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Induction of an ATPase inhibitor protein by propylthiouracil and protection against paracetamol (acetaminophen) hepatotoxicity in the rat

[1]Atreyee Banerjee, [1,3,8]Willem G. Linscheer, [1,6]Hideyuki Chiji, [1,3]Uma K. Murthy, [2,4,7]Chaidong Cho, [3]Jyotirmoy Nandi & [5]Samuel H.P. Chan

Departments of [1]Medicine and [2]Pathology, Veterans Affairs Medical Center and Departments of [3]Medicine and [4]Pathology, SUNY Health Science Center, Syracuse, NY 13210, and [5]Department of Biology, Syracuse University, Syracuse, NY 13244, U.S.A.

1 The purpose of the present study was to test the following hypothesis: propylthiouracil (PTU) treatments of rats induces an increase in the concentration and activity of the mitochondrial ATPase (m-ATPase) inhibitor protein (IF_1). The PTU-induced elevated baseline levels of this inhibitor protein inactivated m-ATPase, and prevented hepatotoxicity by a toxic dose of acetaminophen (AAP) (paracetamol), by maintaining hepatic adenosine 5′-triphosphate (ATP) levels.

2 Male Wistar rats were either gavaged with a toxic dose of AAP alone, or after pretreatment with PTU for periods of 3 and 12 days.

3 Twenty four hours after acetaminophen treatment alone, toxicity was manifested by: an approximately 10 fold increase in serum transaminase levels (serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase); depletion of hepatic reduced glutathione (GSH) and ATP levels; loss of inhibitor protein activity, and extensive pericentral necrosis of the hepatocytes. Propylthiouracil pretreatment for 12 days enhanced the concentration of the following metabolites in the liver: ATP (1.5 fold), ATPase inhibitor protein (IF_1) (4.5 fold), and reduced glutathione (1.3 fold), while the activity of the inhibitor protein increased 2 fold. When the PTU treated rats were challenged with AAP, transaminases were not elevated, and only sporadic areas of necrosis were detected by histological examination of the liver tissue. In contrast to the 12 day treatment with PTU the 3 day treatment had no protection against AAP. No histological evidence of protection was manifested and the transaminases were not different from AAP treated controls. Most of the protective metabolites were depleted.

4 Our findings suggest that PTU-induced increased concentration of inhibitor protein and GSH, are contributing factors in the prevention of hepatotoxicity by maintaining hepatic m-ATP levels and reducing the harmful effect of the toxic metabolite of AAP.

Keywords: Paracetamol (acetaminophen) intoxication; propylthiouracil; ATPase; ATPase inhibitor protein; reduced glutathione; hypothyroidism

Introduction

The adverse effects of a toxic dose of paracetamol (or acetaminophen (AAP), in the U.S.A.) on rat liver have been well documented (Mitchell *et al*., 1973; Potter *et al*., 1973; Linscheer *et al*., 1980).

AAP is metabolized by two non-toxic pathways (sulphation and glucuronidation) and one oxidative and potentially toxic pathway. In the toxic pathway, AAP is oxidized by a cytochrome P-450-dependent enzyme system into a reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI) (Dahlin *et al*., 1984). When more NAPQI is formed than can be conjugated to reduced glutathione (GSH), the unbound NAPQI becomes toxic by binding to macromolecules, including cellular proteins (Dahlin *et al*., 1984). In addition, AAP causes the collapse of the proton gradient across the inner mitochondrial membranes, which inactivates ATP synthase and activates ATPase. As shown by Harman *et al*. (1991) in rat hepatocytes and by Nazareth *et al*. (1991) in rat liver slices, the combined toxic effect of loss of the mitochondrial proton gradient, toxicity of NAPQI and depletion of ATP causes cell death. As shown by Linscheer *et al*. (1980) hepatotoxicity of AAP can be prevented by oral treatment with propylthiouracil (PTU) for a period of 12 days before gavaging a toxic dose of AAP. In addition it was demonstrated that the effect of PTU was multifactorial. PTU increased the hepatic concentrations of GSH (a scavenger of reactive molecules), and adenosine 5′-triphosphate (ATP), an essential fuel for numerous intracellular metabolic processes. Hepatic GSH plays an important role in attenuating the consequences of the electrophilic derivative of AAP by covalent binding to GSH (Tirmenstein & Nelson, 1989). Others (Yamada & Kaplowitz, 1980; Yamada *et al*., 1981) have demonstrated binding of the toxic metabolite NAPQI to PTU itself and proposed a competitive binding between PTU and GSH for GSH transferase. This proposal was opposed by Habig *et al*. (1984). Recently we found that in addition to GSH and ATP, PTU also increased the concentration and the activity of a mitochondrial ATPase inhibitor protein (IF_1).

