Humanin prevents undesired apoptosis of chondrocytes without interfering with the anti-inflammatory effect of dexamethasone in a model of arthritis

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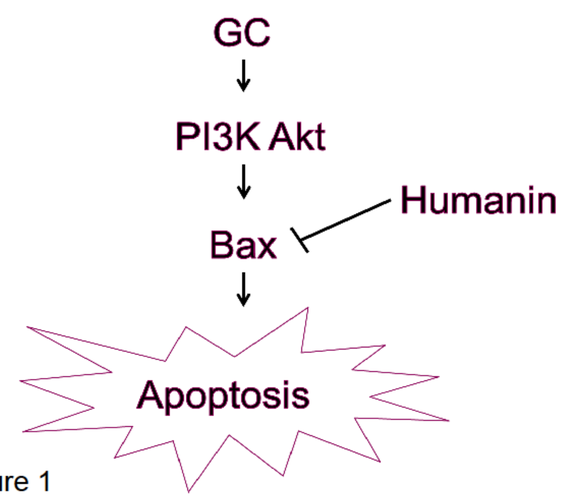

Figure 1

Conclusions

- Humanin prevents GC-induced chondrocyte apoptosis
- Humanin does not interfere with the anti-inflammatory effects of GCs in a mouse model of arthritis

Introduction

Glucocorticoids (GCs) are widely used for treatment of inflammatory and autoimmune conditions. Prolonged use of GCs, however, has several negative side effects, including bone growth impairment in children which is believed to be linked with chondrocyte apoptosis in the growth plate (Figure 1). Humanin, a small mitochondrial derived peptide has shown promising effects in rescuing chemotherapy-induced growth impairment as well as apoptosis in growth plate chondrocytes by suppressing Bax.

Aims

- Study if a synthetic analog to humanin, [Gly^{14}]-HNG (HNG) can rescue from dexamethasone (Dexa) induced apoptosis in articular cartilage
- Study if HNG interferes with the anti-inflammatory effect of Dexa in an *in vivo* model of Collagen type II-Induced Arthritis (CIA).

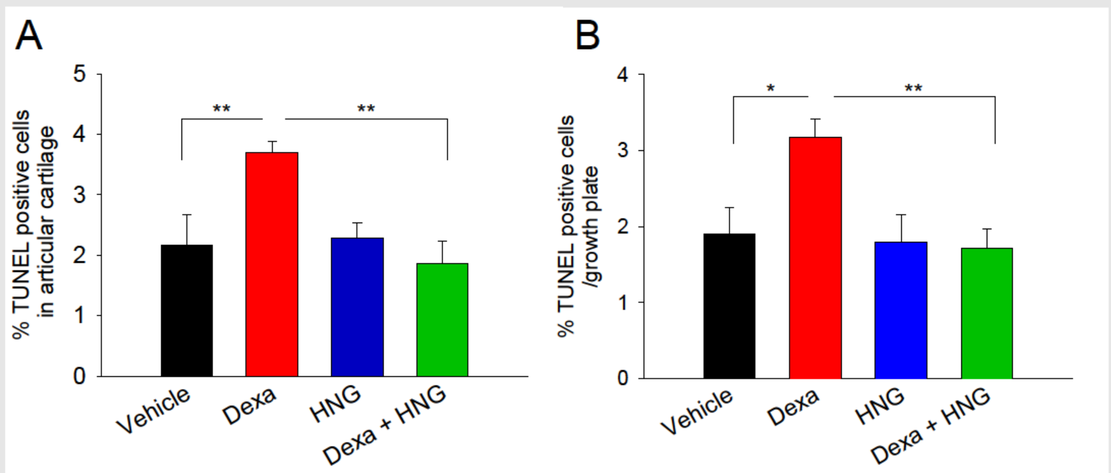

Figure 2
TUNEL analysis of articular cartilage (A) and growth plate cartilage (B) in femur from CIA mice.

