Resveratrol, a component in red wine: A possible new therapeutic agent for low back pain?

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INTRODUCTION:
Discogenic back pain is a major health problem with limited treatment options. Conservative treatment does often not 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, agents that would prevent a proinflammatory situation upon injection into the disc 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 grape skins and red wines, was not only shown to be antimutagenic and anticarcinogenic, but also anti-inflammatory in various cell types. In bovine intervertebral disc cells, resveratrol was able to increase proteoglycan synthesis\(^1\). However, it is unclear whether resveratrol has also an anti-inflammatory effect on human intervertebral disc cells, thus presenting a potential therapeutic agent for patients with low back pain. Therefore, the aim of this study was to analyze whether resveratrol can reduce the levels of proinflammatory cytokines. In addition, effects on the levels of matrix degrading enzymes were also investigated as a reduction would be clinically beneficial as well.

METHODS:
Intervertebral disc tissue was received from patients undergoing spinal surgery (n=4) after informed consent was obtained. The study was approved by the ethics committee.

Cells were isolated by enzymatic digestion and expanded in monolayer up to passage 3. Before stimulation, cells were rendered serum-free for 2 hours, then prestimulated with recombinant IL-1\(\beta\) (5 ng/ml) to increase levels of proinflammatory cytokines and matrix degrading enzymes. After 2 hours, resveratrol (5 \(\mu\)M or 50 \(\mu\)M) was additionally added to all but one flask and cells were incubated for 18 hours before isolation of RNA.

Using real-time RT-PCR, gene expression in cells that were stimulated with resveratrol (and preincubated with IL-1\(\beta\)) was compared to cells only preincubated with IL-1\(\beta\). Additionally, completely untreated cells were analyzed to verify that preincubation with IL-1\(\beta\) induced expression of chosen genes. Following genes were analyzed: IL-1\(\beta\), IL-6, IL-8 (proinflammatory cytokines) and MMP1, MMP3, MMP13, (matrix degrading enzymes). Changes in gene expression were quantified using the \(\Delta\Delta Ct\)-method (data shown as Mean ± SEM) and statistically evaluated (resveratrol treatment after prestimulation versus IL-1\(\beta\) treatment alone) using a One-Way ANOVA with a significance level of \(p<0.05\).

RESULTS:

Prestimulation with IL-1\(\beta\):
Prestimulation with IL-1\(\beta\) caused increased expression of the genes analyzed in this study.

Treatment with resveratrol:
At the lower concentration (5 \(\mu\)M), resveratrol exhibited minor or no effects. However, at the higher concentration (50 \(\mu\)M), resveratrol caused a significant reduction in several of the relevant proinflammatory cytokines and matrix degrading enzymes. In detail, the following effects could be observed:

After prestimulation with IL-1\(\beta\), IL-6 expression was increased 38 fold compared to untreated conditions (ctrl) and significantly reduced to 9 fold by treatment with 50 \(\mu\)M resveratrol (see Figure 1). For IL-8, a 13 fold increase could be observed that was reduced to 5 fold (\(p<0.05\)). No effects of resveratrol could be observed for IL-1\(\alpha\) expression.

After prestimulation with IL-1\(\beta\), MMP1 expression was increased 49 fold compared to untreated conditions (ctrl) and significantly reduced to 12 fold by treatment with 50 \(\mu\)M resveratrol (see Figure 2). For MMP3, a 60 fold increase could be observed that was reduced to 17 fold (\(p<0.05\)). For MMP13, a 13 fold increase could be observed that was reduced to 6 fold (\(p<0.05\)).

DISCUSSION:
In this in vitro cell culture study, resveratrol at a concentration of 50 \(\mu\)M was able to reduce increased levels of proinflammatory cytokines and matrix degrading enzymes that are known to be present in degenerated painful discs. As diffusion patterns and bioavailability in the disc in vivo are unclear to date, animal studies will be necessary to verify the observed in vitro effects in vivo.

Nevertheless, treatment with 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 that involve higher risks.

REFERENCES:
1) Li et al, Spine 2008 Nov 15;33(24):2586-95

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