available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.elsevier.com/locate/biochempharm

Fibroblast heterogeneity in collagenolytic response to colchicine

Berit Mathisen [a], *Thrina Loennechen* [b], *Tobias Gedde-Dahl* [c,d], *Jan-Olof Winberg* [a,*]

[a] *Department of Biochemistry, Institute of Medical Biology, University of Tromsø, 9037 Tromsø, Norway*
[b] *Department of Pharmacology, Institute of Pharmacy, University of Tromsø, 9037 Tromsø, Norway*
[c] *Dermatological DNA Laboratory, Department of Dermatology and Research Institute for Internal Medicine, The National Hospital, Oslo, Norway*
[d] *Institute of Forensic Medicine, University of Oslo and The National Hospital, Oslo, Norway*

ARTICLE INFO

Article history:
Received 26 September 2005
Accepted 21 November 2005

Keywords:
Matrix metalloproteinases
Collagenase
Tissue inhibitors of matrix metalloproteinases
Colchicine
Fibroblast heterogeneity
Drug response

Abbreviations:
MMPs, matrix metalloproteinases
TIMPs, tissue inhibitors of MMPs
SBTI, soybean trypsin inhibitor
CLCS, collagenase colchicine sensitivity

ABSTRACT

Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) are important in various physiological and pathological conditions, including those that involve homeostasis of collagen. Drug induced regulation of MMP-1, other MMPs and TIMPs is critical in treatment of various diseases, e.g. the use of the plant alkaloid, colchicine. One possible factor that might explain the failure in colchicine-treatment of some patients is interindividual variability on the cellular level. To investigate the possible individual heterogeneity in response to colchicine, we studied the effect of colchicine-induced synthesis of collagenase from 32 different human skin fibroblast strains derived from both healthy individuals as well as individuals with different skin diseases. We showed that colchicine induced an increased synthesis of collagenase in 22 of 32 cases. This heterogeneity occurred in fibroblasts from healthy as well as diseased individuals. To determine if colchicine also affected the fibroblast synthesis of gelatinase, stromelysin and tissue inhibitors of MMPs, we investigated several individuals from a single family. The results showed that both colchicine responsive and non-responsive fibroblasts with respect to collagenase synthesis responded to colchicine by an increased stromelysin synthesis, while the synthesis of gelatinase and TIMP-1 were unaffected. As a whole, our results indicate that individual heterogeneity in collagenase response to colchicine treatment may partly explain some of the controversial results obtained with colchicine as a drug.

1. Introduction

The matrix metalloproteinases (MMPs) constitute a family of at least 25 different mammalian zinc and calcium-dependent enzymes, of which skin fibroblasts can express several types [1–5]. The enzyme family includes collagenases (MMP-1/-8/-13), gelatinases (MMP-2/-9), stromelysins (MMP-3/-10/-11), matrilysins (MMP-7/-26) as well as membrane-type MMPs

* *Corresponding author*. Tel.: +47 776 45488; fax: +47 776 45350.
E-mail address: janow@fagmed.uit.no (J.-O. Winberg).
0006-2952/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2005.11.018

(MT-MMPs) and other MMPs [4,5]. The activity of MMPs is regulated at the transcriptional, translational and post-translational levels. Most of the MMPs are synthesized in their latent pro-form, and must be converted to their active forms in the extracellular space. During activation, either parts or the entire N-terminal pro-domain are removed. This process can be performed by various agents in vitro, including *p*-aminophenylmercuric acetate (APMA), SDS, urea, chaotropic agents, heat treatment and by proteinases [6]. Once activated, the activity of MMPs can be regulated by endogenous inhibitors such as α2-macroglobulin and tissue inhibitors of MMPs (TIMPs) [7].

Together, the MMPs are able to degrade most extracellular matrix proteins, as well as regulating the activity of serine proteinases, growth factors, cytokines, chemokines and cell receptors [8]. Thus, MMPs have complicated biological functions playing a role in modulating normal cellular behaviour, cell–cell communication as well as in pathological conditions [8]. Among the naturally occurring processes that involve MMPs are wound healing and tissue remodelling, development, growth and maintenance, and examples of pathological conditions where MMPs and tissue breakdown is involved include various types of arthritis, skin diseases, oral diseases, multiple sclerosis, diabetes mellitus, cardiovascular diseases, tumor growth and angiogenesis, invasion and metastasis of tumor cells [1–5,8].

