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Melanocortin peptides protect chondrocytes from mechanically induced cartilage injury

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ABSTRACT

Introduction: Mechanical injury can greatly influence articular cartilage, propagating inflammation, cell injury and death – risk factors for the development of osteoarthritis. Melanocortin peptides and their receptors mediate anti-inflammatory and pro-resolving mechanisms in chondrocytes. This study aimed to investigate the potential chondroprotective properties of α-MSH and [DTRP^8]-γ-MSH in mechanically injured cartilage explants, their ability to inhibit pro-inflammatory and stimulate anti-inflammatory cytokines in *in situ* and in freshly isolated articular chondrocytes.
Methods: The effect of melanocortins on *in situ* chondrocyte viability was investigated using confocal laser scanning microscopy of bovine articular cartilage explants, subjected to a single blunt impact (1.14 N, 6.47 kPa) delivered by a drop tower. Chondroprotective effects of α-MSH, [DTRP^8]-γ-MSH and dexamethasone on cytokine release by TNF-α-activated freshly isolated articular chondrocytes/mechanically injured cartilage explants were investigated by ELISA.
Results: A single impact to cartilage caused discreet areas of chondrocyte death, accompanied by pro-inflammatory cytokine release; both parameters were modulated by α-MSH, [DTRP^8]-γ-MSH and dexamethasone. Melanocortin pre-treatment of TNF-α-stimulated freshly isolated chondrocytes resulted in a bell-shaped inhibition in IL-1β, IL-6 and IL-8, and elevation of IL-10 production. The $MC_{3/4}$ antagonist, SHU9119, abrogated the effect of [DTRP^8]-γ-MSH but not α-MSH on cytokine release.
Conclusion: Melanocortin peptide pre-treatment prevented chondrocyte death following mechanical impact to cartilage and led to a marked reduction of pro-inflammatory cytokines, whilst prompting the production of anti-inflammatory/pro-resolving cytokine IL-10. Development of small molecule agonists towards melanocortin receptors could thus be a viable approach for preventing chondrocyte inflammation and death within cartilage and represent an alternative approach for the treatment of osteoarthritis.

1. Introduction

Osteoarthritis (OA) is a disease affecting load-bearing joints, characterized by self-perpetuating low-grade inflammation and degradative processes within the articular cartilage of affected joints. It is a leading cause of disability affecting almost every age group, with prevalence increasing dramatically over the age of 50, affecting ~60% of people in this age group. With increasing age, obesity and longer life spans, OA represents an ever-increasing socio-economic burden [1], for which at present there is no cure.

Abbreviations: ACTH, adrenocorticotropic hormone; CLSM, confocal laser scanning microscopy; DMARDs, drug modifying anti-rheumatic drugs; ECM, extracellular matrix; GPCR, G-protein coupled receptor; IL, interleukin; MC, melanocortin receptor; MMP, matrix metalloproteinases; NSAIDs, non-steroidal anti-inflammatory drugs; OA, osteoarthritis; POMC, pro-opiomelanocortin; RA, rheumatoid arthritis; α-MSH, alpha-melanocyte-stimulating hormone.

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Traumatic joint injuries are a major risk factor for the development and progression of OA [2] and increase the risk of arthritis 5- to 17-fold [3,4]. Knee traumas, in particular, represent over 40% of all sports injuries [5–7] and often result from traffic accidents with surgical restoration of joint stability not preventing future arthritis development [8–11]. The incidence of post-traumatic arthritis is therefore high – creating insistent demand for pharmacological intervention, directed at limiting the progression and propagation of destructive processes taking place in the early stages post-injury [2,12].

In a healthy joint, the smooth surface provided by articular cartilage promotes near frictionless joint movement allowing the joint to withstand tensile and compressive forces arising from movement [13]. Mechanical loading within physiological limits is an essential stimulus for chondrocytes to produce extracellular matrix (ECM), capable of withstanding normal levels of stress and is responsible for triggering the synthesis, exportation and degradation of ECM components – collagen and proteoglycans [14]. However, when the joint/cartilage experiences mechanical stresses above the normal physiological range and/or frequency, such as in impact trauma, this results in significant chondrocyte death attributed to mechanical necrosis [15] and apoptotic processes [16,17] that could trigger the development of OA [18].

The effect of impact trauma on the functionality and metabolism of chondrocytes is receiving increasing attention [16,17,19,20], because within mature articular cartilage, chondrocytes do not generally undergo cell division [21,22]. Additionally, OA is featured by reduced cellularity [21,23–29], a fact that is thought to contribute to the inability of the remaining chondrocytes to maintain normal matrix synthesis, thereby contributing to cartilage degradation [30].

