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Biochemical Pharmacology 66 (2003) 917–926
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Depletion of Kupffer cell function by gadolinium chloride attenuates thioacetamide-induced hepatotoxicity Expression of metallothionein and HSP70

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Received 13 February 2003; accepted 1 June 2003

Abstract

Kupffer cell function plays an important role in drug-induced liver injury. Thus, gadolinium chloride (GD), by selectively inactivating Kupffer cells, can alleviate drug-induced hepatotoxicity. The effect of GD was studied in reference to metallothionein and heat shock proteins expression in an *in vivo* model of liver necrosis induced by thioacetamide. Rats, pre-treated or not with GD (0.1 mmol/kg), were intraperitoneally injected with thioacetamide (6.6 mmol/kg), and samples of blood and liver were obtained at 0, 12, 24, 48, 72 and 96 hr. Parameters related to liver damage, Kupffer cell function, microsomal FAD monooxygenase activity, oxidative stress, and the expression of metallothionein and HSP70 were determined. GD significantly reduced serum myeloperoxidase activity and serum concentration of TNFα and IL-6, increased by thioacetamide. The extent of necrosis, the degree of oxidative stress and lipoperoxidation and microsomal FAD monooxygenase activity were significantly diminished by GD. The effect of GD induced noticeable changes in the expression of both metallothionein and HSP70, compared to those induced by thioacetamide. We conclude that GD pre-treatment reduces thioacetamide-induced liver injury and enhances the expression of metallothionein and HSP70. This effect, parallel to reduced levels of serum cytokines and myeloperoxidase activity, demonstrates that Kupffer cells are involved in thioacetamide-induced liver injury, the degree of contribution being approximately 50%.

Keywords: Gadolinium chloride; Kupffer cells; Thioacetamide hepatotoxicity; Metallothionein; HSP70

1. Introduction

Different aspects of liver pathophysiology are produced by alteration of hepatic non-parenchymal cell function [1]. Among the non-parenchymal liver cells, Kupffer cells are the macrophages residing in the sinusoids of the liver. This makes Kupffer cells the first macrophage population to come into contact with noxious material (bacteria, virus, tumour cells or drugs). Kupffer cell function plays an important role in the patogenesis induced by hepatotoxic compounds [2]. In the mechanism by which Kupffer cell increase drug-induced liver injury, the generation of superoxide anion by macrophage NADPH oxidase, as well as the release of cytokines and proteases play an important role [3]. Thus, blocking of Kupffer cells function by gadolinium chloride (GD), significantly reduces the hepatotoxicity [4].

Exposure to drugs, either *in vivo* or *in vitro*, causes the induction of stress proteins. Among these proteins, HSP70 and metallothioneins are included in the cell defence systems. Metallothionein, a small metal-binding, sulfhydryl-rich, readily inducible protein, is involved in hepatocellular proliferation after partial hepatectomy in rats [5,6], and it has been demonstrated that its expression is enhanced by chemicals that induce oxidative stress [6–10]. Metallothionein is a scavenger of hydroxyl radicals *in vitro* and cells with high levels of metallothionein are resistant to radiation. However, recent data [11] have shown that clofibrate, a peroxisome proliferator that induces oxidative stress, does not induce the expression of metallothionein. Induction of metallothionein is an important cellular adaptative mechanism in response to environmental aggression [12]. Because one-third of its amino acid residues are cysteines,

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0006-2952/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0006-2952(03)00443-X

metallothionein provides a nucleophilic sink and binding electrophiles. Metallothionein may also act as a free radical scavenger to protect against oxidative stress.

Heat shock proteins (HSPs) induction confers protection against diverse forms of cellular injury. Induction of these proteins represents an essential and highly conserved cellular response to stress stimuli. Schiaffonati and Tiberio [13] examining the expression of HSP70 in a model of liver injury, found that the induction of HSP70 expression occurs rapidly and transiently and is preceded by the activation of HSF-DNA binding activity. Yamasaki *et al.*, studied the effects of drugs on HSPs induction [14] and other authors [15] found, in ethanol-induced oxidative stress, that the close relationship between oxidative stress and HSP70 mRNA, but not HSP70 protein, may probably be due to the binding of HSP70 protein to other damaged proteins which reduce free HSP70 protein levels and lead to an increased HSP70 expression.

The acute liver injury induced by a necrogenic dose of thioacetamide (TA) is characterised by a severe perivenous necrosis [16–19]. The necrosis develops as a consequence of the biotransformation of TA through the microsomal flavin-dependent monooxygenase [20]. The reactive metabolites responsible for TA hepatotoxicity are the radicals derived from thioacetamide-*S*-oxide and the reactive oxygen species derived as subproducts in the process of microsomal TA oxidation, both of which can deplete reduced glutathione leading to oxidative stress [21–23].

