Combined Anti-Inflammatory and Anti-AGE Drug Treatments have a Protective Effect on Intervertebral Discs in Mice with Type 1 Diabetes

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INTRODUCTION: Diabetes mellitus type 1 is a systemic disease affecting many organs [1]. Diabetes is an etiologic factor in spinal stenosis, intervertebral disc (IVD) degeneration, and vertebral pathology [2] and these relationships may be associated with increased levels of inflammation and accumulation of advanced glycation end-products (AGEs) [3]. This study determined the effects of type 1 diabetes on vertebral and IVD morphology and evaluated if anti-inflammatory and AGE inhibition drugs could mitigate such effects.

METHODS: Treatment & control mice were maintained for 6.5 months (21 mice, n=6-8 per group). In diabetic animals (DB), kidney disease was induced in 4-8 week old ROP Os/+ mice with 2 weeks of streptozotocin injections, diabetes control was then monitored for 1 month by blood glucose levels (> 200 mg/dl), and animals were maintained another 5 months. Treated (En+Pyr+PPS) ROP Os/+ animals underwent the same diabetes induction protocol but were given 5 months of treatments including: Enalapril (EN, antihypertensive drug commonly used in diabetic patients); Pyridoxamine (PYR, an AGE inhibitor), and Pentosan Polysulphate (PPS, a broad acting anti-inflammatory). Non diabetic ROP Os/+ mice (Control) were also maintained. (Fig. 1)

RESULTS: Compared to control and treated animals, DB mice had decreased bone volume fraction (BVF) and trabecular thickness (Tb.Th., p<0.05), and increased trabecular spacing (Tb.SP.). Both treated and DB mice revealed decreased trabecular number (TB.N.), while En+Pyr+PPS animals maintained bone morphology more closely to control. (Fig. 2)

DISCUSSION: This experimental type 1 Db model induced vertebral bone loss and IVD GAG reduction suggestive of early degeneration, supporting the notion that both vertebrae and intervertebral discs are susceptible to changes during type 1 diabetes [4]. Combined anti-inflammatory and anti-AGE treatment in this model mitigated the effects of diabetes on bone loss and IVD degeneration, highlighting a potential role for such therapies in spinal diseases. Further studies will better characterize the model and evaluate individual drug treatment effects. The direct evidence for the application of anti-inflammatory and anti-diabetic drugs as a positive intervention on spinal pathology has to be further evaluated on different species.

SIGNIFICANCE: This study addresses the relationship between type 1 diabetes and spinal disorders with public health implications for the high prevalence of diabetes in society. Results provide a mechanism for spine related disorders associated with diabetes and suggest that anti-inflammatory and anti-AGE treatments may have efficacy in preventing structural and compositional alterations to vertebrae and IVDs.

REFERENCES:
1: Burner TW, Rosenthal AK, CurrOpinRheumatol; 2009
4: Dayem AE et al.; Scand J Clin Lab Inves; 2011

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Fig. 1: Study design

Following sacrifice, lumbar spines were analyzed with µCT. After decalcification, plastic embedded vertebrae were sectioned for histology (Safranin O/fast green) and immunohistochemistry (ADAMTS-5).

Fig. 2: Representative µCT images of (A) control, (B) treated and (C) diabetic mice.

Table 1: quantitative µCT analysis (n=6-8/group; mean±SD)

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<tbody>
<tr>
<td>DB</td>
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<td>NDB</td>
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<td>EnPyrPPs</td>
<td>0.149±0.016</td>
<td>0.040±0.002</td>
<td>3.667±0.450</td>
<td>0.235±0.032</td>
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Fig. 2: Representative µCT images of (A) control, (B) treated and (C) diabetic mice. Safranin O staining indicated reduced GAG in diabetic IVDs relative to control, but treatment qualitatively returned staining towards control levels.

Fig. 3: Representative image of ADAMTS-5 histology for (A) control, (B) treated and (C) diabetic mice. Arrow marks ADAMTS positive cell. Scale bar = 40 µm.

Cells immune-positive for ADAMTS-5 were found in greatest number in the outer annulus fibrosus of DB mice, suggestive of a mechanism for loss of GAG (Figure 3).