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Influence of aminoguanidine on parameters of liver injury and regeneration induced in rats by a necrogenic dose of thioacetamide

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1 When aminoguanidine, a nucleophilic hydrazine compound, was administered to rats (50 mg kg^{-1} body wt) 30 min before a necrogenic dose of thioacetamide (500 mg kg^{-1} body wt), significant changes related to liver injury and hepatocellular regeneration were observed.

2 The extent of necrosis was noticeably less pronounced, as detected by the peak of serum aspartate aminotransferase activity. Depletion of hepatic glutathione (GSH) and the increase in malondialdehyde concentration as markers of oxidative stress, produced by thioacetamide metabolism, were significantly diminished. However, the activity of microsomal FAD monooxygenase, the system responsible for thioacetamide oxidation, did not show significant alterations. Antioxidant enzyme systems involved in the glutathione redox cycle, such as glutathione reductase and glutathione peroxidase activities, slightly decreased following aminoguanidine pretreatment.

3 Primary cultures of peritoneal macrophages from control rats, when incubated in the presence of serum collected following thioacetamide intoxication, showed a significant decrease in nitric oxide (NO) release at 24 h, that was more pronounced in the group pretreated with aminoguanidine. However, the sharp and progressive increase in macrophage NO release, when incubated in the presence of serum obtained at 48, 72 and 96 h, were increased by aminoguanidine-pretreatment.

4 The cell population involved in DNA synthesis sharply increased in both groups at 48 h of intoxication, although the values at 0, 24, 72 and 96 h were markedly higher in the group pre-treated with aminoguanidine. Polyploidy at 72 and 96 h of intoxication was delayed by the effect of aminoguanidine and a progressive increase in the hypodiploid hepatocyte population, which reached 16% of the total at 96 h, was observed.

5 These results indicate that a single dose of aminoguanidine before thioacetamide administration, markedly diminished the severity of the liver injury by decreasing oxidative stress and lipoperoxidation, but hepatocellular regeneration was apparently unaffected probably due to an enhanced mitogenic activity.

Keywords: Aminoguanidine; hepatic necrosis; hepatic regeneration; thioacetamide; nitric oxide synthase; oxidative stress

Introduction

The acute liver injury induced by a necrogenic dose of thioacetamide is characterized by a severe perivenous necrosis immediately followed by hepatocellular regeneration (Cascales *et al*., 1992; Díez-Fernández *et al*., 1993, 1996a; Sanz *et al*., 1998a). Liver damage is initiated by biotransformation of the hepatotoxic agent by the microsomal FAD-monooxygenase system (Chieli & Mavaldi, 1984) and is followed by oxidative stress, lipoperoxidation (Cascales *et al*., 1991; Sanz *et al*., 1998b), increases in cytosolic calcium (Díez-Fernández *et al*., 1996b) and changes in DNA ploidy and distribution in the cell cycle phases (Díez-Fernández *et al*., 1993). The liver is capable of recovering from damage or loss of up to 90% of its mass by means of proliferative activity, restoring it to normal size. There is an increasing interest in the ability of mammalian liver to grow after the loss of its own cells due to the action of hepatotoxic substances (Mehendale, 1991; Steer, 1995). The proliferative response immediately following thioacetamide-induced cytotoxicity provides a useful model to study cell proliferation in liver and to consider the factors which trigger and stop cell division in response to chemical attack (Díez-Fernández *et al*., 1993; Mangipudy *et al*., 1995).

The nucleophilic compound aminoguanidine has been found to decrease the development of complications in experimental animal models of diabetes and been proposed as a potential protective agent in man (Brownlee *et al*., 1986; Hames *et al*., 1991; Ou *et al*., 1993; Schleicher *et al*., 1997). Moreover, aminoguanidine has received much attention as an inhibitor of nitric oxide synthase (NOS) due to the early recognition of its selectivity towards inducible NOS, its low acute toxicity, and its potential clinical usefulness. There are numerous reports on the beneficial effects of aminoguanidine in various experimental models of inflammation and shock (Cross *et al*., 1994; Joshi *et al*., 1996; Szabó *et al*., 1997; Gardner *et al*., 1998; Shiomi *et al*., 1998). It has also been described that compounds, such as aminoguanidine, that act as iNOS inhibitors and peroxynitrite scavengers may be useful anti-inflammatory agents (Szabó *et al*., 1997).

