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ORIGINAL PAPER

Myeloperoxidase and elastase are only expressed by neutrophils in normal and in inflammed liver

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Abstract Myeloperoxidase (MPO) is involved in acute and chronic inflammatory diseases. The source of MPO in acute liver diseases is still a matter of debate. Therefore, we analysed MPO-gene expression on sections from normal and acutely damaged [carbon tetrachloride-(CCl_4) or whole liver γ-Irradiation] rat liver by immunohistochemistry, real time PCR and Western blot analysis of total RNA and protein. Also total RNA and protein from isolated Kupffer cells, hepatic stellate cells, Hepatocytes, endothelial cells and neutrophil granulocytes (NG) was analysed by real time PCR and Western blot, respectively. Sections of acutely injured human liver were prepared for MPO and CD68 immunofluorescence double staining. In normal rat liver MPO was detected immunohistochemically and by immunofluorescence double staining only in single NG. No MPO was detected in isolated parenchymal and non-parenchymal cell populations of the normal rat liver. In acutely damaged rat liver mRNA of MPO increased 2.8-fold at 24 h after administration of CCl_4 and 3.3-fold at 3 h after γ-Irradiation and MPO was detected by immunofluorescence double staining only in elastase (NE) positive NGs but not in macrophages (ED1 or CD68 positive cells). Our results demonstrate that, increased expression of MPO in damaged rat and human liver is due to recruited elastase positive NGs.

Keywords Myeloperoxidase · Kupffer cells · Liver · Neutrophile granulocytes

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Introduction

Myeloperoxidase (MPO), a heme protein, is a major component of azurophilic granules of neutrophil granulocytes (NGs). Optimal oxygen-dependent microbicidal activity depends on MPO as the critical enzyme for the generation of hypochlorous acid and other toxic oxygen products (Nauseef et al. 1988). The native enzyme has a molecular weight of 120 kDa (Zgliczynski and Stelmaszynska 1975). The MPO is a tetrameric molecule consisting of a pair of heavy- (~59 kDa) and light chains (~13.5 kDa) and two iron atoms (Olsson et al. 1972). There are two more components separated by electrophoresis at 40 and 20 kDa which consist of the autocatalytic products of the MPO (Taylor et al. 1992). The peroxidase activity of the enzyme is dependent on this chlorine group (Nauseef 1988). Through various enzymatic reactions within the NGs and interactions with other activated NGs, the MPO produces reactive oxygen species. The MPO in the lysosome causes the destruction of ingested organisms by binding to the walls of the microorganisms and locally producing hypochlorous acid. In the extracellular and

intravascular spaces secreted MPO also bacteria, viruses, or even the body's own tumor cells. The MPO is an important marker for differentiating acute myelogenous leukaemia from acute lymphoblastic leukaemia (Bennett et al. 1976). Multiple forms of MPO have been isolated from human myeloid leukaemia HL-60 cells (Yamada et al. 1981; Morita et al. 1986). MPO detection also has been used as a marker of neutrophil infiltration into tissues (Haqqani et al. 1999; Mcconnico et al. 1999). Reactive oxygen species produced by MPO are involved in the processes resulting in tissue damage. Hypochlorous acid is known to oxidize sulfhydryl and thioether groups of proteins (Winterbourn 1985; Arnhold et al. 1993). MPO products are also involved lipid peroxidation. Peroxidation by hypochlorous acid is favored by the presence of hydroperoxides previously accumulated in lipid material (Panasenko et al. 1997; Panasenko and Arnhold 1999). MPO is thought to be involved in the pathology of diseases such as atherosclerosis, cancer, multiple sclerosis, rheumatoid arthritis and Alzheimer's disease (Podrez et al. 1999; Nagra et al. 1997; Reynolds et al. 1999; Jolivalt et al. 1996; Weiss 1989; Daugherty et al. 1994; Malle et al. 2000).

The expression of MPO in Kupffer cells (KCs) has been reported in a few publications (Brown et al. 2001; Nahon et al. 2009; Rensen et al. 2009) but remains a matter of debate. Other reports claim that monocytes express MPO. Again this observation is controversial. Owen et al. (1994) and Akiyama et al. (1983) reported the presence of two different monocyte populations, one with and without MPO expression. Brennan et al. (2001) have shown that murine macrophages in atherosclerotic lesions do not express immunoreactive MPO. Several investigators have reported that MPO is not detectable after the differentiation of monocytes into macrophages (Kumar et al. 2004; Cribb et al. 1990; Nakagawara et al. 1981).

The hepatocellular inflammatory reaction induced by CCl_4-administration and γ-Irradiation is characterized principally by monocytes/macrophages and to a lesser degree lymphocytes and granulocytes (Knittel et al. 1999; Imhof and Dunon 1995).

The expression of MPO in normal and isolated parenchymal and non-parenchymal cells of normal rat liver, and acutely injured rat liver induced by either CCl_4-administration or γ-Irradiation was assessed in the present investigation. MPO-expression was identified only in granulocytes.

Materials and methods

Animals

Male Wistar rats weighing of about 170–200 g body weight were purchased from Harlan–Winkelmann (Brochen, Germany). They were maintained under standard conditions with 12-h light and dark cycles and allowed ad libitum access to fresh water and food pellets consisted with the university's policies and guidelines for the care and use of laboratory animals. This research use of rats was reviewed, approved, and overseen by the local ethics committee of the University of Goettingen as well as the public authority on animal welfare. The Human liver sections were used as well for the immunohistochemical studies and were obtained with full I. R. B. approval.

Antibodies

Rabbit polyclonal antibody against human MPO, mouse monoclonal anti-human macrophage CD68, Swine Anti-Rabbit Immunoglobulin HRP and Goat Anti-Mouse Immunoglobulin horseradish peroxidase were purchased from DakoCytomation (Berlin, Germany). Mouse monoclonal antibody against human MPO was purchased from Labgen CTS where located. Rabbit polyclonal antibody against human neutrophil elastase (NE) was purchased from Calbiochem (Bad Soden, Germany). Mouse monoclonal antibody ED1 against rat monocyte/macrophage was purchased from Serotec (Düsseldorf, Germany). Mouse monoclonal antibody against ß-actin, Anti-Mouse IgG (FITC conjugate) and Goat Anti-Rabbit IgG (TRITC conjugate) were purchased from Sigma (Steinheim, Germany).

