EPITHEORESE KLINIKES FARMAKOLOGIAS
KAI FARMAKOKINETIKES, INTERNATIONAL
EDITION 13: 111-112 (1999)

Effect of Prothymosin α on Production of Metalloproteinases and Tissue Inhibitors of Metalloproteinases by Human Synovial Fibroblasts

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INTRODUCTION

Prothymosin α (ProTα) is a highly acidic (pI 3,5) polypeptide of mol. mass 12,5 kDa, first isolated from rat thymus as a putative precursor of thymosin α_1 (Tα$_1$), which is contained at the NH_2-terminus. It is expressed in many tissues, including synovial membrane, and is highly evolutionary conserved. It plays an extracellular and an intracellular role, but its function still remains unclear. ProTα is found intracellularly, localised in the nucleus and its function is related with cell growth, proliferation and apoptosis. ProTα has also been detected in serum of normal individuals and presents in vitro many immunopharmacological properties. It increases the PHA-induced proliferation of human peripheral blood mononuclear cells (PBMC) by enhancing the IL-2R expression in these cells. It also increases HLA-DR surface expression and mRNA accumulation in cultured human monocytes and human B cells. ProTα induces the increased secretion of IL-1β and TNF-α by blood monocytes from colorectal tumor patients in culture, while the levels of TGF-β and PGE_2 in the cell culture supernatants are significantly decreased. It also antagonizes the inhibitory effect of TGF-β on the expression of adhesion molecules by IL-1β activated HUVECs. ProTα receptors have been recognised on PBMC.

It is known that in many arthritides inflammatory or not, such as rheumatoid arthritis (RA) and osteoarthritis (OA) the synovial cells are activated. They secrete several cytokines and growth factors such as IL-1, IL-6, TNF-α, TGF-β, GM-CSF, EGF etc., a number of degradative enzymes including matrix metalloproteinases (MMPs), plasminogen activators, cathepsins and the tissue inhibitors of metalloproteinases (TIMPs). MMPs is a family of mammalian zinc-dependent metallopeptidases, consisting from at least 19 homologous enzymes. They are able to degrade almost all the extracellular matrix (ECM) components. MMPs have been implicated in remodelling of connective tissue, but are also thought to play a crucial role in the joint destruction seen in RA and OA. The most MMPs are usually expressed as zymogens. Their production are specifically regulated at the levels of gene expression by several cytokines and growth factors and their activities are controlled post-translationally in the extracellular space via co-ordinated activation of secreted proenzymes as well as through interaction of MMPs with TIMPs. Increased levels of the secreted enzymes or a failure of regulation of

their extracellular activity is probably an intrinsic part of the RA or OA process.

OBJECTIVE

Significant amount of ProTα has been detected from our group in synovial fluid from patients with RA or OA. MMPs and TIMPs levels in the synovial fluid are regulated by cytokines and growth factors. There is a disturbance in their balance in RA and OA, therefore in the present study the effect of ProTα in the production of MMPs and TIMPs by synovial fibroblasts was studied.

METHODS

Synovial membranes were collected from patients with RA or OA, which subjected to total knee arthroplasty. Fibroblasts were isolated by mild treatment of membranes with bacterial collagenase and cultured in DMEM. Fibroblast cultures in passage five were used. The incubation of cells in the presence of ProTα was performed in serum-free DMEM, containing 0,2% lactalbumin hydrolysate, for 48h. The DNA content of the cell layer was measured fluorometrically, using 4', 6-diamidino-2-phenylindole (DAPI). In the conditioned media the levels of MMPs and TIMPs were determined. The determination of MMP-2 was performed by gelatin-zymography, of MMP-1 and TIMP-2 by dot-immunobinding technique, using specific monoclonal or polyclonal antibodies and of TIMP-1 by ELISA, using specific polyclonal antibodies and human TIMP-1 for coating. The intensity of gelatin lysis bands from the zymograms and of dots in dot-immunobinding technique was measured by a digital image scanner and the image process was performed by the image PC program. Statistic analysis was performed by student t-test.

RESULTS

ProTα was tested in duplicate in synovial fibroblasts cultures at concentration of 8, 40 and 80pmole/ml, corresponding to 0,1, 0,5 and 1 μg/ml. It was found that ProTα at concentration 40 and 80pmole/ml significantly decreases the levels of TIMP-1in both cultures conditioned media; in RA patients 72% ($p<0,05$) and 49% ($p<0,01$) and in OA patients 86% ($p<0,05$) and 84% ($p<0,05$) at concentrations 40 and 80pmole/ml, respectively. The levels of TIMP-2 were remained unaffected in culture of RA patients at any concentration of ProTα, but they decreased in culture of OA patients at concentration of ProTα 40 and 80pmole/ml, with the decreasing at 40pmole/ml to be statistical significant, 51% ($p<0,05$). On the other hand, ProTα at concentration of 80pmole/ml significantly increased the MMP-1 levels in culture conditioned medium of RA patients, 112% ($p<0,05$), while it did not affect the MMP-2 levels in RA or OA patients cultures.

In order to ascertain if any of the N- or C-terminal of ProTα is responsible for its above activities, fibroblasts cultures at the same passage were treated with $Tα_1$ (1-28 amino acids of ProTα) or with Ct-ProTα (69-109 amino acids) at the same as above concentrations. It was found that $Tα_1$ had not any effect on the levels of MMPs and TIMPs. On the other hand the Ct-ProTα, while did not affect the levels of TIMP-1, MMP-1 and MMP-2, it significantly increased the levels of TIMP-2, 153% ($p<0,05$) and 171% ($p<0,05$), at concentration of 40 and 80pmole/ml, respectively.

CONCLUSIONS

The effect of ProTα on the production of MMPs and TIMPs by synovial fibroblasts resembles that of the proinflammatory cytokines and growth factors, such as IL-1β and TNF-α. Remains to be elucidated if the effect of ProTα is direct, possibly via its receptors, or if it is mediated by other cytokines or growth factors of which the production from fibroblasts is induced by ProTα.

The intact structure of ProTα is needed in order to express its activity on fibroblasts.

Removing a fragment from ProTα molecule, may lead in a change of the remaining part conformation and consequently of its activity.

ProTα may be implicated in pathogenesis of RA and OA.

Acknowledgments. This study was supported by the Research Committee of the University of Patras, grant K. Karatheodoris.