Steroids 76 (2011) 1474–1482

Contents lists available at SciVerse ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Glucocorticoids exert context-dependent effects on cells of the joint *in vitro*

Suzi H. Madsen [a,*], Kim V. Andreassen [b], Søren T. Christensen [c], Morten A. Karsdal [a], Francis M. Sverdrup [d], Anne-Christine Bay-Jensen [a], Kim Henriksen [b]

[a] Cartilage Biology and Biomarkers, Nordic Bioscience A/S, Herlev Hovedgade 207, DK-2730 Herlev, Denmark
[b] Bone Biology, Nordic Bioscience A/S, Herlev Hovedgade 207, DK-2730 Herlev, Denmark
[c] Department of Biology, The August Krogh Building, University of Copenhagen, Universitetsparken 13, DK-2100 Copenhagen OE, Denmark
[d] Center for World Health and Medicine, Saint Louis University, Doisy Research Rm 355, 1205 Carr Ln, Saint Louis, MO 63104, USA

ARTICLE INFO

Article history:
Received 21 June 2011
Received in revised form 27 July 2011
Accepted 28 July 2011
Available online 10 August 2011

Keywords:
Prednisolone
Dexamethasone
Chondrocytes
Osteoclasts
Osteoblasts
Ex vivo model

ABSTRACT

Introduction: Glucocorticoids are known to attenuate bone formation *in vivo* leading to decreased bone volume and increased risk of fractures, whereas effects on the joint tissue are less characterized. However, glucocorticoids appear to have a reducing effect on inflammation and pain in osteoarthritis. This study aimed at characterizing the effect of glucocorticoids on chondrocytes, osteoclasts, and osteoblasts.
Experimental: We used four model systems to investigate how glucocorticoids affect the cells of the joint; two intact tissues (femoral head- and cartilage-explants), and two separate cell cultures of osteoblasts (2T3-pre-osteoblasts) and osteoclasts ($CD14^+$-monocytes). The model systems were cultured in the presence of two glucocorticoids; prednisolone or dexamethasone. To induce anabolic and catabolic conditions, cultures were activated by insulin-like growth factor I/bone morphogenetic protein 2 and oncostatin M/tumor necrosis factor-α, respectively. Histology and markers of bone- and cartilage-turnover were used to evaluate effects of glucocorticoid treatment.
Results: Prednisolone treatment decreased collagen type-II degradation in immature cartilage, whereas glucocorticoids did not affect collagen type-II in mature cartilage. Glucocorticoids had an anti-catabolic effect on catabolic-activated cartilage from a bovine stifle joint and murine femoral heads. Glucocorticoids decreased viability of all bone cells, leading to a reduction in osteoclastogenesis and bone resorption; however, bone morphogenetic protein 2-stimulated osteoblasts increased bone formation, as opposed to non-stimulated osteoblasts.
Conclusions: Using highly robust *in vitro* models of bone and cartilage turnover, we suggest that effects of glucocorticoids highly depend on the activation and differential stage of the cell targeted in the joint. Present data indicated that glucocorticoid treatment may be beneficial for articular cartilage, although detrimental effects on bone should be taken into account.

1. Introduction

Glucocorticoids (GCs) are used to overcome inflammatory conditions, such as inflammatory bowel diseases and rheumatoid arthritis [1]. In addition, intra-articular injections of GCs have been tested for their ability to reduce inflammation and pain in osteoarthritis (OA) and appear to have a reducing effect on both parameters [2]. Furthermore, OA chondrocytes express fewer glucocorticoid receptors (GR) than normal chondrocytes, indicating the importance of glucocorticoid stimulation of normal cartilage [3]. However, GC treatment in children and adolescents is associated with severe growth retardation caused by apoptosis of the growth plate cartilage, especially in the proliferative zones of the cartilage [4,5]. With respect to the effects on articular chondrocytes, *in vitro* and *ex vivo* studies of chondrocytes are contradicting and highly context dependent [6–9].

