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Artículo original

Phenobarbital pretreatment increases thioacetamide induced necrosis and post-necrotic regeneration in rats

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ABSTRACT

One of the most important events related to drug interactions is the ability of some drugs to induce hepatic microsomal monooxygenases and to increase the toxicity of other drugs. A single intraperitoneal dose of thioacetamide was administered to rats (500 mg/Kg) to induce liver necrosis and regeneration; and on this experimental model the influence of intraperitoneal phenobarbital administration (80 mg/Kg/day) for five days before thioacetamide was studied. The results show that phenobarbital pre-treatment increased liver damage induced by thioacetamide, as detected by increases in serum enzymatic activities, levels of total bilirubin, and by the extent of the necrotic area in the perivenous acinar region. The higher liver injury was parallel to the higher rate of tissue regenerative response as demonstrated by the rate of DNA synthesis in hepatocytes and the level of α-fetoprotein in serum. We can conclude that phenobarbital pre-treatment enhanced hepatotoxicity and hepatocellular regeneration, but did not change either acinar location or timing of liver injury and regeneration induced by thioacetamide. Moreover, the proliferative response as well as the changes in diploid and polyploid populations, were more pronounced in phenobarbital pretreated hepatocytes.

Key Words: Phenobarbital.—Thioacetamide.—Liver injury.—Liver regeneration.

RESUMEN

El pretratamiento con fenobarbital incrementa la necrosis y la regeneración postnecrótica inducida por tioacetamida

Uno de los aspectos más importantes de las interacciones de fármacos es la habilidad de algunos de ellos de inducir las monooxigenasas microsomales hepáticas e incrementar la toxicidad de otras drogas. Se administró a las ratas una dosis única de tioacetamida por vía intraperitoneal (500 mg/Kg de peso) para provocar una necrosis y regeneración hepática; sobre este modelo experimental se estudió la influencia de la administración intraperitoneal de fenobarbital (80 mg/Kg/día) durante los cinco días previos a la administración de la tioacetamida. Los resultados muestran que el pretratamiento con fenobarbital incrementó el daño hepático provocado por la tioacetamida, como se demuestra por los incrementos de las actividades enzimáticas en suero, niveles totales de bilirrubina y por la extensión del área necrótica en la región acinar perivenosa. Este mayor daño hepático fue paralelo al incremento de la respuesta regenerativa del tejido como queda demostrado por el aumento de la síntesis de DNA y por el nivel de α-fetoproteína en suero. Podemos concluir que el pretratamiento con fenobarbital aumenta la hepatotoxicidad y la regeneración hepatocelular, sin embargo, no modifica ni la localización acinar ni el patrón temporal del daño hepático y regeneración inducida por la tioacetamida. Además, tanto la respuesta proliferativa como los cambios en las poblaciones diploides y poliploides, fueron más pronunciados en los hepatocitos pretratados con fenobarbital.

Palabras clave: Fenobarbital.—Tioacetamida.—Daño hepático.—Regeneración hepática.

INTRODUCTION

It is well known that phenobarbital (PB) induces P450-dependent metabolic processes in liver tissue (1, 2). This inductive response has a major impact on drug interactions (3, 4). Thus, PB potentiates the hepatotoxicity of cocaine by increasing the severity of liver damage and shifting the intraacinar injury from the perivenous to the periportal area of the liver acini (3). PB also potentiates the hepatotoxicity of thioacetamide and enhances the activity of flavin-containing monooxygenase (4). Infliction of toxicant-induced injury is accompanied by a parallel tissue repair stimulation response, which allows the organism to overcome the injury up to a threshold dose. Beyond this threshold, a diminished or delayed tissue repair response allows an unrestrained progression of injury. The

stimulation of liver repair through hepatocyte proliferation has been shown to permit the recovery from massive and normally lethal liver injury. The concept of toxicodynamic interaction between inflicted injury and stimulated tissue repair offers opportunity to fine-tune many pharmacological aspects.

Thioacetamide (TA) is a potent hepatotoxic agent which, when administered at doses of 500 mg/Kg to rats, originates a severe hepatocellular perivenous necrosis (5, 6). The initiation of the chain of cellular events leading to TA-induced liver necrosis is due to the generation of reactive metabolites: S-oxide and sulfone, obligatory intermediates in the process of microsomal oxidation of TA (7-9). The selective destruction of perivenous hepatocytes and the immediate proliferative state of the remaining liver cells, have been used as an experimental model by which to study the hepatic response against the aggressive attack of a hepatotoxic drug. Thus, this response presents a double aspect: the hepatocellular necrosis and the post-necrotic regeneration linked to the restoration of liver function (10, 11).

Phenobarbital (PB) induces efficiently the transcription of several isoforms of cytochrome P450 (12), and, consequently, has the ability to enhance the activity of hepatic drug metabolizing enzymes, which can result in the increased metabolism and toxicity of drugs (2, 13).

The aim of the present study was to clarify experimentally whether PB, potentiating the hepatotoxicity and the amplification of the hepatic injury induced by TA, also affects the proliferative regenerating state immediately triggered following the necrosis, as well as to visualize the location and extent of the necrotic area on liver slices. Accordingly, rats were treated or not with PB for five days previous to administration of TA. To follow the time course of events, samples of blood, liver and hepatocytes were obtained at 0, 12, 24, 48, 72 and 96 h of TA intoxication. Morphological study, parameters of liver injury, and those related to TA metabolism were obtained. The proliferative post-necrotic response was assayed by evaluating the mitotic index on glass slide mounted sections of liver, as well as by determining the serum levels of a-fetoprotein and the ploidy and DNA distribution in the cell cycle phases in isolated hepatocytes by flow cytometry.