In a previous article we described that NOS expression and NO release play a role in the pathophysiological mechanisms that trigger post-necrotic hepatocellular regeneration following thioacetamide administration (Díez-Fernández *et al*., 1997). On the basis of these results, the action of aminoguanidine, administered prior to a hepatotoxin, was investigated on parameters related to liver necrosis (oxidative stress and lipoperoxidation) and hepatocellular regeneration (DNA synthesis and ploidy).

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Methods

Animal treatment and processing of the samples

Adult male Wistar rats 2 months old (180–220 g), supplied with food and water *ad libitum* and exposed to a 12-h light-dark cycle, were given a single dose of thioacetamide intraperitoneally (500 mg kg^{-1} body wt) freshly dissolved in 0.9% NaCl. Control rats received 0.5 ml of 0.9% NaCl (Cascales *et al*., 1992). Animals were injected intraperitoneally with aminoguanidine (50 mg kg^{-1} body wt) 30 min before the thioacetamide intoxication. Rats were killed by cervical dislocation and samples were obtained at 0, 12, 24, 48, 72 and 96 h. Blood was collected from the heart and kept at 4°C for 24 h, centrifuged at 3,000 r.p.m. for 5 min and serum was obtained as the supernatant, as described previously (Díez-Fernández *et al*., 1997). Liver samples were quickly freeze-clamped *in situ* (using stainless tongs cooled in liquid nitrogen) and kept at −80°C until processed. Each experiment was repeated four times (four rats) and followed the international criteria for the use and care of experimental research animals outlined in the 'Guide for the Care and Use of Laboratory Animals' published by the National Institute of Health (N.I.H. 1985).

Isolation of hepatocytes

Hepatocytes were isolated by the collagenase perfusion technique as described (Seglen, 1993; Díez-Fernández *et al*., 1996b). The yield of hepatocytes from thioacetamide-treated animals at 24 h of intoxication (maximum necrosis) was approximately 50% and 80% for non-treated and aminoguanidine pretreated animals, when compared to their respective controls. The viability (>90%) of isolated hepatocytes was assessed by trypan blue exclusion as previously described (Díez-Fernández *et al*., 1997).

Preparation and culture of macrophages

Macrophages were obtained from the peritoneal cavity of control rats and seeded on plastic dishes at $1 \times 10^6/cm^2$. Experiments were carried out in phenol-red free DMEM supplemented with 0.5 mM arginine and 10% fetal calf serum (FCS) as described by Díez-Fernández *et al*. (1997). Ten per cent of serum samples obtained from rats non-pretreated and aminoguanidine-pretreated following thioacetamide, previously sterilized by filtration with 0.2 μM filters, were added to macrophage cultures as described (Sanz *et al*., 1998b).

Enzyme assays

As a marker of necrosis, serum enzyme activity of aspartate aminotransferase was spectrophotometrically measured at 340 nm in the presence of α-ketoglutarate, aspartate, NADH and malate dehydrogenase. FAD monoxygenase was determined in the microsomal fraction obtained as previously described (Cascales *et al*., 1991) by the method of Sum & Kasper (1982). Glucose-6-phosphate dehydrogenase was assayed in hepatic soluble fraction spectrophotometrically at 340 nm in the presence of glucose-6-phosphate and NADPH as previously described (Sanz *et al*., 1997). Glutathione reductase and glutathione peroxidase were also determined in hepatic soluble fraction following the respective methods already described (Goldberg *et al*., 1987; Günzler *et al*., 1974).

Metabolite assays

Parameters related to oxidative stress and lipoperoxidation, malondialdehyde and glutathione levels, were determined in samples of liver as described previously (Díez-Fernández *et al*., 1993). Malondialdehyde was assayed following the method of Nieaus *et al*. (1969). Glutathione (GSH) concentration was measured following the method of Griffith (1980). NO was measured by the Griess method according to Green *et al*. (1982) as described (Díez-Fernández *et al*., 1997). Protein was measured according to the method of Bradford (1975) using bovine serum albumin as standard.

Flow cytometry analysis

DNA content was obtained from 10^6 isolated viable cells stained with propidium iodide following the multistep procedure of Vindelov *et al*. (1983). The emitted fluorescence of the DNA-propidium iodide complex was assayed in a FACScan flow cytometer (Becton-Dickinson).

Chemicals

Enzymes were from Boehringer (Mannheim, Germany). Substrates, coenzymes and other chemical and biochemicals were from Sigma (St. Louis, MO) or Merck (Darmstadt, Germany) Kinesis-50 was from Bio Rad (Richmond, CA).