As it is generally accepted that the Kupffer cell function is involved in the severity of liver damage induced by drugs, and that induces a selective blockade of Kupffer cell function, the purpose of the present study was to investigate the effect of GD on an experimental model of liver injury induced by a single necrogenic dose of thioacetamide which results in pericentral liver necrosis. Groups of rats were pre-treated or not with GD 24 hr before thioacetamide, and animals were sacrified at different time interval, in order to follow the sequence of events involved in the processes of liver injury, necrosis and inflammation, as well as those related to oxidative stress. Parameters related to Kupffer cell function as well as the expression of both metallothionein and HSP70 in liver were also determined.

2. Material and methods

2.1. Reagents

Enzymes were obtained from Boehringer Mannheim. Substrates and coenzymes were from Sigma Chemical Co. Standard analytical grade laboratory reagents were obtained from Merck. [α-^{32}P]dCTP (3000 Ci/mmol) and multiprimer DNA-labelling system kit were purchased from Amersham. Metallothionein cDNA was kindly provided by Dr. Naganuma.

2.2. Animals and treatment

Male adult Wistar rats 2 months old (200–220 g) were obtained from PANLAB, and acclimated to our animal room for 2 weeks before use. Throughout these 2 weeks rats were supplied with food (SanderSa) and water *ad libitum*, exposed to a 12 hr light–dark cycle and given intraperitoneally a single necrogenic dose of thioacetamide (6.6 nmol/kg body weight) freshly dissolved in 0.9% NaCl. The dose of thioacetamide was chosen as the highest dose with survival above 90% [21–23]. GD pre-treatment was performed 24 hr before thioacetamide. GD was dissolved in 0.9% NaCl and administered in a tail vein (0.1 mmol/kg body weight). Untreated animals received 0.5 mL of 0.9% NaCl. Animals were sacrified and samples of blood and liver were obtained at 0, 12, 24, 48, 72 and 96 hr following thioacetamide [24]. Experiments were performed on two different groups: rats treated with a single dose of TA and rats pre-treated with GD and treated with a single dose of TA (GD + TA). Each experiment was performed in duplicate from four different animals and followed the international criteria for the use and care of experimental animals outlined in *The Guiding Principles in the Use of Animals in Toxicology* adopted by the Society of Toxicology in 1989.

2.3. Determination of enzymes and metabolites

Enzymatic determinations were carried out in serum or in subcellular fractions (soluble and microsomal) of liver homogenates in the optimal conditions of pH, temperature and substrate and cofactor concentrations. Aspartate aminotransferase (AST) and isocitrate dehydrogenase (ICDH) activities were determined in serum. AST (EC 2.6.2.1) activity was assayed following the method of Rej and Horder [25]. ICDH (EC 1.1.1.39) was determined as described previously [26]. Serum mieloperoxidase (EC 1.11.1.7) was assayed in serum using the BIOXYTECH® MPO-EIA™ Kit (Oxis Research™) following the method described by Klebanoff [27]. Serum TNFα and IL-6 levels were also assayed in serum using the Biotrak™ [(r)TNFα] ELISA system and Biotrak™ [(r)IL-6] ELISA system, respectively (Amersham Pharmacia Biotech). Microsomal flavin-dependent monooxygenase (FADm; EC 1.14.13.8) activity was assayed in the microsomal fraction of liver obtained as previously described [22], following the method of Sum and Kasper [28]. NADPH-generating enzymes such as glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) and malic enzyme (ME; EC 1.1.1.39) were determined following the methods described by Deutsch [29] and by Outlaw and Springer [30], respectively. Reduced and oxidised glutathione were determined in liver homogenates, following the method of Hissin and Hilf [31]. Thiobarbituric acid reactive substances (TBARS) were assayed in liver homogenates, following the method of Nieaus *et al.* [32].

2.4. RNA extraction and Northern blot analysis of metallothionein

Total RNA was extracted following the guanidinium thiocyanate/phenol reagent method [33]. Twenty micrograms RNA was submitted to Northern blot analysis being electrophoresed on 0.9% agarose gels containing 0.66 M formaldehyde, transferred to Gene Screen^{TM} membranes and cross linked to membranes with UV light. Hybridisation was carried out as described by Amasino [34]. The relative level of mRNA transcript was determined using metallothionein cDNA probe [35] labelled with [α^{32}P]dCTP using a multiprimer DNA-labelling system kit (Amersham). Quantification of the films was performed by a laser densitometer (Molecular Dynamics) using the hybridisation with an 18S ribosomal RNA probe as an internal standard. The variability in the measurement of fold increase in mRNA, after quantification by scanning densitometry from the filters, was not greater than 15%.

2.5. Immunoblotting for detection of HSP70 protein

Total protein extracts was obtained as follows: 100 mg liver tissue was homogenated in 1 mL lysis buffer containing 10 mM Tris–HCl, 200 mM NaCl, 1 mM EGTA, 0.5% Nonidet P-40, 5 mM β-mercaptoethanol, 5% glycerol, 1 mM $MgCl_2$ and the protease inhibitors 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 40 μg/mL aprotinin and 4 μg/mL leupeptin.

