Microscopic evaluation of pro–MMP–9 immunohistochemical reaction correlates to the mean signal intensity of cytoplasmic antigen expression – a study of regenerating liver after treatment with interferon α2b

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Abstract

The aim of this study was to evaluate the effect of IFNα2b on pro–MMP–9 antigen expression in regenerating liver by using two methods and verify the correlation between results obtained by these methods. The study was performed in a model of hepatectomized rats. Group I was treated with IFNα2b and group II was injected with NaCl. Microscopic analysis comprised counting of pro–MMP–9 immunopositive cells, while the second method comprised mean signal intensity analysis of pro–MMP–9 immunohistochemical reaction by using Matlab. The results revealed that IFNα affects liver regeneration through modulatory effect on pro–MMP–9 antigen expression. Moreover, we found that antigen expression was regulated in a different manner in heterogeneous liver areas. We also conclude that the analysis of mean signal intensity performed by Matlab constitutes an interesting tool for evaluation of cytoplasmic expression of antigens that belong to matrix metalloproteinase family.

Keywords: liver regeneration, matrix metalloproteinase, interferon, antigen expression, mean signal intensity

1. Introduction

The liver is a highly unique organ in that it retains the ability to regenerate after injury until an organ of similar weight is formed (Fausto et al, 1994; Rudolph et al, 1999; Koniaris et al, 2003). Organ remodeling is composed of strictly controlled multiple processes that comprise cell proliferation, migration, extracellular matrix (ECM) degradation and synthesis. In the early stages of liver regeneration, hepatic histology differs significantly from normal. Hepatocytes are grouped into nonvascularized clusters of 12 to 15 cells, with the amount of extracellular matix reduced as a consequence of hepatocyte proliferation without concomitant extracellular matrix synthesis. Later in the regenerative process, hepatocytes proliferation decreases and ECM synthesis is induced that with formation of novel vascular branches result in restoration of normal hepatic tissue (Martinez–Hernandez et al, 1991; Fausto et al, 1994; Malik et al, 2002; Michalopoulos et al, 2005; Olle et al, 2006).

MMPs are a family of zinc endopeptidases produced by a variety of cell types. The family includes at least 21 members with proteolytic activity toward a number of different ECM components (Bueno et al, 2000; Giannelli et al, 2002; Marinosci et al, 2005; Olle et al, 2006). MMPs are secreted in latent form and then require cellular activation by the complex membrane type 1 MMP (MT–1 MMP) together with the tissue inhibitor of MMPs (TIMP)–1 and/or–2 (Nagase, 1997; Marinosci et al, 2005). MMP–9 also known as gelatinase owing the ability to degrade collagens, including types I, III, IV, and VI. It has been shown that MMP–9 is a crucial factor in liver regeneration (Matrisian, 1992; Knittel et al, 1999; Knittel et al, 2000; Marinosci et al, 2005; Olle et al, 2006).

IFNα classified as a type I interferon, represents a family of more than 20 different proteins that are produced mainly by leukocytes and exhibit diverse antiviral, immunomodulatory and antiproliferative effects (Kocić et al, 1998; Favre et al, 2001; Thomas et al, 2003; Gao et al, 2004). Many of its actions are exerted by the interaction of the IFNα receptor–kinase complex with STAT transcription factors, which, once activated, form multimers that bind to the regulatory elements of cytokine-inducible genes (Favre et al, 2001). IFNα was initially discovered because of its ability to interfere with viral replication (Theocharis et al, 1997; Gao et al, 2004). At present, the use of IFNα in combination with ribavirin represents the gold standard for the treatment of chronic hepatitis C. Its therapeutic effect on liver tissue in terms of reduction of fibrosis is also considered (Duchatelle et al, 1998; Bueno et al, 2000; Marinosci et al, 2005).

The aim of this study was to evaluate the effect of IFNα2b on the expression of pro–MMP–9 antigen in regenerating liver and to verify whether the antigen expression evaluated by microscopic analysis correlates to the values of mean signal intensity of pro–MMP–9 immunohistochemical reaction performed by Matlab software.

2. Materials and Methods

2.1 Animals and Treatment: The study comprised 40 three months old male Wistar rats (Medical University of Silesia, Katowice, Poland), weighing 280 ± 300g each. The animals were maintained on standard rat chow and water *ad libitum* until the day before surgery. The animals were kept in the room temperature (25°C, 12L:12D photoperiod). Randomly selected animals were assigned into two groups: I – Interferon (n=20), and II – Control (n=20).

At the beginning of the study the animals of group I were subcutaneously injected with 0,5 ml of IFNα (solution of 5MU/100 ml 0,9% NaCl, Intron A, Schering–Plough) and the animals of group II were injected with 0,5 ml of 0,9% NaCl, respectively.

The dose of IFNα2b corresponded to a dose used in human therapy calculated for rat body weight. 24h after injections the animals were subjected to partial hepatectomy (PH, 70% of liver tissue excised) (Higgins et al, 1931), under ketamine anesthesia (100 mg/kg, intraperitoneally). 24h after PH, the animals of group I were injected with the second dose of IFNα, and the animals of group II with the second dose of 0,9% NaCl. The animals of both groups were sacrificed under ketamine anesthesia in subgroups of five per group at 48h, 72h, 96h and 120h post–operatively. Experiments were started between 7:00 and 9:00 AM. All animal experiments were conducted in accordance with local institutional guidelines for the Care and Use of Laboratory Animals. Small portions of regenerating liver tissue were removed, fixed in 4% formaldehyde and embedded in paraffin. Liver tissue sections were assigned for immunohistochemistry.

