, actually the 10–30% of it, while the inner free margin of the meniscus is avascular and nourished by the diffusion of synovial fluid (Arnoczky and Warren, 1982; Musumeci et al., 2012b).

Meniscal cells are generally considered to be a cross between chondrocytes and fibroblasts, known as fibrochondrocytes, due to the fact that they exhibit characteristics of both fibroblasts and chondrocytes. These cells are very important for tissue homeostasis as they synthesize, maintain and/or degrade the extracellular matrix (ECM), that is primarily composed of type I collagen (90–95%). The other matrix components include proteoglycans, elastin, water and other collagen types (Belzer and Cannon, 1993; Musumeci et al., 2012c).

Aquaporins (AQPs) are some of the cellular proteins that have been found in fibrocartilage and similar tissues such as the intervertebral disc (IVD). AQPs are specific protein channels for water transport that have been shown to perform an important role in tissue compression strength in relation to load (Borgnia et al., 1999; Ishibashi et al., 2000; Verkman and Mitra, 2000; Richardson et al., 2008). Water represents one of the most important substances in the human body and the meniscus is composed approximately of

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0040-8166/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tice.2012.10.004

75% water. The family of AQPs may be subdivided into two groups based on structural and functional differences. Group 1 members (AQP0, 2, 4, 6, and 8) are primarily water selective channels, whilst group 2 members (AQP3, 7, 9, and 10), or "aquaglyceroporins", transport glycerol and other small molecules, including urea (Verkman, 2009; Wang et al., 2003). AQPs expression has been studied in various tissues and organs such as kidney, respiratory tract, brain, eye, gastrointestinal tract (Ma and Verkman, 1999; van Os et al., 2000). In Meckel's cartilage (Wang et al., 2003), they exert an important role in water movement across the membrane, for active near-isosmolar fluid absorption/secretion, neuronal signal transduction, cell metabolism and proliferation (Verkman, 2009). In particular AQP1 has been investigated in articular chondrocytes (Mobasheri et al., 2004), chondrocyte-like cells of human IVD (Richardson et al., 2008), temporomandibular joint disc (Loreto et al., 2012a,b) human synovitis (Mobasheri et al., 2010) and in chondrocytes of patients with either rheumatoid arthritis (Trujillo et al., 2004) or OA (Meng et al., 2007). Over 70% of the total tissue weight in the cartilage matrix consists of water, and in early-stage OA, there is an increase in water content through swelling (Dijkgraaf et al., 1995).

Clinical evidence in OA joint suggests that joint swelling, accompanying chronic inflammation, is caused by synovitis, retention of synovial fluid in the joint cavity and edema, resulting from circulatory disturbance. Therefore, we hypothesized that AQP1 may be involved in joint swelling and edema formation. Thus, the purpose of the present study is to measure the expression of AQP1, through immunohistochemistry, immunocytochemistry and Western blot, in experimentally induced osteoarthritic (OA) knee menisci and to provide new insights into the physiological processes of meniscus degeneration.

## 2. Materials and methods

### 2.1. Animals

Thirty-six, two-month-old male albino Wistar rats (Charles River Laboratories, Wilmington, MA, USA), with a body average weight of 200 ± 20 g were used and housed singly in stainless steel cages during the entire pre- and postoperative stabilization period and were permitted free cage activity without joint immobilization. The animals were maintained at 20–22 °C, with a relative humidity of 40–60% and a photoperiod of 12 = 12 h, light and dark. All surgical procedures for anterior cruciate ligament transection (ACLT) were performed in accordance to the method previously described by Grigolo et al. (2009). The 36 animals were distributed in two different groups: 12 rats for control group (without ACLT) and 24 rats for OA group (articular cartilage of both distal femoral epiphyses was submitted to ACLT to induce OA model and left untreated). The animals at 3 months underwent medial or lateral meniscectomy. All the animals after the meniscectomy (control group and OA group) were sacrificed under general anesthesia by an intravenous lethal injection of Tanax (Hoechst Roussel VetGmbH, Wiesbaden, Germany). Both femurs were explanted, cleaned from soft tissues and the samples were used to perform histomorphometric evaluations. Meniscus after meniscectomy was used to perform histological, immunohistochemical, immunocytochemical and Western blot evaluations. For the *in vivo* study we used the menisci from 12 rats with OA and 6 control rats (6 OA rats and 3 control rats for histological and 6 OA rats and 3 control rats for immunohistochemical analyses). For the *in vitro* study we used the menisci from 12 rats with OA and 6 control rats (6 OA rats and 3 control rats for immunocytochemical and 6 OA rats and 3 control rats for Western blot evaluations). Each experiment was repeated 3 times for each rat. All procedures were conformed to the guidelines of the Institutional Animal Care and Use Committee of the University of Catania.