Unfortunately, prolonged GC therapy has also been associated with bone loss and fractures, resulting in severe osteoporosis [10,11]. In this scenario, GCs cause significant reductions in bone formation, as well as a transient acceleration of bone resorption,

Abbreviations: IGF-I, insulin-like growth factor 1; BMP-2, bone morphogenetic protein 2; OSM, oncostatin M; TNF-α, tumor necrosis factor-α; GC, glucocorticoid; OA, osteoarthritis; RANKL, receptor activator of nuclear factor kappa-B ligand; OPG, osteoprotegerin; PRED, prednisolone; DEX, dexamethasone; MMP, matrix metalloproteinase; PBS, phosphate buffered saline; M-CSF, macrophage colony-stimulating factor; α-MEM, α-modified eagle medium; ELISA, enzyme-linked immunosorbent assay; TRAP, tartrate-resistant acid phosphatase; sGAG, sulfated glycosaminoglycans.

* Corresponding author. Tel.: +45 44 52 52 52; fax: +45 44 52 52 51.
E-mail addresses: shm@nordicbioscience.com (S.H. Madsen), kan@nordicbioscience.com (K.V. Andreassen), stchristensen@bio.ku.dk (S.T. Christensen), mk@nordicbioscience.com (M.A. Karsdal), fsverdrup@charter.net (F.M. Sverdrup), acbj@nordicbioscience.com (A.-C. Bay-Jensen), kh@nordicbioscience.com (K. Henriksen).

0039-128X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2011.07.018

leading to fragility fractures [12], which especially occur in high-turnover bone compartments, such as vertebrae [13]. *In vivo*, this effect is mediated via induction of osteoblast and osteocytes apoptosis, as well as a less well-understood activation of osteoclasts [1,14–17]. Interestingly, a mouse study showed that the detrimental effect of GCs on bone formation was mediated through the osteoclasts; whether it was dependent on bone resorption was not clear [17]. *In vitro* studies of the effects of GCs on osteoclasts have shown highly context dependent effects, i.e., both inhibitory and activating effects were observed [17–19]. One possible explanation for these contradicting results may lay within the heterogeneity of the model systems used, which comprise both mouse and human cells in either purified or mixed cell populations [17–19]. In osteoblasts and their precursors, GCs are known to stimulate Receptor Activator of Nuclear Factor Kappa-B ligand (RANKL), while reducing osteoprotegerin (OPG) [18], potentially explaining the transient activation of bone resorption observed *in vivo*. GCs also possess the ability to stimulate *in vitro* osteoblastogenesis in cell cultures [20,21], although separate studies show that GCs may arrest osteoblastogenesis and introduce apoptosis [22,23], illustrating the complex and context dependent nature of GCs.

Finally, GC signaling in osteoblasts and osteocytes was shown to be involved in the pathogenesis of inflammatory arthritis, demonstrating the importance of cellular cross-talk in the joint in relation to effects of GCs [24]. So, whether GCs administered *in vivo* affect both bone and cartilage, or just bone, is presently not clear [25,26]. Especially in OA, a disease of both bone and cartilage [27,28], the effects of GCs in the context of a whole joint need to be studied in much further detail.

In this study, we used four highly robust cell systems, namely murine femoral head explants [29], bovine articular cartilage explants [30], $CD14^+$ derived human osteoclasts [31] and the 2T3 osteoblast cell line [32], to characterize the effects of the GCs – prednisolone (PRED) and dexamethasone (DEX) – on cells of the joint. Furthermore, we cultured the cells under different conditions to shed light on the context dependency of the effects of GCs.

2. Experimental

2.1. Ex vivo models

2.1.1. Murine femoral heads

The experiment was performed in accordance with relevant guidelines and regulations from the Ethical Committee, as mice were euthanized before the experiment started. Femoral heads from 12-week-old outbred Naval Medical Research Institute (NMRI) female mice (Charles River, Germany) were isolated and cultured accordingly to Madsen et al. [29]. Femoral heads were cultured for 3 weeks, in the presence or absence of different factors: the anabolic factor Insulin-like Growth Factor I (IGF-I) [100 ng/ml] (Sigma, DK), the catabolic cytokines Oncostatin M (OSM) [10 ng/ml] (Sigma, DK) + Tumor Necrosis Factor-α (TNF-α) [20 ng/ml] (R&D Systems, UK) and PRED [100 nM] (Sigma, DK).