2.2 Immunohistochemistry: Immunohistochemical staining was performed using a three stage indirect immunoperoxidase technique. Tissue was rehydrated in graded alcohols, rinsed in a bidistilled water, and boiled in a citrate buffer (pH 6,0; microwave) for 10 minutes.

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Subsequently slides were incubated with 1,5% hydrogen peroxide in methanol. After washing in PBS, tissue sections were incuvated for 20 minutes in blocking solution (Normal Serum, Novostain Super ABC Kit, universal, NCL-ABCu, Novocastra Lab., U.K.). Mouse anti–rat pro–MMP–9 antibody (95kDa collagenase IV, gelatinase B, MAB 13421, Chemicon Int. USA) at 1:200 dilution was applied for 1h incubation. Next, the tissues were incubated with biotinylated IgG (uABC, Novocastra Lab., U.K.) for 30 minutes followed by 30 minutes incubation with avidin conjugated to horseradish peroxidase (uABC, Novocastra Lab., U.K.).

In the next step 15 minutes incubation with DAB Substrate–chromogen (Novocastra) was performed. After a final wash the slides were counterstained with a Mayer's hematoxylin for 1 minute, washed in tap water, dehydrated in graded alcohols, and mounted in DPX medium.

For all specimens reference liver tissue sections were sequentially cut and processed identically to experimental sections except of primary antibody incubation (Matkowskyj et al, 2000; Matkowskyj et al, 2003). Each reference liver tissue section was used as a negative control for immunohistochemical reaction. Liver tissue from rat injected with CCl_4 solution (200µl CCl_4/100g m.c., subcutaneous injection) was a positive control for each run.

2.3   **Microscopic analysis of pro–MMP–9 immunopositive cells:** Liver tissue sections were assessed independently by 2 experienced pathologist (magnification 200 x, 1000x Nikon *E600*). Pro–MMP–9 immunopositive cells located around 10 periportal (PPA) and 10 pericentral (PCA) regions per one liver specimen (2 sections/specimen) were counted and the images of cells were acquired by analogue TV camera (GP–KR222E, Panasonic) coupled with the frame–grabber (752 x 548 pixels) and saved as TIFF files. Liver sections that contained pro–MMP–9 immunopositive cells around 10 periportal (PPA) and 10 pericentral (PCA) regions were marked as "EXPERIMENTAL" (EXP) for purposes of further analyses. To define corresponding regions within "REFERENCE" (REF) liver sections (primary antibody omitted) we performed morphometric analysis of pro–MMP–9 immunopositive cells (table 1). Minimal and maximal values of measured parameters served as a criterion for identification and acquisition of corresponding cells around 10 PPA and 10 PCA regions within REF sections. Further, pro–MMP–9 immunopositive cells from EXP liver sections (10 PPA and PCA per one liver specimen) as well as defined by morphometric analysis cells from REF liver sections (10 PPA and 10 PCA per one liver specimen) served for analysis of mean signal intensity of pro–MMP–9 immunohistochemical reaction.

2.4 Mean signal intensity analysis of pro–MMP–9 antigen expression: The parallel regions assigned to analysis comprised 10 PPA and 10 PCA regions in EXP and REF sections per one liver specimen (fig. 1a). Each PPA and PCA region comprised 200 000 µm^2 surrounding portal area, and central vein, respectively (fig. 1b). In the next step, regions of interest (ROI) of all acquired cells were defined. In this purpose the GIMP software was used (fig. 1c). Each ROI was of size M x N (15 x 15 pixels) and comprised cell cytoplasm area. The pairs of EXP and REF images were created within each PPA and PCA region of EXP and corresponding REF sections. EXP image file contained ROIs within cytoplasm of cells expressing pro–MMP–9 antigen that were defined in PPA/PCA of EXP section. Subsequently, REF image file contained ROIs within cytoplasm of cells defined by morphometric analysis that were localized in parallel PPA/PCA regions of REF sections. Both files were assigned for analysis by using Matlab software (MathWorks, Natick, US). In summary, 100 images per 10 PPA and 100 per 10 PCA (per one specimen) were assigned for analysis. The absolute value of gray–level differences between pixels in the ROI's of EXP and REF images was used for the estimation of the amount of antibody–generated chromogen inside parallel regions in the liver.

The chromogen signal was measured as follows:

$F(\mathbf{x}_{chromogen}) = | f(\mathbf{x}_{experiment}) - f(\mathbf{x}_{reference}) |,$

where f(•) represent the grey levels of pixels on EXP and REF ROI, respectively, and F(•) is the chromogen signal value.

