THE EFFECTS OF CANNABINOIDS ON CHONDROCYTE GLYCAN BREAKDOWN
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Aim: The aim of this study was to determine whether cannabinoids have direct effects on chondrocyte nitric oxide production and cartilage proteoglycan breakdown which may be chondroprotective.

Methods: The effects of cannabinoids anandamide and R-(+-) win 55, 212-2 (win-55), both agonists of cannabinoid receptors CB1 and CB2, were studied on chondrocyte nitric oxide (NO) production and cartilage proteoglycan breakdown. Bovine articular chondrocytes were used to study NO production. These were prepared by sequential enzymic digestion and plated directly into 24-well plates at high density (2 x 10^5/cm^2). After 5 days they were incubated with win-55 (0.01 - 10 µM) or anandamide (1-100 µM) in the presence or absence of interleukin 1α (IL-1α) (100 U/ml) for 48 h. NO production was determined using cultured bovine nasal cartilage explants, unstimulated or stimulated to resorb with IL-1α. Nitric oxide production was determined using cultured bovine nasal cartilage explants, unstimulated or stimulated to resorb with IL-1α (500U/ml) in the presence or absence of win-55 (10-100 µM).

Results: Both anandamide and win-55 inhibited IL-1 stimulated NO production, win-55 being the more potent inhibitor producing inhibition at 1-10 µM compared with 50-100 µM for anandamide. Preliminary studies have also shown inhibition of IL-1 stimulated cartilage proteoglycan breakdown by win-55 at 10 and 100 µM.

Conclusions: Our studies indicate that cannabinoids may be chondroprotective, possibly in part by inhibition of NO production. Other workers have previously shown anti-arthritic effects of cannabinoids in animal models of arthritis which our observations suggest could be mediated by effects on chondrocyte metabolism.

PA69
MITOCHONDRIAL RESPIRATORY ACTIVITY IS ALTERED IN OA HUMAN ARTICULAR CHONDROCYTES: A NEW APPROACH TO UNDERSTAND OA
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Introduction: Osteoarthritis (OA) is associated with matrix degradation, nitric oxide (NO) production and chondrocyte death by apoptosis. Increasing lines of evidence suggest a key role for mitochondria in apoptosis and ageing.

Aims: To analyze the biochemical parameters to define the Activity of Mitochondrial Respiratory Chain Complexes (AMRCC) in human chondrocytes from normal and OA cartilage. To study the aged-related changes on AMRCC and to examine the effect of NO on AMRCC.

Materials and Methods: Nineteen normal and 53 OA cartilages were used (ages: 40-90 years). The activities of NADH CoQ oxidoreductase (Complex I), succinate dehydrogenase (Complex II), Doccylubiquinol: ferricytochrome c oxidoreductase (Complex III), cytochrome c oxidase (Complex IV) and citrate synthase were measured using Beckman DU-650 spectrophotometer to monitor the reduction of ferricyanide (Complex I), 2,6-dichlorophenolindophenol (Complex II), cytochrome c (Complex III), oxidation of cytochrome c (Complex IV) and appearance of free coenzyme A (citrate synthase). Variation on mitochondrial membrane potential was measured by 5,5′, 6, 6′-tetrachloro-1,1′, 3, 3′-tetraethylbenzimidazole carbocyanide iodide (JC-1) using flow cytometry. Statistical analysis was carried out using U-Mann Whitney and Pearson’s tests.

Results: In base on citrate synthase activity, OA chondrocytes have more mitochondria than normal chondrocytes (13.6%). However, the AMRCC is altered on OA chondrocytes. The activity of complex II is decreased by 3.7.6% in OA cells with regard to normal cells. These differences between OA and normal cells are independent of age. A comparative analysis by classes of age from 40 to 70 and from 70 to 90 between normal and OA chondrocytes shows a reduction in activity of complex II (about 37%). The analysis of correlation between ageing and mitochondrial activity on normal and OA chondrocytes did not show any significant association. The administration of NO as Sodium NitroPrusside (SNP), causes a reduction in activity of complex I (32.8%) and IV (25.5%) on normal chondrocytes. Furthermore, NO decreases the mitochondrial membrane potential by a 24% quantified by flow cytometry.
Conclusions: These findings suggest that the alteration of complex II activity may contribute to pathological process in OA. NO is involved in pathological development of OA because it modulates the mitochondrial respiratory activity and induces a mitochondrial depolarization.