Protein concentrations were determined by the method of Bradford [36] (Bradford reagent, Sigma). Total protein extracts were boiled in equal volumes of loading buffer (125 mM Tris–HCl, pH 6.8, 4% SDS, 20% glycerol and 10% 2-mercaptoethanol). Protein levels were then analysed by Western blot. Aliquots containing equal amounts of protein (20 μg) were loaded onto a precast ready gel 12% Tris–HCl (BioRad). Proteins were separated electrophoretically and transferred to polyvinylidene difluoride (PVDF) membranes (Hybond-P, Amersham Life Science) using the BioRad Electrophoretic Transfer Cell. For immunoblotting, membranes were blocked with 10% non-fat dried milk in TPBS for 2 hr. The primary antibody employed was goat polyclonal antibody against HSP70 (sc-1060) (Santa Cruz Biotechnology Inc.). After washing, appropriate secondary antibody (anti-goat IgG-peroxidase conjugated from Santa Cruz) was applied for 1 hr. Blots were washed, incubated in commercial enhanced chemiluminescence reagents (ECL; Amersham) and exposed to chemiluminescence film. Quantification of the films was performed by a laser densitometer (Molecular Dynamics).

2.6. RT–PCR analysis of HSP70

For RT–PCR, total RNA (1 μg) was subjected to random primed first-strand cDNA synthesis in 40 μL reactions composed of 50 mM Tris–HCl, 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 1 mM dNTPs (each), 50 ng of random hexamer, 0.5 IU/μL Mo-Mu-LV reverse transcriptase (Superscript Pre-Amplification System; Gibco-BRL, Life Technologies). The reactions were incubated for 60 min at 42° and terminated at 65° for 15 min. The first-strand cDNAs were subsequently amplified by PCR; β-actin cDNA was used as an internal control. The sequences of the primers were as follows: HSP70 sense: 5′-CTC GTG CGT GGG CGT GTT CC-3′; HSP70 antisense: 5′-TCG CCC TTG TAG TTC ACC TG-3′ [37]; β-actin sense: 5′-TAC AAC CTC CTT GCA GCT CC-3′; β-actin antisense: 5′-GGA TCT TCA TGA GGT AGT CAG TC-3′ [38]. The PCR reaction mixture contained PCR buffer (20 mM Tris–HCl, pH 8.4, 50 mM KCl), 1.5 mM $MgCl_2$, 100 μM dNTPs (each), 0.4 μM primers and 0.0025 U/μL Taq polymerase in a final volume of 50 μL. The number of PCR cycles was adjusted to avoid saturation of the amplification system (94° for 30 s, 59° for 1 min and 72° for 30 s (25 cycles) for HSP70 and 94° for 30 s, 58° for 30 s and 72° for 30 s (24 cycles) for β-actin) with a final elongation at 72° for 10 min. Amplification products were visualised on 1.8% agarose gels containing ethidium bromide (1 μg/mL): HSP70 product, 285 bp; β-actin product 630 bp. A 100 bp DNA ladder was used as marker. The products were quantified by laser densitometer.

2.7. Statistical analysis

The results were calculated as the means ± SD of four experimental observations in duplicate (four animals). Differences between groups were analysed by an ANOVA following Snedecor F ($\alpha = 0.05$). Student's t-test was performed for statistical evaluation as follows: (a) all values against their control; (b) differences between two groups GD + TA vs. TA.

3. Results

3.1. Parameters of liver necrosis

Liver damage induced by xenobiotics is characterised by the release in serum of hepatic enzymes due to necrosis of hepatocytes. AST and ICDH are two enzymes used as markers of necrosis. AST is randomly distributed in the hepatic acinus, while ICDH is mainly located in the perivenous acinar region. The increase in both activities was detectable at 12 hr of thioacetamide administration and reached the maximum at 24 hr (Fig. 1). The extent of necrosis induced by thioacetamide was detected by a peak of 30 and 45 times the basal values, for AST and ICDH activities, respectively. When rats were pre-treated with GD the peak values at 24 hr were reduced to 15 and 16% for AST and ICDH activities, respectively. However, at 48 hr of intoxication the differences due to GD were 56 and 43%