The chromogen signal energy is proportional to the square root of the summation of the squares of the F(•) values calculated for each pixel of ROI. In 24-bit color image, the red, green and blue components are represented as 8-bit integer numbers. The chromogen signal energy $f_b(•)$ can be expressed as:

$$f_b(\mathbf{x}) = \left[\sum_{i=1}^{M} \sum_{j=1}^{N} [f_c(i,j)]^2 \right]^{1/2} + \left[\sum_{i=1}^{M} \sum_{j=1}^{N} [f_z(i,j)]^2 \right]^{1/2} + \left[\sum_{i=1}^{M} \sum_{j=1}^{N} [f_n(i,j)]^2 \right]^{1/2}$$

where f_c, f_z f_n are the grey levels of R,G,B components of pixel p(i,j).

The mean signal value ("intensity") of pro–MMP–9 immunohistochemical reaction was performed as chromogen signal energy divided by the number of all evaluated pixels. These mean signal intensity values, calculated for the ROIs analysed were reported in arbitrary units intensity/pixel (*I/pix*) (Matkowskyj et al 2000). Method used to measure mean signal intensity of cytoplasmic antigen expression in pro–MMP–9 immunopositive cells by using Matlab software is depicted in table 2.

In statistical analysis of multiple comparisons and between every time–point (48h, 72h, 96h, 120h) the Kruskal–Wallis test was used (in tables 2 and 3 noted as ANOVA). Post–hoc test for non–parametric analyses for multiple comparisons (double-sided) was performed. The different variances were managed between variance among groups of animals in different time–points of experiment in two heterogenous areas separately (periportal and pericentral) and in two groups separately (Interferon and Control). In all instances, data are expressed as the means ± standard deviation (SD). Differences were considered to be significant at p<0,05. Statistical analysis was performed using Statistica 6.0 PL.

3. Results

Pro–MMP–9 antigen expression was evaluated by two methods. First, estimation of the number of pro–MMP–9 immunopositive cells within periportal and pericentral regions was performed. Further, we performed the analysis of mean signal intensity of pro–MMP–9 immunohistochemical reaction within collected immunopositive cells. The second analysis was conducted in serially cut liver sections to create pairs of EXP and REF images. EXP image file contained ROIs within cytoplasm of cells expressed pro–MMP–9 antigen that were defined in PPA/PCA of experimental section. REF image file contained ROIs within cytoplasm of cells defined by morphometric analysis that were localized in parallel PPA/PCA region of reference section. Collected images were analyzed by Matlab software. Both analyses were performed in sections from liver excised 48h, 72h, 96h and 120h after surgery. To answer whether pro–MMP–9 expression evaluated by the number of immunopositive cells correlate to the values of mean signal intensity measured by computer method we evaluated Spearman's R coefficient.

3.1 Pro–MMP–9 antigen expression in periportal and pericentral regions of regenerating liver treated with interferon α2b–comparison between particular time–points after PH: In both liver regions pro–MMP–9 expression peak was observed at 48h after PH and it was significantly higher as compared to control group (p<0,01). Similarly, at 72h after PH, antigen expression was significantly higher as compared to control (p<0,01). In contrary, a decrease of antigen expression was found at later stages after PH with the lowest antigen expression at 120h post–operatively and it was observed in both liver regions (p<0,01) (table 3, 4; figure 2 –5).

3.2 Pro–MMP–9 antigen expression in periportal and pericentral regions of regenerating liver treated with interferon α2b–comparison between heterogeneous regions of the liver: Pro–MMP–9 antigen expression obtained at each time–point after PH in periportal areas was significantly higher as compared to its expression observed in pericentral areas (p<0,01) (table 3, 4; figure 6, 7).

3.3 Spearman's R coefficient evaluation: Spearman's R analysis revealed strong correlation between the number of pro–MMP–9 immunopositive cells and mean signal intensity values in both liver regions.

In particular, the highest correlation was observed in periportal and pericentral regions of group treated with IFNα2b. Relatively lowest correlation was observed in periportal and pericentral regions of control group (table 5).

4. Discussion

To date, it has not been elucidated whether pro–MMP–9 expression is modulated by interferon α during liver regeneration. To answer this question we evaluated pro–MMP–9 antigen expression by employing two methods.

Microscopic analysis comprised evaluation of the number of pro–MMP–9 immunopositive cells in periportal and pericentral regions of regenerating liver. Additionally, antigen expression was evaluated by measurement of mean signal intensity of immunohistochemical reaction by using Matlab software. Subsequently, we estimated Spearman's coefficient to verify whether results obtained by microscopic and computer methods correlate to each other.

Our analyses revealed that IFN α significantly induced expression of pro–MMP–9 antigen at 48h post surgery followed by gradual decrease at later time–points and such profile of expression was noted in both liver regions i.e. around portal and central veins. The effect of IFN α at 96h and 120h after PH was very weak where pro–MMP–9 expression was under the level observed for control group. Similar expression profile of pro–MMP–9 antigen was observed by Haruyama et al (2000). In contrary, several other studies shown that matrix remodeling play a role in the early stages of hepatic regeneration (Liu et al, 1994; Olle et al, 2006). Kim et al (2000) demonstrated that the induction of pro–MMP–9 is observed around 30 minutes after PH, while the active form was detected between 3h and 6h after PH. Others observed that the gelatinase was activated within 6 to 12 hours after PH, during the window of growth factor stimulus (Rudolph et al, 1999; Knittel et al, 2000; Mohammed et al, 2005). It was sugessted that pro–MMP–9 activation after partial hepatectomy contributes to priming hepatocytes for proliferation by modulation of the matrix environment in the remnant liver (Kim et al, 1997; Kim et al, 2000; Olle et al, 2006).

