Aggrecanase Activity and Proteoglycan Release from Human Articular Cartilage as modulated by Tetracyclines

+1Steinmeyer, J; ²Kordelle, J; ³Stuerz, H
¹University Hospital Giessen & Marburg, Giessen, Germany
Senior author  juergen.steinmeyer@ortho.med.uni-giessen.de

ABSTRACT INTRODUCTION:
The degradation of aggrecan during osteoarthritis (OA) is catalyzed by increased activities of matrix metalloproteinases and members of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motif) family. ADAMTS4 and ADAMTS5, also called aggrecanase-1 and aggrecanase-2, have been identified in cartilage and were shown to cleave aggrecan at five different sites both in vitro and in vivo. Four cleavage sites are located between the aggrecan globular domains G2 and G3 while one cleavage occurs between the globular domain G1 and G2 (Glu737 – Ala734).

Identification of agents able to reduce the activity of proteoglycans has long been a therapeutic goal for inhibiting or slowing down the degradation of cartilage. Several studies have implicated that tetracyclines, in addition to their antimicrobial properties, can slow down the progression of cartilage damage both in animal models of OA as well as in humans (1,2). In search for the underlying mechanisms we previously reported that minocycline and doxycycline can not only inhibit the activity of MMP-1 but also the expression of MMP-1, MMP-3 and iNOS by bovine articular chondrocytes (3,4). In this line, the present in vitro-study was designed to examine for the first time whether tetracyclines also possess an inhibitory potential on the activities of ADAMTS4 and ADAMTS5 and, 2. can thus prevent interleukin-1 (IL-1) inducible proteoglycan loss from human articular cartilage.

METHODS:
1. Determination of aggrecanase activity: The activity of recombinant human (rh) ADAMTS4 and rhADAMTS5 were determined using rh aggrecan interglobular domain (rhAggrecan-IGD; mdbioscience) as a substrate. Briefly, after proteolytic cleavage of the rhAggrecan-IGD by 30 nM rhADAMTS4 (F213-A579 from mdbioscience or F213-R695 from R&D) or rhADAMTS5 (S262-F632 from R&D), an aggrecan peptide with the N-terminal sequence ARGGSVI was released, which was then quantified using two monoclonal anti-peptide antibodies. The %inhibition was calculated from a standard curve established with untreated controls (N=3).

2. Proteoglycan loss and viability of treated cartilage explants: Full-thickness cartilage explants of the lateral compartment of the femoral condyle. Each experimental condition was repeated five times using explants from different patients (N=6).

RESULTS:
Doxycycline, minocycline and tetracycline dose-dependently inhibited the activity of rhADAMTS4 (Fig. 1) and rhADAMTS5 (data not shown).

In order to determine whether the in vitro inhibition of aggrecanases can also be demonstrated in vivo, human cartilage explants were cultured for 11 days in nutrient media (Fig. 2). Here, doxycycline, minocycline and tetracycline, even when tested at a high concentration of 100 µM, did not inhibit the IL-1-induced increase in release of proteoglycan loss from cartilage explants into the nutrient media (Fig. 2). This lack of inhibitory potential was seen both in mild and moderately (data not shown) affected human OA cartilage explants.

DISCUSSION:
Our investigation show for the first time that tetracyclines possess an inhibitory potential on the activities of both rhADAMTS4 and rhADAMTS5. However, a marked inhibition of these metalloproteinases was only observed at concentrations lying several times over their therapeutic plasma levels reported to be in the range of 2-7 µM. Furthermore, no inhibitory effect of tetracyclines on any proteoglycanolytic activities within IL-1-treated human cartilage explants could be demonstrated even when tested at a very high concentrations of 100 µM. This might be due to an impaired diffusion and/or enhanced binding of these drugs to cartilage extracellular matrix. In conclusions, tetracyclines appear to have no inhibitory potential to inhibit any aggreganolytic activities within mild or moderately affected OA cartilage tissue at therapeutic achievable plasma levels.

REFERENCES:

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