University of Medicine and Pharmacy of Tîrgu Mureş
Doctoral School

PhD Thesis abstract

Study of biochemical factors with regulatory role in the metabolism of cartilage tissue

Scientific Supervisor: **Prof. Dr. Daniela-Lucia Muntean**

PhD Candidate: **Csifo (Vajda) Enikő**

Articular disorders are characterized by their complex pathogenicity, diagnostic, and treatment pitfalls. Definition of osteoarthritic pathological events encounter difficulties, since the disease involves the participation of three major elements: cartilage, synovium, and subchondral bone. Metabolic alterations of these anatomical components influence each other, resulting in sophisticated interactions.

Diagnosis is established by radiography, a technique which does not detect early stages of the disease. In this context, finding specific biomarkers of osteoarthritis, that reflect initial stages, is a real challenge in research in the field. Definition, and clinical use of such representative markers would be an important step in the development of new therapeutic strategies.

Objectives: the primary objective of the thesis is the study of etiopathogenic factors in human knee osteoarthritis, and correlation analysis of these parameters with disease severity in order to determine their potential diagnostic, and prognostic role in osteoarthritis.

The second part of the study aims to achieve an experimental model of osteoarthritis in rats. In the model created, the cytokine profile, and its variations during, and after anti-inflammatory treatment with meloxicam were analysed. The effect of meloxicam on cartilage degradation was also studied. A HPLC method has been developed for the determination of meloxicam in rat plasma, serving to study the pharmacokinetics of meloxicam, and to establish therapeutic schemes for the treatment of iodoacetate generated rat osteoarthritis.

The general part of the thesis presents the current state of the art regarding osteoarthritis: pathology, biomarkers, experimental models, treatment, use of meloxicam in the treatment of this disease.

The experimental part consists of appropriate studies of the proposed objectives.

The first part of the research focuses on the study of metabolic aspects in etiopathogenesis of human knee osteoarthritis by analysing relevant biochemical parameters of cartilage degradation (enzyme MMP-8, cytokines TNF-α, and IL-6) or subchondral bone metabolism (OPG-osteoprotegerin, OC-osteocalcin), and exploring their correlation with disease severity (assessed by IKDC score).

Neutrophil collagenase MMP-8 showed significantly increased concentrations in serum, and synovial fluid of patients with knee osteoarthritis or meniscus injuries compared to the healthy group. A statistically significant negative correlation was observed between synovial levels of the enzyme, and IKDC score (high MMP-8 values were associated with lower IKDC scores - advanced disease). Therefore, it was concluded that synovial MMP-8 plays an important role in pathogenesis of osteoarthritis, and this parameter might be a diagnostic, and prognostic biomarker in osteoarthritis.

TNF-α concentrations were characterized by higher values in groups with joint disease than in control group, and the levels of this cytokine between the two biological fluids showed a significant positive correlation. The

association described between serum cytokine concentrations, and IKDC score reveals the importance of TNF-α in the development of osteoarthritis.

Cytokine IL-6 showed increased concentrations particularly in synovial fluid of patients with osteoarthritis, but it was not associated with IKDC severity score.

Although it is known, that inflammatory cytokines trigger the production of degradative enzymes, correlations between the concentrations of cytokines TNF-α, IL-6, and MMP-8 enzyme were not found in this study.

Study of specific markers of bone metabolism showed higher levels of osteocalcin in early stages of osteoarthritis, suggesting an increased bone remodelling, as a possible compensatory mechanism to prevent cartilage destabilization. In advanced stages of the disease bone metabolism decreases, marked by lower values of OC.

OPG showed significantly higher local concentrations than the systemic ones, highlighting its implication in knee osteoarthritis. The negative correlation described between synovial MMP-8, and OPG in patients with small IKDC score leads to the idea that in advanced stages of osteoarthritis cartilage degradation (increased MMP-8) is associated with bone resorption (low OPG).

The second experimental part consisted of a comprehensive study on the effect of meloxicam anti-inflammatory treatment on metabolic, and etiopathogenic factors in a rat osteoarthritis model.

Development, and partial validation of a HPLC method for the determination of meloxicam in rat plasma were achieved. Reverse phase HPLC method is based on the use of internal standard (piroxicam), it is simple, fast (8 minutes), economical, and it is characterized by adequate validation parameters (specificity, linearity, accuracy, precision). Therefore, that it is suitable for quantification of meloxicam in rat plasma during pharmacokinetic determinations.

Pharmacokinetic study was carried out in order to determine the meloxicam treatment schedule in rats, generating the following results: administration of a single dose of 1 mg/kg bw lead to maximum plasma concentrations at 8 hours, followed by a slow elimination with long half-life time (15 h). Based on these facts it was concluded that meloxicam will be administered once a day, estimating the time required to achieve stationary concentration to 5 days. Treatment period was set to 4 weeks.

Osteoarthritic model was performed by intra-articular administration of a single dose of 4 mg sodium monoiodo-acetate to Wistar rats. The success of the experimental model was proved by histological examination of cartilage tissue samples, biochemical analysis of collagen type II degradation product (CTX-II), and cytokine profile (IL-6, TNF-α, and IL-10) characterization.

In this model of osteoarthritis, cytokine IL-6 variations were the most relevant, TNF-α, and IL-10 showing only minor changes.

Study of the effect of meloxicam treatment in osteoarthritis led to the conclusion that different doses (0,2 mg/kg bw, and 1 mg/kg bw) can exert divergent influences on the IL-6 serum levels. Suppression observed following administration of low dose was associated with an increase in IL-6 concentrations (potential stimulation) during treatment with high dose.

The significantly low histological score obtained in the group treated with small dose of meloxicam, respectively an important downward trend in the high-dose group, indicate the efficacy of treatment with both doses of the anti-inflammatory drug.

Biochemically, a more expressed decrease of C-terminal telopeptide of type II collagen suggests a better efficacy of high dose. Meloxicam has a beneficial metabolic effect on collagen type II, by supressing its catabolism, probably due to inhibition of the enzymes responsible for the degradation (matrix metalloproteinases).

Keywords: osteoarthritis, IKDC score, pro-, and anti-inflammatory cytokines, matrix metalloproteinase-8, rat experimental model, meloxicam, histological score, C-terminal telopeptide of type II collagen (CTX-II).