Statistical analysis

Values were obtained as the means ± s.d. of four different experimental observations (four animals). Student's *t*-test was performed for statistical evaluations and the statistical significance considered as $P < 0.01$.

Results

Parameters related to liver injury

To detect the time course of liver injury, aspartate aminotransferase activity was determined in samples of serum obtained at 0, 12, 18, 24, 48, 72 and 96 h after thioacetamide administration to non-pretreated and aminoguanidine-pretreated rats. In previous studies (Díez-Fernández *et al*., 1993) thioacetamide, when administered to rats, produced a peak of necrosis at 24 h of intoxication. In the present study the increases in the enzyme activity, at the moment of maximum necrosis, was 19 times that of control (Figure 1). However, when aminoguanidine was administered to rats 30 min before thioacetamide, the necrotic process initiated earlier, but the peak of necrosis was significantly less pronounced, reaching, at 24 h of intoxication, values only six times the respective control.

Time course of FAD monooxygenase activity

The activity of the FAD monooxygenase system was assayed in the microsomal fraction of liver homogenates as the enzyme involved in thioacetamide oxidation. Figure 2 shows that in the sequential process of liver injury and regeneration induced by thioacetamide, the activity of FAD monooxygenase underwent significant changes: an early and significant increase at 12 h of intoxication followed by a marked decrease at 24 h (the peak of necrosis) and a second peak at 48 h. However, aminoguanidine-pretreatment did not essentially affect the pattern of

changes induced by thioacetamide in the activity of this system.

Parameters related to oxidative stress: levels of glutathione and malondialdehyde

Reduced glutathione was assayed in samples of liver obtained from thioacetamide-intoxicated non-treated and aminoguanidine-pretreated rats (Figure 3). The time course of reduced glutathione concentration is shown in Figure 3a. In non pretreated rats thioacetamide induced a progressive decrease in hepatic glutathione, showing the lowest concentration at the moment of maximum necrosis at 24 h (42% of control, $P<0.001$). However, the decrease was significantly less pronounced in livers of aminoguanidine pre-treated rats (86% of control).

As a parameter of lipoperoxidation, the concentration of malondialdehyde was determined in samples of liver obtained from non pretreated and aminoguanidine-pretreated rats. In previous studies we have described that thioacetamide, when administered in a sublethal dose to rats, significantly increases the hepatic concentration of malondialdehyde, a metabolite related to lipoperoxidation, which is a consequence of glutathione depletion (Cascales *et al*., 1991; Díez-Fernández *et al*., 1993). To see the effect of aminoguanidine pretreatment, the time-course of malondialdehyde level following thioacetamide was assayed. Figure 3b shows that in livers of thioacetamide intoxicated rats, malondialdehyde peaks at 24 h with values more than doubled (210% of control, $P<0.01$), while in the group pretreated with aminoguanidine, malondialdehyde was only slightly increased (130% of control).

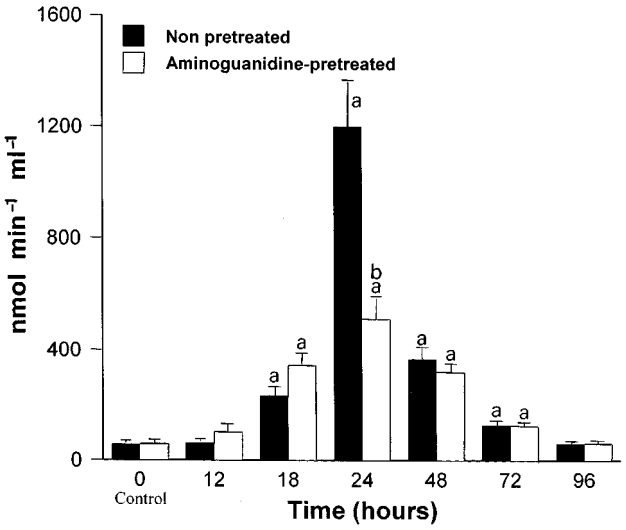

Figure 1 Effect of aminoguanidine pre-treatment on serum aspartate aminotransferase activity in rats following thioacetamide administration. Samples of serum were obtained from rats at 0, 12, 18, 24, 48, 72 and 96 h after thioacetamide administration. Groups of rats were untreated or pre-treated with aminoguanidine 30 min before the administration of thioacetamide. The activity was calculated spectrophotometrically at 340 nm and are expressed as nmol of substrate transformed per min per ml of serum. Results are the mean ± s.d. of four experimental assays (four animals). [a]Differences against control (0 h); [b]Differences between pretreated and non-pretreated. $P<0.01$.

