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Immunoexpression of constitutive and inducible cyclooxygenase isoforms in distinguishing and accessory structures of synovial joints in rat foetuses

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Joint formation is a developmental process regulated by various factors including bone morphogenetic proteins, transforming and growth factors, etc. Recently, a high expression of cyclooxygenase (COX) isoforms in the foetal cartilaginous elements was also revealed. On the other hand, various joint and skeletal abnormalities were seen in laboratory animal and human offspring, exposed in utero to several COX inhibitors. Immunoexpression of constitutive (COX-1) and inducible (COX-2) cyclooxygenase isoforms was evaluated in various articular structures of untreated and unfamiliar 21-day-old male rat foetuses. Both COX isoforms were detected in the articular cartilage and joint capsule, as well as in the intra-articular disc of the temporomandibular joint and meniscus of the knee joint. COX-1 immunostaining was revealed in the anterior and posterior cruciate ligament of the knee joint and the labrum of the hip and shoulder, whereas COX-2 immunoreactivity in those structures was not found. It could be concluded that both constitutive and inducible COX isoforms are physiologically expressed in various structures of synovial joints in rat foetuses at the end of prenatal development. (Folia Morphol 2009; 68, 2: 59–64)

Key words: articular surface, articular disc, joint capsule, cyclooxygenase, ligament, labrum, meniscus, synovial joint

INTRODUCTION

Synovial joints are the most common type of skeletal connections in mammals. They are formed by three distinguishing structures: articular surfaces, the joint capsule, and the cavity. The surfaces are covered with the articular cartilage, while internal aspect of the capsule is lined with the synovial membrane, which secretes synovial fluid. The external, fibrous layer of the capsule blends with the periosteum or perichondrium of the adjoining bones depending on their developmental stage. Furthermore, each joint is supported by accessory elements such as disc, labrum, ligament, or meniscus. In some species, the intra-articular tendon (e.g. the tendon of the long head of the biceps brachia in humans) could also be seen [33]. All these mesenchyma-derived structures develop during the early stages of foetal life [22, 23, 32], and their formation is regulated by various factors including bone morphogenetic proteins 2 and 4 (BMP2, BMP4), connexin 32 and 43,

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transforming growth factor β1 (TGFβ1), growth differentiation factor 5 and 6 (GDF5, GDF6), as well as such important factors as noggin, chordion, Wnt14, β-catenin, Cux-1, Barx1, and Wisp [1]. In recently published papers [3, 4, 10, 29] a high expression of cyclooxygenase (COX) isoforms in the foetal cartilaginous elements was also revealed on gene and/ /or protein levels. On the other hand, various joint and skeletal abnormalities were seen in laboratory animal and human offspring exposed in utero to several COX inhibitors [2, 24].

Unlike the well-identified characteristic of COX isoform expression in the epiphyseal cartilage, their localization in other articular structures is still unknown. The aim of the study was to evaluate the immunoexpression of constitutive and inducible COX isoforms in distinguishing and selected accessory structures of the synovial joints in untreated rat foetuses.

MATERIAL AND METHODS

The study was performed on material collected from untreated control groups during previous teratological investigations that were handled according to international teratological procedures and were fully approved by the Local Bioethical Committee.

Sexually mature albino Wistar CRL:(WI)WUBR rats, obtained from a commercial breeder (Warsaw--Rembertow, Poland), were used. The rats were acclimatised for at least 2 weeks, and housed and maintained in an animal care facility. On mating days, the females weighing 200–250 g were placed in cages with the males (5:2) for approximately 14 hours. The following morning, a vaginal smear was performed to determine if copulation had occurred. The day when sperm was found was designated gestation day 1 (GD1). Sperm positive females were randomly taken for the study group. No xenobiotics were administered during the study.

On gestation day 21, twelve randomly selected pregnant females were sacrificed. The foetuses were delivered by caesarean section, sexed, and examined routinely [6]. One randomly selected male foetus from each litter was fixed in 10% buffered formalin, embedded in paraffin (without decalcification), sectioned routinely in sagittal plates at 5 μm, and then stained routinely with haematoxylin and eosin. When well-developed articular structures were found, the following 4 μm sections were taken for immunohistochemistry.