The importance of IF_1 in preventing ischaemia of heart muscle was shown previously by Rouslin (1988) and Rouslin and Broge (1989). The role of IF_1 in preserving ATP

[6]Present address: Associate Professor, Department of Food Science and Nutrition, Fuji Womens' College, Hanakawa Minami 4-5, Ishikaricho, Hokkaido 061-32, Japan.
[7]Present address: Dianon Systems, 200 Watson Boulevard, Stratford, CT 06497, USA
[8]Author for correspondence at: VAMC, 800 Irving Ave, Syracuse NY 13210, USA.

concentrations and, therefore, in the protection of the hepatocytes has not been clarified. IF_1 is the natural mitochondrial ATPase inhibitor protein located in a membrane bound complex, which regulates ATP synthesis by its ATP synthase activity as well as the hydrolysis of ATP by ATPase activity. IF_1, reported by Pullman and Monroy (1963), is a small protein (MW10,500), originally isolated from beef heart mitochondria. During ischaemia of the heart muscle, ATP synthase activity is inactivated by the destruction of the proton gradient between the inner mitochondrial membrane and the cytoplasm. Inactivation of ATP synthase leads to the activation of ATPase. However, IF_1 is expressed by the activation of ATPase. This has an inhibiting effect on ATPase activity by conjugation of IF_1 to ATPase. This complicated protective mechanism against ATP depletion is effective at protecting heart muscle against ischaemia (Schwerzmann & Pedersen, 1986; Rouslin, 1988; Rouslin & Broge, 1989). It has been shown that AAP also eliminates the proton gradient and thereby the ATP synthase activity, which has similar effects of ATPase and IF_1. The purpose of the present study was to evaluate the role of IF_1 in the multifactorial protection of PTU against AAP toxicity.

Animals

Male Wistar rats (Charles River Laboratory, Wilmington, MA) weighing 225–250 g were used in all studies. All rats received humane care in compliance with the Veterans Affairs guidelines. The rats were housed in individual wire cages in an air-conditioned laboratory with a 12 h light (6 h 00 min to 18 h 00 min) and a 12 h dark cycle. They were fed ground rat chow (Prolab RMH 3200, Agway, Syracuse, NY). The rats were divided randomly into 4 treatments groups.

Group 1 Rats were gavaged with a toxic dose of AAP (1 g kg^{-1} body weight (BW)) suspended in an isotonic saline solution containing gum of acacia (10 mg ml^{-1}). The volume of the gavaged AAP was kept constant for all rats (2.5 ml). The rats were then fasted, but had free access to water. They were killed by decapitation 5, 24 and 48 h post AAP treatment. The rats in the 48 h group were fed at 24 h.

Group 2 Rats ingested 0.0375 g of PTU per day, mixed with rat chow for 3, 5, 8 and 12 days, and were then killed.

Group 3 Rats were gavaged with AAP (1 g kg^{-1} BW) after being treated with PTU for either 3 or 12 days (see above). They were then fasted and killed at 24 h.

Group 4 (control) Rats were fed chow, but did not receive either PTU or AAP medication and were killed on the same day as the rats in the experimental groups.

Methods

Chemicals

PTU, carbonylcyanide m-chlorophenylhydrazone (CCCP), polyoxyethylene ether (Lubrol-WX), ATP, $MgSO_4$, oligomycin, Tris.HCl, 2-[N-morpholino] ethanesulphonic acid (MES), trichloroacetic acid (TCA), sucrose, phosphoglycerate phosphokinase, glyceraldehyde phosphate dehydrogenase, NADH and GSH were obtained from Sigma (St. Louis, MO); K_2SO_3 from Aldrich Chemicals Co. (Milwaukee, WI); amplified alkaline phosphatase (AP) Immuno Blot assay kit, containing affinity purified Goat anti-rabbit IgG (H + L) AP conjugate, from Bio-Rad. Laboratories (Richmond, CA) and an Autoprobe III kit (which uses a wide spectrum universal Peroxidase-based Immunohistochemical detection system) from Biomedia corp. (Foster City, CA). All chemicals and reagents were of the highest purity grade available. Purified IF_1 and IF_1 antibodies were prepared in our laboratory.

Analytical procedures

Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were analysed by standard laboratory methods (Hørder *et al*., 1981) after collection of blood from each control and experimental rat.

