

## Short Communication

# The Effect of Interferon $\beta$ -1a on MMP-2 and MMP-9 Proteolytic Activity

(IFN  $\beta$ -1a / MMP-2 / MMP-9 / zymography)

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**Abstract.** MMP-2 and MMP-9 play a significant role in the development of numerous diseases mainly with the inflammatory pathogenesis. One of the drugs exerting an effect on metalloproteinases is IFN- $\beta$ . Previous studies showed that IFN  $\beta$ -1b can decrease MMP synthesis and moreover inhibit their proteolytic activity. The aim of our study was to analyse the influence of recombinant IFN  $\beta$ -1a (identical with natural IFN- $\beta$ ) on MMP-2 and MMP-9 activities. The gelatinolytic activity was evaluated with zymography in sera obtained from 10 healthy donors. After electrophoresis gels were incubated with or without IFN  $\beta$ -1a (2000 U/ml) for 18 h. We noticed a significant decrease of MMP-2/72 kDa ( $P = 0.0283$ ) and the augmentation of MMP-9/92 kDa ( $P = 0.0042$ ) activities after incubation with interferon. The elevation of MMP-9/92 kDa activity suggests that IFN  $\beta$ -1a can exhibit proinflammatory features besides well-known anti-inflammatory properties.

## Introduction

Metalloproteinase (MMP) MMP-2 and MMP-9 are  $Zn^{2+}$ -dependent endopeptidases that degrade compounds of extracellular matrix ECM such as different types of collagen (mainly type IV), elastin, and laminin (Chandler et al., 1997). MMP-2 and MMP-9 disrupt the subendothelial basal membrane; therefore, they are responsible for the facilitation of leukocyte migration across the vessel wall and the development of the inflammatory process (Opdenakker et al., 2001). Both enzymes play a significant role in numerous physiological and pathological processes. An example is multiple sclerosis (MS), where MMP-2 and MMP-9 are involved in blood-brain barrier (BBB) disruption. MMP-9 seems to be associated with the active MS,

whereas MMP-2 is more specific for chronic progressive MS (Avolio et al., 2003). Recombinant interferon  $\beta$ -1a (IFN  $\beta$ -1a) exerts immunomodulatory properties during MS treatment, decreases gadolinium-enhancing lesions of the white matter on magnetic resonance imaging (MRI) scans and restores the BBB mainly due to downregulation of MMP-9 expression (Avolio et al., 2005). Previous studies showed that IFN  $\beta$ -1b can also inhibit MMP-2 and MMP-9 activity at the posttranslational level, probably through interaction between the drug and the enzyme (Kieseier and Hartung, 2004). IFN  $\beta$ -1b displays some differences in comparison with IFN  $\beta$ -1a (a slightly different amino acid structure and lack of the glycosylated moiety). The structure of IFN  $\beta$ -1a is consistent with natural human IFN- $\beta$  with a high glycosylation ratio. The aim of our study was to evaluate the direct effect of IFN  $\beta$ -1a on serum MMP-2 and MMP-9 gelatinolytic activity.

## Material and Methods

The present work was performed in accordance with the ethics standard of Helsinki Declaration and approved by local Ethics Committee. All individuals gave informed consent. The serum samples were obtained from 10 healthy donors (female/male: 6/4, mean age  $\pm$  SD:  $24 \pm 1.2$  years).

### *Biochemical procedures*

After centrifugation the serum was stored at  $-30^{\circ}\text{C}$  for two weeks. MMP-2 and MMP-9 activity was determined by gelatin zymography according to the method described by Azeh et al. (1998) with modifications. Briefly, the samples consisting of 9  $\mu\text{l}$  of diluted serum (1/50 with redistilled water) + 3  $\mu\text{l}$  of sample buffer with 10% sodium dodecyl sulphate (SDS) were separated on 10% polyacrylamide gel with 0.05% gelatin type A from porcine skin (G2500, Sigma-Aldrich, Poole, Dorset, UK). After electrophoresis, washing was carried out for two 30-min periods with buffer 50 mM Tris-HCl, pH 7.2, containing 10 mM  $\text{CaCl}_2$ , 0.02%  $\text{NaN}_3$  and 2.5% Triton X-100. The incubation with or without 2000 U/ml IFN  $\beta$ -1a (Rebif, Serono International, Geneva, Switzerland) was performed for 18 h at  $37^{\circ}\text{C}$  in the above buffer but with 1% Triton X-100. Gels were stained with 0.1% Coomassie

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Abbreviations: BBB – blood-brain barrier, CNS – central nervous system, ECF – extracellular fluid, Gd-enhancing – gadolinium enhancing, IFN- $\beta$  – interferon  $\beta$ , IL-1 $\beta$  – interleukin 1 $\beta$ , MMP-2 – matrix metalloproteinase 2, MMP-9 – matrix metalloproteinase 9, TNF- $\alpha$  – tumour necrosis factor  $\alpha$ .