MATERIAL AND METHODS

Chemical reagents

Enzymes and collagenase were obtained from Boehringer Mannheim. Substrates, coenzymes and propidium iodide were from Sigma Chemical. Standard Analytical grade Laboratory Reagents were obtained from Merck.

Animals and sampling

Male Wistar Rats aged 2 months (200-225 g) were supplied with food and water *ad libitum,* and exposed to a 12 h light-dark cycle. The following groups were established: (A) rats were pretreated intraperitonally with 0.9% NaCl daily for five days before a single sublethal dose of TA (500 mg/Kg body weight) freshly dissolved in 0.9% NaCl. (B) rats were pretreated intraperitoneally with PB freshly dissolved in 0.9% NaCl (80 mg/g body weight) daily for five days before TA administration. (C), rats were pretreated with PB daily for five days, and instead of TA, this group received NaCl 0.9%. The control of each group refers to samples obtained at 0 h (before TA administration in group A and B, and before NaCl administration in group C). Each experiment was performed in duplicate from four animals, and followed the international criteria outlined in the «Guide for the care and use of laboratory animals» published by the National Institutes of Health (NIH publication n.º 80-83, revised 1985).

Processing of the samples

In order to clarify the sequential changes during the different stages of liver injury and regeneration, samples were obtained from control (0h) and at 12, 24, 48, 72, and 96 h following TA intoxication in groups A and B, and following NaCl in group C. Rats were sacrificed by cervical dislocation and samples of liver were obtained and processed as previously described (14). Blood was collected from hearts and kept at 4ºC for 24 h, centrifuged at 3000 rpm for 15 min,

and serum was obtained as the supernatant. Liver samples were obtained for morphological and mitotic index analysis. Another group of rats was used for hepatocyte isolation.

Histological study

Rat liver pieces untreated and pretreated with PB following thioacetamide intoxication were fixed in 10% formaldehyde embedded in paraffin, sectioned (5 μm) and stained with hematoxilin and eosin. The mitotic index was calculated on glass slide mounted liver sections as the ratio of number of mitosis per cell density in the perivenous and midzonal acinar regions according to the method of Simpson *et al.* (15).

Enzyme and metabolite assays

Enzyme determinations were carried out in serum and in the microsomal fraction of the liver homogenates in the optimal conditions of pH and temperature, and with substrates and cofactors at saturation. Several determinations were carried out in serum of rats: Isocitrate dehydrogenase (nm/min/ml serum) was determined spectrophotometrically at 340 nm in the presence of isocitrate and NADPH (16); total bilirubin (μg/ml serum) was determined spectrophotometrically at 578 nm with sulfanilic acid diazoreagent in the presence of caffeine (17). α-fetoprotein (ng/ml serum) was detected by enzymatic immunoassay of particles (MEIA) as described by Wespie (18) modified by Barnes *et al.* (19). Proteins were evaluated by the method of Bradford (20).

Thiobarbituric acid reactive substances (TBARS) were expressed as mmol/g of fresh liver. Liver pieces were homogenized in trichloroacetic acid and the supernatant was treated with thiobarbituric acid. Samples were heated at 90°C for 15 min, centrifuged and the absorbance was measured at 535 nm as described by Niehaus and Samuelsson (21).

In the microsomal fraction of liver, obtained as previously described (4), the activities of flavine-containing moonooxygenase (FMO) and the *O*-dealkylation of pentoxiresorufin, as enzyme marker

of cytochrome P-450 2B, were determined. FMO activity, expressed as nmol/min/mg protein, was assayed spectrophotometrically at 420 nm by measuring the N, N-dimethylaniline oxidation (22). Pentoxyresorufin *O*-dealkylase activity, expressed as pmol of resorufin/min/mg protein, was determined as previously described by Honkakoski and Lang (23), by measuring the formation of resorufin using a Perkin-Elmer spectrophotofluorimeter at the excitation and emission wavelengths of 522 and 586 nm, respectively.

Isolation of hepatocytes and flow cytometry analysis

From rats anaesthetized with 50 mg/Kg sodium pentobarbital dissolved in 0.9% NaCl, hepatocytes were isolated by perfusion *in situ* according to the classic collagenase method (24). Cell viability, determined by tripan blue exclusion, was greater than 90%. For the analysis of DNA content and ploidy, 1×10^6 viable cells were stained with propidium iodide following the multistep procedure of Vindelov *et al*. (25). The emitted fluorescence (FL2A) of the DNA-propidium iodide complex was assayed in a FACS-scan flow cytometer (Becton-Dickinson). A double discriminator module was used to distinguish between signals coming from a single nucleus and those produced by nuclear aggregation. Data were analyzed by evaluating single nucleus inputs (10^4 nuclei/assay).

Statistical analysis

The results were calculated as the means ± SD of four experimental observations obtained from four animals. Differences between groups were obtained by analysis of variance followed by Snedecor F ($\alpha = 0.05$). Student t test was performed for statistical evaluations as follows: all values against their control as (a), the PB-pretreated group against untreated as (b). Statistical significance was considered for $p < 0.05$.

RESULTS

PB increases cell proliferation in the liver (26); and, as a result, the ratio liver weight/body weight increases significantly. In our experimental conditions the increase of this radio was 150% (4).