Livers were removed immediately following decapitation. One gram of liver was used for GSH determination. A second section was processed immediately for determination of ATP and for isolation of the mitochondrial fraction. A third small section was fixed in buffered formalin (3% paraformaldehyde and 2% picric acid in 0.1 M PBS solution, pH 7.2) for microscopic examination.

Immunohistochemical staining

Immunohistochemical staining was done on formalin-fixed and paraffin embedded liver tissue. Four sections were cut, deparaffinized, rehydrated, and stained by the streptavidin biotinated complex (ABC) method (AutoProbe III kit from the Biomedia Corp.). A 1:50 dilution of the polyclonal antibody to IF_1 was used as the primary antibody. Pre-immune serum from the same animal was used as control.

Isolation of hepatic mitochondria

Liver mitochondria were isolated according to the method of Pedersen *et al*. (1978) and stored in 1–2 ml of ice-cold 0.25 M sucrose buffer containing 20 mM Tris HCl, pH 7.4. Mitochondrial protein was determined by the method of Lowry *et al*. (1951).

Measurement of IF_1 activity

Aliquots of the mitochondrial suspension were diluted 10–20 times in a medium (200 μl) containing 0.25 M sucrose, 50 mM MES or 20 mM Tris HCl, 2 mM ATP, 4 mM $MgSO_4$ and 5.0 μM uncoupler (CCCP) at either pH 6.4 or pH 8.0. Incubation of mitochondria at pH 6.4 in the presence of Mg, ATP and CCCP induced inhibition of ATPase activity by IF_1, while at pH 8.0 this inhibition was absent (Sah *et al*., 1993). After 5 min pre-incubation at room temperature, aliquots (20 μl) were transferred to a medium (1 ml) containing 0.25 M sucrose, 20 mM Tris HCl at pH 8.0, 0.004% Lubrol, 5.0 mM K_2SO_3 (removes ADP dependent inhibition of ATPase), with and without oligomycin (5 μg ml^{-1}). The reaction was initiated by the addition of 4 mM ATP. After 2, 4 and 6 min, the reaction (200 μl aliquots) was stopped by adding a buffer (100 μl) containing 10% SDS and 100 mM EDTA. ATPase activity in the disrupted inner mitochondrial membranes was measured by phosphate (P_i) liberation at room temperature (Baginski *et al*., 1967). Lubrol has no effect on ATPase activity or on the interaction of ATPase with added IF_1 in the submitochondrial particles (Chernyak *et al*., 1987). ATPase activity was expressed as μmol P_i mg^{-1} protein min^{-1}.

The ATPase activity measured at pH 8.0 was taken as 100%. The difference between the ATPase activities at pH 8 and pH 6.4 equals IF_1 activity (Figure 1) and was expressed as

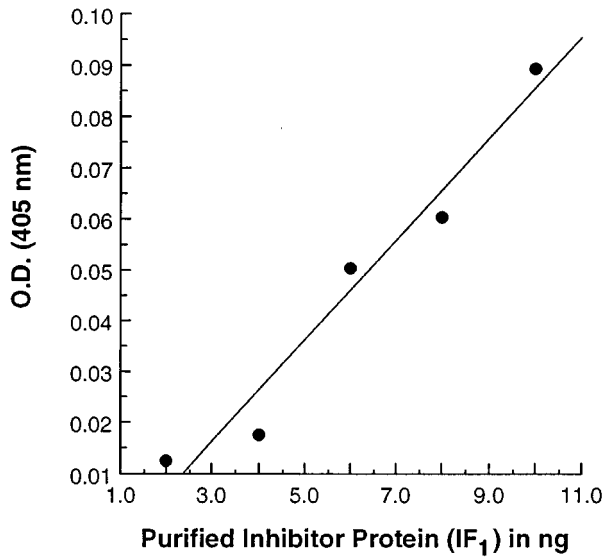

Figure 1 Standard curve of purified inhibitor protein (IF_1): the curve shows a linear relationship between the concentration of purified IF_1 and the O.D. readings. The slope of the regression line was used for calculation of the amounts of IF_1 in the mitochondria of the hepatocytes.

percentage inhibition of the maximum ATPase activity at pH 8.0 (Chernyak *et al*., 1987).

Isolation of IF_1

The mitochondrial fraction from each liver (obtained above) was further diluted to 5–10 mg protein ml^{-1}. Partially purified IF_1 was isolated from the mitochondria by the modified method of Horstman & Racker (1970), as described by LaMarche *et al*. (1992). The IF_1 was stored at −70°C.

Determination of mitochondrial concentrations of IF_1

Enzyme-linked immunosorbent assay (ELISA) was carried out using polyclonal antibodies against IF_1, raised in rabbits as described by Chan & Schatz (1979), Chan & Barbour (1976) and Engvall & Perlmann (1972).