CCl_4-induced liver injury

Rats were administered by gastric gavage 300 µl of a mixture of CCl_4 and corn oil (1:1 by volume) per 100 g body weight. Control rats received the same volume of corn oil alone. Animals were sacrificed 0, 3, 6, 9, 12, 24 and 48 h after CCl_4 administration. Their livers were either immediately frozen in liquid nitrogen or perfused to isolate liver cells. Treated animals (three for each time point) and controls (three for each time point) were sacrificed at 1, 3, 6, 12, 24, and 48 h after CCl_4-administration.

Whole liver γ-Irradiation in vivo

These experiments were performed as described previously (Christiansen et al. 2006): planning computed tomography was performed with a scanner (Somatom Balance; Siemens Medical Solutions, Erlangen, Germany) to delineate the location of the livers of each animal. The margins of the liver were marked on the skin of the animals, and a dose distribution was calculated. The rats were anesthetized with 90 mg ketamine per kilogram of body weight (Intervet, Unterschleissheim, Germany) and 7.5 mg/kg of 2% xylazine (Serumwerk Bernburg, Bern-burg/Saale, Germany) administered intraperitoneally. The livers were irradiated

selectively with 6 mV photons (dose rate of 2.4 Gy/min) using a Varian Clinac 600C accelerator (Varian, Palo Alto, CA); 25 Gy was delivered via an anterior–posterior/posterioranterior (ap/pa) treatment technique. Treated animals (three for each time point) and sham-irradiated controls (three for each time point) were sacrificed at 1, 3, 6, 12, 24, and 48 h after irradiation.

Human liver tissues

Fresh human liver was obtained at the time of liver transplantation for end-stage or fulminant disease or resection of a mass lesion and was immediately stored at −70°C. Archived blocks of diseased human tissues were obtained from the Department of Pathology, Georg August University. This component of study was approved by the Institutional Review Board of Georg August University reviewing Human studies.

Immunohistochemistry and immunofluorescence double staining procedures

Five-micrometer cryostat sections were obtained, airdried, fixed with acetone (−20°C, 10 min), and stored at −20°C. After inhibition of endogenous peroxidase by incubating the slides with PBS containing 180 mg glucose, 5 mg glucose oxidase and 100 µl of 1 M sodium azide, they were treated with fetal calf serum (FCS) for 30 min to minimize nonspecific staining. The sections were incubated in a humidified chamber with the first antibody directed against either MPO, NE, ED1 or CD68 diluted in PBS at 1:100 for 1 h at room temperature. Negative controls were obtained by incubating with isotype-specific mouse/rabbit/goat IgGs instead of the specific primary antibody. After washing, the slides were covered with peroxidase-conjugated anti-rabbit/anti-mouse/antigoat immunoglobulins preabsorbed with normal rat serum to avoid cross-reactivity. Slides were washed and incubated with PBS containing 3,3′-diaminobenzidine (0.5 mg/ml) and H_2O_2 (0.01%) for 10 min to visualize immune complexes. Nuclei were counterstained with Meyer's hemalum solution before the slides and coverslips mounted.

Isolation of liver parenchymal and nonparenchymal cells, conditions of cell culture

Hepatocytes and hepatic stellate cells (HSC) from rat liver were isolated and cultured as described previously (Knittel et al. 1992; Neubauer et al. 1995). Endothelial cells and KC's of normal and injured liver were isolated as described by Knook and Sleyster (1976) with modifications (Armbrust et al. 1993). Centrifugal elutriation was performed to separate different cell populations (Beckman centrifuge J2-21, J-6B rotor at 2,500 rpm) and cells were harvested at the following flow rates: Endothelial cells—12 ml/min, small KCs—28 ml/min and large KCs—55 ml/min. Cells were collected in the respective culture medium (for KCs: M199, 15% FCS), 100 U/ml penicillin, 100 mg/ml streptomycin; for endothelial cells: DMEM, 15% FCS, 1% L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, Seromed, Berlin, Germany) and plated, or further processed for RNA isolation.

Neutrophilic granulocytes were isolated and cultured as described earlier (Boyum 1968).

RNA isolation and quantitative using real time RT–PCR

Total RNA was extracted from cultured cells by guanidine isothiocyanate and purified by caesium chloride cushion ultracentrifugation (Chirgwin et al. 1979). For real-time PCR, reverse transcription of RNA samples was performed using the Superscript kit from Invitrogen (Groningen, Netherlands) and following the manufacturer's instructions. Real-time PCR analysis of cDNA was performed at 60 to 95°C for 45 cycles in the Sequence Detection System of ABI Prism 7000 (Applied Biosystems, Darmstadt, Germany) following the manufacturers' instructions and by using SYBR Green Reaction Master Mix (ABI Prism; Applied Biosystems) and the primers listed in Table 1. All primers were synthesized by Invitrogen. In every RNA sample, β-actin mRNA was measured as the housekeeping gene (Rat 18 s was also measured with very similar results). Values were then compared with those obtained by using the control-RNA obtained from each animal series. The results were normalized to the housekeeping gene and

Table 1 Primer sequences used in this study

Primer	Forword	Reverse
MPO	5′-ACCTACCCCAGTACCGATCC-3′	5′-AACTCTCCAGCTGGCAAAAA-3′
NE	5′-CTTTGAGAACGGCTTTGACC-3′	5′-CACATTGAGCTCTTGGAGCA-3′
CD11b	5′-CACAGTACACCAGAACTGCA-3′	5′-ATCACCAGCTGGCTTAGAC-3′
β-actin	5′-ACCACCATGTACCCAGGCATT-3′	5′-CCACACAGAGTACTTGCGCTCA-3′
Rat 18 s	5′-AAACGGCTACCACATCCAAG-3′	5′-CCTCCAATGGATCCTCGTTA-3′s

fold change expression was calculated by using threshold cycle (C_t) values. For calculation of the relative changes, gene expression measured in control animals sacrificed at the same time points was set at one.