### 2.2. Histomorphometric analysis

Both femurs were explanted, cleaned from soft tissues as previously described (Musumeci et al., 2011d). Histomorphometric analysis was performed on the total number of rats used and specifically on both medial and lateral femoral condyles from untreated and treated animals. Histomorphometry was performed with image analysis, Kontron KS 300 software (Kontron Electronics, Eching bei Munchen, Germany). Two blinded investigators made the analyses. We assumed that the evaluations were correct if there were no statistically different values between the investigators. Fifteen fields randomly selected from each section were analyzed. The semi-quantitative histological grading criteria of Kraus' modified Mankin score were used (Mankin et al., 1971; Kraus et al., 2004). For cartilage degradation was used a new easier inter-observer parameter considered for histopathology assessment systems (Pauli et al., 2012), a comparison between MANKIN system and OARSI system.

### 2.3. Isolation and culture of rat fibrochondrocytes

Rat fibrochondrocytes from OA and non-OA menisci were isolated from the inner-half of the meniscus. The procedures were similar to those used for articular chondrocytes as previously described (Musumeci et al., 2011e) but a few extra isolation steps were needed to free the cells from the more complex ECM. Briefly, after slicing the meniscus, the isolation began with a short digestion in 0.05% hyaluronidase (Sigma Aldrich, St. Louis, MO, USA) for 5 min, then a digestion in 0.2% trypsin for 30 min, followed by overnight incubation in 0.2% collagenase (collagenase type I, GIBCO Grand Island, NY, USA) in α-Modified Eagle's Medium (α-MEM) (Sigma Aldrich, St. Louis, MO, USA) containing 5% fetal bovine serum (FBS; JRH, Lenexa, USA). The suspension was filtered through a 100-μm-nylon filter (FALCON, USA) and washed 3 times with phosphate buffered saline (PBS, GIBCO, Grand Island, NY, USA) containing 100 μg/ml penicillin/streptomycin (GIBCO, Grand Island, NY, USA). The number and size distribution of the isolated cells was determined using a Z2 Coulter Counter and size Analyzer (Beckman Coulter, Inc., Palo Alto, CA, USA). The cells were plated onto separate 10 cm tissue culture dishes at a density of 10,000 cells/cm$^2$. Cells were incubated at 37 °C and 5% $CO_2$ in fibroblasts medium composed of DMEM (GIBCO, Grand Island, NY, USA) containing 10% fetal bovine serum, and 100 μg/ml penicillin/streptomycin. Culture medium was changed twice weekly. Cells were observed with Axiovert 25 Zeiss inverse microscope (Germany) and photographed with a digital camera calibrated means micrometer slide (Power Shot G5 Canon, Japan).

### 2.4. Histology

Samples (femurs and menisci) were rinsed in phosphate-buffered saline (PBS, Bio-Optica, Milano, Italy), fixed in 10% buffered-formalin for 24 h at room temperature (Bio-Optica, Milan, Italy). Following an overnight wash, specimens were dehydrated in graded ethanol, cleared in xylene and paraffin-embedded (Bio-Optica, Milan, Italy), preserving their anatomical orientation. Sections of 4–5 μm thick were cut from paraffin blocks using a microtome and they were mounted on poly-L-lysine coated or silanized slides (Bio-Optica, Milan, Italy) and stored at room temperature. The sections were stained with Hematoxylin and Eosin (H&E, Bio-Optica, Milan, Italy) for general cell identification. The sections were observed with an Axioplan Zeiss light microscope