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INVESTIGATION OF POSSIBLE MECHANISMS OF HEPATIC SWELLING AND NECROSIS CAUSED BY ACETAMINOPHEN IN MICE

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Abstract—Vascular congestion and liver swelling have long been recognized as features of the hepatotoxic effects of acetaminophen (AAP) in mice and rats and have been proposed as contributing factors to the eventual extent of necrosis produced. Neutrophil accumulation in the hepatic microcirculation has been proposed as being responsible for the blockage of hepatic blood flow and thereby the expansion of the region of damage. We therefore determined in mice the effects of hepatotoxic doses of AAP on the messenger RNA for intercellular adhesion molecule-1 (ICAM-1), which is a critical determinant of neutrophil adhesion, activation and ultimately of neutrophil-mediated tissue injury. Hepatotoxic doses of AAP did not upregulate ICAM-1 messenger RNA. However, doses of bacterial lipopolysaccharide (LPS) did cause a rapid and dramatic increase in ICAM-1 message, which was accompanied by a much greater hepatic accumulation of neutrophils, but which led to only scattered single cell necrosis. In addition, we investigated the effects of pentoxifylline (PTX) on AAP-induced vascular congestion and on hepatic necrosis as evaluated histologically and by measurement of plasma transaminase activities. Although PTX has been shown to increase blood cell deformability and improve vascular perfusion in a number of animal models of restricted blood flow, and is used in humans for the treatment of intermittent claudication, we found no decrease in AAP-induced hepatic swelling or in AAP-induced necrosis in response to PTX. With some dosing regimens, PTX-treated animals proved to be slightly more susceptible to AAP, which may be related to the reported potentiation of the cytotoxicities of a number of alkylating anti-cancer drugs by PTX and other methylxanthines. We conclude from these studies that upregulation of ICAM-1 and subsequent adhesion and vascular plugging by neutrophils are not significant determinants of AAP-induced liver swelling and necrosis and that whatever hemorheological advantages PTX might offer in AAP-induced hepatic damage appear to be overshadowed by effects that potentiate the toxic responses.

Acetaminophen (AAP)§ is a commonly used analgesic that is usually safe in therapeutic doses, but when taken in very large amounts can cause massive hepatic necrosis and host death in humans and in animals. The mechanisms involved in the initiation of hepatic damage have been studied extensively in experimental animals and many reviews of the subject are available [1–3]. The injury is generally thought to be initiated by a chemically reactive metabolite that in most respects resembles *N*-acetyl-*p*-benzoquinoneimine (NAPQI) [4–6], although one interpretation of the evidence presently available is that NAPQI is not a significant free intermediate in the metabolism of AAP [7]. The NAPQI-like alkylating metabolite is produced at all doses of AAP, but this metabolite is conjugated efficiently with glutathione and is excreted eventually as the cysteinyl conjugate or the corresponding mercapturic acid unless the cellular capacity to maintain adequate glutathione availability is exceeded, in which case cellular proteins become alkylated by the reactive metabolite(s) [8,9]. Despite or perhaps because of extensive investigation, the mechanisms responsible for the initiation of damage remain controversial and contributions from thiol oxidation and lipid peroxidation receive considerable support [2, 9–11]. It should be noted, however, that hepatic necrosis has never been reported to occur in the absence of alkylation of target tissue protein by metabolites.

Nevertheless, other mechanisms may contribute to the progression of injury, if not to initiation. One interesting hypothesis published recently proposed that accumulation of neutrophils and consequent plugging of the hepatic microvasculature cause the region of necrosis to expand through an ischemic infarct of the periacinar zone [12]. AAP-induced hepatic necrosis initially occurs in the parenchymal hepatocytes immediately surrounding the central vein and then progresses outwardly from this initial lesion [13, 14], which would be consistent with the

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§ Abbreviations: AAP, acetaminophen; ICAM-1, intercellular adhesion molecule-1; LPS, lipopolysaccharide; PTX, pentoxifylline; NAPQI, *N*-acetyl-*p*-benzoquinoneimine; ALT, alanine aminotransferase; SDS, sodium dodecyl sulfate; and SSC, saline sodium citrate.

infarct-progression hypothesis. Significant contributions from similar physiological mechanisms of cell death could help explain some of the reported examples of hepatoprotection that do not measurably alter covalent binding [9, 10, 15].