Histological study

The experimental liver toxicity was confirmed by means of histopathology and serum assays. The histopathological study was carried out to detect the acinar location of necrosis, and to establish the relationship between the histopathological events and the biochemical parameters of liver injury. Figures 1-4 show the liver morphology observed by light microscopy in sections of 5 μm obtained from non PB pretreated (Figure 1 & 3) or pretreated rats (Figures 2 & 4). Figures 1 & 2 (magnification 125), corresponding to rat liver at 24 h following TA, show the necrotic areas surrounding the central vein, and the much more pronounced extent of these areas in liver of PB pre-treated rats (Figure 2). The extent of the necrotic areas was parallel to other parameters of liver injury, such as serum alanine and aspartate aminotransferases, isocitrate dehydrogenase or total bilirubin. PB pretreatment did not affect the intraacinar location of necrosis.

Figures 3 & 4 show the morphology (magnification 625) of rat liver following 48 h of TA administration. Figure 3 corresponds to non PB pretreated liver and Figure 4 corresponds to PB-pretreated liver. In both microphotographs, cells in mitosis are clearly visible.

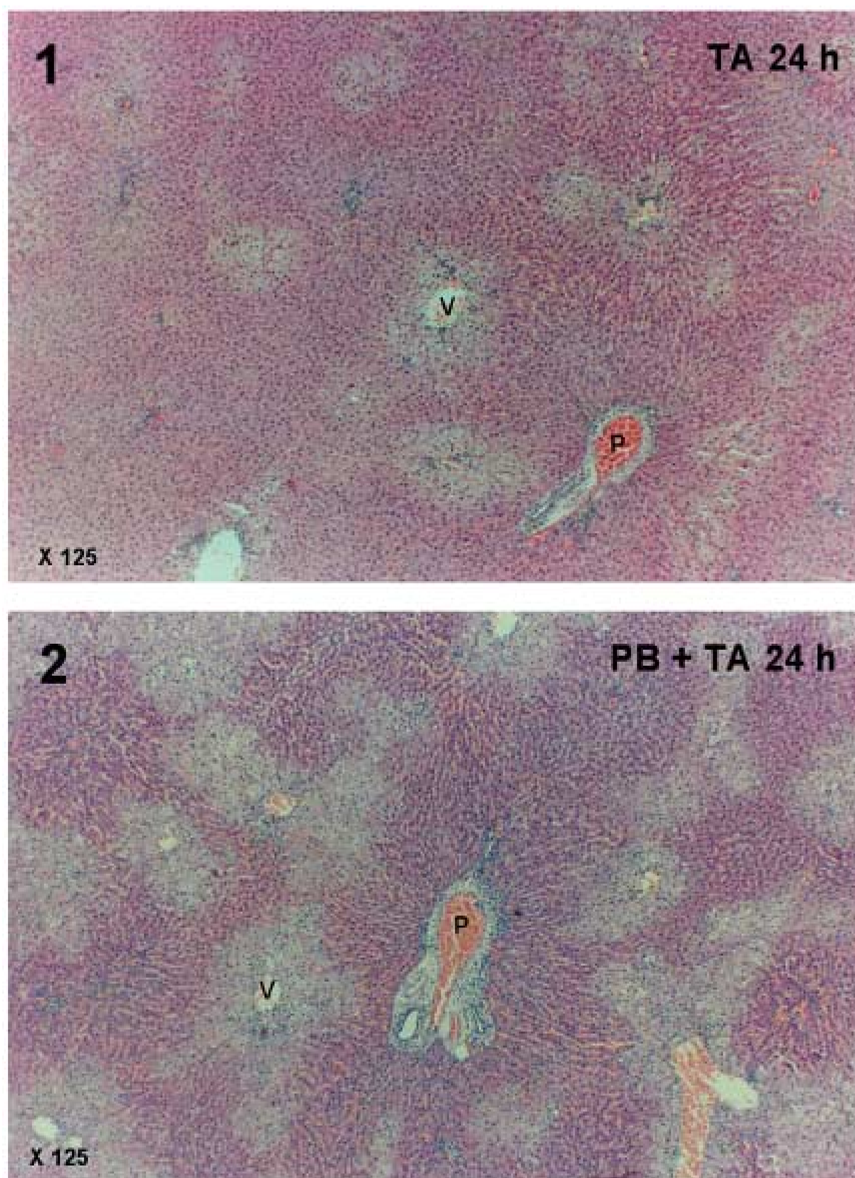

FIGURE 1-2. *Effect of PB-pretreatment on liver morphology following 24 h of TA administration. Liver slices of 5µm were obtained and stained from non PB pretreated (Figure 1) and PB pretreated rats (Figures 2). Figures 1 & 2 correspond to liver slices obtained at 24 h of intoxication and show the areas of necrosis surrounding the venous terminal (x 125).*