To the some extent similar observations were presented in the study of Bueno et al (2000). In this study bile duct ligated (BDL) rat's model was used to evaluate the effect of IFNα2a on MMPs activation. Three bands of gelatin degradation were detected by zymography, corresponding to 65kDa, 75kDa and 95kDa. It has been concluded that IFN α may interact on ECM through MMPs by modulation of its expression and activation.

In another study, Marinosci et al (2005) evaluated pro–MMP–9 expression in naive patients with chronic hepatitis C before and after treatment with PEG–IFNα2b. In contrary, the intensity of staining by anti–pro–MMP–9 antibody performed in liver biopsies decreased in responding patients, whereas no differences were observed in non–responders. To better define the clinical significance of above finding, pro–MMP–9 mRNA levels were measured by real–time PCR in liver biopsies. After therapy, a strong decrease in MMP–9 mRNA was observed in responders, but not in non–responders.

We found that IFN induced significantly the increase of pro–MMP–9 antigen expression 48h after PH, while gradual decrease of antigen expression was observed at later time–points after surgery. Of note, in the present study 48th hour after PH corresponds to 24th hour after subcutaneous injection of IFN. Induction of pro–MMP–9 antigen expression at 24th hour after IFN injection followed by gradual decrease in later stages corresponds to IFNα2b pharmacokinetics and bioavailability (Corssmit et al, 1997; European Medicines Agency, CHMP, London, 2006). Additionally, antagonistic to IFN interactions of growth factors and cytokines, which are abundantly synthesized after partial hepatectomy and play an important role in liver regeneration might play a role (Andus et al, 1991; Fausto et al, 1995). For example, TGFβ$_1$ selectively stimulates proliferation and migration of nonparenchymal cells as well as the synthesis of ECM components both *in vivo* and *in vitro* (Lawrence, 1996; Grasl–Kraupp et al, 1998; Ma et al, 1999; Malik et al, 2002). It was also shown that TGFβ$_1$ reduces the synthesis of MMPs, and reduces their proteolytic activity by increasing the expression of plasminogen activator inhibitor–1 and TIMPs–specific inhibitors of MMPs (Overall et al, 1991). Moreover, cytokines and growth factors interact with IFNα and are involved in IFN signaling in the liver. For instance, Hong et al, (2001) and Gao et al (2004) demonstrated that injections of synthetic TNFα markedly inhibited IFNα–induced signals in the liver, and that TNFα was responsible for suppression of IFNα signaling in the experimental model of liver injury induced by CCl$_4$.

Based on the location of the blood vessels and the direction of the blood flow, the individual liver lobule can be subdivided into an upstream, i. e. periportal, and a downstream i. e. pericentral area. Hepatocytes located in either of the two regions have different, often complementary functions, as indicated by differences in the content and activity of key enzymes and xenobiotic metabolism (Jungermann, 1992,1995; Ferri et al, 2005). According to this notion, we compared the modulative effect of IFNα2b on pro–MMP–9 antigen expression in two structurally and physiologically heterogeneous liver regions. We showed that pro–MMP–9 antigen expression was significantly higher in PPA as compared to PCA at each time–point after PH.

An interesting observation was made in PPA and PCA at 96h and 120h after PH, respectively. Pro–MMP–9 antigen expression decreased in PPA at 96h so dramatic that it reaches lower levels than in control group. In opposite, in PCA antigen expression at 96h after PH was similar in both groups, and decreased at 120h after surgery. Therefore, we assumed that IFN administered to hepatectomized rats modulates the expression of pro–MMP–9 antigen in a different manner that is dependent on the localization along the periportal–pericentral axis in the liver lobule.

The effect of IFNα2b on pro–MMP–9 antigen expression was evaluated by two methods. The number of pro – MMP–9 immunopositive cells was evaluated in PPA and PCA regions at 48h, 72h, 96h and 120h after PH and IFN treatment. The second method comprised mean signal intensity measurement in pro–MMP–9 immunopositive cells and was conducted by using Matlab software. Spearman's rank coefficient was evaluated for results obtained by these two method for PPA and PCA regions, both in interferon and control groups. The highest correlation was observed in periportal and pericentral regions of group treated with IFNα2b. Relatively lower correlation was observed in control group. Mean signal intensity analysis that we performed by using Matlab software was previously described by Matkowskyj et al (2000) who used this method to study aberrantly expressed gastrin–releasing peptide receptor (GRPR) in human colon cancers. This methodology was also used by others to evaluate expression of several receptors related to colitis and colon cancer (Carroll et al, 2000; Marrero et al, 2000; Matkowskyj et al, 2003).

5. Conclusion

The results obtained in this study revealed that IFNα affects liver regeneration through modulatory effect on pro–MMP–9 antigen expression. Moreover, we found that antigen expression was regulated in a different manner in periportal and pericentral regions of liver. Based on present findings we conclude that the analysis of mean signal intensity performed by Matlab constitutes an interesting tool for evaluation of cytoplasmic expression of antigens that belong to matrix metalloproteinase family.