Western blot analysis and SDS–PAGE

Lysates were prepared by homogenization at 4°C in 20 mM Tris–HCl (pH 8.0), 5 mM EDTA, 3 mM EGTA, 1 mM DTT, 1% SDS, 1 mM PMSF, and protease inhibitor cocktail (Sigma–Aldrich Inc.). Samples were cleared by centrifugation at 15,000 g for 15 min at 4°C, and the protein concentration was measured by BCA assay (Pierce, Rockford, IL, USA), using bovine serum albumin, as the standard. Protein lysates were separated on SDS-polyacrylamide gels, electrotransferred to polyvinylidene difluoride membranes (Invitrogen, USA), and probed with primary antibodies overnight. The appropriate peroxidase-conjugated secondary antibodies (DAKO, Glostrup, Denmark) in a dilution of 1:5,000 was then added and incubation continued for 1 h, at room temperature. Bound antibodies were visualized using chemiluminescent substrate (ECL; Amersham-Pharmacia, UK). Equal loading was previously controlled by transient Ponceau S staining.

Results

Expression of MPO in normal rat liver tissue

By indirect immunohistochemical labeling (antibodies against MPO, NE, and ED1), it was possible to detect only a few cells by MPO (Figs. 1a, 2b) and NE (Fig. 1c) and macrophage/KCs by ED1 (Figs. 1b, 2a) in normal rat liver. The MPO^+ and NE^+ cells were distributed diffusely. Significantly more $ED1^+$ cells were detectable as MPO^+ and NE^+ cells. The MPO^+ and NE^+ cells were scattered, mostly round and had little cytoplasm. $ED1^+$, macrophage/KCs had a stellate or spindle shape.

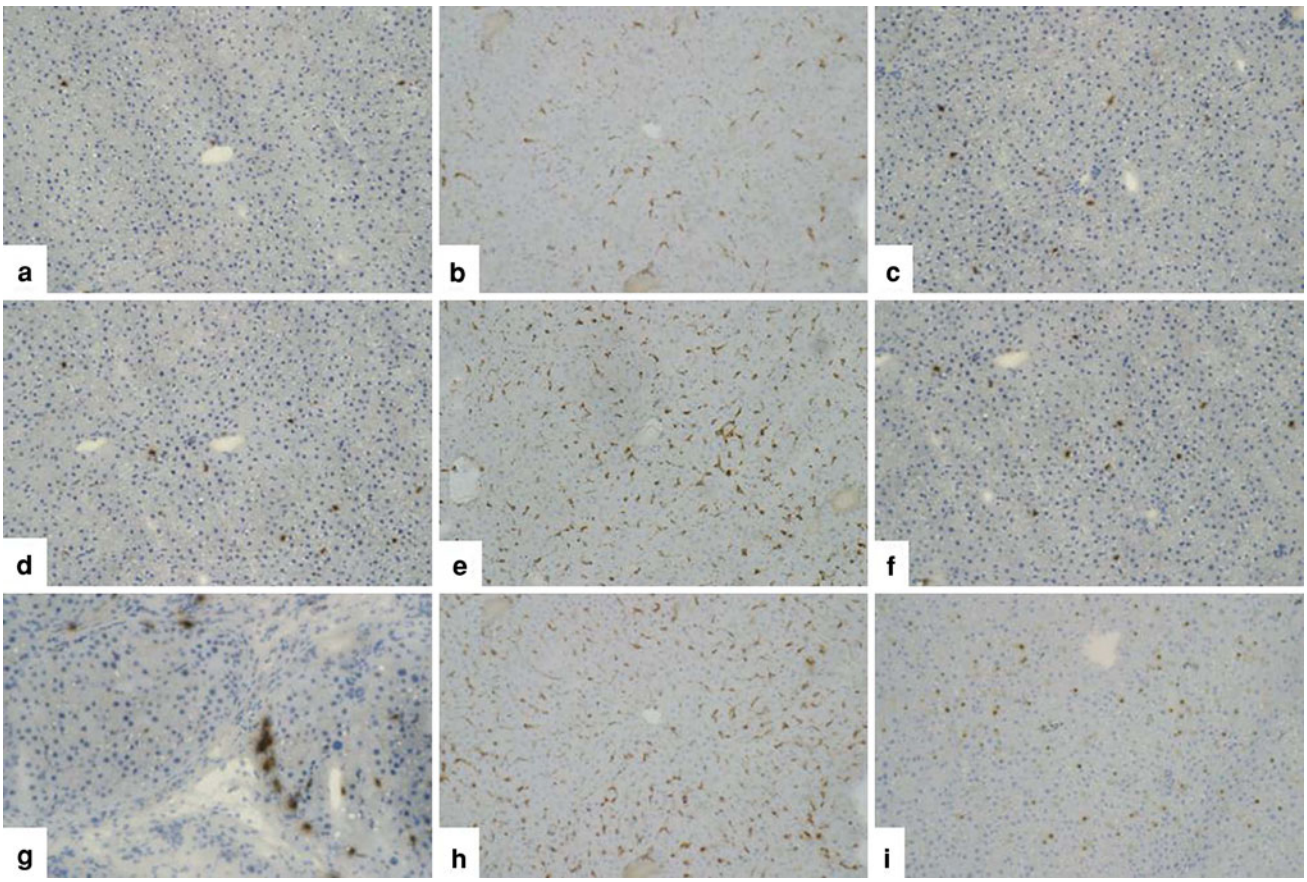

Fig. 1 Immunhistochemical detection of myeloperoxidase (MPO), neutrophil elastase (NE) and ED1 cells in rat liver 6 h after one single oral dose of CCl_4-adminstration induced rat liver injury. MPO^+ cells (**a**), $ED1^+$ cells (**b**) and NE^+ cells (**c**) in control rat liver. MPO^+ cells (**d**), $ED1^+$ cells (**e**) and NE^+ cells (**f**) at 6 h after treatment. The *lower panel* shows MPO^+ cells (**g**), $ED1^+$ cells (**h**) and NE^+ cells (**i**) at 24 h after treatment. Original magnification, ×100. *G* original magnification, ×200 173 × 116 mm (300 × 300 DPI)

