EFFECT OF TYROSINE KINASE INHIBITORS ON THE INDUCTION OF MMP-1, TIMP-1, AND TNF-α GENE EXPRESSION BY COBALT AND CHROMIUM IONS IN HUMAN U937 MACROPHAGES

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INTRODUCTION

The most common cause of failure of total hip arthroplasty is aseptic loosening of the prosthesis. The adverse tissue response to prosthesis wear particles is an important contributor to bone loss around implants. Clinical observations suggest that macrophages are involved in the pathogenesis of aseptic loosening due to bone destruction around the prosthesis (peri-prosthetic osteolysis). Previous reports have suggested that the imbalance of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) activity may contribute to prosthetic loosening (1). However, the mechanisms controlling the activity of these enzymes in the peri-prosthetic environment is unknown.

Cobalt (Co\textsuperscript{2+}) and chromium (Cr\textsuperscript{3+}) ions, two corrosion products of metal-on-metal hip prostheses, are known to induce the production of cytokines, which have the ability to regulate the expression of several MMPs and TIMPs (2). However, few studies have been directed towards the signaling pathways mediating the induction of MMPs and TIMPs by cytokines.

In the present study, we investigated the modulation by Co\textsuperscript{2+} and Cr\textsuperscript{3+} ions on the expression of genes encoding MMP-1, one of the principal proteinases capable of degrading native fibrillar collagens in the extracellular matrix (ECM), and its inhibitor TIMP-1. Their expression was studied in relation to the expression of TNF-α, a cytokine that plays a central role in the induction of implant osteolysis, in order to gain insight into the regulation of ECM degradation and tissue remodeling around hip prostheses.

MATERIALS AND METHODS

Cell Culture - Human U937 macrophages (ATCC, Rockville, MD) were cultured in suspension in DMEM high-glucose supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin. Macrophages were incubated for 24h with 100 μM and 1000 μM Co\textsuperscript{2+} (CoCl\textsubscript{2}, Fisher Scientific, Ville St-Laurent, QC) and Cr\textsuperscript{3+} (CrCl\textsubscript{2}, Sigma-Aldrich, Oakville, ON) ions. Macrophages alone served as a negative control. Incubations were conducted at 37°C in a 5% humidified CO\textsubscript{2} environment. In experiments involving tyrosine kinase inhibitors, genistein (50 μM) and herbimycin A (1 μM) (Sigma-Aldrich, Oakville, ON) were added to the cell suspension 24h prior to the addition of ions as previously described (3).

Gene Expression - The levels of mRNAs were determined by reverse transcription-polymerase chain reaction (RT-PCR). The RT and PCR reactions were performed as previously described (4). Primer sequences used for PCR are shown in the Table 1. GAPDH was used as a housekeeping gene.

MMP-1: 5’ Forward: AAAGGGAATAAGTACTGGG
3’ Reverse: CTGAGGAAAAACCTGGAAGAAC
TIMP-1: 5’ Forward: CAATTCGACCTCGTCATC
3’ Reverse: CACCAGGCTACACTGTTG
TNF-α: 5’ Forward: AAGCCTGATAGCCATGGTACG
3’ Reverse: GAAGACCCCTCCCAGATAGATG
GAPDH: 5’ Forward: GCTCTCAGAACATCATCCTCGCC
3’ Reverse: AGGCCATTCTGTTATGACACAG

Table 1: Primer sequences for PCR.

RESULTS

Our results show that both Co\textsuperscript{2+} (Figure 1) and Cr\textsuperscript{3+} (Figure 2) ions induce the expression of MMP-1, TIMP-1, and TNF-α mRNA in a dose-dependent manner. However, tyrosine kinase inhibitors have different effects on these stimulatory effects. Indeed, genistein has only partial inhibitory effect on MMP-1 and TIMP-1, with even less effect on TNF-α expression. In contrast, herbimycin A completely blocks MMP-1 and TNF-α while partially inhibiting TIMP-1.

DISCUSSION

These results provide for the first time evidence that Co\textsuperscript{2+} and Cr\textsuperscript{3+} ions can act directly on U937 macrophages to stimulate MMP-1 and TIMP-1 expression. Interestingly, this induction was inhibited by genistein and herbimycin A, indicating a requirement for tyrosine kinase activity in this process. This is in agreement with the reported inhibition of MMP-1 by tyrosine kinase inhibitors in fibroblasts induced by three-dimensional collagen (3,5). These studies also reported a more potent effect of herbimycin A compared to genistein.

The relatively weak effect of tyrosine kinase inhibitors on TIMP-1 expression suggests that other signaling pathways are implicated in the regulation of this ECM protein. However, the role of the other protein kinases in the regulation of ion-induced MMP-1 and TIMP-1 expression remains to be determined.

Finally, the effect of tyrosine kinase inhibitors on ion-induced TNF-α expression is very similar to what was observed for MMP-1, suggesting that common pathways are implicated in the modulation of these genes by Co\textsuperscript{2+} and Cr\textsuperscript{3+} ions.

In conclusion, our results suggest that tyrosine kinases play an essential role in the signaling pathways regulating the degradation of collagen by macrophages activated by Co\textsuperscript{2+} and Cr\textsuperscript{3+} ions in the peri-prosthetic environment. This may serve as a target for selective inhibition of periprosthetic osteolysis.

REFERENCES

(5) Langholz part of Orthopaedic Research Society

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