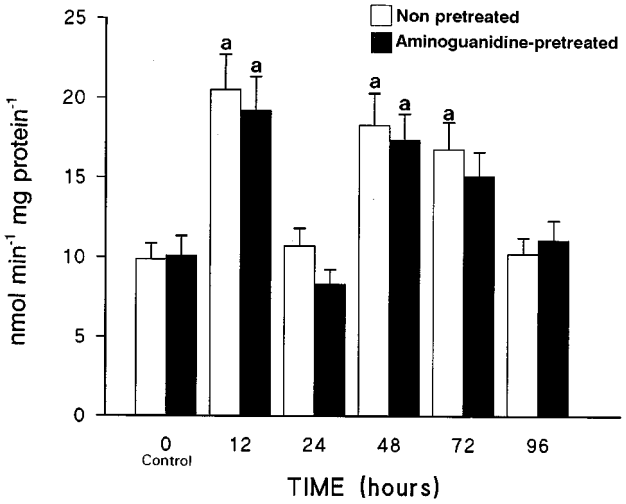

Figure 2 Changes in FAD monooxygenase activity in the microsomal fraction of liver of thioacetamide-treated aminoguanidine pretreated rats. FAD monooxygenase was assayed in the microsomal fraction of liver homogenates obtained from rats at 0 (control), 12, 24, 48, 72 and 96 h of thioacetamide administration. Results are the means ± s.d. of four different observations (four animals). [a]Differences versus control (0 h); [b]Differences against the untreated group. $P<0.01$.

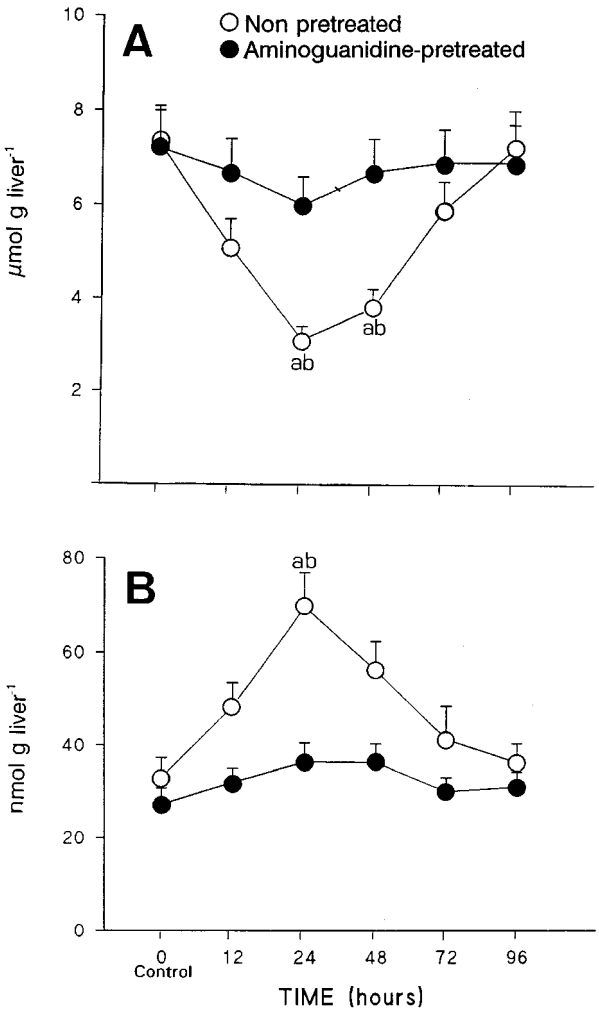

Figure 3 Effect of aminoguanidine-pretreatment on the levels of glutathione and malondialdehyde in liver extracts of thioacetamide-treated rats. (A) Glutathione (GSH) and (B) malondialdehyde concentration were determined in samples of liver at 0, 12, 24, 48, 72 and 96 h of thioacetamide administration. Glutathione is expressed as μmol g liver^{-1}, and malondialdehyde is expressed as nmoles g liver^{-1}, respectively. Results are the means ± s.d. of four different observations (four animals). [a]Differences versus control (0 h); [b]Differences against the untreated group. $P<0.01$.