Monoclonal mouse anti-human antibodies against COX-1 and COX-2 (clones 12E12 and 4H12, respectively; Novocastra; Newcastle, UK) and DakoEnvision^{+TM}/HRP kit (DakoCytomation, Glostrup, Denmark) were applied. The details of the method are described elsewhere [8, 9]. All slides were evaluated using a light microscope (Olympus BX45; Japan).

RESULTS

In the articular cartilages of all the studied joints a positive immunostaining for COX-1 and COX-2 was revealed in all zones (Fig. 1A–F). However, positively stained chondrocytes were found mostly in the deep and external superficial ones. The staining intensity was stronger, especially for COX-1, in the most peripheral part of the cartilage, close to the joint capsule. In all the cases the staining pattern for COX-1 was cytoplasmic, whereas for COX-2 it was nuclear. Distinct COX-1 immunostaining was also seen in chondrocytes in the labrum of shoulder (Fig. 1A) and hip joints.

In all the analyzed joints, strong to moderate COX-1 staining was found in the joint capsule as well (Fig. 1A, C, E). The reaction was seen in the majority of synoviocytes of both types, as well as in fibroblast-like cells of the external aspect of the capsule. COX-2 immunostaining was also revealed in the same types of cells; however, staining intensity was much more variable than for COX-1 (Fig. 1B, D, F). Both nuclear and cytoplasmic COX-2 staining was noted.

In the medial and lateral menisci of the knee joints of all the foetuses, both COX-1 and COX-2 immunostaining was strong and was observed in most chondrocyte-like cells, except those externally situated in close vicinity to the joint cavity (Fig. 1C, D). Positive cells were especially numerous in the most peripheral part of the menisci adjacent to the joint capsule. Both nuclear and cytoplasmic COX-2 staining was seen (Fig. 1D).

In all studied intra-articular discs of temporomandibular joints, COX-1 immunostaining was mild and confined to scattered chondrocyte-like cells (Fig. 1E). COX-2 reaction was even weaker but similarly located (Fig. 1F).

In all the cases, intracapsular ligaments of the knee joints revealed very weak COX-1 immunoreactivity in single fibroblast-like cells (Fig. 1G). COX-2 reaction was negative (Fig. 1H) in all but two cases which showed weakly positive cells in both anterior and posterior cruciate ligaments.