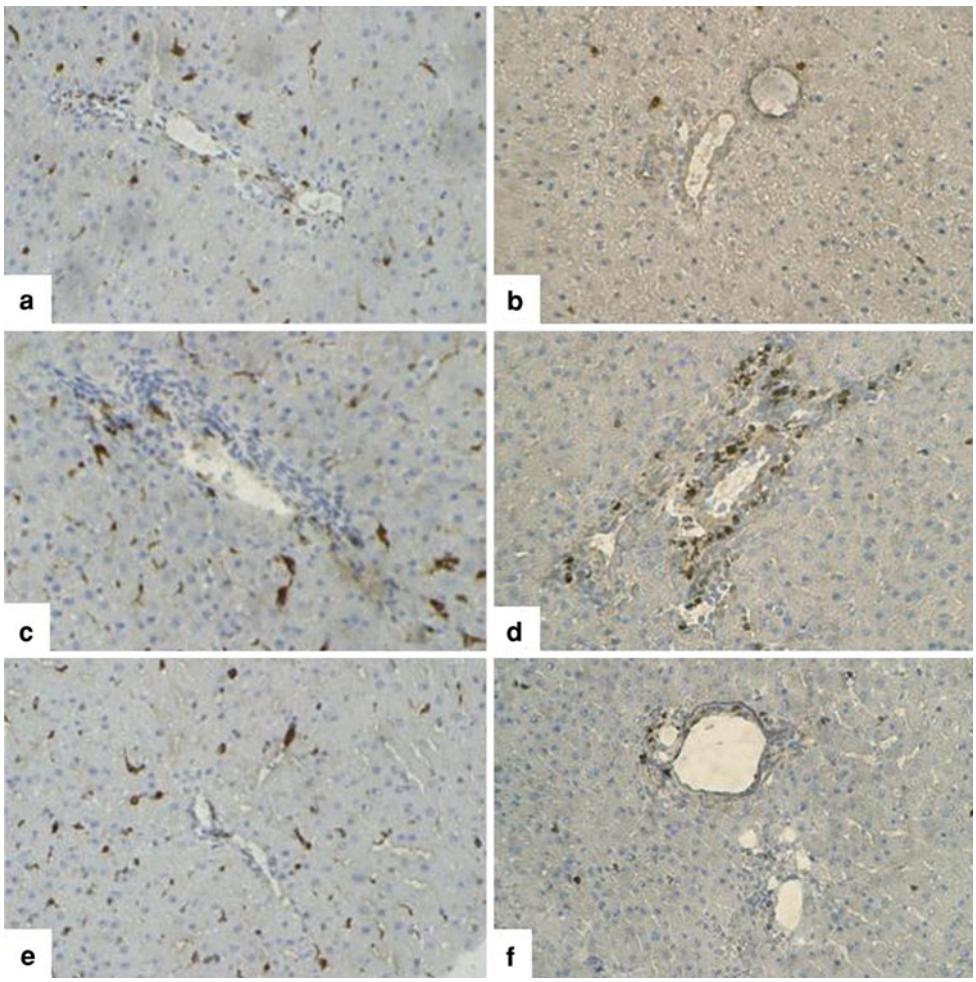

Fig. 2 MPO^+ and $ED1^+$ cells in rat liver after γ-Irradiation induced rat liver injury. $ED1^+$ cells (**a**) and MPO^+ cells (**b**) in control rat liver. $ED1^+$ cells (**c**) and MPO^+ cells (**d**) at 3 h after γ-Irradiation. $ED1^+$ cells (**e**) and MPO^+ cells (**f**) at 24 h after γ-Irradiation. Original magnification, ×100
173 × 174 mm
(300 × 300 DPI)

Expression of MPO-gene in parenchymal and non-parenchymal liver cells

After immunhistochemical labelling the expression of MPO from isolated liver cell populations and NG at the level of RNA and protein by real time PCR and Western blot was assessed. By real time PCR (Fig. 3), the C_t value of MPO in NG was 31.3. The C_t value of MPO in small KCs was 36.7, in large KCs 34.8, in hepatocytes 34.6, in endothelial cells 35.7 and in HSC 36.8. Consistent with the results of real time PCR, expression of MPO by Western blot was evident only from NG (Fig. 4). The parenchymal and non-parenchymal cells of the liver did not express MPO.

Expression of MPO in CCl_4 and γ-Irradiation induced rat liver injury

Two different models of acutely induced liver injury with either CCl_4 or γ-Irradiation were utilized. Indirect immunodetection in liver sections after CCl_4 and γ-Irradiation

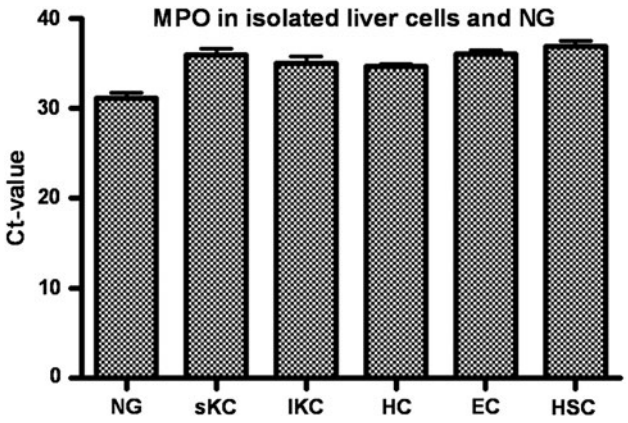

Fig. 3 MPO-gene expression in different cells analyzed by amplification of total RNA extracted from isolated cell populations of normal rat liver. Comparison of C_t values of MPO in NG, small (sKC) and large KC (lKC), hepatocytes (HC), endothelial cells (EC) and hepatic stellate cells (HSC). Results were obtained by real time PCR analysis of total RNA. Results represent three experiments (in duplicate) and mean ± SEM values are shown for each cell type
46 × 34 mm (300 × 300 DPI)

were carried out with the antibodies against MPO, NE and ED1 followed by peroxidase and immunofluorescence double staining.

In the acutely injured rat liver quantitatively more MPO^+ and NE^+ and $ED1^+$ cells were detected than that present in normal rat liver.