2.1.2. Bovine articular cartilage

Bovine knees were bought at the local slaughter house (Slangerup, DK) just after slaughtering. Articular cartilage explants were isolated and cultured accordingly to our previous studies [30,33]. The explants were cultured for 3 weeks, in the presence or absence of different factors: OSM [10 ng/ml] + TNF-α [20 ng/ml], IGF-I [100 ng/ml], PRED [100 nM] and DEX [100 nM] (Sigma, DK).

2.2. In vitro models

2.2.1. Human osteoclast preparation

Human blood was donated from the Danish University Hospital of Copenhagen (has an approval from the Ethical Committee to donate blood to us), and $CD14^+$ monocytes were isolated and differentiated into osteoclasts accordingly to Sorensen et al. [31].

2.2.2. Differentiating osteoclasts

The cells were seeded on plastic or bovine cortical bone slices (Nordic Bioscience Diagnostics, DK) at a density of 100,000 cells/well in a 96-well plate and cultured in phenol red free α-MEM containing 10% serum, P/S, M-CSF [25 ng/ml] (R&D System, DK), RANKL [25 ng/ml] (R&D Systems, DK), either in the presence or absence of DEX [100 nM] (Sigma, DK). Incubation was performed at 37 °C and 5% CO_2. The media were changed every 2nd or 3rd day and saved at −20 °C.

2.2.3. Mature osteoclasts

For experiments with mature human osteoclasts, cells were grown in 75 cm^2 culture flasks for 10 days in phenol red free α-MEM containing 10% serum, P/S, M-CSF [25 ng/ml], and RANKL [25 ng/ml] to induce osteoclastogenesis until the cells had 3–5 nuclei on average. The cells were washed with PBS twice, trypsin was added, and the cells were incubated at 37 °C for approximately 20 min. The cells were scraped off, and re-seeded on plastic or bovine cortical bone slices at a density of 40,000 cells/well in a 96-well plate in the presence of phenol red free α-MEM containing 10% serum, P/S, M-CSF [25 ng/ml], RANKL [25 ng/ml] in the presence or absence of DEX [100 nM] at 37 °C and 5% CO_2. The media were changed every 2nd or 3rd day and saved at −20 °C.

2.2.4. Murine osteoblasts

2T3 pre-osteoblast cells were purchased from Dr. Steven E. Harris at the University of Texas Health Science Center at San Antonio. The murine mesenchymal stem cell line, 2T3, is able to differentiate into osteoblasts under proper predetermined conditions. Osteoblasts were prepared accordingly to Ghosh-Choudhury et al. [32]. To induce bone nodule formation, the 2T3 cells were lifted by trypsin and reseeded in 24-well plates at a density of 10,000 cells/well, and then cultured in the presence of ascorbic acid [100 μg/ml] and β-glycerol-phosphate [4 mM], in the presence or absence of BMP-2 [30 ng/ml] or DEX [100 nM] for 10 days. Incubation was performed at 37 °C, 5% CO_2. The media were replaced every 2nd or 3rd day and saved at −20 °C.

2.3. Concentrations

The concentrations of IGF-I, BMP-2 and OSM + TNF-α were selected accordingly to previous studies [29–31,33,34]. The concentrations of DEX and PRED were selected accordingly to previous studies [5,6,17].

2.4. Cell-survival after culture-period

The viability of the cells (in explants or isolated cells) were measured at the end of the culture period, using the colorimetric dye, alamar blue (Invitrogen, DK), as a 10% solution in the conditioned media, accordingly to the manufacturer's instructions [35].

2.5. Biomarkers

The resorption marker CTX-I (RatLaps®), the bone formation marker Rat/Mouse PINP EIA and the cartilage formation marker PIINP (IDS Ltd., UK) were measured accordingly to the manufacturer's instructions [33]. The competitive ELISA CIIM (Nordic