Determination of ATP in liver tissue

A modification of the method of Adam (1963) was used and is described as follows. Ice-cold whole liver tissue in buffer was homogenized quickly at 0°C. After addition of ice-cold 12% TCA, the homogenate was frozen immediately at −70°C. For determination of ATP, homogenates were thawed on ice, centrifuged at 6,800 *g* for 10 min. The ATP content in the supernatant was measured by an end-point enzymatic ultraviolet assay (Adam, 1963), using phosphoglycerate phosphokinase and glyceraldehyde phosphate dehydrogenase to catalyze ATP to ADP and NADH to NAD^+, respectively by determining the decrease in absorbance caused by oxidation of NADH to NAD^+ at 340 nm in a spectrophotometer, the amount of total ATP content in the liver was calculated and expressed as nmol mg^{-1} protein. In separate experiments the modification of Adam's method was validated by comparing it with Adam's original method (freezing liver tissue *in situ* by liquid N_2 cooled metal clamps, followed by the addition of cold TCA). There was no significant difference between the two methods.

Determination of GSH in liver tissue

GSH in the fresh livers was determined by the method of Ellman (1959), and expressed as nmol mg^{-1} protein. Estimation of GSH is not affected by the presence of GSSG or PTU. No change in the GSH levels was observed in liver samples with and without addition of PTU.

Determination of PTU in liver tissue

PTU concentration in liver tissue of rats was determined by the method of García *et al*. (1995) and expressed as mg g^{-1} liver.

Statistics

Data are expressed as means ± s.e.mean; n = number of rats. To determine the statistical significance for multiple comparisons between the experimental groups and one control group ANOVA was used. Otherwise unpaired Student's t test was used. The significance was accepted at $P<0.05$ and denoted by asterisks: $*P<0.05$, $**P<0.001$, $***P<0.0001$.

Results

The following parameters of toxicity were determined for each group of rats: serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) levels, hepatic ATP, GSH and IF_1 concentrations, and hepatic mitochondrial IF_1 activity (Figures 2–5).

Effect of AAP treatment

We tested previously the effect of AAP for various time periods. The most significant changes in the parameters of toxicity were found at 24 h after the administration of AAP alone.

Figure 2 shows that the concentration of SGOT, and SGPT increased 10 fold for both transaminases. Although the concentration of m-IF_1 did not show a significant change as compared with the untreated rats during the 24 h observation period (Figure 3), activity of IF_1 after an initial rise at 5 h was practically absent at 24 h (Figure 4). The hepatic ATP levels increased during the first 5 h (1.4 fold), AAP also induced partial depletion of hepatic ATP and GSH at 24 h to 50% and 45% of control values (Table 1).

Effect of PTU pretreatment alone on IF_1

The effect of PTU treatment on IF_1 concentrations was measured after 3, 5, 8 and 12 days. No significant change was found at 3 days, but the concentration increased gradually between 5 and 12 days (Figure 5) by 4.5 fold above control at 12 days, while the activity increased only 2 fold above control values (Figure 4).

The effect of PTU treatment (12 days) on SGOT, SGPT, GSH and ATP

The other parameters were determined at 12 days of PTU treatment. There was no change in the SGOT and SGPT levels (Figure 2). Hepatic ATP and GSH concentrations increased 1.4 fold, and 1.3 fold, respectively (Table 1). The hepatic concentration of PTU at 12 days was 1.96 ± 0.19 mg g^{-1} liver.

Effect of PTU pretreatment alone (3 days) In contrast to the 12 days of treatment, 3 days of PTU treatment did not increase GSH and ATP concentrations (Table 1). The hepatic PTU concentration after 3 days was 0.38 ± 0.16 mg g^{-1} liver.

Effect of PTU pretreatment (12 days) + AAP When the PTU rats (treated for 12 days) were gavaged with AAP, the SGOT and SGPT levels were still within control values (Figure 2). The high IF_1 concentration was reduced but not depleted (Figure 3), while IF_1 activity was not different from the untreated controls (Figure 4). The combination of AAP and PTU treatment resulted in a significant decrease of ATP and the GSH levels by approximately 40% and 30%, respectively. However, there was no depletion because these levels were still within the normal range (Table 1).

Effect of PTU pretreatment (3 days) + APP When the rats were treated for 3 days with PTU, and then gavaged with AAP, the parameters of toxicity were similar to the AAP-treated rats (Figures 2 and 3). Hepatic ATP and GSH concentrations were depleted in both groups (Table 1).