In CCl_4-induced liver injury, a diffuse increase in the number of MPO^+ (Fig. 1d, g) and NE^+ (Fig. 1f, i) cells in the liver parenchyma by indirect immunhistochemical staining. The increase on MPO^+ and NE^+ cells was achieved at 24 h after CCl_4 administration (Fig. 1g, i). An increase in the number of $ED1^+$ cells was detectable at 6 and 24 h around the portal vein (Fig. 1e, h).

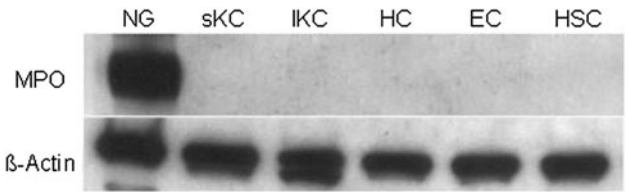

Fig. 4 Western blot analysis of total protein of NG, small KC's (sKC), large KC's (lKC), hepatocytes (HC), endothelial cells (EC) and hepatic stellate cells (HSC). Cells were isolated and cultured for 24 h. Protein was then extracted, 20 g of total protein were separated by SDS–PAGE, and blotted onto PVDF-membranes. The membranes were subsequently incubated with the antibodies against MPO (*upper panel*) and s-actin (*lower panel*). The molecular weight of MPO is 59 and 13.5 kDa. In addition, autocatalytic products (AP) can be seen at 40 and 20 kDa (data not shown). The molecular weight of β-actin is 42 kDa 129 × 41 mm (300 × 300 DPI)

Immunofluorescence double staining with the same antibodies in CCl_4 treated Animals also demonstrated an increase of MPO^+ (Fig. 5b, d), NE^+ (Fig. 5a, c) and $ED1^+$ cells. But the $ED1^+$ cells were not MPO positive. These results were confirmed utilizing real time PCR for MPO (Fig. 6a) and NE (Fig. 6b) gene expression at the RNA level and for MPO at the level of using protein by Western blot analysis (Fig. 7a). The real time PCR analysis showed an increased expression of MPO, 2.0-fold at 6 h and 2.7-fold at 24 h after CCl_4-administration (Fig. 6a). The gene expression of NE showed similar results after 6 h (2.9-fold change) and 24 h (2.0-fold change) after CCl_4-administration (Fig. 6b). An increase of MPO protein at 24 h after CCl_4-administration was confirmed by Western blot (Fig. 7a).

By indirect immunhistochemical labelling, it was possible to detect an early increase in the number of MPO^+ and NE^+ cells around portal vessels of the rat liver after γ-Irradiation. The accumulation of MPO^+ (Fig. 2d) and NE^+ (data not shown) cells reached a maximum at 3 h. The immunofluorescence double staining studies utilizing γ-irradiated rat liver showed an increase in the number of MPO^+, NE^+ and $ED1^+$ cells. The increase of MPO^+ (Fig. 8c, e) and NE^+ (data not shown) cells was detectable principally in the portal area. $ED1^+$ cells were not MPO^+. At the same time, an increased of MPO and NE gene expression at the level of RNA and of MPO at the level of protein was detected by real time PCR analysis and Western blot, respectively. The real time PCR analysis

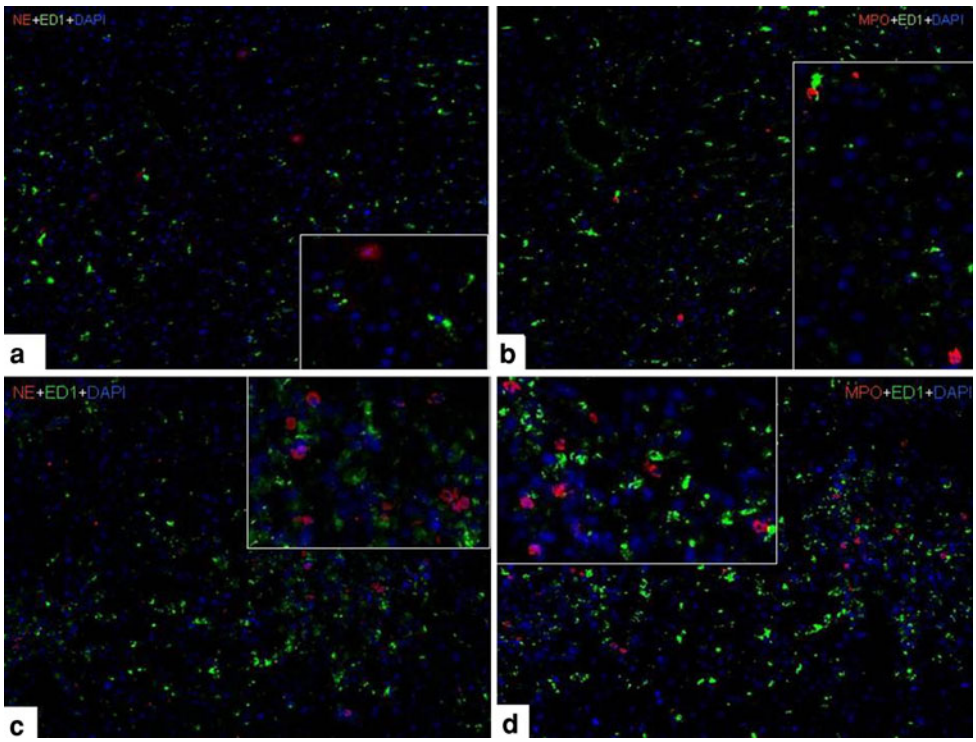

Fig. 5 Double staining of liver sections with monoclonal antibodies directed against MPO or NE (*red*) and monoclonal antibody against ED1 (*green*) followed by fluorescence immunodetection in sections of rat liver at different time points after CCl_4-administration. **a** NE^+ or $ED1^+$ cells in CCl4-induced liver injury at 0 h. **b** MPO^+ or $ED1^+$ cells in CCl_4-induced liver injury at 0 h. **c** NE^+ or $ED1^+$ cells in CCl_4-induced liver injury 24 h after administration. **d** MPO^+ or $ED1^+$ cells in CCl_4-induced liver injury 24 h after administration. Original magnification, ×100 173 × 130 mm (300 × 300 DPI)

