Curcuma extracts and curcumin reduce proinflammatory cytokines and matrix degrading enzymes in human intervertebral disc cells by possibly influencing TLR2 activity

1Quero, L; 2Klawitter, M; 3Klaasen, J; 4Nerlich, A; 5Boos, N; 6Wuertz, K
1University of Zurich, CABMM, Zurich, Switzerland, 2University Hospital Balgrist, Zurich, Switzerland, 3University Hospital Munich-Bogenhausen, Munich, Germany
karin.wuertz@cabmm.uzh.ch

INTRODUCTION:
Although there is certain evidence in the literature that discogenic back pain has a correlation to increased levels of proinflammatory cytokines (e.g. IL-1β, IL-6, IL-8, TNF-α), current treatment does not take these molecular events into account. At the moment, therapeutic options are limited to conservative treatment (e.g. physiotherapy), pain medication (often cortisone) and surgical interventions.

Research in the most recent past has concentrated on the development of minimal-invasive, yet effective new treatment options, covering approaches from cell therapy and gene therapy to anti-inflammatory substances for intradiscal injection. The range of potential anti-inflammatory substances for intradiscal injection is large and choosing the right candidates to work with is often difficult.

Curcuma has been used as a drug in the traditional Indian medicine for centuries and has more recently been demonstrated to be anti-inflammatory, with its effect probably being related to a class of substances called curcuminoids.

The aim of this study was to analyze the effects of curcuma extracts as well as of selected components of curcuma on IL-1β mediated cellular responses of human intervertebral disc (IVD) cells in vitro. Additionally, its mechanism of action was investigated by testing for involvement of Toll-like receptor (TLR) 2, which is known for its frequent regulation in inflammatory diseases.

METHODS:

General experimental design
In order to mimic the situation during painful degenerative disc disease, human IVD cells were pretreated with recombinant IL-1β, thus increasing the levels of proinflammatory cytokines and matrix degrading enzymes. Therafter, different solvents (DMSO, EtOH) were used to prepare curcuma extracts and tested for their ability to reduce catabolic gene expression at different time points.

The presumably most abundant bioactive substance in the most potent extract was chosen based on structure-based solubility and information in the literature (in this study: curcumin) and tested in the same setting, using the time point that showed best results in the experiments using curcuma extracts. A mechanistic investigation, looking at involvement of TLR2 pathway, was performed for this substance as well.

Non-toxicity of all used concentrations was determined beforehand.

Cytotoxicity
Different concentrations of the curcuma extracts (DMSO, EtOH) as well as of curcumin were tested for their cytotoxicity at 6, 18 and 30 hours, using the MTT assay (n=3).

Cell culture/stimulation
Human IVD cells isolated from biopsies that were excised from patients undergoing spinal surgery were expanded up to P3 using standard cell culture techniques. Ethical approval and informed consent was obtained. Expanded IVD cells were cultured in serum-free medium for 2 hours, followed by prestimulation with recombinant IL-1β (5 ng/ml, 2 hours) before treatment with curcuma extracts for 6, 18 or 30 hours (n=5).

Curcuma extracts were prepared by dispersing 320 mg/ml curcuma (Cormick®) in DMSO, followed by (sequential) extraction in EtOH.

RESULTS:

Cytotoxicity:
Curcuma DMSO extracts revealed cytotoxic effects for all time points if concentrations higher than 500 mg/ml were used while no toxic effects could be observed for the EtOH extract up to 1000 mg/ml. Curcumin showed major cytotoxicity at 100 µM. The chosen concentrations of 100 µg/ml (curuma) and 5, 10 and 20 µM (curcumin) were not toxic. DMSO and EtOH did not impair cell viability at the used concentrations.

Gene expression
While the curcuma EtOH extract showed only minor or no anti-inflammatory and anti-catabolic potential, the DMSO extract was able to significantly reduce expression of IL-1β, IL-6 (Fig. 1), MMP1, MMP3, MMP13, with best effects after 6 hours. For very few genes, long incubation times resulted in an increase in gene expression (IL-8, MMP1, IL-1β). Curcumin, which is the most abundant DMSO-soluble bioactive substance of curcuma, showed very similar effects compared to the DMSO extract after 6 hours, with a significant reduction of IL-1β, IL-6 (Fig. 1), IL-8, MMP1, MMP3, MMP13, mostly at the highest concentration. Curcumin as well as curcuma caused an up-regulation of TNF-α.

DISCUSSION:

Our results clearly show that the curcuma DMSO extract as well as the DMSO-soluble compound curcumin can effectively reduce levels of major proinflammatory cytokines and matrix degrading enzymes. However, we noticed an increase in some genes after 30 hours as well as a strong increase in TNF-α gene expression at all time points. Analyzing whether mRNA are transmitted to the protein level is currently ongoing. Ideally, inhibition of catabolic genes would be confirmed on the protein level whereas the increase in TNF-α would be limited to the gene expression level.

Effects were comparable between the curcuma DMSO extract and curcumin, with slightly more uniform results for the single substance. We therefore conclude that the major bioactive substance in curcuma extracts (DMSO) acting on human IVD cells is curcumin.

Anti-inflammatory biological products such as curcumin represent an attractive and safe alternative for the treatment of inflammatory disorders. The results of this in vitro study provide sufficient rationale to investigate the effects of curcumin in an in vivo animal model of disc degeneration and discogenic pain. These biodrugs, if brought into the clinical setting, might hence have the potential to postpone or maybe even prevent surgical interventions by effectively reducing pain.

This study was supported by AOSPINE (SRN 02/103, AOSBRC-07-03).