Results

We found that humanin protects chondrocytes from GC-induced cell death in articular cartilage of femur as well as in the growth plates in CIA mice (Figure 2). Based on the clinical scoring (Figure 3) we observed that humanin in combination with Dexa does not interfere with the desired anti-inflammatory effects of Dexa in the CIA model.

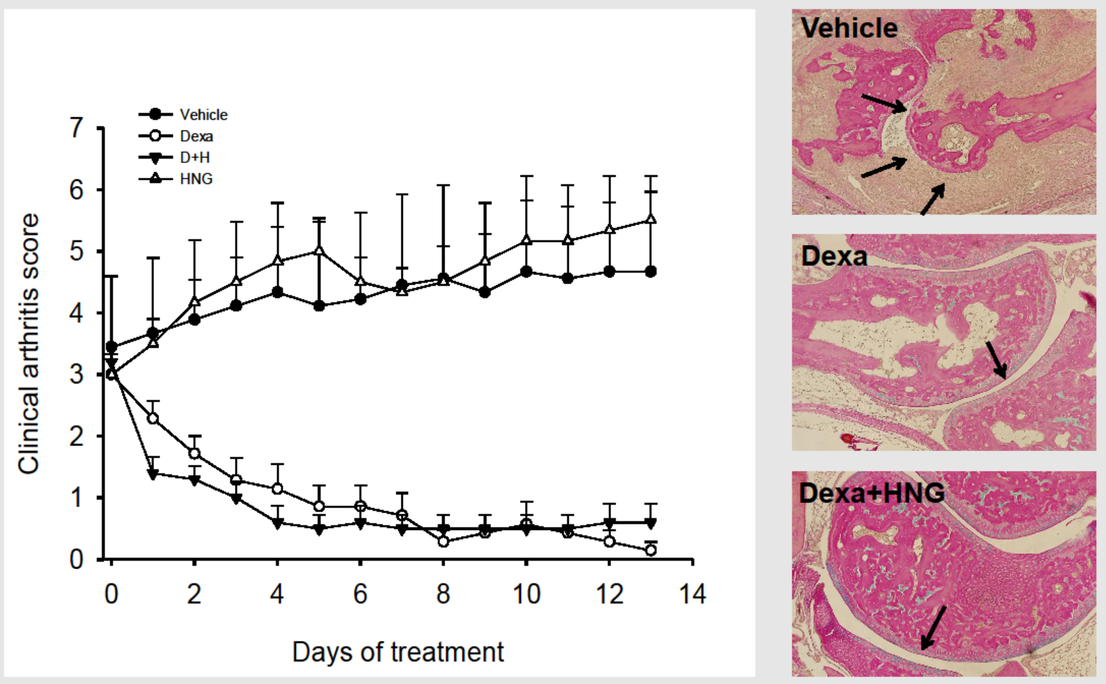

Figure 3
The clinical scores shows that development of arthritis was significantly attenuated with treatment with Dexa and Dexa + HNG, compared with the vehicle treated animals. Histology of the paws from vehicle, Dexa, and Dexa + HNG treated mice. Arrows show massive cell infiltration and joint destruction in the vehicle group compared to the healthy joints in the Dexa and Dexa + HNG groups.

Materials and methods

CIA was induced in DBA/1 mice and the animals were treated with Dexa (0.25 mg/kg/day) with/without HNG (100 µg/kg/day) for 14 days. The animals were observed daily for the presence of arthritis including signs of erythema and swelling of the joints and the paws were scored based on the severity of the swelling.

Femur and paws from all animals were dissected and fixed in 4 % formaldehyde for 24 h. The bones were decalcified with EDTA buffer for 3-4 weeks before dehydration and paraffin embedding. The serial sections were stained with a TUNEL kit (TdT-FragEL, Calbiochem) in order to analyze apoptosis in the growth plate cartilage as well as the articular cartilage.

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