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Parameter measured	Pro–MMP–9 immunopositive cells
	Minimal – maximal values
Cell area [μm^2]	33,39 – 44,40
Nucleus area [μm^2]	13,61 – 15,71
Cell cytoplasm area [μm^2]	17,80 – 29,55
Cytoplasm- nucleus ratio	0,33 – 0,46
Cell perimeter [μm]	21,09 – 25,40
Circularity ratio	0,86 – 0,94

Table 1: Morphometric Analysis of Cells Expressed Pro–MMP–9 Antigen in Periportal and Pericentral Regions of Liver Sections

```
I=115;
    for i = 1:I
disp(sprintf('%i/%i',i,I));
name=sprintf('contr.%i.tiff',i);
a=double(imread(name));
desc=imfinfo(name);
Na(i)=desc.Width()*desc.Height();
name=sprintf('exp.%i.tiff',i);
b=double(imread(name));
desc=imfinfo(name);
Nb(i)=desc.Width()*desc.Height();
ea(i)=norm(a(:,:,1))+norm(a(:,:,2))+norm(a(:,:,3));
eb(i)=norm(b(:,:,1))+norm(b(:,:,2))+norm(b(:,:,3));
    end
ea_m=mean(ea);
ea_s=std(ea);
eb_m=mean(eb);
eb_s=std(eb);
en=abs( (ea_m/sqrt(sum(Na))) - (eb_m/sqrt(sum(Nb))) );
disp('chromogen intensity values for particular homologic regions');
    for  i = 1:I
disp(sprintf('no: %i, ea: %d, eb: %d, Na: %i, Nb:
%i',i,ea(i),eb(i),Na(i),Nb(i)));
    end
  disp('end values');
  disp(sprintf('ea_m: %d +- %d',ea_m,ea_s));
  disp(sprintf('eb_m: %d +- %d',eb_m,eb_s));
  disp(sprintf('en: %d',en))
```

Table 2: Method Used to Measure Mean Signal Intensity of Cytoplasmic Antigen Expression in Pro–MMP–9 Immunopositive Cells by Using Matlab Software

Group (n)	Areas	Mean number of cells ± SD Time points post-operatively				„p" value for time points analysis						ANOVA „p"
		48h	72h	96h	120h	48/72	48/96	48/120	72/96	72/120	96/120	
Interferon	Periportal (10)	18,7 ± 0,26	18,3 ± 0,26	15,00 ± 0,37	14,3 ± 0,41	1,00	0,062	**0,002**	0,523	**0,033**	1,00	**0,001**
	Pericentral (10)	16,1 ± 0,35	15,3 ± 0,37	11,7 ± 0,40	11,2 ± 0,26	1,00	**0,035**	**<0,001**	0,689	0,057	1,00	**<0,001**
Control	Periportal (10)	16,3 ± 0,26	15,7 ± 0,32	18,4 ± 0,32	16,7 ± 0,35	0,940	0,072	1,00	**0,001**	0,112	0,689	**0,001**
	Pericentral (10)	12,3 ± 0,26	11,7 ± 0,37	12,00 ± 0,49	15,1 ± 0,45	0,494	1,00	0,440	1,00	**0,003**	**0,042**	**0,003**

Table 3: Mean Number of pro–MMP–9 Immunopositive Cells in PPA and PCA

Group	Areas	Mean signal intensity ± SD Time points post-operatively				„p" value for time points analysis						ANOVA „p"
(n)		48h	72h	96h	120h	48/72	48/96	48/120	72/96	72/120	96/120	
Interferon	Periportal (10)	72,2 ± 3,39	61,0 ± 1,75	44,4 ± 1,39	39,7 ± 1,8	1,00	**0,045**	**<0,001**	1,00	**0,045**	1,00	**<0,001**
	Pericentral (10)	57,9 ± 1,95	48,4 ± 1,36	35,6 ± 1,38	25,8 ± 1,12	1,00	**0,045**	**<0,001**	1,00	**0,045**	1,00	**<0,001**
Control	Periportal (10)	48,7 ± 1,2	46,1 ± 3,27	52,9 ± 0,90	52,5 ± 1,56	1,00	0,112	0,195	**0,012**	**0,023**	1,00	**0,002**
	Pericentral (10)	39,3 ± 1,16	37,7 ± 0,84	39,1 ± 1,90	47,7 ± 1,85	0,807	1,00	0,253	1,00	**0,003**	0,084	**0,004**

Table 4: Mean signal Intensity of pro–MMP–9 Cytoplasmic Reaction in PPA and PCA [*I/pix*]

GROUP	AREA	SPEARMAN'S R COEFFICIENT
INTERFERON	PPA	0,90
CONTROL	PPA	0,82
INTERFERON	PCA	0,94
CONTROL	PCA	0,66

Table 5: Spearman's R Coefficient Calculated for the Number of pro–MMP–9 Immunopositive Cells and Mean Signal Intensity Values in PPA and PCA

