

## Abstract

**Purpose:** The study was designed to identify synergistic effects of salmon Calcitonin (sCT) with reference to treatments for osteoarthritis using the bovine cartilage explant model in which degradation is induced by a cocktail of three cytokines.

**Methods:** Matrix degradation was quantified by measuring proteoglycan release, collagen release and MMP activity. The pro-inflammatory molecule PGE2 was also measured.

In the first experiment maximum ineffective concentrations of sCT, Galardin, Celebrex®, Dexamethasone, BB-94 and Doxycycline were determined. In the second experiment combinations of sCT along with each of the other drugs were assayed to determine if there were statistically significant synergistic effects of the combinations.

**Results:** Neither Calcitonin (0.5nM) nor Doxycycline (10µM) alone had an effect on collagen release. They changed collagen release minimally from 135 to 133 and 125 ng CTX-II per mg cartilage, respectively. The combination, however, decreased collagen release to 73 ng CTX-II per mg cartilage. This effect is stronger than expected based on the effects of the compounds alone and is therefore synergistic.

A similar effect is observed for the combination of Calcitonin (0.5 nM) with BB-94 (10 nM). Alone they change the collagen release from 135 to 133 and 137 ng CTX-II per mg cartilage, respectively, while the combination reduced the collagen release to 88 ng CTX-II per mg cartilage.

Although none of the other compounds showed synergy with sCT in this study, further experiments to determine a more appropriate range of concentrations for each of the compounds is planned.

**Conclusions:** The combination of sCT with Doxycycline and BB-94 may act synergistically to reduce type II collagen degradation in a bovine cartilage explant model. These results lend support to the concept that a combination therapy to reduce matrix degradation in OA may be developed with very low doses of each of the compounds. Such a combination therapy has the potential to alleviate the safety concerns with high doses of Doxycycline or BB-94.

## Introduction

Osteoarthritis is a multi-modal disease that is known to result in cartilage degradation, loss of subchondral bone, inflammation and pain. It is likely that the ideal DMOAD will eventually be a combination of compounds that has a beneficial effect on these parameters. The aim of the current study was to determine if Calcitonin (sCT) has additive or synergistic effects in combination with other treatments on inhibition of cartilage degradation in the bovine cartilage explant model. Synergism with Galardin, Celebrex® (celecoxib), Dexamethasone, Doxycycline and BB-94 (Batimastat) was tested.

The study consisted of two separate experiments:

In the first experiment the effects of each individual compound were tested in three to four concentrations in order to find the concentration in which each compound has little or no effect on cartilage degradation (data not presented).

In the second experiment the additive or synergistic effects of sCT with the other treatments were determined. The results of the first experiment were used to select the appropriate concentrations of the compounds for the second experiment.

No additive or synergistic effects of Calcitonin and Dexamethasone or Celebrex® were observed at the concentrations used, and hence no data are reported for these compounds.

## Experimental Design

•Full thickness bovine cartilage was obtained aseptically from two metacarpophalangeal joints of approximately 6 month old calves

•4 mm diameter punches were taken from the cartilage and placed in culture dishes with culture medium (DMEM+FCS, penicillin, streptomycin and ascorbic acid)

•Cartilage explants were weighed and explants between 5 and 15 mg were randomly divided into a 96-well culture plate with 1 explant per well and 200 µL culture medium

•Cartilage degradation was induced by addition of TNFα, IL-1α, and OSM at 10 ng/mL each to the culture medium

•Test compounds were then added at different concentrations

•Once weekly the explants were placed in new culture plates with fresh medium, stimuli and compounds

•Additionally, calcitonin was added to the wells twice weekly

•The experiment was terminated after 3 weeks

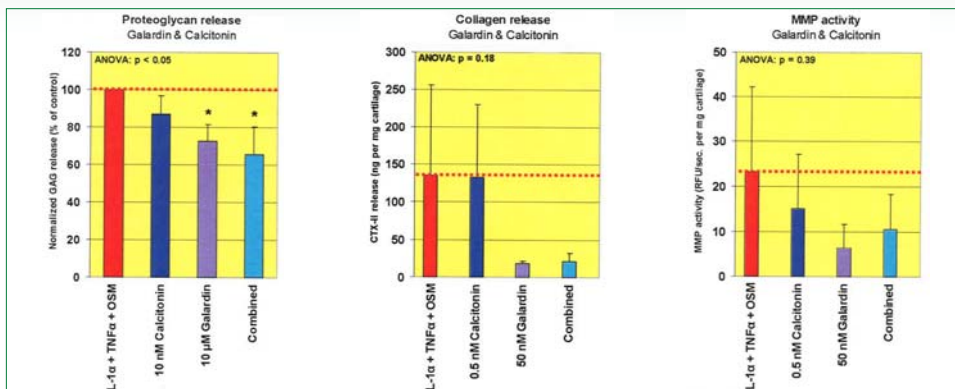
•All test conditions were cultured in quadruplicate and the entire experiment was performed in 3 replicates with cartilage from 3 different animals