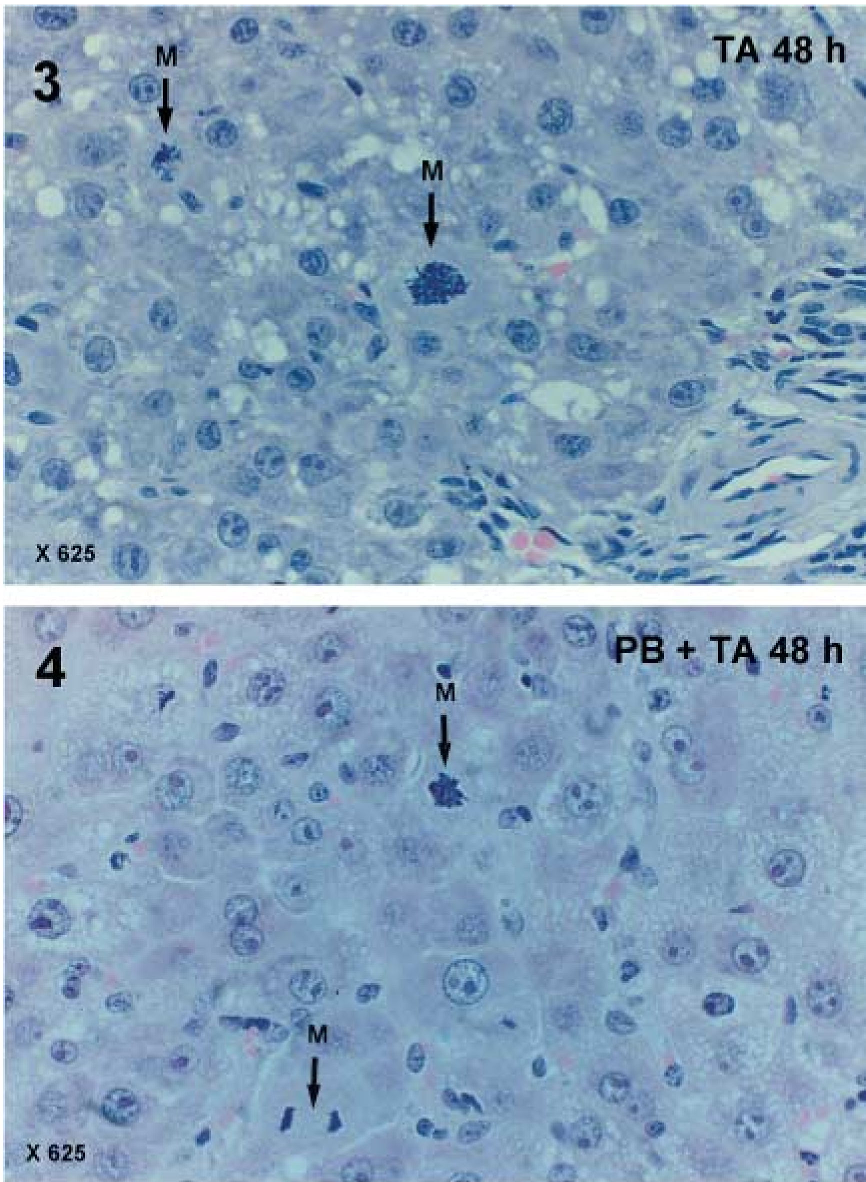

FIGURE 3-4. *Effect of PB-pretreatment on liver morphology following 48 h of TA administration. Liver slices of 5 μm were obtained and stained from non PB pretreated (Figure 3) and PB pretreated rats (Figures 4). Figures 3 & 4 correspond to liver slices obtained at 48 h of intoxication and show cells in mitosis (M) (x 625).*

Mitotic Index (MI)

Mitotic index (MI) is defined as the ratio between cells in mitosis and total cells (15). MI, as a marker of cellular proliferation, was determined on slices of 5 µm obtained from rat liver. Slices were stained with hematoxilin and eosin and cells were observed and mitosis quantified under a light microscope. MI was calculated as the number of mitosis against the total number of cells by analyzing the perivenous area of the hepatic acinus. The highest index of proliferation was located in the vicinity of the necrotic region.

The results obtained by counting the cells in mitosis against total cells are shown in Table 1 and are expressed as MI x 10^3. As hepatocytes from control rats (without treatment) were in the quiescent state, mitosis was very low and MI was undetectable. However, in the case of TA-treated livers, when the peak of cell proliferation was reached at the time point of 48 h of intoxication, the values obtained were 53 ± 3 and 80 ± 6 for non-pretreated and PB-pretreated, respectively. The differences against control were statistically significant. From this time point a progressive decrease was observed in the MI that was still detectable at 96 h of TA administration. PB-pretreatment, without TA, induced a detectable MI, but this effect disappeared progressively when PB was no longer administered.

Table 1. *Effect of PB-pretreatment on mitotic index (MI) in liver slices of rats at 0, 12, 24, 48, 72 and 96 h following TA administration*

Time (h)	PB	TA	PB + TA
		MI x 10^3	
0	2 ± 0.3	Undetectable	2 ± 0.2
12	1 ± 0.1	Undetectable	1 ± 0.1
24	1 ± 0.1	Undetectable	2 ± 0.2
48	Undetectable	53 ± 3 [a]	80 ± 6 [ab]
72	Undetectable	46 ± 4 [a]	60 ± 6 [ab]
96	Undetectable	11 ± 1 [a]	17 ± 2 [ab]

Mitotic index (MI) was calculated as the ratio of number of mitosis per cell density, according to the method of Simpson et al. (1992). Data expressed as means ± SD are mean of 15 values, either the number of cells or the number of mitosis. Statistics were (a) values against control; (b) PB + TA against TA.

Serum parameters of thioacetamide hepatotoxicity

One of the symptoms of liver necrosis is the appearance in serum of hepatic enzymes. Isocitrate dehydrogenase (ICDH) is an NADPH generating enzyme mainly located in the perivenous area of the hepatocytes. ICDH activity is used as a parameter of hepatocellular damage to measure the severity of centrilobular necrosis *in vivo*. Serum ICDH is the best marker for perivenous necrosis since aspartate and alanine aminotransferases are mainly located in the periportal space (27). The effect of PB pre-treatment in ICDH activity on rat serum obtained at 0, 12, 24, 48, 72 & 96 h after TA administration is shown in Figure 5. The peak of enzyme activity in both groups, PB untreated or PB pretreated, was observed at 24 h, but in the case of PB-pretreated samples a significant increase was detected, which indicates that the extent of necrosis induced by thioacetamide was markedly increased by the effect of PB-pretreatment. This enhanced severity of TA-induced injury by the effect of PB was also described in previous results from our group (4) and from others (28).