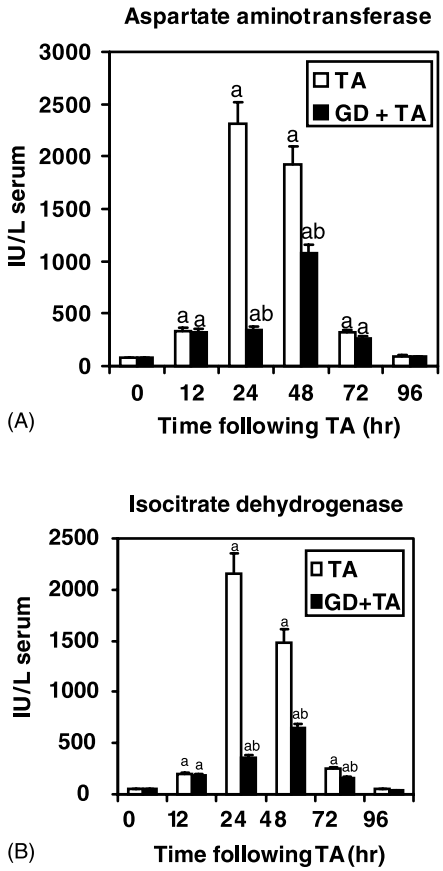

Fig. 1. Effect of GD pre-treatment on aspartate aminotransferase (A) and isocitrate dehydrogenase (B) activities in serum of rats intoxicated with one sublethal dose of thioacetamide. Samples were obtained at 0, 12, 24, 48, 72 and 96 hr following thioacetamide (TA). The results, expressed as IU/L of serum, are the mean ± SD of four determinations in duplicate from four rats. Differences against the respective control are expressed as (a) and differences due to GD are expressed as (b), $P < 0.05$.

for these two enzyme activities, respectively, which indicate that GD delays thioacetamide-induced liver injury, since the maximum of necrosis appeared at 48 hr of intoxication. No effects were detected on serum activities when GD was administered without thioacetamide (data not shown).

3.2. Parameters related to Kupffer cells function

In serum of rats intoxicated with thioacetamide, with or without GD pre-treatment, parameters related to Kupffer cells function such as TNFα and IL-6 and mieloperoxidase activity were assayed. In Table 1 are shown that following thioacetamide the level of these two cytokines increased markedly in serum at 24 hr of intoxication, and when GD was pre-administered, both cytokines were significantly lowered and appeared at 48 hr of intoxication. This decrease and delay of cytokine release was parallel to that found in the levels of serum AST and ICDH activities shown in Fig. 1.

Mieloperoxidase is an enzyme component of Kupffer cells and also a marker of macrophage function [39]. Its

Table 1
Effect of GD pre-treatment on the levels cytokines TNFα and IL-6 in serum of rats intoxicated with thioacetamide

Time following TA (hr)	TNFα (pg/mL)		IL-6 (pg/mL)	
	TA	GD + TA	TA	GD + TA
0	–	–	–	–
12	75 ± 7	–	60 ± 6	–
24	2 ± 0.2	25 ± 3*	30 ± 3	6 ± 0.5*
48	–	–	5 ± 0.4	–
72	–	–	–	–
96	–	–	–	–

The results, expressed as pmol/mL of serum, are the mean ± SD of four determinations in duplicate from four rats. Differences between GD pre-treated or non-pre-treated rats are expressed as $^{*}P < 0.05$.

activity, assayed in serum of rats, increased sharply at 48 and 72 hr of thioacetamide intoxication reaching at 48 hr 3-fold the basal values. However, when GD was pre-administered, no changes in mieloperoxidase activity were detected in any of the samples assayed (Fig. 2).

3.3. Parameters related to microsomal biotransformation of thioacetamide. Activity of microsomal flavin-dependent monooxygenase

Following thioacetamide, FADm activity was induced and this induction was earlier than changes in serum enzymes activities that characterise necrosis [23]. As thioacetamide is metabolised in liver by this microsomal system, the effect of GD pre-treatment was analysed to investigate whether the metabolism of thioacetamide was affected in these conditions. Figure 3 shows FADm activity determined in the microsomal fraction of rat liver following thioacetamide pre-treated or not with GD. In previous experiments of our group using the same model of liver injury [22,23], it was demonstrated that FADm activity increased at 12 hr of thioacetamide intoxication and decreased at 24 hr, when necrosis reaches the peak. GD

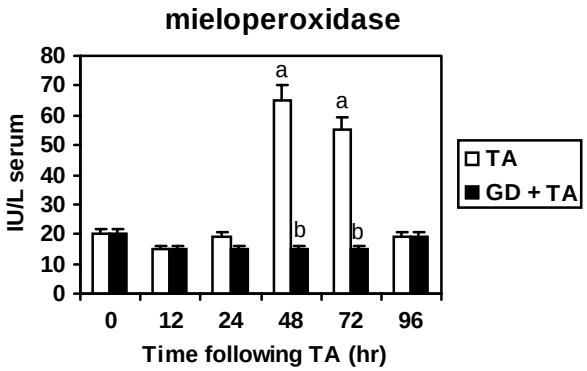

Fig. 2. Effect of GD pre-treatment on mieloperoxidase activity in serum of rats intoxicated with a sublethal dose of thioacetamide. Samples were obtained at 0, 12, 24, 48, 72 and 96 hr following thioacetamide (TA) results, expressed as IU/L of serum, are the mean ± SD of four determinations in duplicate from four rats. Differences against the respective control are expressed as (a) and differences due to GD are expressed as (b), $P < 0.05$.