Immunohistology

Immunohistology of liver tissue of the control rats showed faint yellow-brown IF_1 antibody staining of a few, mostly pericentrally located hepatocytes (Figure 6a). In contrast, the livers of the PTU treated rats (12 days) showed wide zones of much greater intensity of the IF_1 antibody stain (brown colour) around the central veins (Figure 6b), while the rats receiving AAP and PTU in combination had a smaller zone of

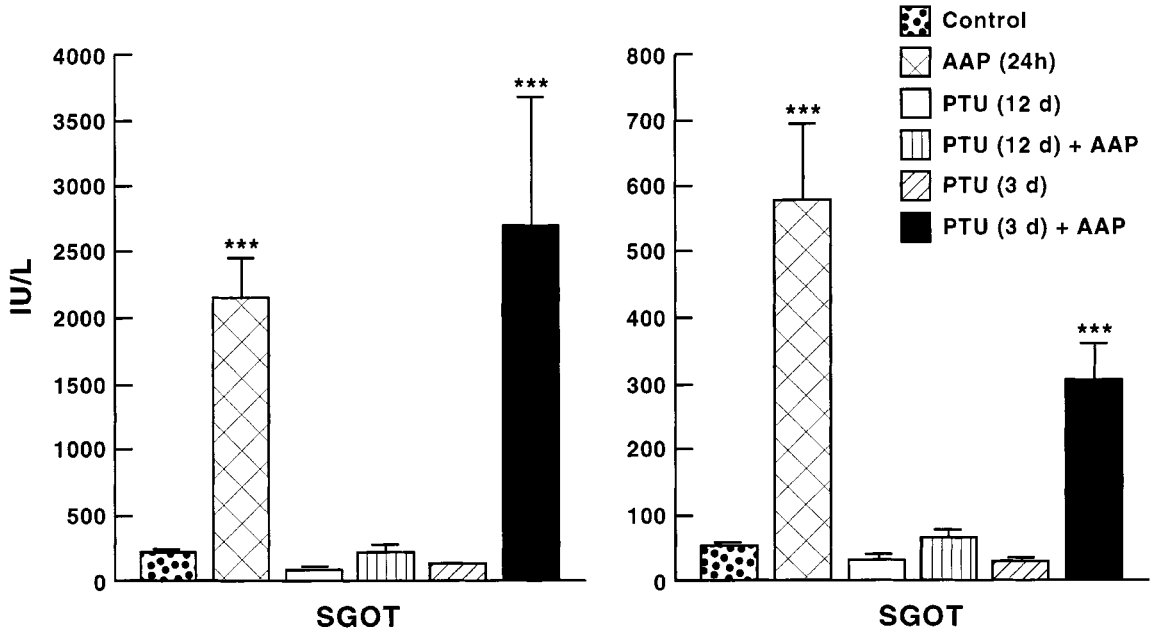

Figure 2 The effect of AAP on SGOT and SGPT levels: levels are expressed as international units per litre. Ten fold increases of both transaminases were observed in control rats 24 h after AAP administration. Similarly, 12 fold increases were observed 24 h after AAP treatment of rats pretreated with PTU for 3 days. In contrast, there was no significant difference between the transaminase of normal controls and PTU rats (12 days pretreatment) gavaged with AAP.

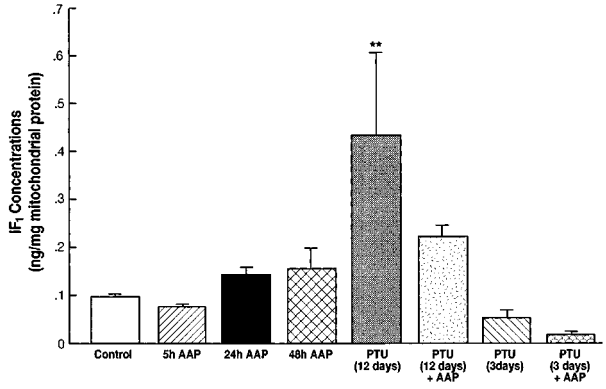

Figure 3 Effect of AAP on IF_1 concentration: the concentration of IF_1 in the AAP-treated control rats did not change significantly, but in the rats treated with PTU for 12 days there was a 4.5 fold increase compared with controls. When this PTU group was challenged with AAP, IF_1 concentration decreased significantly. However, there was no depletion of IF_1 because its concentration was still 2 fold higher than controls. Treatment for 3 days with PTU had no effect on IF_1 concentration.