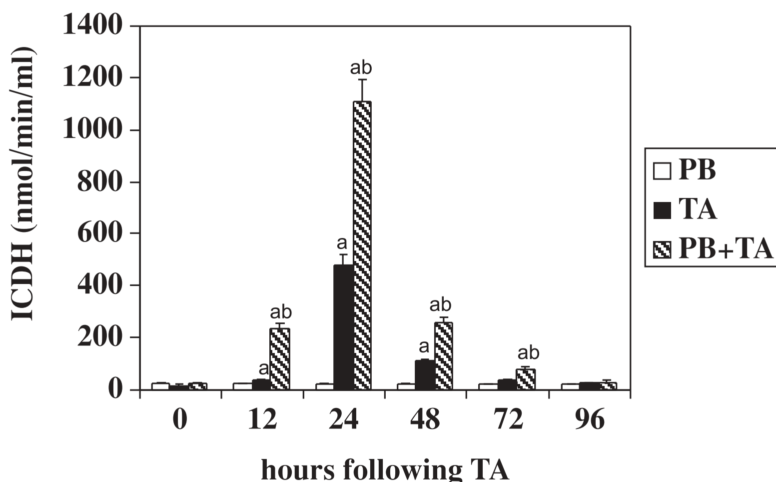

FIGURE 5. *Effect of PB pre-treatment on ICDH activity in rat serum. Samples of blood were obtained from rats untreated or pretreated with PB at 0, 12, 24, 48, 72 and 96 h following TA administration. Results are expressed as nmol per min per ml of serum and are the mean ± SD of four experimental determinations from four rats. Statistics were (a) values against control; (b) PB + TA against TA.*

The levels of total bilirubin (µg/ml) in serum obtained from PB untreated and PB-pretreated rats were determined at 0, 12, 24, 48, 72 and 96 h following TA administration. The release of bilirubin to serum is a marker of liver disease. In our experiments the levels of bilirubin increased markedly with a profile parallel to that obtained for ICDH activity, although the peak of serum bilirubin when compared to that of ICDH was delayed, and appeared at 48 h of intoxication (Figure 6).

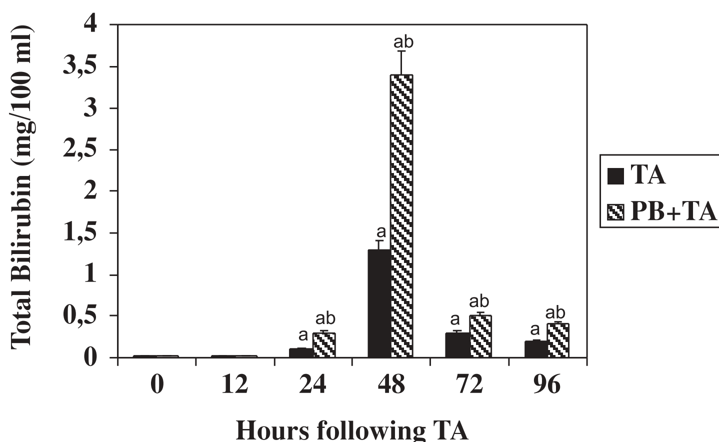

FIGURE 6. *Effect of PB pre-treatment on the levels of total bilirubin in rat serum. Samples of blood were obtained from rats untreated or pretreated with PB at 0, 12, 24, 48, 72 and 96 h following TA administration. Results are expressed as mg/100 ml of serum and are the mean ± SD of four experimental determinations from four rats. Statistics were (a) values against control; (b) PB + TA against TA.*

The concentration of α-fetoprotein (AFP), expressed as ng/ml was assayed on serum of rats that underwent or not a PB pre-treatment at 0, 12, 24, 48, 72 and 96 h following TA (Figure 7). AFP is a glycoprotein whose gene expression occurs in the yolk sac, in the fetal liver and gut and in the adult liver during regeneration and tumorigenesis (29). Our results show that a significant increase in AFP was detected in rat serum at 12 h of TA intoxication, which remained high until 72 h. In serum of PB-pretreated rats the increase in AFP following TA was higher and remained high until 96 h of intoxication.

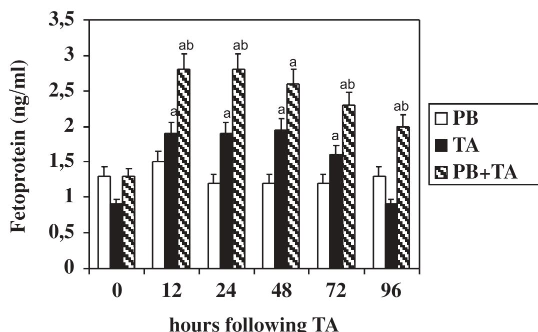

FIGURE 7. *Effect of PB pre-treatment on the levels of α-fetoprotein in serum of rats. Samples of blood were obtained from rats untreated or pretreated with PB at 0, 12, 24, 48, 72 and 96 h following TA administration. Results are expressed as ng/ml of serum and are the mean ± SD of four experimental determinations from four rats. Statistics were (a) values against control; (b) PB + TA against TA.*

An enhancement in the concentration of TBARS is a marker of oxidative stress and lipoperoxidation, which is a consequence of drug metabolism. Figure 8 shows the levels of TBARS determined in liver homogenates of rats at 0, 12, 24, 48, 72 and 96 h of TA administration either in PB untreated or pretreated rats. TBARS increased significantly at 24 of intoxication. In PB-pretreated liver, the levels of TBARS levels were higher and differences against untreated were significant.

