Effect of Leptin Alone and in Combination with IL1β on Human Chondrocytes in a Pellet Culture System

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ABSTRACT

Osteoarthritis is the most common cause of disability among elderly, with obesity being a risk factor. The mechanical force on load-bearing joints in obese patients is known to induce Osteoarthritis development, however, low load-bearing joints in obese patients have also been found to develop Osteoarthritis. Leptin is a systemic hormone, associated with body-weight, and therefore may be the link between obesity and Osteoarthritis. This study aimed to investigate the effect of leptin on primary chondrocyte metabolism in a pellet culture system. The pellets were treated with IL1β or leptin (0.1-10μg/ml) or IL1β and leptin (0.1-10μg/ml) for 21 days. During that period, sulfated glycosaminoglycans (sGAGs) and hydroxyproline released in culture media and remaining in the pellets, as well as the expressions of ACAN, COL2A1, COL1A1, MMP3, MMP13 genes and MMP3, MMP13 enzymes were measured. Additionally, sGAGs and collagen accumulation in the extracellular matrix was determined by histological analysis. Leptin (1.0-10μg/ml) was able to reduce the ECM molecule contents, both sGAGs and collagen, through up-regulation of MMPs expression, down-regulation of ACAN expression and induction of the dedifferentiation stage of chondrocytes. The effect of 10μg/ml leptin was similar to IL1β, the main cytokine involved in cartilage degradation. Interestingly, leptin had an additive effect with IL1β on the reduction of pellet ECM molecule contents. This study shows that leptin can induce cartilage
breakdown by down regulation of ECM molecules and up regulation of protease enzymes and has an additive effect with IL1β on cartilage degradation.

Keywords: Osteoarthritis, Chondrocyte, IL1β, Leptin, Pellet culture

INTRODUCTION

Osteoarthritis (OA) is the most common cause of musculoskeletal system disability among elderly worldwide (Musumeci et al., 2015). The pathogenesis of OA involves an imbalance in the synthesis and degradation of cartilage tissue, primarily the extracellular matrix (ECM) containing type II collagen which encoded from COL2A1 gene and proteoglycans such as aggrecan which is a main proteoglycan found in cartilage and encoded from ACAN gene. Cartilage tissue degradation is mediated by protease enzymes, matrix-metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs), which are induced by pro-inflammatory cytokines, mainly IL1β, TNFα and IL6. MMP3 and MMP13 are main enzymes that can degrade ECM of cartilage. Additionally, OA is also associated with body weight due to increased mechanical force on joints, resulting in the development of OA in load-bearing joints, such as the knees, especially in obese patients. Interestingly, obesity is also a risk factor for the progression of OA in low-weight bearing joints, such as the hands, which cannot be explained by increased mechanical force on the joints (Yusuf et al., 2010). Cytokines, including leptin, that systematically increase obesity may be involved in the weight associated development of OA.

Leptin, a 16-kDa peptide hormone produced primarily by adipose tissue, is responsible for the metabolism of energy, with ability to regulate appetite and the consumption of energy (Ahima et al., 1996; Trayhurn, 2005). Studies have found higher levels of leptin in both obese subjects and OA patients compared to normal-weight and non-OA patients, respectively. Serum leptin concentration was found to be correlated with body weight, with higher levels in obese subjects (31.3±24.1 ng/ml) compared to normal-weight subjects (7.5±9.3 ng/ml) (Considine et al., 1996). In OA patients compared to non-OA patients, leptin levels were higher in the peripheral blood (18±13 ng/ml and 73±61 ng/ml in normal and OA subjects, respectively) and in the synovial fluid (2.05 ng/ml, with a range of 1.0-4.6 ng/ml, and 4.40 ng/ml, with a range of 0.5-15.8 ng/ml, in normal and OA subjects, respectively) (Otero et al., 2006; Ku et al., 2009; de Boer et al., 2012).

While there have been numerous investigations on the effect of leptin on cartilage metabolism, the results remain inconclusive. The contradictory reports on the effect of leptin may result from numerous factors, including the use