AN N-TERMINAL LINK PROTEIN PEPTIDE STIMULATES BIOSYNTHESIS OF COLLAGEN BY EXPLANTS OF HUMAN ARTICULAR CARTILAGE

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Introduction: Nanomolar concentrations of a synthetic unglycosylated 16 amino acid peptide (LN), identical in sequence to the N-terminus of human link protein, can stimulate proteoglycan (PG) synthesis in explant cultures of human articular cartilage from a wide range of subjects[1-2]. In this present study, we have investigated the effects of LN on collagen synthesis. The results showed that LN was able to up-regulate synthesis of type II collagen concomitantly and to an equal degree with that of PG in cartilage explant culture. The peptide was effective in both the presence and absence of fetal bovine serum.

Material and Methods: LN (DHLSDNYTLDHDRAIH) was synthesized at the Charing Cross and Westminster Medical School, UK. Cartilage was from macroscopically normal femoral condyles or tibial plateaus of patients undergoing bone replacement or amputation. Full depth cartilage was diced and cultured in DMEM or DMEM plus 2% fetal calf serum. The medium was supplemented with 1% glutamate and 0.01% ascorbic acid. After 48 h, cultures were treated for 4-10 days with LN, which was diluted in PBS containing 0.1% endotoxin-free bovine serum albumin. The medium was changed every 24 h and the cartilage labeled with 20 µCi/ml L-[2,3-^{3}H]proline or dual labeled with 20 µCi/ml [^{35}S]sulfate and 20 µCi/ml L-[2,3-^{3}H]proline for the last 24 h of incubation, with the labeling medium containing 50 µg/ml of β-aminopropionitrile. Explants were extracted with 4 M guanidine HCl and residues digested with cyanogen bromide (CNBr) in 70% formic acid or explants directly digested with CNBr. Extracts and digests were separated from free isotopes by chromatography on PD-10 columns and total incorporation of isotopes determined. Aliquots were hydrolyzed in 6 N HCl at 108 °C for 24 h and analyzed for radioactivity of incorporated hydroxy[^{3}H]proline by chromatography on Dowex 50W. The remaining samples were purified on DEAE cellulose and analyzed for collagen type by electrophoresis on 7% (collagen chains) or 12% (CNBr peptides) SDS polyacrylamide gels. All experiments were performed in at least triplicate and data analyzed using the Student's *t*-test.

Results: When cartilage explants from a 14 year old subject were treated with a range of concentrations of LN for 4 days in the presence of 2% serum, collagen synthesis, measured as the conversion of [^{3}H]proline into hydroxy[^{3}H]proline, was stimulated in a dose dependent manner (Figure 1). The optimal stimulation of collagen synthesis by LN was reached at a concentration of 10 ng/ml and this dose was therefore used in subsequent experiments, with a range of subjects of different ages. Synthesis of collagen was higher in the presence of serum than in its absence, but LN was able to up-regulate this still further by amounts ranging from 25% to 120% in subjects aged from 8 years to 36 years old (all $P<0.05$). There was no discernible effect of age of tissue on the degree of up-regulation. In some experiments samples from the same tissue were tested both with and without serum. The degree of stimulation by LN was almost identical in each case, although the overall rate of synthesis was higher when serum was present. Collagen synthesis was up-regulated equally as well during periods of incubation ranging from 4 to 10 days. To determine if LN had similar effects on both collagen and PG synthesis, explants from a 16 year old subject were incubated for 4 days with LN in serum containing medium and dual labeled with [^{35}S]sulfate and [^{3}H]proline. Synthesis of both PG and collagen was up-regulated by 25% ($P<0.05$), showing that LN was able to stimulate synthesis of both the major components of cartilage matrix equally (Figure 2). This was consistent with our findings with [^{3}H]proline labeling experiments that in most cases, when collagen synthesis was up-regulated, total [^{3}H] incorporation, a measure of total protein synthesis, was also up-regulated (data not shown). Electrophoresis and autoradiography showed that newly synthesized collagen chains as well as their CNBr peptides exhibited a band pattern identical to that of type II collagen (data not shown).

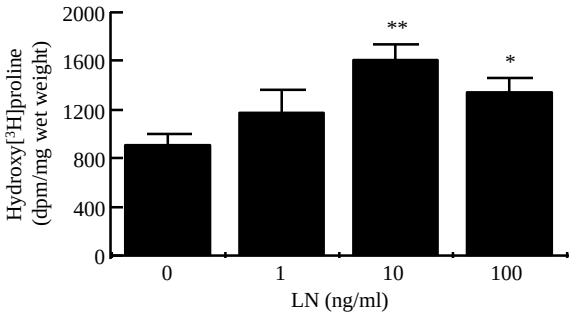

Figure 1. Cartilage from a 14 year old subject was treated with LN for 4 days in the presence of 2% serum and labeled with [^{3}H]proline for the final 24 h. Incorporation of hydroxy[^{3}H]proline was expressed as mean ± SD of triplicates. *: $P<0.05$, **: $P<0.01$.

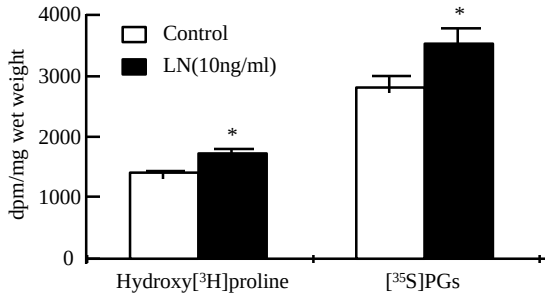

Figure 2. Cartilage from a 16 year old subject was treated with LN for 4 days in the presence of 2% serum and dual labeled with [^{3}H]proline and [^{35}S]sulfate for the final 24 h. Incorporation of isotopes was expressed as mean ± SD of triplicates. *: $P<0.05$.

Discussion: We have shown recently that physiological concentration of LN markedly up-regulated synthesis of normal PGs by explants of human articular cartilage cultured in the presence and absence of serum [1-2]. The present studies showed that the synthesis of type II collagen, the predominant collagen of normal articular cartilage, was also up-regulated by LN in explant cultures from a wide range of subjects. Furthermore, LN was able to up-regulate synthesis of collagen by a similar degree to that of PG. These results suggest that LN may have a general regulatory role in the synthesis of cartilage matrix.

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References:

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