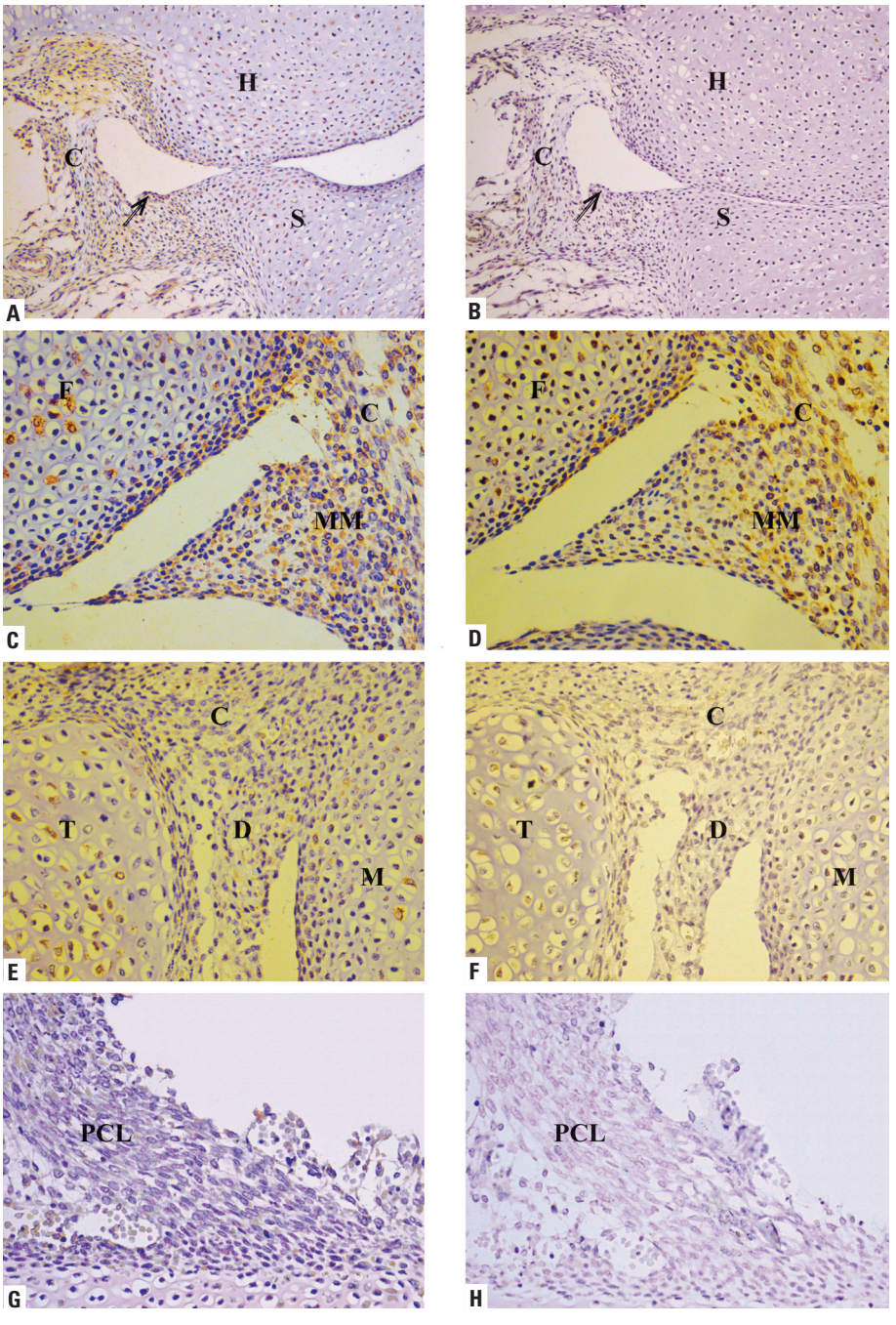

Figure 1. Immunoexpression for COX-1 (**A, C, E, G**) and COX-2 (**B, D, F, H**) in structures forming the shoulder (A, B), knee (C, D, G, H) and temporomandibular (E, F) joints. C — joint capsule, D — articular disc, F — femur, H — humerus, MM — medial meniscus, PCL — posterior cruciate ligament, S — scapula, T — temporal bone, M — mandible, the arrow points the glenoidal labrum; objective magnification A, B: 10 ×; C–H: 20 ×).

DISCUSSION

The presented data indicate physiological expression of the constitutive and inducible COX isoforms in distinguishing and accessory structures of the synovial joints in foetuses of Wistar rats. Using the same methodology, expression of both isoforms were previously revealed in kidneys [7], lungs [5], and the pancreas [8], as well as various parts of the digestive tract [9], obtained from foetal and/or juvenile rats. High expression of the inducible COX isoform was also reported by other authors in the offspring of rats and other laboratory animals at the end of the pregnancy and in the first day of postnatal life [29, 31]. According to Stanfield et al. [29]

and Streck et al. [31], expression of COX-1 is detected in foetal tissues throughout the pregnancy, while COX-2 is revealed mostly at the beginning and at the late gestational stage. It is postulated that high expression of inducible isoform is a consequence of significant changes in maternal hormonal status in those critical developmental periods [11].

In our previous study [10] cytoplasmic immunohistochemical reaction for COX-1 was localized in the epiphyseal and articular chondrocytes of long bones, as well as in cells of the periosteum and perichondrium. A similar localization was revealed for COX-2; however, as in the current study, both nuclear and cytoplasmatic reactions were observed. It is worth mentioning that immunoreactivity and gene expression of both COX isoforms were unchanged even in animals exposed to high doses of non-selective COX inhibitors that cased foetotoxicity.