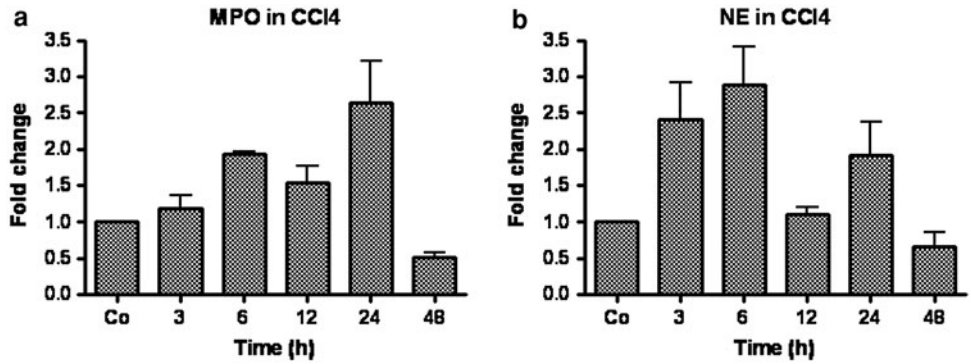

Fig. 6 Fold change of mRNA expression of MPO (**a**) and NE (**b**) after CCl_4-administration induced rat liver injury at different time points. Real time PCR was normalized by using two housekeeping genes: β-actin and 18 s RNA. Results represent mean ± SEM value of three experiments (in duplicate) compared with controls for each time point 173 × 62 mm (600 × 600 DPI)

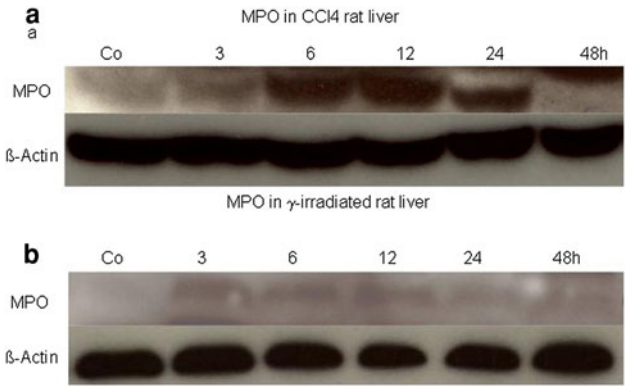

Fig. 7 Western blot analysis of MPO of total protein in acute liver injury induced by CCl_4 (**a**) and γ-Irradiation (**b**). Protein was extracted from livers of control (Co) rats or 3, 6, 12, 24 and 48 h after one single oral dose of CCl_4-adminstration or γ-Irradiation induced rat liver injury. 20 µg of total protein were separated by SDS–PAGE, and blotted onto PVDF-membranes. The membranes were subsequently incubated with the antibodies against MPO (*upper panel*) and β-actin (*lower panel*). The molecular weight of the MPO is 59 and 13.5 kDa. In addition, autocatalytic products (AP) can be seen at 40 and 20 kDa (data not shown). The molecular weight of β-actin is 42 kDa 160 × 90 mm (300 × 300 DPI)

showed an increased MPO gene expression 3.3-fold changes at 3 h and 2.5-fold change at 6 h after γ-Irradiation (Fig. 9a). NE gene expression increased 3.3-fold change at 3 h and 5.3-fold change at 6 h after γ-Irradiation (Fig. 9b). These results were confirmed by Western blot for MPO (Fig. 7b). CD11b-gene expression (Fig. 10), a marker for NG showed similar results.

Expression of MPO in acutely injured human liver

The results so far presented documented the expression of MPO in normal and acutely injured rat liver. To examine the expression of MPO in acutely injured human liver, indirect immunohistochemistry and immunofluorescence double staining studies were performed using histological sections of acute alcohol-associated injury. Indirect immunohistochemistry using antibodies against MPO (Fig. 11a) and NE (Fig. 11b) showed positive staining only within small, round cells with barely cytoplasm. What was striking was the presence of staining in the immediate vicinity of the cells, which probably represents extracellular secreted MPO and NE. Using immunofluorescence double staining with antibodies against MPO and CD-68, $CD\text{-}68^+$ cells did not express MPO (Fig. 12a). This result was confirmed using antibodies against NE and CD-68. $CD\text{-}68^+$ cells do not express NE (Fig. 12b). In Fig. 12 (sequential sections) there are MPO^+ and $CD\text{-}68^+$ cells (Fig. 12a) and NE^+ and $CD\text{-}68^+$ cells (Fig. 12b) can be seen.

Discussion

In this study, the expression of the MPO-gene in normal rat liver and isolated parenchymal and non-parenchymal rat liver cells, as well as in acutely induced rat liver either by CCl_4-administration or γ-Irradiation was assessed. Human liver with acute alcohol induced injury was studied also. In normal rat liver immunohistochemically detectable MPO was detected only in single elastase positive NGs. MPO was not expressed in either parenchymal or non-parenchymal liver cells. Isolated mononuclear phagocytes did not express MPO, at either the level of RNA or at the level of protein. In contrast, Granulocytes were strongly positive for MPO expression to both RNA and protein.

During acute liver damage, induced by CCl_4-administration or single dose whole liver γ-Irradiation, an increase in the expression of MPO was observed that peaked 24 h after administration of CCl_4 and 3 h after γ-Irradiation. In acutely injured human liver, MPO expression was detected immunohistochemically only in granulocytes.