Enzyme systems involved in the glutathione redox cycle

Figure 4 shows the activities of glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase assayed in the soluble fraction of liver homogenates. Glutathione peroxidase and glutathione reductase activities increased significantly following thioacetamide treatment with peaks at 72 h (271% of control, $P<0.01$ and 854% of control, $P<0.01$, respectively). Aminoguanidine pretreatment did not significantly affect the pattern of change of these two enzyme activities throughout the processes of liver injury and regeneration induced by thioacetamide. In the post-necrotic regeneration processes induced by thioacetamide, glucose-6-phosphate dehydrogenase sharply increased at 48 h to 534% of control ($P<0.01$), an increase described in previous studies of our group, both at the level of enzyme activity and mRNA (Díez-Fernández *et al.*, 1996a). The results obtained in the aminoguanidine-pretreated group showed a significantly lower increase (228% of control; $P<0.01$) at 48 h and a delayed restoration of this enzyme activity.

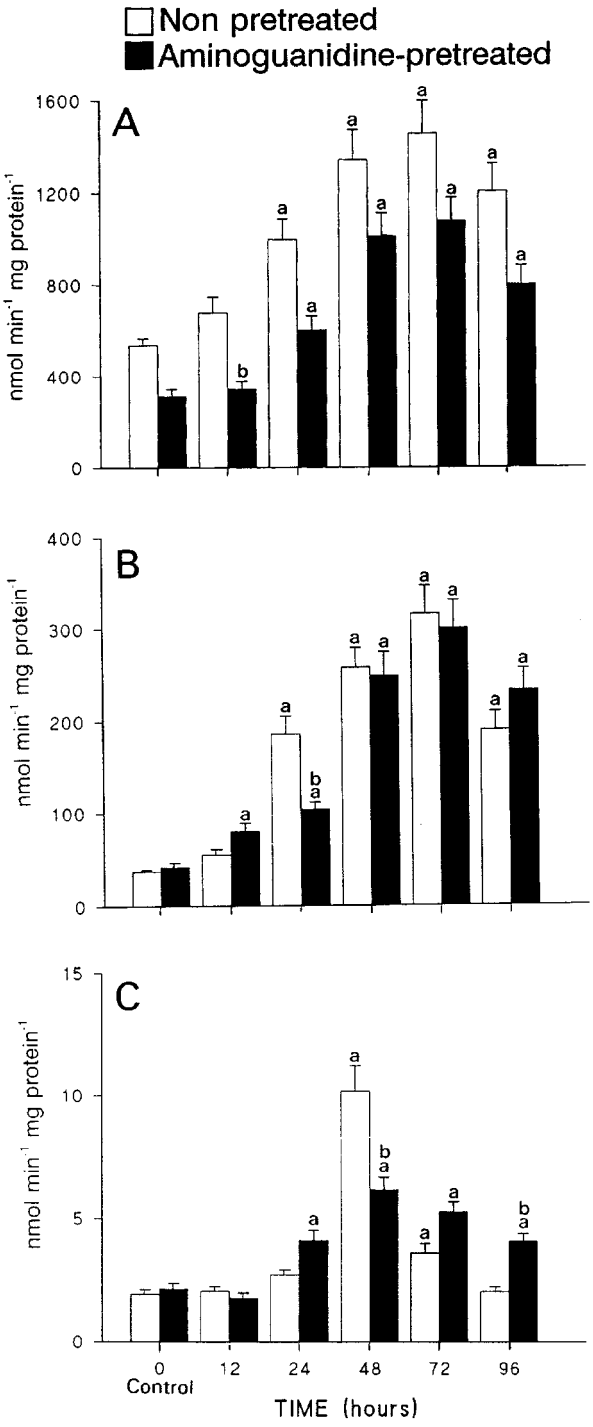

Figure 4 Effect of aminoguanidine-pretreatment on enzymes related to glutathione-redox cycle: (A) glutathione peroxidase, (B) glutathione reductase and (C) glucose-6-phosphate dehydrogenase on liver of thioacetamide-treated rats. Results are expressed as nanomoles min^{-1} mg $protein^{-1}$ and are the mean $\pm$ s.d. of four experimental observations (four animals). [a]Differences versus control (0 h); [b]Differences against non pretreated group. $P<0.01$.

NO release in ex vivo *peritoneal macrophages from control rats after incubation with serum from thioacetamide-treated rats*

We investigated the ability of serum collected at 0 (control) 12, 24, 48, 72 and 96 h after thioacetamide intoxication both in non-pretreated and aminoguanidine-pretreated rats, to induce NO release in primary cultures of peritoneal macrophages from control rats. Figure 5 shows that significant time-dependent changes in NO release were observed in cultures incubated in the presence of serum from thioacetamide-treated rats (Díez-Fernández *et al.*, 1997). Minimum values of NO release at 24 h of intoxication (12% of the control; $P<0.01$) and progressive increases at 48, 72 and 96 h were detected in macrophages cultures. There are significant differences between both groups at 0 and 12 h of thioacetamide intoxication (decreased NO release following aminoguanidine-pretreatment) and at 48 and 72 h (increased NO release following aminoguanidine-pretreatment).