Unlike our results, Brochhausen et al. [4] observed COX-2 immunoexpression only in proliferative, prehypertrophic and hypertrophic zones of the tibial epiphyseal cartilage in four-week-old Sprague-Dawley rats. Much weaker staining was found in chondrocytes of the reserve zone. Chondrocytes of the articular cartilage showed high expression only in transitional and deep zones, while no staining was found in the apical zone. However, similar to our results, a homogeneous COX-1 expression was revealed in both epiphyseal and articular cartilages. Based on in vivo and in vitro observations, authors have postulated that strong expression of COX-2 in the proliferative zone of the epiphyseal plate and lack of staining in the resting zone suggests a COX--2-mediated pathway in autocrine and/or paracrine regulation of chondrocyte proliferation. Such a role of COX-2 is supported by some reports on pro-proliferative activity of prostaglandin E2 (PGE2), a cyclooxygenase dependent eicosanoid, in cultured epiphyseal chondrocytes [3, 21, 26].

There is a limited number of reports, based mostly on samples taken from mature individuals, on physiological expression of constitutive and inducible isoforms in the joint capsule. Yashida et al. [34] observed COX-1 immunostaining in the same cells of the synovial layer of normal temporomandibular joints and joints with internal derangement. A similar intensity and localization of staining was noted for COX-2 in normal samples. However, a diffuse expression pattern of COX-2, observed mostly on the surface layer of the hypertrophic synovial membrane, was revealed in samples taken from "sick" people [35]. Both COX isoforms were also recently detected in unstimulated human synovial cells of the knee joint [27]. Similar to other studies, the authors reported that COX-2 expression was increased in cases of pathological joint changes. They also confirmed the pioneering observation of Crofford et al. [12] that COX-1 expression could be alerted by joint injury. An overexpression of COX-2 found in synoviocytes and fibroblast-like synovial cells — obtained from joint capsule and synovial fluid, respectively — is typical for rheumatoid arthritis and other rheumatoid diseases [13, 30]. According to Lee at al. [19], in rheumatoid arthritis and osteoarthritis an intense COX-1 staining is seen in the synovial lining cells, while slight and moderate in stromal fibroblast-like cells and vascular endothelial cells. However, the most intense immunoreactivity for COX-2 was found in inflammatory cells. It should also be noted that reports regarding lack of *COX-2* expression in the mature unstimulated synovium also exist [14, 20].

Except for our previous article [10], no information about COX isoform expression in foetal synovial joint accessory structures was found in the available literature. However, *COX-2* expression was physiologically detected in the medial collateral and anterior cruciate ligaments of the knee joint and in both menisci in female rabbits [15–18]. A significant 160.5% increase of COX-2 mRNA levels in both studied ligaments was reported in pregnant dams at the end pregnancy when compared with non-pregnant females [15]. Transcripts for COX-2 were 61% and 50% of control values in the medial menisci of primigravida (first-time pregnant immature animals) and third-time multiparous animals, respectively, while COX-2 mRNA levels were 150% and 188% of control values in the lateral menisci of primigravida and multiparous dams, respectively [18]. Moreover, a 170% elevation of the gene expression was found in animals with ligament injury performed eight weeks before insemination [17]. Furthermore, Sairyo et al. [25] reported a high expression of *COX-2* and other inflammation-related genes (i.e., TNF-α, IL-1, IL-6, IL-8, and IL-15) in the hypertrophied lumbar ligamentum flavum in the spinal canal stenosis in adult humans. A weak correlation between COX-2 expression and ligament thickness was also found. However, a positive COX-2 immunoexpression was revealed mostly in vascular endothelial cells and chondrocyte-like cells, while fibroblasts did not show any immunoreactivity. Based on those observations, authors have postulated that endothelial cells are the main source of the COX-2 that is responsible for

the scarring and fibrosis of the ligament that led to its secondary hypertrophy. A similar pathomechanism of ligament damage, however without COX expression evaluation, was previously reported for the medial collateral ligaments of the knee joints in rabbits [28].

There are only two papers [34, 35] that explain COX expression in the intra-articular discs. COX-1 was physiologically detected only in fibroblast-like cells and some endothelial cells in the mature human disc of the temporomandibular joint. Such cells were also stained, together with chondrocyte-like cells, in discs with internal derangement; however, a large number of new capillaries were revealed [34]. The same characteristic was found for COX-2 [35].

CONCLUSIONS

Constitutive and inducible cyclooxygenase isoforms are physiologically detected in synovial joints of Wistar CRL:(WI)WUBR rat foetuses.

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