Figure Legends

Figure 1: Processing of liver sections for analysis in Matlab. Parallel regions of experimental and sequentially cut reference rat liver sections (a); The example region of PCA in experimental and reference liver sections (b); Definition of regions of interest (ROI) within pro–MMP–9 immunopositive cells in EXP sections (left) or in cells defined by morphometric analysis in serially cut REF sections (right). The pairs of EXP and REF images were created within each PPA/PCA region of experimental and corresponding reference sections. Each ROI was of size M x N (15 x 15 pixels) and comprised cell cytoplasm area. ROI were defined by using GIMP software (c).
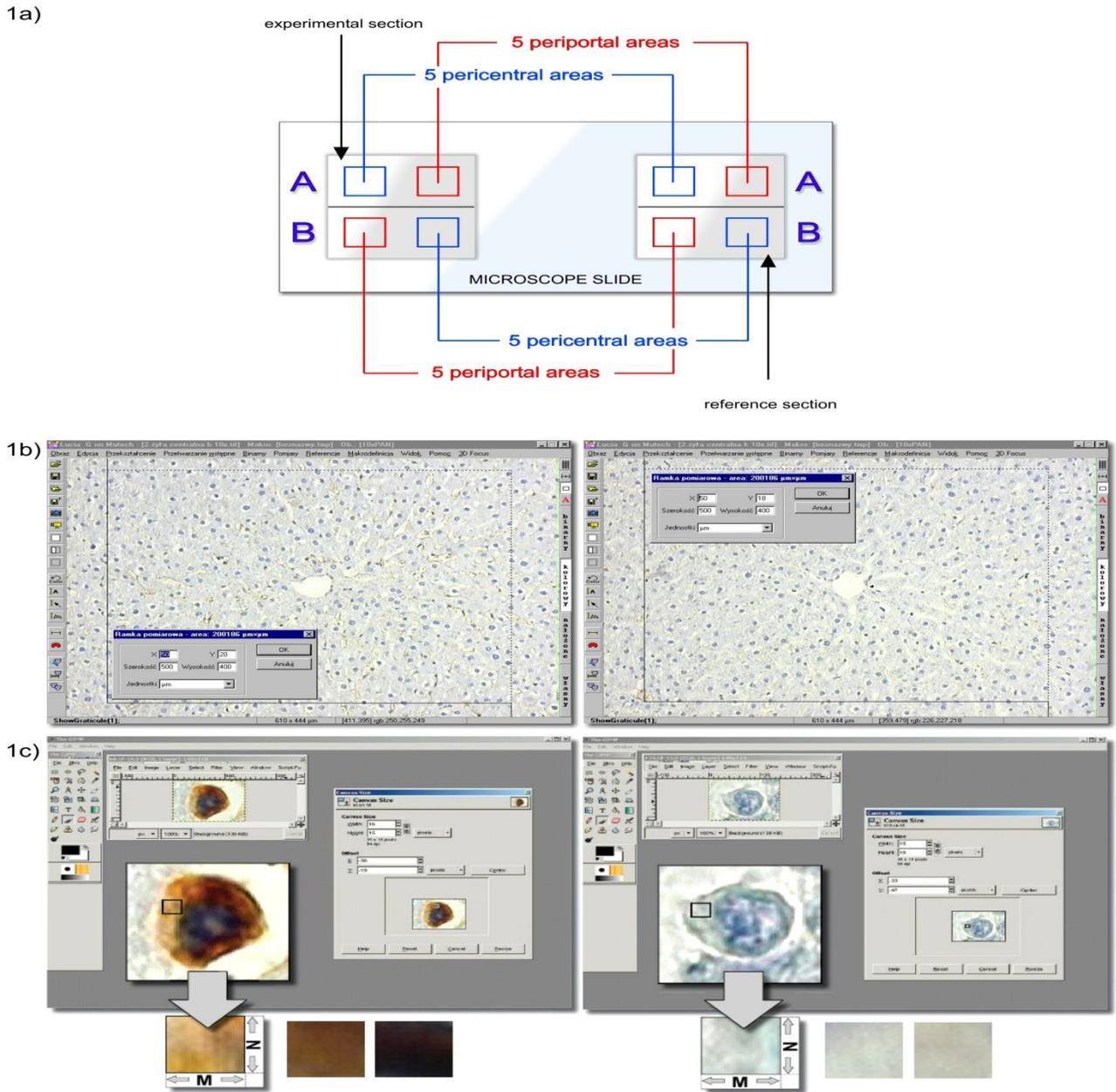
Figure 1.