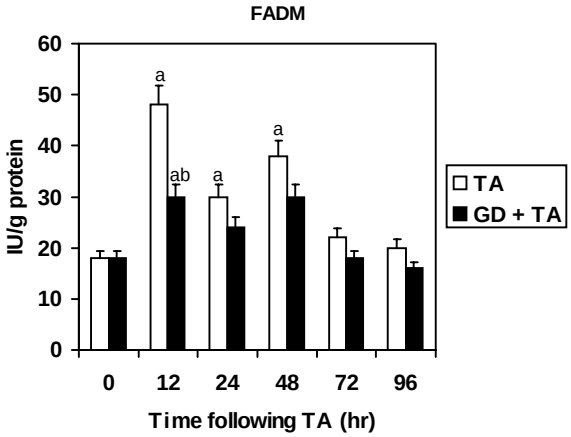

Fig. 3. Effect of GD pre-treatment on microsomal FAD monooxygenase activity in the microsomal fraction of liver of rats intoxicated with a sublethal dose of thioacetamide (TA). Samples were obtained at 0, 12, 24, 48, 72 and 96 hr following thioacetamide. The results, expressed as IU/g of protein, are the mean ± SD of four determinations in duplicate from four rats. Differences against control are expressed as (a) and differences due to gadolinium are expressed as (b), $P < 0.05$.

pre-treatment did affect significantly the pattern of changes induced by thioacetamide in FADm activity, which indicates that the attenuating effect on liver damage produced by GD pre-treatment is partially due to changes in thioacetamide biotransformation.

3.4. Parameters related to oxidative stress

The ratio GSH/GSSG is used as a marker of oxidative stress. Figure 4A shows that the changes in this redox ratio following thioacetamide are detected by a sharp decrease at 24 hr of intoxication with levels near of half (52%, $P < 0.05$) of the basal values. At 72 hr of intoxication the ratio GSH/GSSG recovers its normal values. GD pre-treatment attenuated the decrease and delayed the minimum value at 48 hr with a significant decrease to 69% ($P < 0.05$) of the control. Differences due to GD were significant at 24 hr of intoxication.

In order to demonstrate the effect of GD pre-treatment on lipid peroxidation, TBARS were determined and the results are shown in Fig. 4B. The noticeable increase in lipid peroxidation by the effect of thioacetamide reached a maximum at 24 hr of intoxication with values of 280% ($P < 0.05$) the control. However, GD pre-treatment reduced significantly this enhancement to 160% ($P < 0.05$). Differences due to the effect of GD were significant at 24 and 48 hr.

3.5. Effect of GD pre-treatment on soluble NADPH-generating enzymes

The activated state of Kupffer cells is accompanied by an elevated uptake and phosphorylation of glucose [40]. Glucose through the pentose phosphate pathway generates NADPH. Accordingly, G6PDH and ME, as NADPH generating enzymes, were determined in the soluble fraction of liver of rats following thioacetamide, pre-treated or not with GD. Figure 5 shows that the changes induced by thioacetamide on these soluble enzyme activities were not significantly affected by the GD pre-treatment.

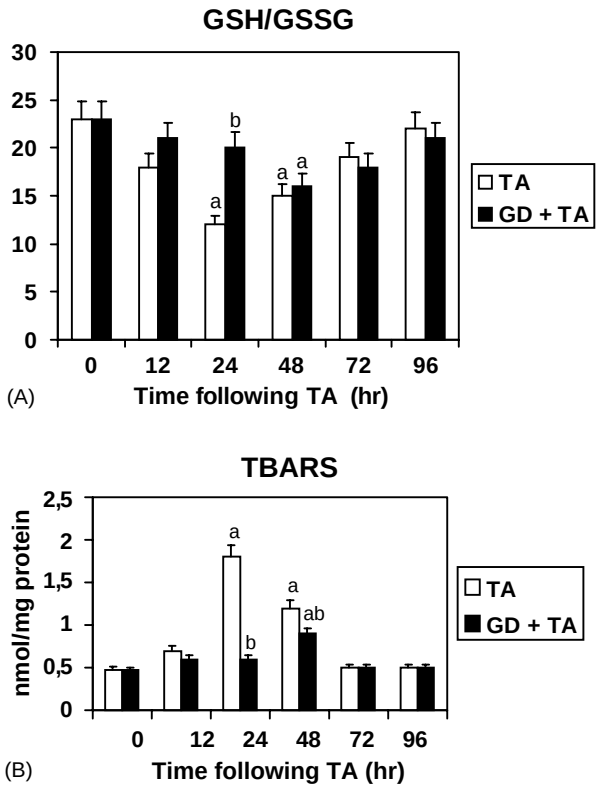

Fig. 4. Effect of GD pre-treatment on GSH/GSSG ratio (A) and thiobarbituric acid reactive substances (TBARS) concentration (B) in liver homogenates of rats intoxicated with a sublethal dose of thioacetamide (TA). Samples were obtained at 0, 12, 24, 48, 72 and 96 hr following thioacetamide. Basal values for GSH and GSSG were 40.0 ± 3.8 and 1.7 ± 0.2 nmol mg/protein. The results, expressed as the ratio GSH/GSSG and nmol of peroxides per mg of protein, respectively, are the mean ± SD of four determinations in duplicate from four rats. Differences against the respective control are expressed as (a) and differences due to GD are expressed as (b), $P < 0.05$.