Colchicine is an alkaloid that disrupts the microtubular organisation, arrests cells in mitosis and affects other microtuble-dependent functions [9]. Due to its anti-fibrotic, anti-mitotic, anti-inflammatory and anti-metastatic activities, colchicine has been therapeutically used in the treatment of various diseases, such as gout, cirrhosis, sclerosis, aquired epidermolysis bullosa, Behcet's syndrome, Mediterranean fever and Sweets syndrome as well as in various cancers [10–20]. It has been shown that colchicine alters the expression of various MMPs (among these MMP-1), TIMPs, collagen I and other proteins in a variety of cells and tissues [21–27]. In diseases with a deposition of collagen, treatment with this kind of agents that reduce the synthesis of collagen and/or increases the synthesis of collagenases appears to be beneficial. However, in many cases the therapeutic effect of colchicine has been questioned, and sometimes treatment has resulted in severe side effects [10].

The rationale for therapeutic use of colchicine varies with the type of disease, and hence the lack of effect may be manifold. In cases where the aim is to alter the collagen homeostasis, one might expect the lack of clinical responsiveness to colchicine in some patients to be due to interindividual heterogeneity in the cellular expression of collagenases in response to colchicine. One of us (TGD) has during the last 40 years studied the genetics of various skin diseases, especially a family of blistering disorders called epidermolysis bullosa [28,29], and has therefore during the years established skin fibroblast cultures from many individuals with skin diseases as well as from healthy individuals. In the present work, we have used this material to determine if there is an interindividual heterogeneity in skin fibroblasts response to colchicine treatment with respect to the synthesis of collagenase.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA), colchicine, trypsin, soybean trypsin inhibitor (SBTI), calf thymus DNA, 3,5-diaminobenzoic acid, azocasein, casein, secondary antibodies, gelatin (Bloom 300), acid-soluble calf skin and rattail collagens were from Sigma. Culture media and fetal calf serum were from Flow Laboratories and GIBCO. Quickscint 294 was from Zinsser Analytic Ltd. Tritiated sodium borohydride and ^{14}C-formaldehyde were from Amersham. The polyvinylidene difluoride membranes used for western blotting were from Millipore, the CDP-star chemiluminiscence substrate and AP protein marker detection pack for Western blotting were from New England Biolabs. 2-Methoxy-2,4-diphenyl-3(2H)-furanone (MDPF) was from Fluka. SDS-PAGE molecular weight standards were from BioRad. Human recombinant TIMP-1 and proMMP-2 was from Oncogene and Chemicon, respectively.

2.2. Subjects

Thirty-two fibroblast cell lines were investigated, and these were obtained from 13 healthy persons, 11 patients with junctional epidermolysis bullosa (JEB: eight Gravis-Herlitz and three inversa cases), two with recessive pseudojunctional epidermolysis bullosa (REBPJ; now renamed to epidermolysis bullosa simplex with muscular dystrophy, EBS-MD [30], six with Ehlers-Danlos Syndrome (four with type III, one of type II and one of type IV). The two EBS-MD patients belong to a two generation family (NEB 1) with nine children of which eight were available for this study, and NEB1-9 and NEB1-11 are the EBS-MD children [30,31]. Three of the JEB Herlitz patients were siblings and one of the healthy controls their mother (Family EB 107).

2.3. Fibroblast cultures

Skin fibroblast cultures were established from biopsies taken from medial or lateral aspect of upper arms, as the biopsy site did not affect the collagenase production [32]. Cells from the established skin fibroblast cultures (passages 4–10) were subcultivated in disposable plastic culture dishes or flasks in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, 20 mM HEPES buffer (pH 7.2–7.4), 100 IU/ml penicillin, 100 μg/ml streptomycin, nonessential amino acids (100 times dilution) and 2.0 mM L-glutamine at 37 °C in a humidified 5% CO_2 atmosphere.

To determine the effect of colchicine on the matrix metalloproteinase expression, approximately 1.5×10^5 cells were seeded per cluster 6 well to obtain optimal conditions [26]. After the cell layers were washed three times with Hank's balanced salt solution, the cultures were maintained for 48 h in serum-free DME medium containing varying concentrations of colchicine. Before freezing, half of the harvested media was made 10 mM with $CaCl_2$ and 100 mM with Hepes buffer (pH 7.5) while the other half was made 0.2% BSA/100 mM Hepes (pH 7.5)/10 mM $CaCl_2$. The former solution was used in the determination of collagenase activities, gelatin zymography and real-time reverse gelatin zymography, while