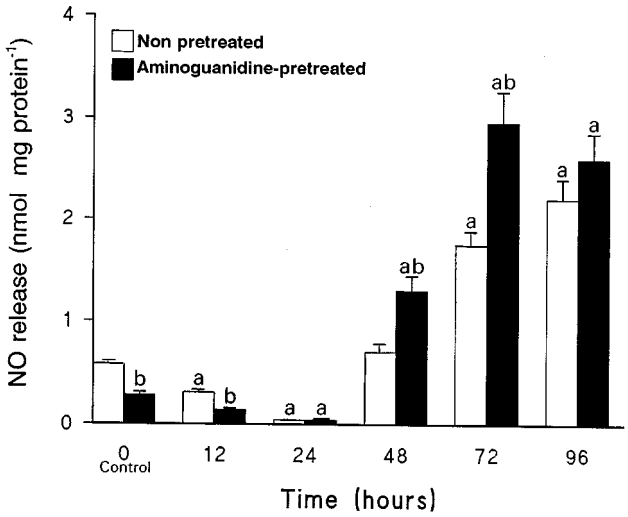

Figure 5 NO release by *ex vivo* macrophages cultures incubated in the presence of serum collected following thioacetamide intoxication. Serum from aminoguanidine-pretreated rats and non-pretreated rats were obtained at 0 (control), 12, 24, 48, 72 and 96 h of intoxication with thioacetamide. Primary cultures of peritoneal macrophages from control animals were incubated for 24 h in the presence of serum (10% v:v). The release of NO was measured by the Griess method (Green *et al.*, 1982). Results are expressed as nmol mg $protein^{-1}$ and are the means $\pm$ s.d. of four different observations (four animals). [a]Differences versus control; [b]Differences against the untreated group. $P<0.01$.

The effect of aminoguanidine on hepatocyte ploidy following thioacetamide.

Following thioacetamide administration without pretreatment, liver cells exhibited marked variations in the pattern of DNA distribution which can be summarized as: a sharp decrease at 48 h in polyploid (tetraploid and octoploid) populations, (from 51.2% to 7.5% and from 4.4% to 0.9%, respectively) followed by the restoration of the initial values at 96 h (Table 1). Alterations in DNA distribution profile were detected in hepatocytes of rats pretreated with aminoguanidine 30 min before intoxication when compared to non pretreated. These changes were as follows: a delay in the recovery of DNA ploidy together with the appearance of a hypodiploid peak, characteristic of apoptosis. The progressive increase in hypodiploid population was parallel to the lower recovery of polyploid populations. The hypodiploid population was quantified and reached 16% of the total population at 96 h. At 24 h, aminoguanidine-pretreatment caused an increase in the cell population in G2-M phase. Changes in the octoploid peak can also be observed in Table 1. Tetraploid and octoploid populations abruptly decreased at 48 h of thioacetamide intoxication, and increased later showing at 96 h values above the initial. Aminoguanidine-pretreatment delayed the restoration of polyploidy. Due to the heterogeneous acinar distribution of hepatocytes and because previous studies of our group (Díez-Fernández *et al*., 1993) have shown that many of the hepatocytes destroyed in perivenous necrosis are mainly tetraploid, the higher yield of hepatocytes in aminoguanidine-pretreated rats has to influence the pattern of DNA ploidy in these liver cells. Thus, in the present results, the decrease in tetraploid population is much higher in hepatocytes from untreated rats (51.2% to 28.5%; $P<0.01$) when compared to those from aminoguanidine-pretreated (58.6% to 51.5%).

Aminoguanidine-pretreatment induced DNA synthesis to the same extent as the untreated group, however in this case the increase against the control was only sixfold due to the higher initial level of DNA synthesis, induced apparently by the mitogenic effect of aminoguanidine. This enhanced DNA synthesis activity induced by aminoguanidine (1.4% versus 0.4%, at 0 h) was accompanied by the appearance of a hypodiploid population that progressively increased from 0.9% to 15.9% at 96 h of intoxication.