3.6. Effect of GD pre-treatment on HSP70 and metallothionein expression in liver of rats following the intoxication of thioacetamide

Figure 6 shows the levels of HSP70 mRNA (A) and protein (B) assayed by RT–PCR and Western blot analysis, respectively. HSP70 protein increased by the effect of thioacetamide and GD pre-treatment produced a higher enhancement, except at 24 hr, which were significant at 48 and 72 hr of intoxication. The pattern of changes observed in HSP mRNA were similar to those of protein, except at 24 hr of intoxication.

Figure 7 shows the levels of metallothionein mRNA assayed by Northern blot analysis, which sharply increased following thioacetamide and this increase was

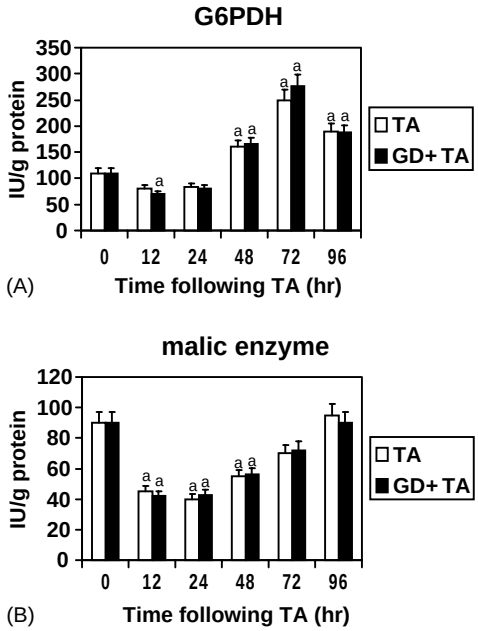

Fig. 5. Effect of GD pre-treatment on glucose-6-phosphate dehydrogenase (A) and malic enzyme (B) activities in the soluble fraction of liver of rats intoxicated with a sublethal dose of thioacetamide (TA). Samples were obtained at 0, 12, 24, 48, 72 and 96 hr following thioacetamide. The results, expressed as IU/g of protein, are the mean ± SD of four determinations in duplicate from four rats. Differences against the respective control are expressed as (a) and differences due to GD are expressed as (b), $P < 0.05$.

more pronounced in all samples pre-treated with GD. Changes in the expression of this protein were inversely proportional to the extent of damage

4. Discussion

Kupffer cells play an important role in the hepatic response to injury; they display upregulated scavenger functions and produce various inflammatory mediators including cytokines and reactive oxygen species [41]. The activation of these cells is accompanied by a higher requirement of glucose [40], which through the pentose phosphate pathway generates NADPH necessary for GSSG reduction. Although thioacetamide induced the activity of NADPH generating enzymes [42], GD pre-treatment did not produce any change.

Kupffer cells and infiltrating neutrophils contribute to liver injury in different experimental models of hepatotoxicity [43,44]. In our experiments, GD significantly attenuates liver damage as well as oxidative stress and lipoperoxidation induced by thioacetamide. This attenuation is parallel to Kupffer cell function since serum mieloperoxidase activity and the levels of cytokines (TNFα and IL-6) were significantly reduced. Thus, it is clear that the mechanism of this protection seems to result from a diminished generation of ROS and inflammatory and cytotoxic mediators (cytokines and proteases) released from Kupffer cells.

GD protects the liver from a number of toxicants that require biotransformation to elicit toxicity [45,46]. Badger *et al.* [47] demonstrated in hepatocytes isolated from GD pre-treated rats, that the P-450 activity was reduced and that the susceptibility of hepatocytes was altered. It has also been shown that hepatic injury induced by ischemia/reperfusion is modulated by the Kupffer cells [48]. Our results demonstrate that the activity of FADm, the microsomal system involved in thioacetamide metabolism, was significantly affected by GD pre-treatment, which led us to consider that the GD attenuating effect on thioacetamide toxicity could be partially due to a reduced thioacetamide biotransformation.

In our experiments GD apparently has no effect on basal FADm activity, but it has an inhibiting effect in the induction of FADm activity due to TA itself. In previous studies [21,23] we demonstrated that TA induces its own biotransformation by enhancing the activity of FADm. In the results of the present paper, the TA-dependent induction of FADm was diminished by the effect of GD, and consequently the biotransformation of TA and free radical generation. At 24 hr, when the maximum of necrosis appears, we detect a decrease in FADm, which is due to the fact that this system is mainly located in the injured intra-acinar area.

We cannot discard the possibility that GD influences the uptake of thioacetamide into the liver. However, we have two parameters, and both of them can by themselves explain the attenuation of the injury: the specific blockade of Kupffer cells function (demonstrated by less MPO activity), and the lesser rate of biotransformation (due to a diminished induction of FAD monooxygenase activity).

