Analysis of the expression of selected biomarkers of periprosthetic osteolysis in macrophage-like cells treated with the neuropeptide CGRP

Periprosthetic osteolysis constitutes the main reason for untimely implant failure after total joint arthroplasty. In that process particulate wear debris derived from the implant is phagocytised by macrophages. This in turn leads to an inflammatory response in which the production of various pro-inflammatory cytokines induces osteoclast-mediated bone resorption. An osteoprotective influence of the neuropeptide calcitonin gene-related peptide (CGRP) has been described. In the present study, the effect of CGRP on the catabolic impact of both ultra-high molecular weight polyethylene (UHMWPE) particles and bacterial lipopolysaccharides (LPS) was analysed *in-vitro*.

Monocytic cells of the THP-1 cell line were differentiated into macrophage-like cells using phorbol-12-myristate-13-acetate (PMA). Afterwards, the cells were stimulated with either UHMWPE particles (cell-to-particle ratios of 1:100 and 1:500) or LPS (1 µg/ml) for distinct periods of time (6/24/48 h) in order to generate virtually osteolytic conditions. Simultaneously, the cells were treated with the neuropeptide CGRP (10^{-8} M). The mRNA expression of both receptor activator of nuclear factor κB (*RANK)* and tumour necrosis factor (*TNF)-α* was determined by quantitative RT-PCR. RANK protein was detected employing western blot analysis. Secreted protein levels of TNF-α as well as of the interleukins (IL)-1β, IL-6 and IL-10 were quantified in cell culture supernatants using a human TNF-α ELISA or a human Bio-Plex Cytokine Assay, respectively.

Levels of RANK mRNA and protein in macrophage-like cells were very low and did not increase following virtually osteolytic activation. Instead, stimulation of the cells with either particles or LPS led to a time- and stimulus-dependent increase in TNF-α mRNA and protein levels as well as to an enhanced secretion of IL-1β and IL-6 ($p \leq 0.049$). CGRP time-dependently inhibited *TNF-α* mRNA expression as elicited by low particle numbers or LPS ($p \leq 0.020$) while it suppressed both particle- (1:100 and 1:500) and LPS-induced TNF-α secretion ($p \leq 0.043$). A pronounced inhibitory effect of CGRP on LPS-induced cytokine production at 24 h of incubation could also be observed with IL-1β and IL-6 ($p \leq 0.002$).

CGRP temporarily impairs the osteolysis-associated production of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 *in-vitro*. This result is consistent with the previously reported osteoprotective properties of the neuropeptide. Thus, CGRP as a therapeutic agent might potentially enhance the life-time of implants following total joint arthroplasty.