The red wine component resveratrol shows promising potential for the treatment of back pain in vitro and in vivo
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INTRODUCTION:
Discogenic back pain is a major health problem with limited treatment options. Conservative treatment is often unable to provide sufficient pain relief, while surgical treatment options have high complication rates. Although the causes of discogenic back pain are not totally understood, it is believed that increased levels of proinflammatory cytokines (that are produced by disc cells) diffuse to the outer parts of the disc where they have the potential to irritate nerves. Therefore, injectable anti-inflammatory substances could serve as new and useful minimal-invasive treatment options with reduced risks compared to classical surgical interventions.

The phytoalexin resveratrol, a natural polyphenol found in red wine, was not only shown to be anti-mutagenic and anti-carcinogenic, but also anti-inflammatory in various cell types. In bovine intervertebral disc (IVD) cells, resveratrol was able to increase proteoglycan synthesis. However, it is unclear whether resveratrol also has an anti-inflammatory effect on human IVD cells, thus representing a potential therapeutic agent for patients with low back pain.

Therefore, the aim of this study was to analyze the anti-inflammatory and anti-catabolic potential of resveratrol in an in vitro IVD cell culture model as well as in an in vivo rodent animal model. In addition, this study aimed to identify the molecular mechanisms underlying the observed effects.

METHODS:

In vitro cell culture study:
IVD tissue was received from patients undergoing spinal surgery after informed consent was obtained according to the requirements of the local ethics committee. Expanded IVD cells in P2 to P3 were serum-starved for 2 hours, followed by prestimulation with recombinant IL-1β (5 ng/ml, 2 hours) before treatment with resveratrol (5 µM or 50 µM, 18 hours).

The effects of resveratrol on IL-1β mediated cellular responses were investigated on the gene expression level of IL-1β, IL-6, IL-8, TNF-α, MMP1, MMP3 and MMP13 using real-time RT-PCR (ΔΔCT-method, reagents from Applied Biosystems®). Statistical analysis was performed using a One-Sample T-test with a significance level of p < 0.05.

In vitro pathway study:
Electrophoretic mobility shift assay (EMSA) for NF-κB (Panomics®) as well as immunofluorescence (IF) staining for p65 (subunit of NF-κB) (antibody from Santa Cruz®) was performed on cells treated with 50 µM resveratrol and 5 ng/ml IL-1β for 60 minutes.

In vitro animal study:

Twelve Sprague-Dawley rats were divided into two study groups:

<table>
<thead>
<tr>
<th>Group Name</th>
<th>Treatment</th>
<th>Nr. of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP</td>
<td>Nucleus pulposus+Saline</td>
<td>6</td>
</tr>
<tr>
<td>NP+Resv</td>
<td>Nucleus pulposus+Resveratrol 50 µM</td>
<td>6</td>
</tr>
</tbody>
</table>

The left L5 nerve root and dorsal root ganglion were surgically exposed and treated with nucleus pulposus tissue (approximately 0.1 mg) that was harvested from a coccyeal vertebral disc. Resveratrol (50 µM) was added in one group and each animal was compared to its preoperative behaviour using von Frey filament tests (statistics: ANOVA, p < 0.05).

RESULTS:
In vitro cell culture study:
At the lower concentration (5 µM), resveratrol exhibited minor or no effects. However, at the higher concentration (50 µM), resveratrol caused a significant inhibition of the IL-1β mediated inflammatory and catabolic response. After setting IL-1β-induced gene expression to 100% for each gene, resveratrol was able to reduce expression of IL-6 (82%), IL-8 (68%), MMP1 (53%), MMP3 (52%) and MMP13 (48%) (Fig. 1). Resveratrol had no effect on IL-1β and TNF-α expression. Protein levels are currently being investigated.

In vitro pathway study:
Both, EMSA and IF staining indicated that resveratrol didn’t exhibit its anti-inflammatory and anti-catabolic effects via the NF-κB pathway. With both techniques, we didn’t find any indication that resveratrol was able to reverse the IL-1β induced nuclear translocation of p65 (= NF-κB activation) (Fig. 2).

In vitro animal study:
Mechanical withdrawal thresholds of both groups were reduced compared to preoperatively, with a less pronounced effect in the resveratrol treated group (NP+Resv) compared to the NP group. This effect was present up to day 14, but not anymore at day 21 (Fig. 3).

DISCUSSION:
In this study, resveratrol at a concentration of 50 µM was able to reduce increased levels of proinflammatory cytokines and matrix degrading enzymes on the gene expression level without acting on the transcription factor NF-κB (alternative pathways are currently being investigated). As these proteins are known to be elevated in degenerated painful discs, the pain-reducing effect of resveratrol was tested in an animal model. Results indicate that resveratrol has the potential to reduce pain behaviour in rodents, however, this effect could only be observed up to day 14. In a future study, the possibility to prolong the pain-reducing effect of resveratrol by using a controlled release system will be tested in the established animal model.

Based on the results so far, resveratrol may be of great benefit for patients with discogenic back pain as it has the potential to intervene in the pain-provoking mechanism in a minimal-invasive manner, thus preventing or at least postponing surgical interventions.

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