Xenobiotics may act directly on hepatocytes causing toxicity by interacting with target molecules and may also act indirectly through activating phagocytic cells. The active phagocytes participate in the pathogenesis of tissue injury by releasing, among others, inflammatory cytokines that upregulate the expression of adhesion molecules. Tissue damage initiates an inflammatory response characterised by an accumulation of macrophages at the site of injury [49]. In our case the effect of GD on thioacetamide hepatotoxicity can be considered from two points of view: by blocking Kupffer cell function and through inhibiting thioacetamide biotransformation, thus reducing its ability to activate phagocytic cells.

Exposure of living organisms to any kind of environmental stress rapidly induces the synthesis of the shock/stress proteins that can provide either an immediate protection or participation in cell repair. The expression of HSP70 and metallothionein significantly increased by the effect of thioacetamide, and this enhancement was even more pronounced in the case of GD. These results can be considered a response to adverse conditions, representing a potential mechanism of defence against disease.

The present results demonstrate that thioacetamide enhances the activity of Kupffer cells by increasing the

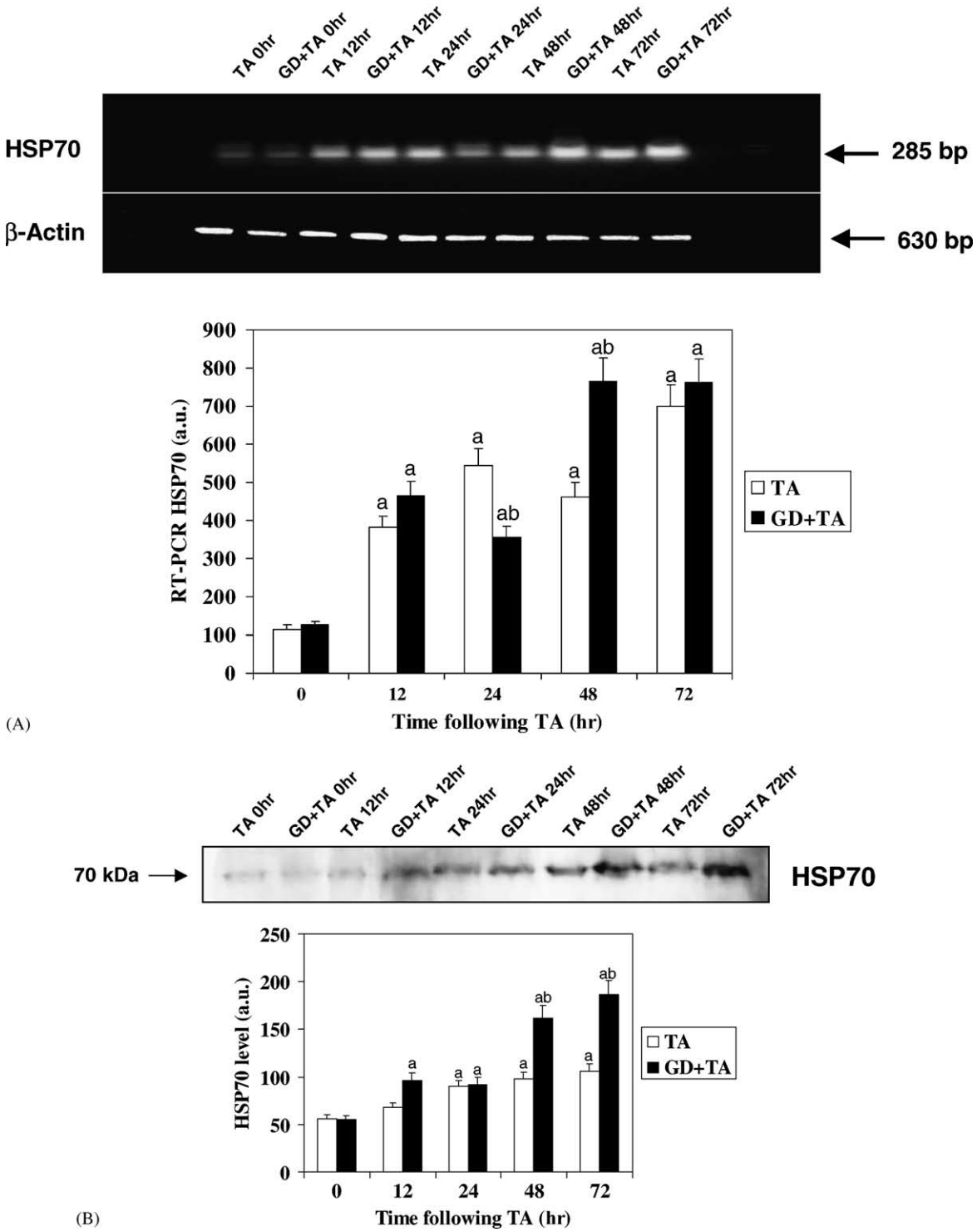

Fig. 6. Effect of GD pre-treatment on the levels of HSP70 protein (A) and HSP70 mRNA (B) assayed by Western blot and RT–PCR analysis, respectively, in liver homogenates of rats intoxicated with a sublethal dose of thioacetamide. Samples were obtained at 0, 12, 24, 48 and 72 hr. The results, expressed in arbitrary units are the mean ± SD of four determinations from four rats. Differences against the respective control are expressed as (a) and differences due to GD are expressed as (b), $P < 0.05$.

extent of necrosis and oxidative stress and by increasing the expression of stress proteins metallothionein and HSP70. Detected also was a thioacetamide-induced release of TNFα and IL-6 and mieloperoxidase activity. In the liver these cytokines and the activity of mieloperoxidase are mainly produced by Kupffer cells and are involved in the program of liver injury by promoting an inflammatory response. The blockade of Kupffer cell function by GD appears to result in a disruption of a part of the sequence of events leading to hepatotoxicity.