Figure 2: Mean Number of Pro–MMP–9 Immunopositive Cells in 10 Periportal Areas. Values Presented as Mean ± SD
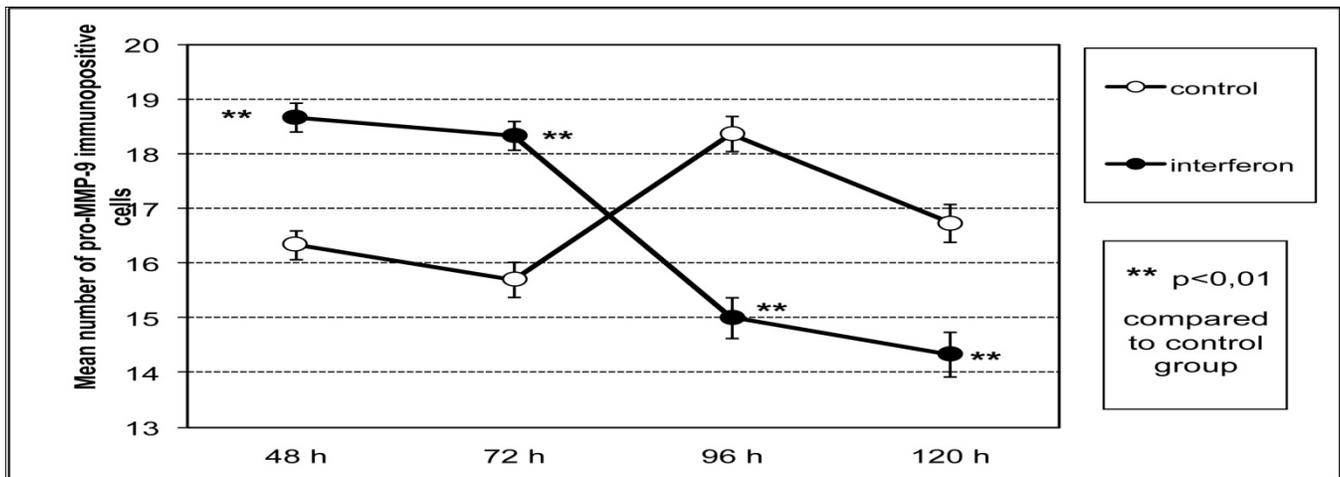
Figure 3: Mean Signal Intensity of Pro–MMP–9 Cytoplasmic Reaction in 10 Periportal Areas [*I/Pix*]. Values Presented as Mean ± SD
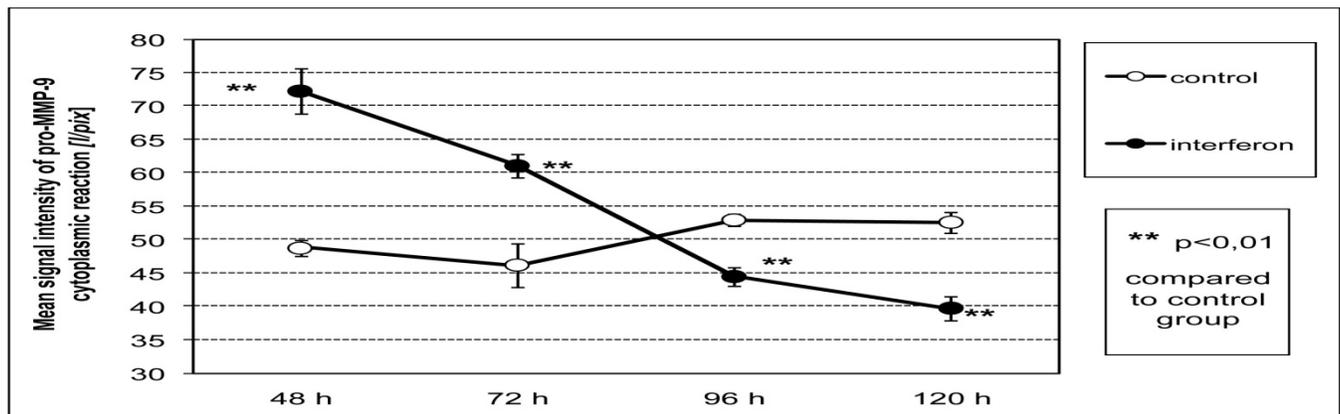