Discussion

Thioacetamide-induced liver injury is a well established area of considerable pharmacological interest, since reactive oxygen species and free radicals, generated in the microsomal drug oxidation, participate in the mechanisms of cell death (Cascales *et al*., 1991; Mehendale *et al*., 1994; Sanz *et al*., 1998b). In the present study thioacetamide hepatotoxicity was used to investigate the effect of a single dose of aminoguanidine on the multistep events involved in liver damage and regeneration. In previous reports we described that when thioacetamide was administered to rats, necrosis developed and peaked at 24 h of intoxication, and that a synchronous proliferative response was immediately initiated, reaching a peak of DNA synthesis at 48 h (Díez-Fernández *et al*., 1993; 1996a; Sanz *et al*., 1998b). The post-necrotic proliferative response, after experimental liver cell death, constitutes an interesting area in which to study the factors involved in the regulation of hepatocyte proliferation.

The results obtained in the present paper provide evidence that aminoguanidine, when administered prior to thioacetamide, significantly decreases the liver necrogenic effects of the hepatotoxin. This beneficial effect of aminoguanidine-pretreatment could not be attributed to actions of this compound on hepatic inducible nitric oxide synthase (iNOS), since we have recently reported that NOS activity and iNOS mRNA levels are only detected in the post-necrotic proliferating hepatocytes (Díez-Fernández *et al*., 1997). However, it also has been reported that NO is produced by constitutive NOS isoforms present in Kupffer cells (Díez-Fernández *et al*., 1997) and in endothelial cells and that guanidines can scavenge part of the peroxynitrite produced by residual iNOS activity or from NO produced by constitutive NOS isoforms (Szabó *et al*., 1997).

Following this line, we tested the ability of the serum collected at several times following thioacetamide treatment from aminoguanidine untreated and pretreated rats to induce NOS in cultures of macrophages obtained from control rats. We used peritoneal macrophages as a cell system very sensitive to growth factors and cytokines (Laskin 1992; Laskin *et al*., 1995; Laskin & Pendino 1995). NO release by peritoneal macrophages incubated *ex vivo* with serum of thioacetamide-treated rats, non-treated or pretreated with aminoguanidine, clearly shows that aminoguanidine exerts its NOS inhibiting effect very early (at 0 and 12 h of intoxication) and that NO

Table 1 Quantitative analysis of hepatocyte population for DNA distribution and ploidy following thioacetamide intoxication in control and aminoguanidine-pretreated rats

Time following thioacetamide (h)	*Hypodiploid* $<2C$	*Diploid* $2C$	*DNA synthesis* $2C\rightarrow4C$	*Tetraploid* $4C$	*DNA synthesis* $4C\rightarrow8C$	*Octoploid* $8C$
Non-pretreated						
0 (control)	–	43.0±4.3	0.4±0.03	51.2±4.9	1.0±0.08	4.4±0.5
24	–	69.5±7.0	1.0±0.09	28.5±2.2[a]	1.2±0.09	4.0±0.6
48	–	78.0±6.8	12.1±1.0[a]	7.5±0.6[a]	1.0±0.07	0.9±0.1[a]
72	–	81.0±7.4	2.5±0.2[a]	12.5±0.9[a]	0.9±0.07	2.2±0.2[a]
96	–	25.8±3.2	0.6±0.03	60.0±5.2	0.9±0.07	11.7±1.1[a]
Aminoguanidine-pretreated						
0 (control)	0.9±0.1[b]	37.0±3.2	1.4±0.1[b]	58.6±5.2	0.8±0.06	1.3±0.08
24	1.2±0.1[b]	40.0±2.8[b]	1.8±0.16	54.5±5.0[b]	0.8±0.07	1.7±0.21
48	2.2±0.2[ab]	71.0±5.8[a]	12.6±1.1[a]	11.5±1.0[ab]	1.4±0.10	1.3±0.09
72	6.4±0.6[ab]	75.0±6.2[a]	4.6±0.6[ab]	7.1±0.6[ab]	2.9±0.2[ab]	4.0±0.3[ab]
96	15.9±0.1[ab]	50.3±4.2[b]	3.0±0.2[ab]	20.9±1.8[ab]	3.0±0.2[ab]	6.9±0.6[ab]

Results are expressed as the percentage of hepatocyte distribution in the cell cycle phases and are the mean±s.d. of four experimental observations from two rats. [a]Differences versus control (0 h). [b]Differences between aminoguanidine-pretreated and non-pretreated. $P<0.01$.

release by macrophages increases when incubated in the presence of serum from aminoguanidine untreated and pretreated animals obtained at 48, 72 and 96 h of thioacetamide intoxication which indicates that, at that time, aminoguanidine is not present in serum.