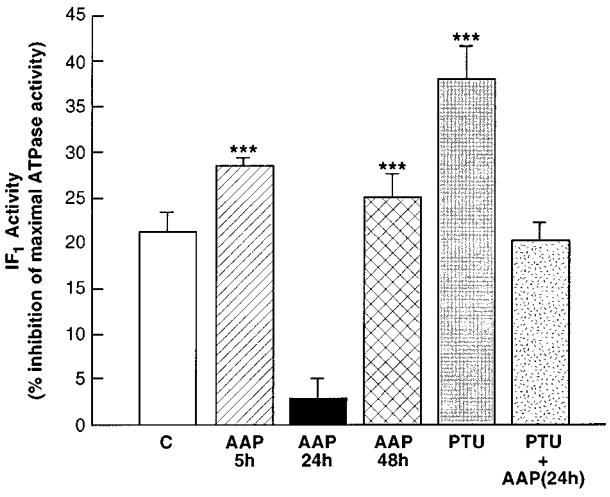

Figure 4 Effect of AAP, PTU (12 days) and PTU + AAP on IF_1 activity: results are shown 24 h after AAP administration alone and in combination with PTU for 24 h. PTU alone (12 days) increased IF_1 activity 2 fold above control levels.

Table 1 Effect of AAP treatment on hepatic ATP and GSH levels in rats with and without PTU

Treatment	*Hepatic ATP* (nmol mg^{-1}) protein	*Hepatic GSH* (nmol mg^{-1} protein)
Control ($n=11$)	13.5 ± 1.2	29.1 ± 1.7
AAP (5 h) ($n=6$)	20.7 ± 0.8***	19.8 ± 1.2***
AAP (24 h) ($n=11$)	6.9 ± 0.4***	13.3 ± 0.4***
PTU (12 days) ($n=8$)	19.3 ± 1.1***	36.9 ± 2.1**
PTU (12 days) + AAP (24 h) ($n=8$)	11.7 ± 1.6, NS	25.9 ± 1.7, NS
PTU (3 days) ($n=5$)	13.3 ± 0.5, NS	30.8 ± 1.4, NS
PTU (3 days) + AAP (24 h) ($n=8$)	8.7 ± 0.8***	20.8 ± 1.1***

Results are expressed as means $\pm$ s.e. *Represent P value designation for statistical differences between experimental and controls (see statistics). NS = not significant.

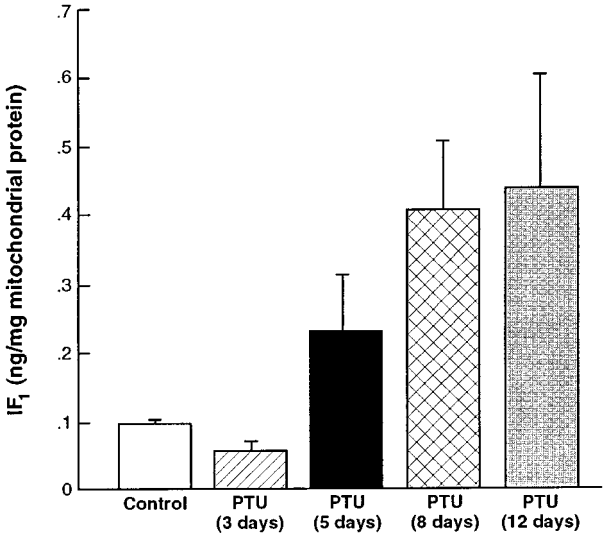

Figure 5 Effect of PTU on IF_1 concentration over a period of 12 days: the concentration increased gradually between 3 and 12 days. A 4.5 fold increase over controls was observed at 12 days ($P<0.01$). The results suggest increase of synthesis of IF_1 although a slower rate of hydrolysis cannot be excluded.

pericentrally located brown staining with minimal cell destruction, mostly located around the central veins (Figure 6c). The IF_1 positive staining of the livers of rats treated with AAP only was very light and extensive pericentral necrosis was evident (Figure 6d).

Discussion

The mechanism by which PTU protects the liver against the multifactorial toxic effects of AAP is not well understood. Our previous studies suggested that the protective effect of PTU treatment of rats for 12 days consisted of several defense mechanisms of the liver against the hepatotoxic effect of AAP: (1) high levels of hepatic GSH concentrations are most likely induced by increased synthesis, as suggested by Figure 5 and as reported previously (in abstract form; Raheja *et al*., 1984). Although GSH is important for survival of the hepatocytes, induced depletion of GSH by diethylmaleate did not cause necrosis or a rise in the transaminases (Raheja *et al*., 1983). (2) Decreased formation of NAPQI by competition of PTU and AAP for the oxidative pathway (Raheja *et al*., 1985). (3) Binding of NAPQI to the PTU molecule (Yamada *et al*., 1981). Protection was still present when hypothyroidism was caused by thyroidectomy and not by PTU (Linscheer *et al*., 1980). (4) When hypothyroidism was prevented in the PTU rats by administration of T3, a thyroid hormone, no necrosis was observed (Raheja *et al*., 1982). (5) Prevention of ATP depletion; although ATP is an essential fuel for numerous metabolic reactions, Martin & McLean (1995) showed that ATP depletion alone did not cause cell damage.