Blue R-250 in 30% ethanol and 10% acetic acid and destained in 30% ethanol and 10% acetic acid. MMP-2 and MMP-9 activity was detected as clear bands on the blue background. Enzymes were identified by co-localization with a molecular mass standard (Fermentas, SM0441). Quantification of zymograms was done using a digital camera (resolution 4.2 Mpx) and ZERO-Dscan Image Analysis System v. 1.0. (Scanalytics, Billerica, MA). MMP-2 and MMP-9 activity was expressed as the optical density (OD) of the substrate lysis zone.

### Statistics

To compare MMP-2 and MMP-9 activity after incubation with vs. without IFN  $\beta$ -1a, Wilcoxon matched pairs test was used. Statistically significant values were considered when  $P < 0.05$ . Data are expressed as mean and SD. Statistical analysis was performed with the use of the computer-assisted statistical program GraphPad InStat v. 3.06.

### Results and Discussion

Gelatinolytic activity was detectable at molecular weight of 72 kDa (corresponding to pro-MMP-2) and 92 kDa (pro-MMP-9). Low activities were noticed at 130 kDa (MMP-9 heterodimer) and at approximately 200 kDa (MMP-9 homodimer), but for further analysis only direct pro-forms of the active enzymes were used. We noticed that after incubation of zymographic gels with 2000 U/mL of IFN  $\beta$ -1a the proteolytic activity at 72 kDa (pro-MMP-2) decreased significantly ( $P = 0.0283$ ), whereas interferon had the opposite effect on MMP-9/92 kDa (increase of activity,  $P = 0.0042$ ) (Fig. 1).

At present, IFN  $\beta$ -1a is used in MS treatment – a chronic inflammatory disease of the central nervous system (CNS) characterized by demyelination, focal T-cell and macrophage infiltration, axonal injury, and loss of neurological functions (Bar-Or et al., 1999). MMPs act on several phases of MS development, disrupt the BBB

(the increase of lymphocyte migration into CNS), and they also take part in the degradation of myelin shield proteins (Chandler et al., 1997). Previous studies demonstrated that IFN- $\beta$  slows down T-cell migration across BBB by suppressing the MMP-9 expression (Hartung and Kieseier, 2000). Moreover, IFN  $\beta$ -1b exhibits inhibitory effects on MMP-2 and MMP-9 activity at the post-translational level (Kieseier and Hartung, 2004). In this study we investigated IFN  $\beta$ -1a influence on MMP-2 and MMP-9 activity at the protein level. Our observations of the effect of IFN  $\beta$ -1a on MMP-2 activity (72 kDa form) are consistent with previous observations (decrease of activity), but the drug unexpectedly augmented the gelatinolytic activity at 92 kDa (pro-MMP-9). This finding suggests that IFN  $\beta$ -1a could also exhibit proinflammatory effects. The concentration of IFN  $\beta$ -1a (2000 U/ml) applied in this experiment was significantly higher than the concentration that is normally present in serum during therapy of MS. It is therefore difficult to predict the influence of interferon on MMP-9 proteolytic activity in natural *in vivo* conditions. We do not know whether the enhanced MMP-9 activity is connected with the presence of glycosylated moiety (common feature of natural IFN- $\beta$  and MMP-9) or other properties of IFN  $\beta$ -1a. Further studies for evaluation of the interactions between metalloproteinases and interferon are needed.

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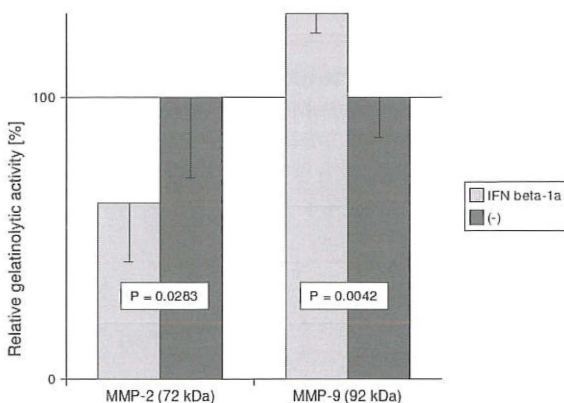


Fig. 1. Relative serum gelatinolytic activity. IFN  $\beta$ -1a had opposite effects on both gelatinases. MMP-2 (72 kDa) activity decreased (38.5%) whereas MMP-9 (92 kDa) increased (30.1%) after incubation with IFN  $\beta$ -1a. Mean activity (with SD) of MMP-2 and MMP-9 developed with standard buffer (-) are expressed as 100%.