Assessing Disease Modification in the Guinea Pig Model of Spontaneous OA

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INTRODUCTION:
Currently assessment of efficacy of Disease Modifying Osteoarthritic Drugs (DMOADs) in animal models of Osteoarthritis (OA) such as the surgical-induced anterior cruciate ligament in dog (1) and rabbit, and the spontaneous guinea pig (Dunkin Hartley) and mouse (STR/OR) has utilised macroscopic analysis of cartilage lesion quantity and histological analysis of cartilage quality. Whilst some evidence of efficacy has been observed, these readouts have limitations including imprecision and lack of applicability to human clinical trials.

The aim of this study was to compare and contrast four different efficacy methodology readouts in a single study assessing the effect of a metalloproteinase inhibitor (MPI) on joint integrity in the guinea pig model of spontaneous OA. The readouts included: (1) cartilage volume and hydration using Magnetic Resonance Imaging (MRI); (2) macroscopic assessment of cartilage lesion damage, using digital densitometry of Indian ink stained cartilage; (3) conventional histology of cartilage; and (4) biomarker, urinary type II collagen crosslink.

METHODS
In vivo: Male, Dunkin Hartley guinea pigs (9 months of age) were dosed for 66 days with vehicle (20% (v/v) DMSO, 60% (v/v) PEG4000, (n=16)) or 0.3mg/kg/day of a MPI (n=10) via an osmotic mini-pump. All work was performed under license to the UK Home Office.

Magnetic Resonance Imaging (MRI): Fat-suppressed MR images covering the entire left knee were acquired under halothane anaesthesia pre- and post-dosing on a 4.7T Varian ‘Inova’ (2). Three blinded segmenters used in-house software to determine cartilage volume on the medial tibial plateau (MTP) of the left knee. MTP cartilage hydration was determined by calculating the signal intensity of segmented cartilage relative to muscle. For both measures change from pre and post-dosing was determined.

Histopathology: Following MRI, the left leg was fixed in 10% formalin, paraffin embedded and 10µm step sections stained with Haemotoxylin and Eosin (H&E). Cartilage degeneration was assessed, based on modified Mankin scoring system (3).

Macroscopic Assessment: The right leg was removed post study and stored at -20°C. The joint was thawed by immersion in phosphate buffered saline (PBS) and the tibia and femur separated. Menisci and soft tissues were removed from the tibia, which was cut to approximately 3cm in length and mounted vertically in a petri dish. The tibial plateau of each bone was painted with 20% (v/v) Indian ink in PBS approximately 3cm in length and mounted vertically in a petri dish. The joint was then immersed in PBS and the MTP cartilage hydration using Magnetic Resonance Imaging (MRI) was measured using a modified Mankin scoring system (3).

RESULTS
A comparison of efficacy by the four described readouts is summarised in Table 1. 3D MR in vivo imaging of the MTP allowed accurate segmentation and longitudinal cartilage volume change to be calculated over the study duration. The volume of MTP cartilage of vehicle treated animals decreased. Conversely there was a statistically significant increase in cartilage volume of those animals continuously dosed with an MPI. The level of hydration in the MTP cartilage showed a decrease over the study duration, whereas the level of hydration of the vehicle treated group remained unchanged. However, these groups were not significantly different from one another.

DISCUSSION
Of the four methodologies tested for assessing efficacy in this study, cartilage volume and hydration change using 3D in vivo MRI proved to be sensitive and informative to monitor focal areas of disease. Macroscopic assessment was visually powerful and clearly indicated the efficacy of the treatment. However, as this was a terminal technique, the precise extent of damage at the beginning of the study was not known. This coupled with the high level of inter-animal variation in the extent of damage compromised the readout. Histology suffers the same problem as macroscopic assessment in that it is a terminal technique. In additional it is difficult to ensure that the region of focal damage is scored for each animal due to the difficulty in orienting the joint precisely for perpendicular sectioning and the impracticality of sectioning the entire joint.

The levels of CTX release at this mature age are very low and impracticality of sectioning the entire joint.

Table 1

<table>
<thead>
<tr>
<th>Readout</th>
<th>Vehicle (n=16)</th>
<th>MPI (n=10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI: Change in MTP cartilage volume (mean±sem, mm³)</td>
<td>-0.47±0.08</td>
<td>0.28±0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MRI: % Change in MTP cartilage hydration (mean±sem)</td>
<td>0.84±1.6</td>
<td>-4.43±2.14</td>
<td>0.062</td>
</tr>
<tr>
<td>Macroscopic Damage (mean±sem, pixels)</td>
<td>14183±1473</td>
<td>11480±1948</td>
<td>0.142</td>
</tr>
<tr>
<td>Histology Score (mean±sem)</td>
<td>2.6±0.2</td>
<td>2.6±0.2</td>
<td>Not Sig</td>
</tr>
<tr>
<td>(i) Cartilage degeneration</td>
<td>2.4±0.2</td>
<td>2.3±0.3</td>
<td>Not Sig</td>
</tr>
<tr>
<td>(ii) Erosion/ulceration</td>
<td>1.2±0.2</td>
<td>1.3±0.2</td>
<td>Not Sig</td>
</tr>
<tr>
<td>(iii) Chondrocyte proliferation/clustering</td>
<td>6.2±0.9</td>
<td>6.1±1.2</td>
<td>Not Sig</td>
</tr>
</tbody>
</table>

REFERENCES

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