Our data suggest that the combination of depleted GSH and ATP is associated with cell death. Since there is little known about the effects of PTU on ATP metabolism in relation to the toxic effects of AAP, we decided to analyse the mechanism by which PTU prevents ATP depletion.

Metabolism of ATP in the mitochondria is regulated by a reversible interaction of a membrane bound (F_1F_0-ATPase) enzyme complex (Schwerzmann *et al*., 1986). Synthesis of ATP in the mitochondria by ATP synthase depends on a proton gradient across the inner mitochondrial membrane. One of the toxic effects of AAP is the destruction of the proton gradient, which inactivates ATP synthase and simultaneously activates ATPase. However, activated ATPase then expresses IF_1 activity. ATPase is inhibited by conjugation of the activated IF_1 to ATPase. Inhibition of ATPase and preservation of ATP is dependent on the concentration of IF_1.

At high concentrations, as in heart muscle, the reversible binding of the naturally occurring IF_1 protein to the β-subunits of F_1-ATPase has a protective role in the prevention of cell destruction in ischaemia, since it inhibits ATP hydrolysis. Unlike heart muscle, liver tissue of rats has, according to our results and Chernyak *et al*. (1987), a low concentration of IF_1. Activity of IF_1 after an initial rise at 5 h was practically negligible at 24 h after AAP administration (Figure 3). Histology showed extensive hepatic necrosis in AAP-treated rats (Figure 6d). This correlated with the simultaneous depletion of GSH and ATP levels in the liver (Table 1).

Although the concentrations and activity of IF_1 were low in the controls, they are a contributing factor in the suppression of the toxicity of a therapeutic dose of AAP in man. Pretreatment for 12 days with PTU gradually increased the IF_1 concentrations to 4.5 fold of control values (Figures 4 and 5). IF_1 activity did not correlate precisely with the IF_1 concentration. Similar observations have been made by Bruni *et al*. (1979).

Immunohistology of the PTU-treated rats (12 days) supported the biochemical measurements of IF_1 by showing high density of the IF_1 antibodies, located primarily around the central veins (Figure 6b); the sites especially vulnerable to AAP-induced necrosis. The high levels of IF_1 after PTU treatment decreased after AAP administration, but were maintained at 2 fold control values. This is consistent with the immunohistology (Figure 6c). The presence of only sporadic small islands of necrosis was in agreement with the other parameters (Figure 2 and Table 1), including the transaminases, which remained within the normal range (Figures 2, 6d and Table 1).

In contrast with the 12 day PTU treatment, PTU treatment for 3 days did not elevate the levels of ATP, GSH or IF_1 in the hepatocytes (Table 1 and Figure 5). Moreover, when these rats were challenged with AAP, there was no evidence of protection against AAP-induced hepatotoxicity (Figure 2 and Table 1).

Histology showed extensive pericentral necrosis similar to AAP-treated controls (data not shown). Our data suggest that the hepatotoxic effects of AAP cannot be averted by a short term treatment period (3 days) with PTU, and that PTU treatment seemed to be effective when all the defence mechanisms were stimulated after a prolonged treatment period (12 days).

It is of interest to note that PTU binds with the toxic metabolite of AAP by direct chemical interactions and probably contributes to the protection against AAP hepatotoxicity (Yamada *et al*., 1981). High concentrations of PTU in the hepatic parenchyma after 12 days may therefore explain the better protection of PTU treatment over thyroidectomy.