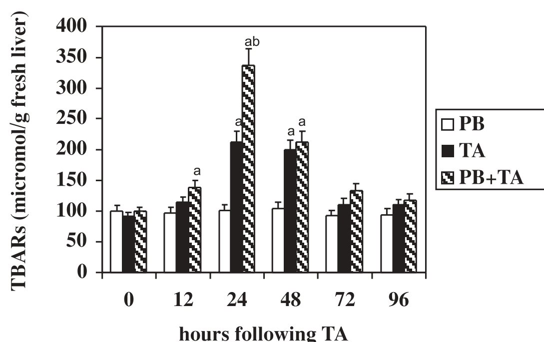

FIGURE 8. *Effect of PB pre-treatment on the levels of TBARS concentration in rat liver homogenates. Samples of liver were obtained from rats untreated or PB-pretreated animals at 0, 12, 24, 48, 72 and 96 h following TA administration. Results are expressed as μmol/g of liver and are the means ± SD of four experimental observations from four rats. Statistics were (a) values against control; (b) PB + TA against TA.*

Parameters related to thioacetamide oxidative metabolism

As TA is not toxic *per se,* its intermediary metabolism is obligatory for its hepatotoxicity. Microsomal monooxygenases are responsible for its oxidation in sulfoxide and sulfone derivatives. In a previous study of our group, it was demonstrated that the basal activity of FMO underwent age-related changes (11, 30). After TA administration FMO activity was induced as early as at 12 h of intoxication preceding the peak of necrosis, and the peak of lipoperoxidation. Figure 9 shows the activity of this enzyme system (nmol/min/mg protein), which underwent biphasic changes showing a marked decrease at 24 h, just at the time point of necrosis. Necrosis destroyed the perivenous area, the area where FMO is mainly located. PB-pretreatment markedly increased the activity of this enzyme system and the increase was detected even in samples of control livers.

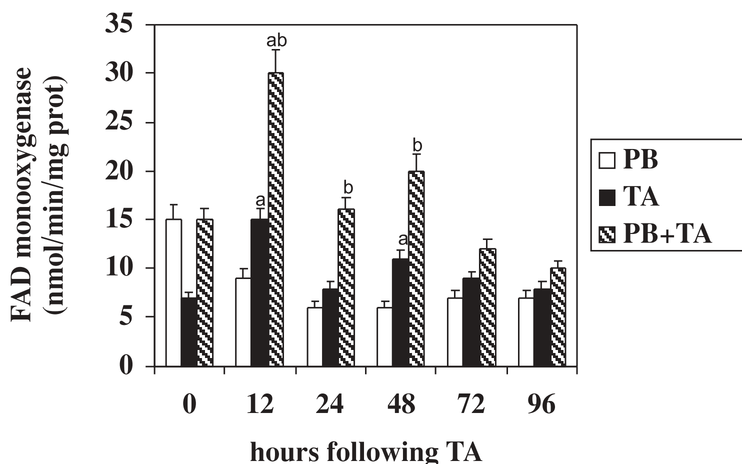

Figure 9. *Effect of PB pre-treatment on the activity of FMO in rat liver homogenates. Samples of liver were obtained from rats untreated or PB-pretreated animals at 0, 12, 24, 48, 72 and 96 h following TA administration. Results are expressed as nmol/min/mg of protein and are the means ± SD of four experimental observations from four rats. Statistics were (a) values against control; (b) PB + TA against TA.*

Figure 10 shows the effect of PB pre-treatment on *O*-dealkylation of pentoxiresorufin expressed as pmol/min/mg protein, determined,

as a marker of CYP 2B activity, in the microsomal fraction of homogenates from rat liver obtained at 0, 12, 24, 48, 72 and 96 h following TA administration. In the experimental conditions of the present study, PB pretretment increased by more than six fold the activity of this enzyme. However, this enhanced activity decreased at 12 and 24 h of PB withdrawal when TA was not administered, in such way that at 48 h the levels reached the basal values (data not shown). TA by itself did not exert any effect on this enzyme activity.

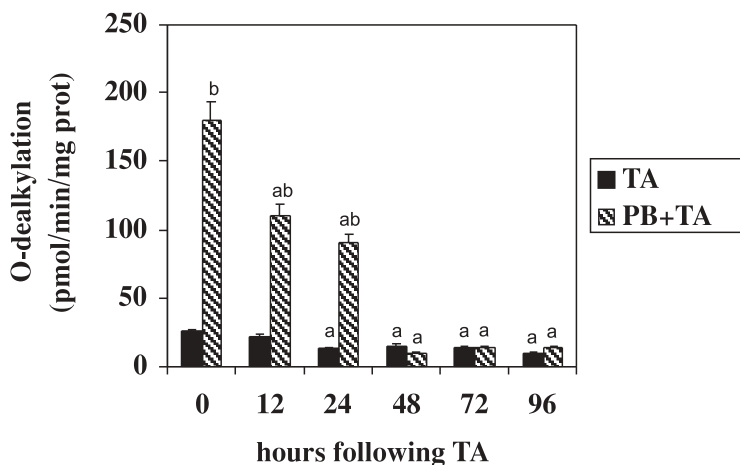

FIGURE 10. *Effect of PB pretreatment on the O-dealkylation of pentoxiresorufin in rat liver homogenates. Samples of liver were obtained from rats untreated or PB-pretreated animals at 0, 12, 24, 48, 72 and 96 h following TA administration. Results are expressed as pmol/min/mg protein and are the means ± SD of four experimental observations from four rats. Statistics were (a) values against control; (b) PB + TA against TA.*