Impact injury is associated with increased production of pro-inflammatory cytokines by affected chondrocytes [31]. Abnormal mechanical forces cause adult chondrocytes to initiate production of a large number of pro-inflammatory mediators including the cytokines TNF-α and IL-1β [18,32], which in combination with reactive oxygen species and lipid-derived inflammatory stimuli (including prostaglandins and leukotrienes) increase the catabolic activity of and ultimately kill chondrocytes distant from the impact-injury site [33–35]. This eventually leads to impaired ECM synthesis, cartilage degradation and, ultimately, development of OA [36,37].

Presently there is no treatment for OA, capable of reducing the degradation of cartilage or improving its function. Current treatment relies largely on conservative pain management strategies (analgesics and non-steroidal anti-inflammatory drugs (NSAIDs)). These are only temporarily effective with numerous side effects, and if unsuccessful leave expensive joint replacement surgery as the last resort. To find highly effective drugs with an enhanced safety profile for OA treatment is imperative. Development of compounds displaying both anti-inflammatory effects along with pro-resolving/chondroprotective properties represents an exciting therapeutic strategy.

Unravelling the mediators that provide tissue protection and restoration of homeostasis and developing peptide-based drugs targeted at the resolution phase of inflammation is an exciting concept [38,39]. Amongst a host of such mediators are the melanocortins. The melanocortin peptides have long been shown to display anti-inflammatory effects from the early seminal studies by Lipton demonstrating their anti-pyretic effects [40] and their potency – they are 25,000× more potent than paracetamol [41]. Over the last four decades a substantial body of evidence has exposed their beneficial effects in models of asthma [42–44], inflammatory bowel disease [45–47], cardiovascular disease [48–53], and neuroprotection [54–56] to name just a few areas.

Within the arthritic field, the melanocortin system has been evaluated in patients with RA and juvenile chronic arthritis; increased α-MSH levels were detected in synovial fluid, with a correlation suggesting that higher levels of α-MSH decrease the level of inflammation observed [57]. These important findings highlighted the prospect of harnessing the anti-inflammatory effects of α-MSH for arthritic diseases and soon after the beneficial effects of the peptide were proven in a model of adjuvant-induced arthritis [58], while more recently, it was found to be beneficial in models of gouty and rheumatoid arthritis (RA) [59–62]. However, only a handful of studies have evaluated their effects in chondrocytes and OA [63,64]. This surprising lack of interest in evaluating the potential of these molecules as a treatment for OA may stem from the fact that although inflammation is considered causal to both RA and gouty arthritis, OA has historically been perceived as simply a condition of natural 'wear and tear', with inflammation not regarded as a major contributor in the development of the pathology. Nevertheless, this initial viewpoint is currently changing [32,64].

Melanocortin peptides are derived from the larger, pre-cursor pro-opiomelanocortin (POMC) protein [65,66] and exert their effects *via* the activation of melanocortin receptors/adenylate cyclase/cAMP signalling pathway [65]. Although five melanocortin receptors (MC) have been identified, all positively coupled to adenylate cyclase *via* Gs and activate cAMP pathways, the anti-inflammatory effect of melanocortin peptides has been found to be mediated primarily *via* MC_1, MC_3 and MC_5 [65,66].

Here, we have demonstrated for the first time the ability of melanocortins to limit the progression of mechanical impact-induced chondrocyte death. In addition, the peptides inhibited the resulting production of pro-inflammatory cytokines in both *in situ* and TNF-α-stimulated freshly isolated articular chondrocytes, while promoting the release IL-10, thereby aiding in the resolution of inflammation and conferring further protection against cartilage damage.

2. Materials and methods

Unless specified otherwise, all reagents were purchased from Sigma–Aldrich Inc. (Poole, UK).

2.1. Cartilage impact studies

2.1.1. Cartilage dissection

Four metacarpophalangeal joints from different 18–24 month old cows (obtained from local abattoir) were skinned, rinsed in water, and joint capsules opened under aseptic conditions within 12 h of slaughter. Full-depth, healthy cartilage, excluding the subchondral bone, was harvested from the flat, load-bearing articular surfaces between the condylar ridges of each joint. Cartilage explants were cultured individually in HEPES-buffered high-glucose Dulbecco's Modified Eagle's Medium (DMEM, 280 mOsm/kg:H_2O, abbreviated to 'mOsm', pH 7.4; Gibco®, Life Technologies, Paisley, UK), supplemented with Penicillin (50.0 U/mL) and Streptomycin (50.0 μg/mL) at 37 °C, and 5% CO_2 in the absence of foetal calf serum (FCS; Invitrogen, Paisley, UK) and cultured within 24 h or used for impact studies [67].

2.1.2. Mechanical loading of tissue

A vertical drop tower previously shown to cause impact damage to cartilage explants in aseptic conditions [30], was used to deliver a single defined impact (137 g weight dropped from a height of 10 cm), equivalent to 1.14 N, 6.47 kPa (assuming linear acceleration), to individual bovine articular cartilage explants [30]. The Isolated, pre-weighed articular cartilage explants (~5 mm^2) were incubated (within 24 h of dissection) in 1.0 mL serum-free DMEM