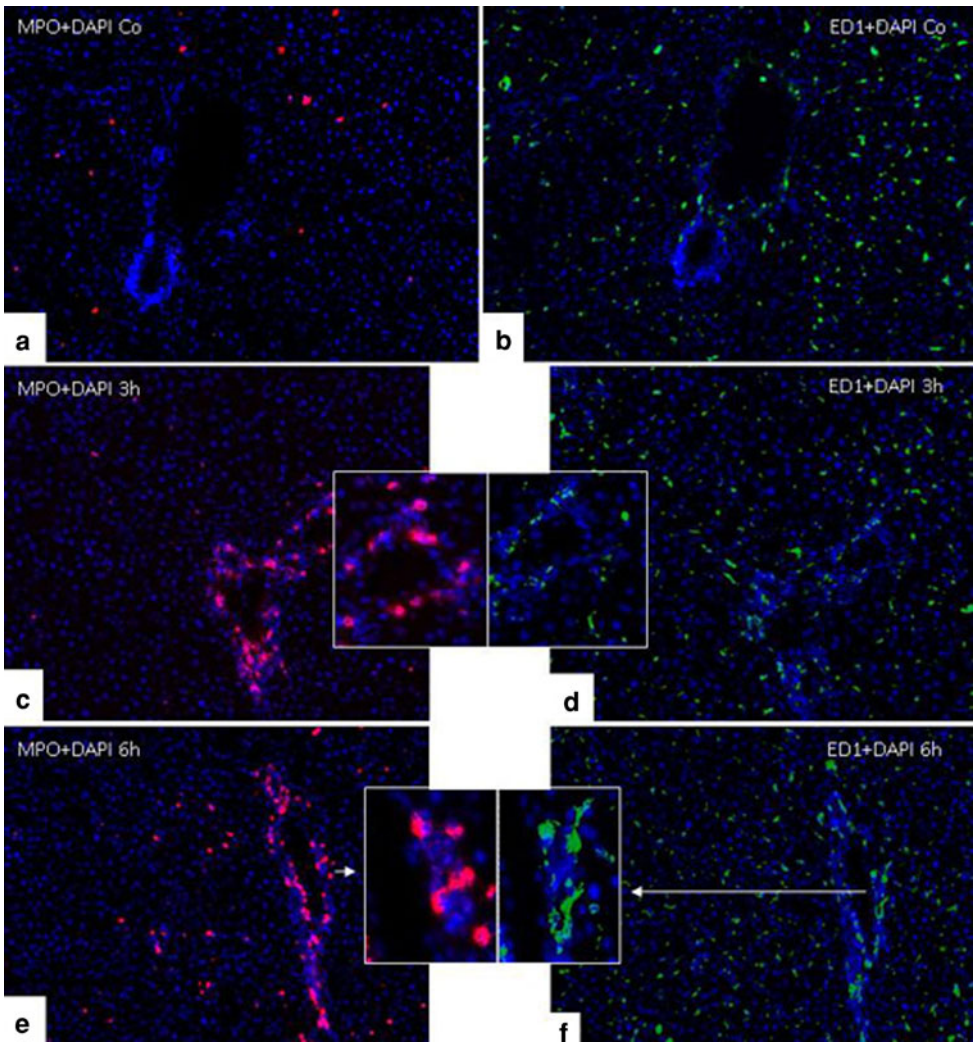

Fig. 8 Immunofluorescence staining of liver sections after γ-Irradiation at different time points with monoclonal antibodies directed against MPO (*red*) and monoclonal antibody against ED1 (*green*). MPO^+ cells (**a**) or $ED1^+$ cells (**b**) in control liver. MPO^+ cells (**c**) and $ED1^+$ cells (**d**) at 3 h after γ-Irradiation. MPO^+ cells (**e**) and $ED1^+$ cells (**f**) at 6 h after γ-Irradiation. Original magnification, $\times 100$
15 × 16 mm (600 × 600 DPI)

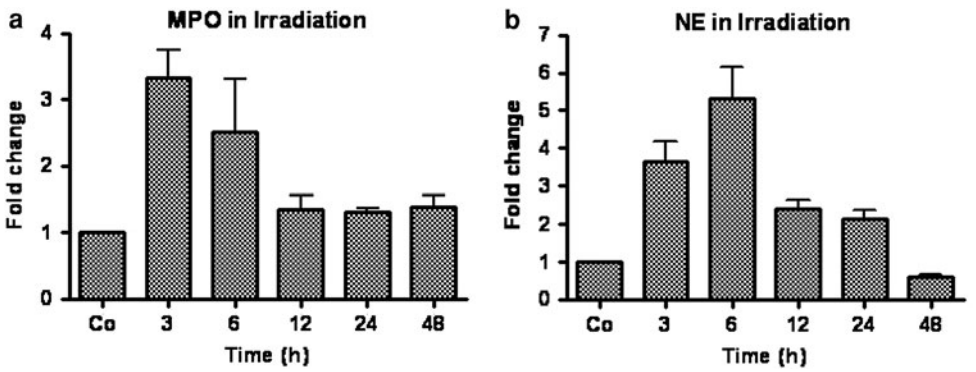

Fig. 9 Fold change of mRNA expression of MPO (**a**) and NE (**b**) after γ-Irradiation induced rat liver injury at different time points. Real time PCR was normalized by using two housekeeping genes: β-actin and 18 s RNA. Results represent mean ± SEM value of three experiments (in duplicate) compared with controls for each time point
173 × 62 mm
(600 × 600 DPI)

Immunofluorescence double staining of rat liver clearly showed that MPO was not detectable in $ED1^+$ cells.

Brown et al. (2001) reported the expression of MPO in resident macrophages principally KCs, using immunohistochemical methods. In the present work, this finding could not be confirmed. In contrast, using an antibody against MPO only small round cells that are NG documented by NE staining were positive for MPO. $ED1^+$ or $CD68^+$ cells were negative for MPO. Analysis of the expression of MPO in parenchymal and non-parenchymal liver cells at the RNA level showed no expression of MPO.