Hepatic regeneration is characterized by the appearance in serum of growth factors and cytokines (Webber *et al*., 1994; Fausto *et al*., 1995; Steer 1995), some of which have the ability to induce iNOS in macrophages (Moncada *et al*., 1991). Our results show that the ability of serum of post-necrotic animals to induce NO release in cultured macrophages was higher in the aminoguanidine-pretreated rats. This enhancement could be a response to serum stimulus also involved in hepatic regeneration, since in previous studies we showed that NO release plays a role in the post-necrotic regenerating process (Díez-Fernández *et al*., 1997). We suggest that a long lasting effect of aminoguanidine increases the expression of serum growth stimulus. Further experiments should be performed to investigate the effect of aminoguanidine in continuous doses during liver injury and post-necrotic regenerating liver.

The significant decrease in hepatic injury induced by the pretreatment of aminoguanidine was reflected in the parameters related to lipoperoxidation and depletion of glutathione, and showed some effect on the activities of enzymes involved in the glutathione redox cycle. However the activity of the enzyme system responsible for thioacetamide oxidation, microsomal FAD monooxygenase, did not show any change with aminoguanidine and no changes were observed in parameters concerning hepatocellular regeneration, such as DNA synthesis. Taken together, these results suggest that activation of the drug can be dissociated from the regenerative process and that aminoguanidine exerts its protective effect by preventing reactive oxygen species reacting with nucleophiles in the cells, thus preventing glutathione depletion and lipid peroxidation. Our results agree with recent studies that describe the antioxidant effect of aminoguanidine significantly inhibiting the hepatotoxicity induced by endotoxemia (Shiomi *et al*., 1998) or by hepatotoxic agents (Gardner *et al*., 1998). Aminoguanidine inhibits the peroxinitrite-induced oxidative processes in two ways: selectively inhibiting iNOS expression, NO release and consequently peroxinitrite formation, and directly scavenging it and consequently neutralizing the peroxinitrite-triggered oxidative processes.

Regarding post-necrotic regeneration, the peak of DNA synthesis was similar in both groups, although it is necessary to point out that the initial DNA synthesis levels were significantly higher due to the effect of aminoguanidine, indicating that in our experiments this compound also exerts a mitogenic action, also described by Kallio *et al*. (1997), which can lead to liver hyperplasia. This hyperplasia, induced by aminoguanidine, was accompanied by the appearance of a hypodiploid (apoptotic) population that increased progressively during the whole process (0 to 96 h). The mechanisms by which pretreatment with aminoguanidine develops apoptosis may be as a response to maintain the normal number of cells. The fact that iNOS inhibition was associated with apoptosis has been recently described (Billiar & Harbretch 1997).

We conclude that pretreatment with aminoguanidine, markedly diminishes the severity of liver injury induced by a necrogenic dose of thioacetamide, by decreasing oxidative stress and lipoperoxidation, while the extent of hepatocellular post-necrotic regeneration, took place parallel to non-pretreated group. Furthermore, aminoguanidine exerts a mitogenic effect, detected by the increase in the hepatocyte population in S phase of the cell cycle from 0 to 96 h, following thioacetamide administration. Moreover, the mitogenic action of aminoguanidine was accompanied by the appearance of a hypodiploid population (apoptotic) as a possible response against hepatocyte overproduction and liver hyperplasia. Thus, we can clearly deduce that aminoguanidine exerts its effects by decreasing the extent of necrosis. However, necrosis is not the only mode of cell death and in our experiments apoptosis progressively increased throughout the experiment.

Independent of the precise mechanisms of the aminoguanidine effects, which still remain to be fully established, the present observations open important therapeutic possibilities of this drug for many cases of oxidative stress-induced cell injury.

The role of NO release induction in liver injury, associated with acute inflammatory results, is an attractive area in the field of pharmacological research, since NO release can either be protective or damaging. On the one hand, non-selective NOS inhibition should be extremely toxic to the liver during endotoxemia (Vos *et al*., 1997), and on the other hand, the selective inhibition of iNOS could have organ-protective effects, significantly reducing hepatic damage in haemorrhagic liver injury (Billiar & Harbretch 1997). Thus, from a therapeutical point of view, the challenge is to eliminate the excess of NO while preserving the basal NO level in order to maintain the vasoprotective effects of this bioregulatory molecule. Aminoguanidine could represent an interesting drug to modulate NO availability reducing tissue damage and providing therapeutic benefits in a number of inflammatory disorders.

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