•Proteoglycan (GAG) content was determined in the pooled medium and explants using the Blyscan® colorimetric assay (Biocolor, Belfast, UK)

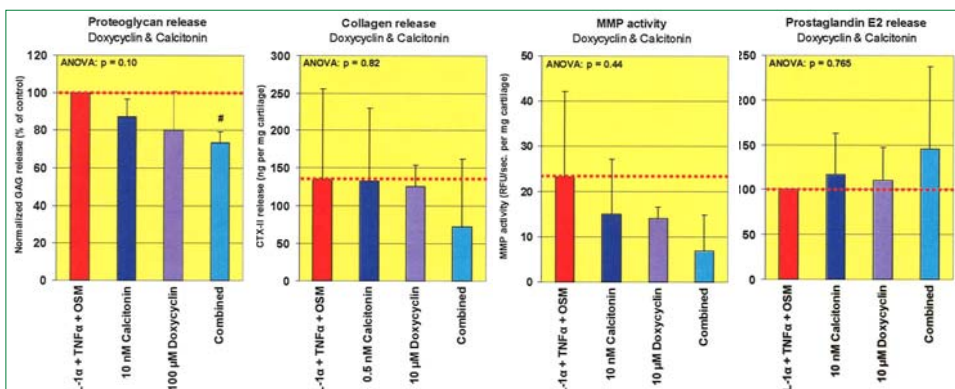
•Collagen was measured in the pooled medium using the CTX-II ELISA assay (Pre-clinical Cartilaps®, Nordic Bioscience)

•General MMP activity was determined in the pooled medium using the fluorogenic substrate TNO211-F

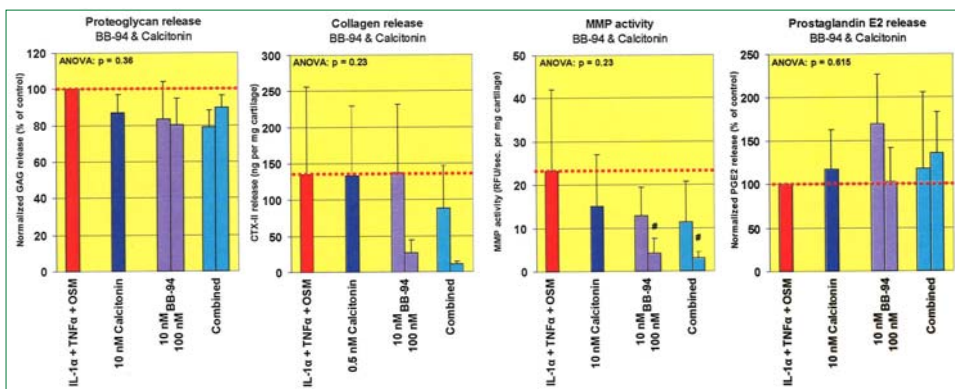
•PGE2 was determined in the pooled medium using a PGE2 ELISA from R&D Systems



**Figure 1.** The effect of Calcitonin in combination with Galardin on cartilage degradation and PGE2 release in bovine cartilage explants. The three graphs show the effect of Calcitonin and Galardin, alone and in combination, on the three outcome parameters: proteoglycan release, collagen release and MMP activity. Each bar represents the mean of three individual paws with the standard deviation.



**Figure 2.** The effect of Calcitonin in combination with Doxycycline on cartilage degradation and PGE2 release in bovine cartilage explants. The four graphs show the effect of Calcitonin and Doxycycline, alone and in combination, on the four outcome parameters: proteoglycan release, collagen release, MMP activity and PGE2 release. Each bar represents the mean of three individual paws with the standard deviation.



**Figure 3.** The effect of Calcitonin in combination with BB-94 on cartilage degradation and PGE2 release in bovine cartilage explants. The four graphs show the effect of Calcitonin and BB-94, alone and in combination, on the four outcome parameters: proteoglycan release, collagen release, MMP activity and PGE2 release. Each bar represents the mean of three individual paws with the standard deviation.

## Results and Conclusions

•Calcitonin and Galardin each decreased the proteoglycan release from 100% to 87% and 72%, respectively. The combination treatment resulted in an additive decrease in proteoglycan release to 65%. The possible benefit of the combination on other parameters remains to be determined at lower concentrations of the two compounds.

•The combination of Calcitonin and Doxycycline seemed to have a larger effect on all outcome parameters than the compounds alone. The strongest effect of this combination was observed on collagen release. The effect of Calcitonin or Doxycycline alone on collagen release is minimal, a decrease from 135 to 133 and 125 ng CTX-II per mg cartilage, respectively. In combination, an inhibition of the release to 73 ng CTX-II per mg cartilage is observed, suggesting a synergistic effect.

•A synergistic effect was also observed for the combination of Calcitonin and BB-94 on collagen release and MMP activity.

•To demonstrate possible additive or synergistic benefits of Calcitonin with other compounds or other parameters, these experiments will be expanded to study a wider range of concentrations to determine more accurately the maximum ineffective concentration of each of the compounds. Additionally, other models of OA will be explored.

## Acknowledgement

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