The liver is known to be an important site for the clearance of NG (Peters et al. 1985; Farstad et al. 1991; Lovas et al. 1996). Various investigations have shown that endotoxemia selectively upregulates hepatic P-selectin and active phagocytosis of apoptotic circulating NG is limited

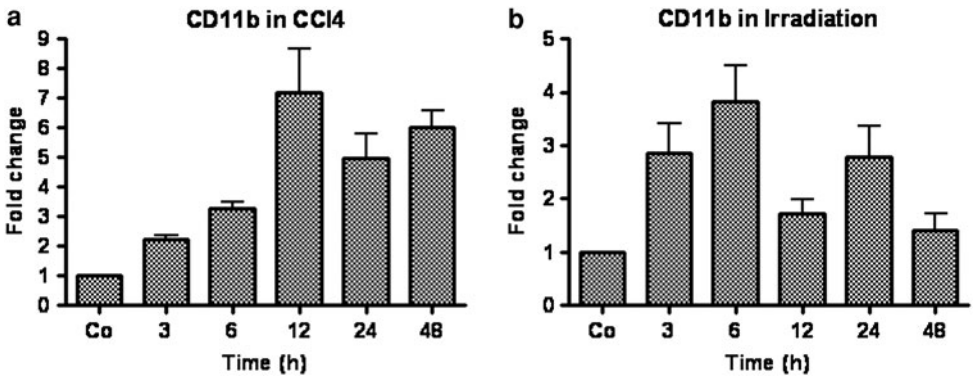

Fig. 10 Fold change of mRNA expression of CD11b after CCl_4-administration and γ-Irradiation induced rat liver injury at different time points. Real time PCR was normalized by using two housekeeping genes: β-actin and 18 s RNA. Results represent mean ± SEM value of three experiments (in duplicate) compared with controls for each time point
173 × 60 mm
(600 × 600 DPI)

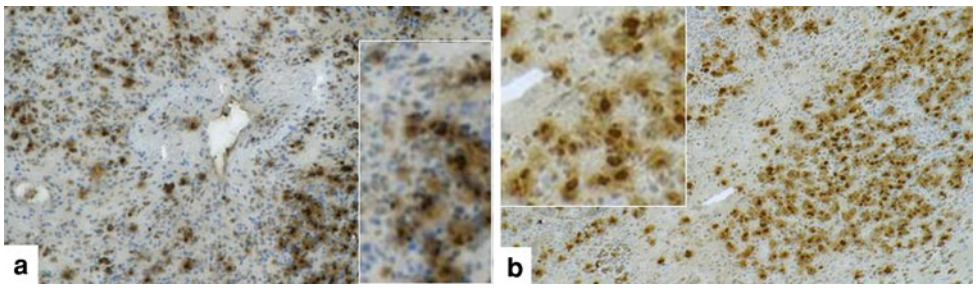

Fig. 11 Indirect immunodetection of MPO (**a**) or NE (**b**) in sections of acute alcohol-toxic injured human liver. Sections were stained with antibodies against MPO or NE followed by peroxidase staining. Original magnification, ×100
173 × 49 mm
(300 × 300 DPI)

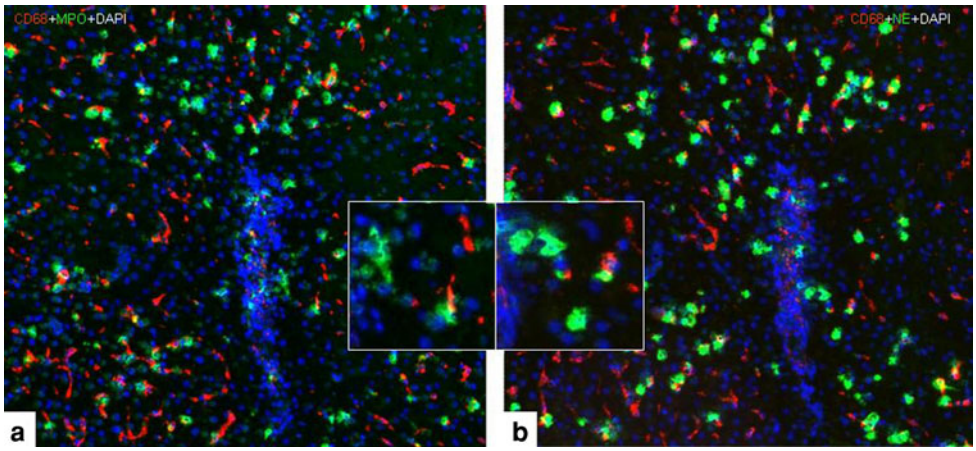

Fig. 12 Double staining of acute alcohol-toxic injured human liver sections with antibodies directed against MPO and NE (*green*) and antibody against CD68 (*red*) followed by fluorescence immunodetection. MPO^+ or $CD68^+$ cells (**a**) and NE^+ or $CD68^+$ cells (**b**). Original magnification, ×100
46 × 20 mm (600 × 600 DPI)

to the liver. The complete absence of endothelial P-selectin results in neutrophilia (Frenette et al. 1996; Johnson et al. 1995; Mayadas et al. 1993). However, the phagocytotic capability of the KCs is diminished also (Shi et al. 1998).

In the damaged liver, adherent NG are metabolically active and transmigrate through the sinusoidal and microvascular endothelium following the release of extracellular matrix-degrading enzymes (Jaeschke and Farhood 1991; Jaeschke et al. 1991; Jaeschke and Smith 1997). The result is a pronounced inflammatory damage to the tissue. Activated NG generate reactive oxygen radicals and release proteases, which damage the endothelium and parenchymal cells directly (Jaeschke et al. 1990). NG emigrates quickly in inflamed tissue, engulf and destroy microorganisms and foreign antigens and ultimately undergo apoptotic death and phagocytoses by macrophage (Haslett 1992; Savill et al. 1989). Within the liver, two different populations of macrophages can be identified and functionally separated (Armbrust and Ramadori 1996) with newly immigrated mononuclear phagocytes manifesting MPO-positivity not present in terminal expended cells (Armburst and Ramadori 1995). 24–48 h after administration of CCl_4, a strong immigration of inflammatory macrophages is observed in the pericentral area of the liver and these newly recruited monocytes are MPO-negative.

KCs represent approximately 10% of the liver cells and 80% of the tissue macrophage population (Armbrust et al. 1993; Hoedemakers et al. 1995) and play an important role in the defence mechanisms of the body. If KCs representing 10% of liver cells were to express MPO, either at the

RNA or protein level, they should have been immunostained positive for MPO. In this study no MPO expression was evident in KCs. The MPO protein within the liver is due to the presence of newly recruited NGs.

This increase of newly recruited MPO positive NGs at the site of liver injury underscores the important role of NGs in the inflammatory reaction that occurs during the multistep process of liver injury and repair.

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