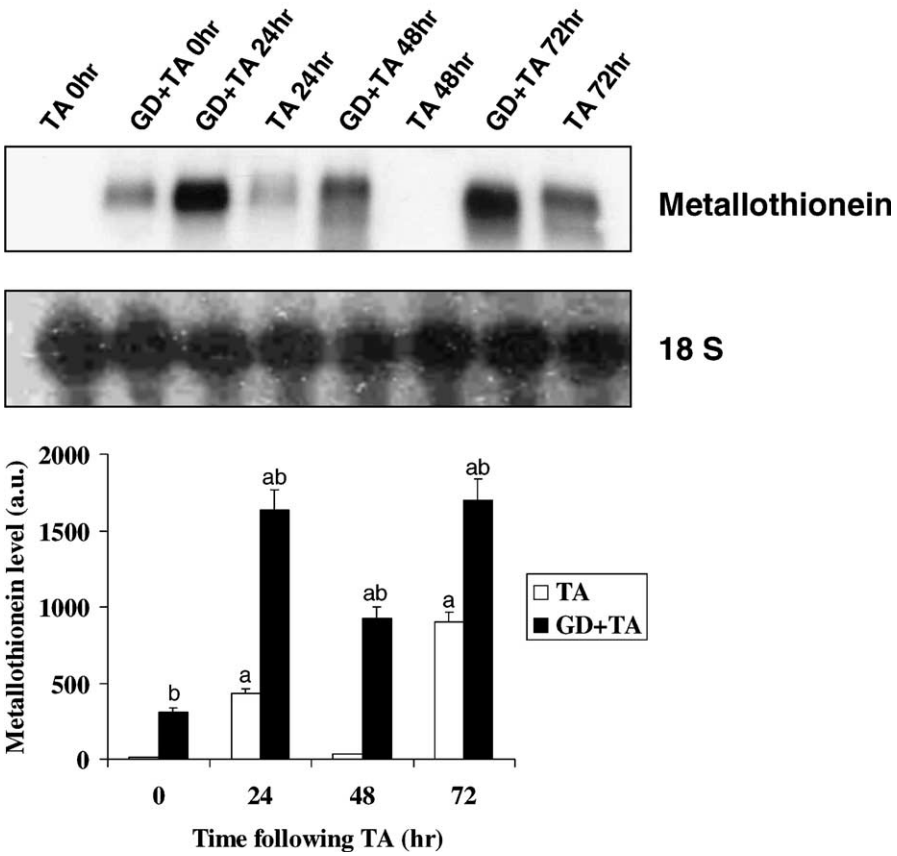

Fig. 7. Effect of GD pre-treatment on the levels of mRNA metallothionein assayed by Northern blot, respectively, in liver homogenates of rats intoxicated with a sublethal dose of thioacetamide (TA). Samples were obtained at 0, 12, 24, 48 and 72 hr. The results, expressed in arbitrary units, are the mean ± SD of four determinations from four rats. Differences against the respective control are expressed as (a) and differences due to gadolinium are expressed as (b), $P < 0.05$.

It is well known that neutrophils contains large amounts of MPO when activated and recruited into the liver in response to TNF generated by Kupffer cells. However, at 48 hr following TA, the peak that we observe in MPO activity cannot be due to neutrophils since these cells, whose half life is 6 hr [50], were activated at 12 hr in response to TNF generation by Kupffer cells. Apparently, the peak of MPO activity at 48 hr of TA administration is not due to neutrophils, but to the Kupffer cells. Thus, the inactivation of MPO by the effect of GD should be only at the level of Kupffer cells.

We conclude that GD pre-treatment significantly attenuates thioacetamide-induced hepatotoxicity. The mechanism by which this attenuation is verified is both by direct inhibiting of Kupffer cell function and through inhibiting thioacetamide biotransformation, thus reducing the ability of this hepatotoxic compound to activate phagocytic cells. The higher expression of HSP70 and metallothionein can also be involved. In our experimental conditions, the degree of this attenuation is close to 50%. The modulation of Kupffer cell function by GD may serve as a potential target for therapeutics and could be useful for preventing liver damage induced by drugs.

Acknowledgments

The authors would like to thank Dolores Velasco for her valuable technical assistance and Erik Lundin for his help and advice in the preparation of the manuscript. We are also very grateful to Naganuma for providing us generously with metallothionein cDNA.

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