**Results:** Significant regional variation was observed in susceptibility to the effects of IL-1. Differences were apparent in chondrocyte expression of the two receptor types; IL-1RI and IL-1RII expression was related.

**Table 1. IL-JR expression on chondrocytes isolated from OA cartilage**

<table>
<thead>
<tr>
<th>Expression</th>
<th>IL-1RI</th>
<th>U-IRII</th>
<th>P value</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Of cells (%)</td>
<td>2.4±0.6</td>
<td>10.9±1.3</td>
<td>&lt;0.001</td>
<td>R=0.53, p=0.005</td>
</tr>
<tr>
<td>R/cell</td>
<td>461±144</td>
<td>1468±396</td>
<td>&lt;0.001</td>
<td>R=0.39, p=0.013</td>
</tr>
</tbody>
</table>

No significant correlation was found between GAG loss from cartilage and expression of either IL-1RI or IL-1RII on chondrocytes.

**Table 2. Relationship between GAG loss and IL-JR expression**

<table>
<thead>
<tr>
<th>Expression</th>
<th>IL-1RI</th>
<th>IL-1RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of cells (%)</td>
<td>R=0.069, p=0.077</td>
<td>r=0.096, p=0.559</td>
</tr>
<tr>
<td>R/cell</td>
<td>R=0.11, p=0.050</td>
<td>r=0.93, p=0.573</td>
</tr>
</tbody>
</table>

**Discussion:** Significant regional differences in cartilage susceptibility to the effects of IL-1 were found. However, the absence of any significant relationship between the IL-1R expression and IL-1b-induced GAG release from cartilage suggests that factors other than chondrocyte receptor expression influence the response of cartilage to the effects of this cytokine.

**Reference**

**PA72**

**EXPRESS 1ON OF TRANSFORMING GROWTH FACTORS AND THEIR RECEPTORS IS DIFFERENTIALLY MODULATED BY REACTIVE OXYGEN SPECIES AND NITRIC OXIDE IN HUMAN ARTICULAR CHONDROCYTES**

**Aim:** To assess the effects of two antioxidants, N-monomethyl-L-arginine (LNMMA) and N-acetylcysteine (NAC) on the expression of TGF-β1, β2 and β3 and their receptors I and II, using LPS-treated human chondrocytes.

**Methods:** Human chondrocytes were treated for 48h with LPS (10 μg/ml), L-NMMA (0.5 mM) or NAC (1 mM). Nitrite levels were assayed using the Griess method. The expression of NO synthase (iNOS), TGF-β1, β2, β3, TGF-β1-RI and II, was determined by semi-quantitative RT-PCR.

**Results:** LPS induced a dramatic increase of both NO and iNOS mRNA levels. L-NMMA abolished NO production without affecting iNOS mRNA levels. In contrast, NAC strongly synergized with LPS to stimulate NO synthesis. LPS did not significantly alter TGF-β1 expression whereas L-NMMA inhibited its production. TGF-β2 mRNA level was markedly reduced by LPS alone, or with both L-NMMA and NAC. TGF-β3RII expression was slightly increased by the combination LPS+L-NMMA or LPS+NAC whereas that of TGF-β3 and TGF-β3RII was reduced in these conditions.

**Conclusions:** ROS differentially control the TGF-β system, depending on the isoform; TGF-β1 mRNA level depends on NO whereas that of TGF-β2 is regulated by ROS and TGF-β3 seems