Flow Cytometric Analysis

The sequence of post-necrotic proliferation of hepatocytes was also detected in isolated liver cells at different time points (0, 3, 12, 24, 48, 72 and 96 h) following thioacetamide, by measuring the ploidy and DNA distribution in the cell cycle phases. In DNA histograms (Figure 11) the values are expressed as the relative number of cells against the fluorescence (FL2A) emitted by the propidium iodide-DNA complex, obtained by flow cytometry. In Figure 12 is shown the

quantification of the DNA histograms relative to the hepatocyte populations: diploid (2C), poliploid (4C + 8C) and in phase of DNA synthesis, S1 (G1 → G2) + S2 (G2 → G4). Remarkable changes were obtained at 48 h, such as a 12 (TA) and 17 (PB + TA) fold increase in DNA synthesis and parallel but opposite changes in diploid and tetraploid populations. 4C diminished parallel to the increase in 2C. The peak of hepatocellular regeneration was at 48 h, when the highest synthesis of DNA was detected, and at this moment the ploidy profile appeared equal to that of fetal hepatocytes.

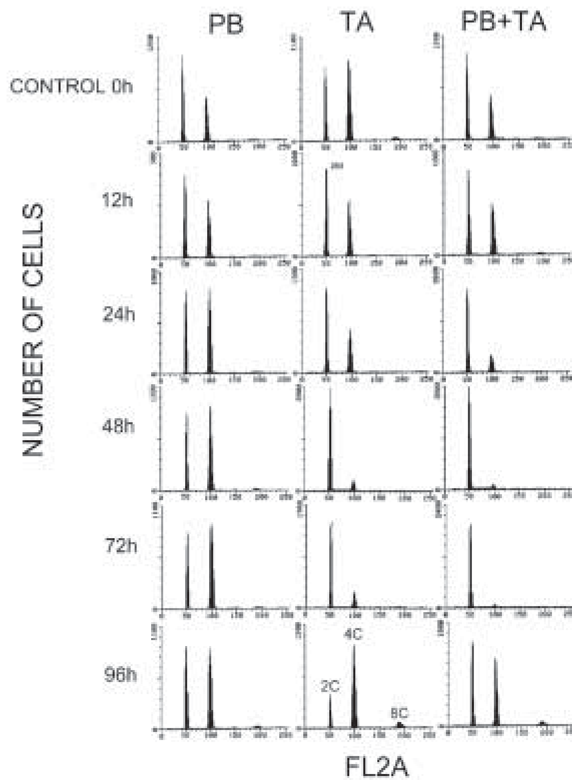

FIGURE 11. *Histograms of DNA content and ploidy by flow cytometry. The relative number of stained cells (vertical axis) was plotted against DNA ploidy. 2C for diploid, 4C for tetraploid and 8C for octoploid populations (horixontal axis). To follow the time course of alterations in genomic DNA distribution, hepatocytes were obtained from PB untreated or pretreated at 0, 3, 12, 24, 48, 72 and 96 h following TA. Isolated and trypsinized hepatocytes were processed as indicated in the kit kinesis for distribution of DNA in the cell populations in the cell cycle. Quantitave analysis is shown in Figure 12.*

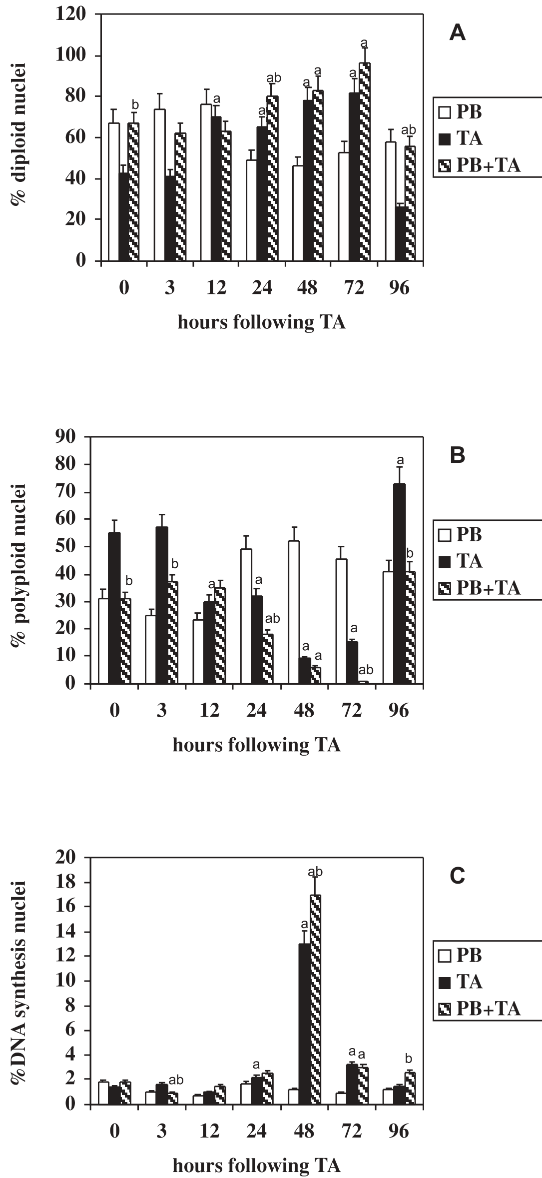

FIGURE 12. *Quantitave analysis of DNA histograms in Figura 11. (A) diploid (2C) population; (B) cells synthesizing DNA and (C) tetraploid and octoploid populations (4C + 8C). Results are expressed as percentage of total are the means ± SD of four experimental observations from four rats. Statistics were (a) values against control; (b) PB + TA against TA.*