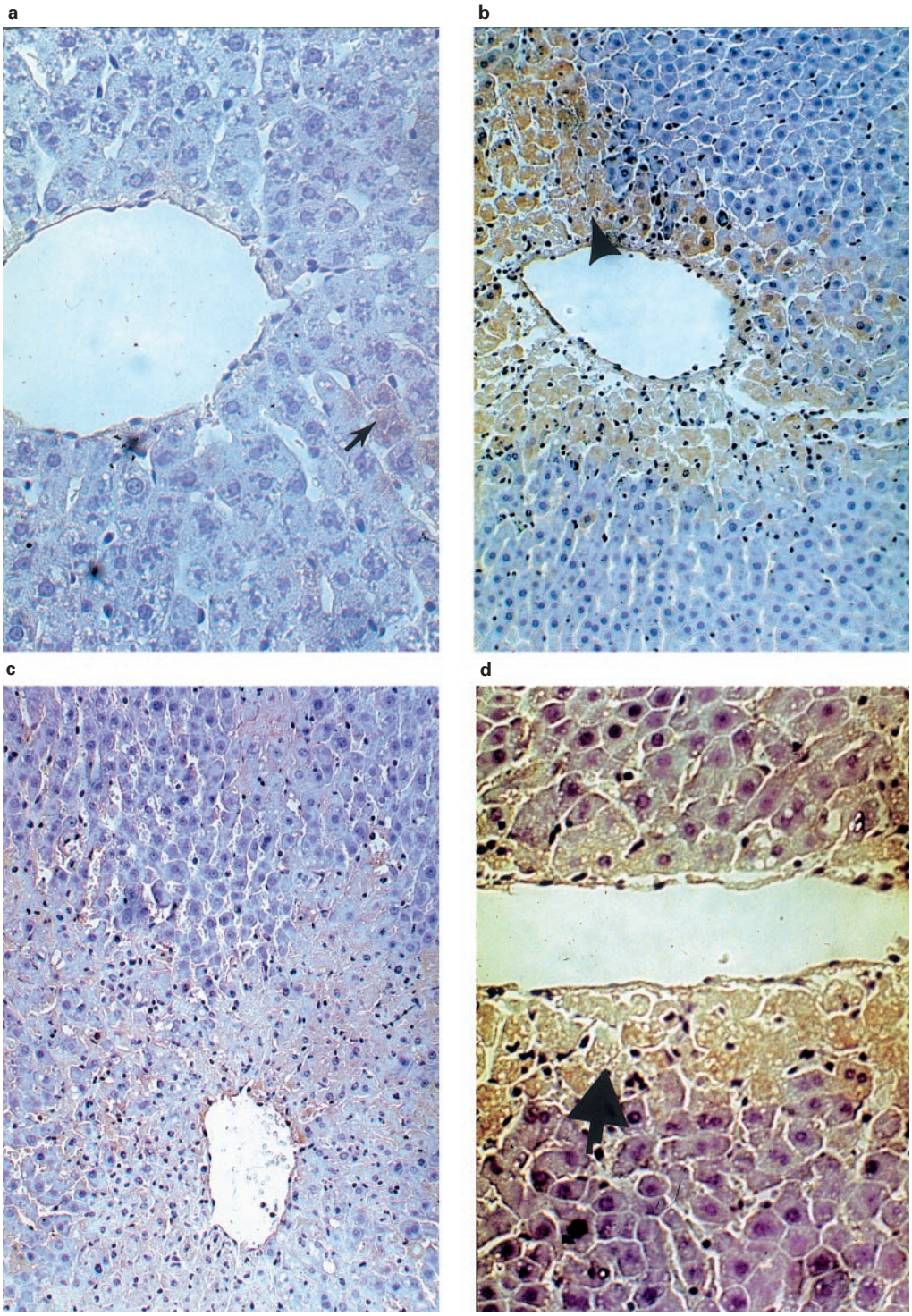

Figure 6 (a) Effect of immunohistochemical stain on the formalin-fixed and paraffin embedded liver tissue, with a 1:50 dilution of the antiserum to IF_1: the liver of the control rats had a very low concentration of IF_1, and was localized primarily in a narrow zone around the pericentral veins (brown colour) (see arrow). (b) In contrast, in the liver of the PTU-treated rat (12 days), many pericentral hepatocytes showed distinctly IF_1 positive cytoplasm, and the brown colour was more diffuse indicating a much higher IF_1 concentration. (c) Rat livers of AAP-treated rats (24 h) showed a much lesser intensity of the immunohistochemical staining and extensive pericentral necrosis was evident. (d) Rats receiving a combination of AAP and PTU had more intense pericentral staining with minimal cell destruction, located close to the central veins. Scale bar = 40×100.

Conclusions

Based on our present and previous studies, we observed that hepatic necrosis occurs when there is a simultaneous deficiency of GSH and ATP. The underlying mechanism of the preservation of ATP by PTU is attributed to its induction of increased concentrations and activity of mitochondrial IF_1, resulting in inhibition of ATPase activity. This is probably one of the most effective of the multiple defence mechanisms against AAP-induced hepatotoxicity.

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