**Figure 4: Mean Number of Pro–MMP–9 Immunopositive Cells in 10 Pericentral Areas. Values Presented as Mean ± SD**
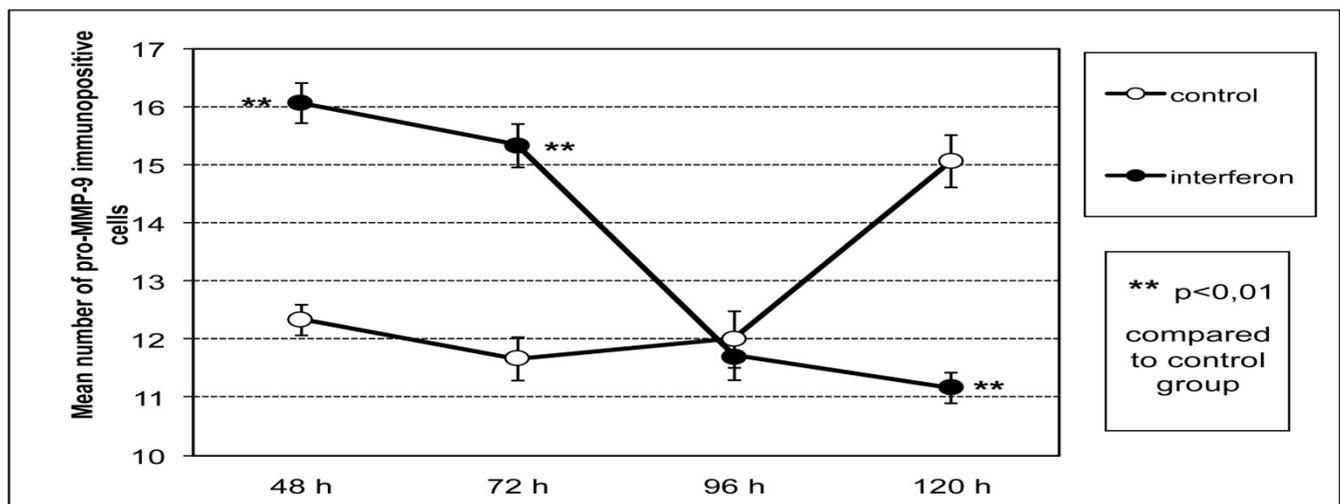
Figure 5: Mean Signal Intensity of Pro–MMP–9 Cytoplasmic Reaction in 10 Pericentral Areas [*I/Pix*]. Values Presented as Mean ± SD
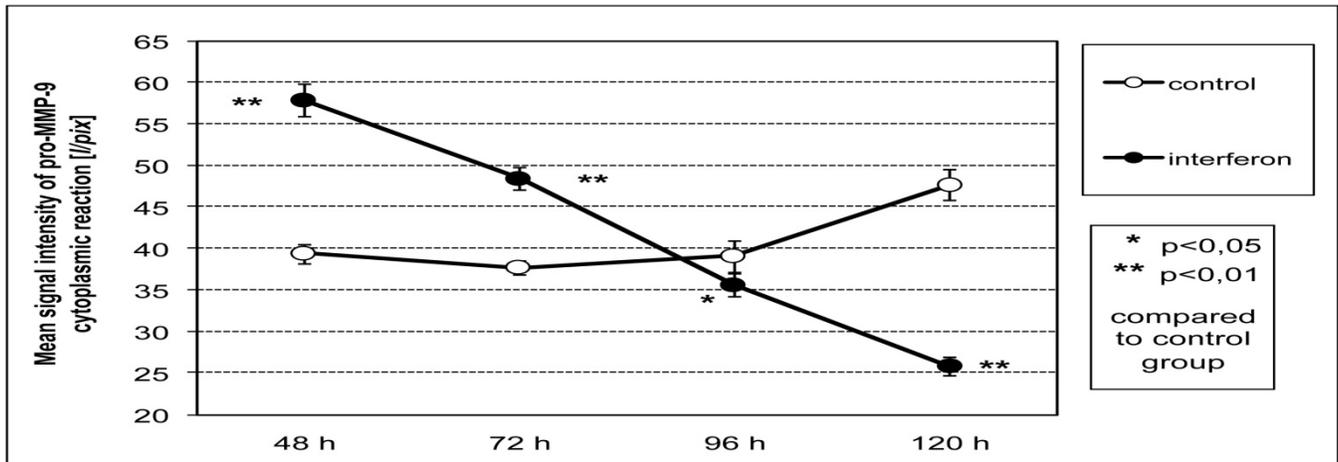

Figure 6: Mean Number of Pro–MMP–9 Immunopositive Cells. Comparison between Periportal and Pericentral Areas in Group Treated with Ifnα2b and in Control. Values Presented as Mean ± SD
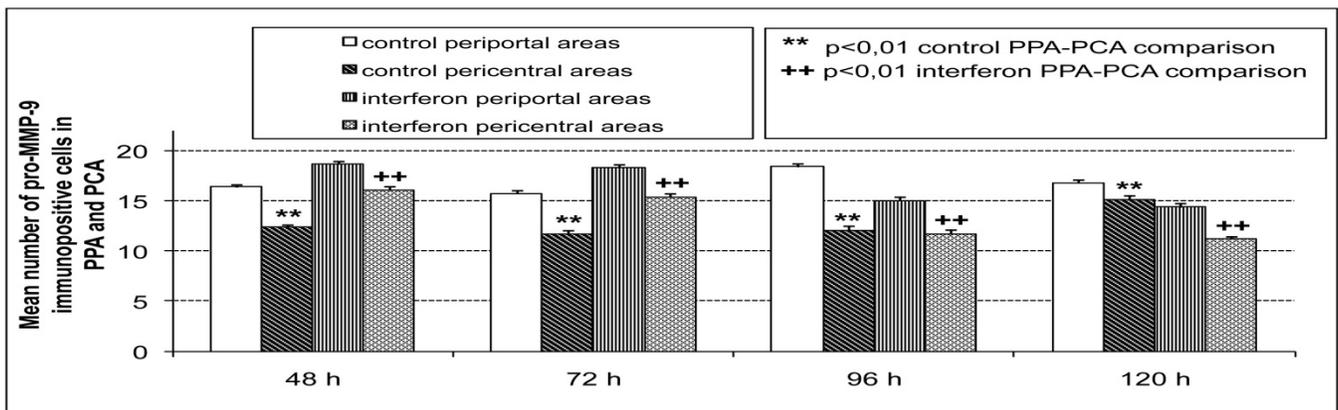

Figure 7: Mean Signal Intensity of Pro–MMP–9 Cytoplasmic Reaction [*I/Pix*]. Comparison between Periportal and Pericentral Areas in Group Treated with Ifnα2b and in Control. Values Presented as Mean ± SD