DISCUSSION

In response to the action of hepatotoxic drugs, the liver develops hepatocellular necrosis, which is immediately followed by hepatocellular regeneration and restoration of the liver function (31-33). In the present investigation the effect of a previous treatment with PB was assayed on liver necrosis and hepatocellular regeneration induced by TA. The location of the liver injury induced by TA in the perivenous acinar region is caused by the higher abundance of smooth endoplasmic reticulum in this area and the greater ability to oxidize xenobiotics and to produce reactive intermediates. The extent of the necrotic area was larger in the case of PB-pretreatment, but the intraacinar location of injury apparently did not change. It is well known that PB induces the proliferation of the smooth endoplasmic reticulum, which is mainly located in the perivenous area (34). PB has the ability to induce the expression of several isozymes of the cytochrome P450-dependent microsomal system, such as P450 2B. One aim of this study was to evaluate the interaction between PB and TA, against the hepatocellular damage and post-necrotic hepatocyte proliferation. TA is a well known necrogenic agent, which is apparently metabolized by the FMO (35, 36). The necrogenicity of TA is a consequence of its biotransformation in free radical derivatives, by the microsomal monooxygenases mentioned previously. As PB induces the proliferation of the smooth endoplasmic reticulum, it may also induce all the microsomal systems that are attached to their membranes such as FMO and CYP. PB also induces the expression of drug metabolizing enzymes, mainly those that are cytochrome P 450 2B dependent. This induction affects drug metabolism and clearance, drug toxicity and carcinogenicity (26).

The results obtained by our group in the present and previous studies demonstrate that PB pretreatment increases FMO activity. The higher degree of necrosis induced by PB-pretreatment may be due to the enhancement of enzyme activities of both FMO and CYP2B. The contribution of each of these two systems to TA metabolism, needs to be clarified in future studies at the level of transcription and translation by using specific inhibitors.

Analyzed in the present study was the influence of PB on TA-induced liver injury and regeneration both at morphological and at flow cytometric levels. On liver slices of 5 μm stained with hematoxilin & eosin, the following observations were made: first, that PB-pretratment does not modify the intraacinar location of the necrotic area induced by TA, which indicates that the microsomal monooxygenase system responsible for TA biotransformation are selectively located in the perivenous region. Other hepatotoxic agents, such as cocaine, when administered to PB-pretreated mice, the location of liver injury shift rom the perivenous to the periportal area (3). Second, that PB-pretreatment increases the severity of the damage, which can be clearly observed in Figures 1 & 2, obtained by light microspopy. As TA is not toxic *per se,* the increase in the extent of toxicity demonstrates that the microsomal systems, responsible for its oxidative metabolism, are induced by PB-pretreatment (4).

These results are in agreement with those obtained in serum as parameters of necrosis. The assay of serum isocitrate dehydrogenase showed that the time point of maximum necrosis appeared at 24 h following thioacetamide in both experimental groups: TA and PB + TA. However, the extent of damage was significantly more pronounced in the PB-pretreated group. Other parameters of liver injury, such as bilirubin and TBARS, showed parallel results.

Liver regeneration is an essential process that permits the repair of the hepatic tissue and the restoration of liver function following liver injury or surgical resection. In our experiments, the extent of liver regeneration induced by TA was parallel to the severity of liver injury. By flow cytometry was determined the distribution of hepatocyte populations with respect to DNA ploidy and DNA synthesis. Population in S phase peaked at 48 h of intoxication, both in PB untreated and pretreated groups. These results agree with those obtained by counting the mitosis as mitotic index. Our results, obtained by flow cytometry, permitted us to detect, not only the population that undergoes DNA replication, but also the various stages of cell proliferation: G1 (2C) and G2 (4C + 8C). In this way it can be observed that when regeneration processes starts, diploid population increases, while tetraploid and octoploid decreases (10), and the profile of liver cell ploidy appears similar to that found in fetal hepatocytes. PB-pretreatmet not only increased DNA synthesis

but also affected the other stages of the cell cycle. AFP levels, another parameter related to the proliferative state of the cell, underwent significant changes due to PB-pretreatment.

On the basis of the present results, it is concluded that PB administration to rats previous to TA intoxication, potentiates the hepatotoxicity and the post-necrotic response induced by TA, but has no influence on the location of the intraacinar injury. The recovery of organisms suffering from severe liver damage depends heavily on the ability of the remaining hepatocytes to proliferate and replace the liver function. Death of the organism usually occurs when the regenerating ability of the liver is compromised owing to heavy damage to the liver. The current approach to therapy aims only to block additional liver injury from hepatotoxicants or hepatic disease. If hepatocellular regeneration and tissue repair could be stimulated after hepatic damage by a therapeutically compatible mechanism, the prevention of death arising from serious liver damage is a distinct possibility.

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