We therefore investigated the effects of hepatotoxic doses of AAP in the steady-state levels of messenger RNA for intracellular adhesion molecule-1 (ICAM-1). Neutrophil adherence to endothelial cells is a key initial component of the process leading to neutrophil accumulation, activation and neutrophil-mediated organ injury [16, 17]. The initial adhesive interaction between neutrophils and endothelial cells usually involves members of the selectin gene superfamily, followed by the interaction between the neutrophil β_2 integrins CD11a/CD18 and/or CD11b/CD18 with ICAM-1, which is a member of the immunoglobulin gene superfamily [18]. The interaction with ICAM-1 is important in reinforcing the initial interaction and is essential for the development of more than a transitory adhesion [19]. ICAM-1 gene expression is controlled primarily at the level of transcription and upregulation of this gene has been shown to lead to increases in ICAM-1 protein and to increased neutrophil adhesion [18, 20].

Apart from the question of the contributions of neutrophil adhesion to vascular plugging and extension of AAP-induced necrosis, the profound disturbances in hepatic blood flow during the process of AAP-induced injury are unquestionable [14, 21]. We therefore also investigated whether pentoxifylline (PTX) might ameliorate the putative hypoxic-ischemic component of AAP-induced hepatic necrosis through improvement in microvascular circulation. PTX is used clinically for treatment of patients with intermittent claudication, a condition that is characterized by acute extremity pain during minimal exertion and is thought to be caused by impaired blood flow and oxygen delivery. A number of effects of PTX on determinants of blood flow have been identified, including improved neutrophil and erythrocyte deformability [22, 23], increased production of prostacyclin and increased endothelium-dependent relaxation [24, 25], inhibition of neutrophil adhesion to endothelium [26, 27], and inhibition of superoxide release by neutrophils [28, 29]. The potential benefits of these effects on tissue damage secondary to restricted blood flow are readily apparent and it is notable that prostacyclin administration has been shown to protect against AAP-induced hepatic damage in mice [15].

MATERIALS AND METHODS

Materials. Male ICR mice were purchased from Harlan Industries (Houston, TX). PTX lipopolysaccharide (LPS) (*Salmonella equi*), AAP and kits for measuring plasma alanine aminotransferase (ALT) activities were purchased from the Sigma Chemical Co. (St. Louis, MO).

Animals and drug treatments. Male ICR mice weighing between 20 and 30 g were studied. The mice were fasted for 18 hr, then pretreated with an i.p. injection of either PTX (50 or 100 mg/kg) or an equal volume of saline. One hour later the mice were dosed with either 250 or 400 mg/kg of AAP dissolved in saline, 2 mg/kg of LPS dissolved in saline, or saline, all given i.p. (0.5 cc volume/20 g mouse). The mice were killed at various times up to 24 hr after the second drug under pentobarbital anesthesia (100 mg/kg, i.p.). At the time of sacrifice, blood was obtained by heart puncture, and the entire liver was removed and weighed. After weighing, portions of the liver were excised for subsequent RNA isolation, and for histopathological analysis. The liver tissue for RNA isolation was placed in liquid nitrogen immediately, and stored at −80°. The tissue for light microscopy was immersed in 10% buffered formalin.

RNA preparation. Total liver RNA was isolated by a modification of the method of Chirgwin *et al.* [30]. In brief, approximately 0.25 g of frozen liver sample was homogenized in 3 mL of 4 M guanidine isothiocyanate and 0.15 mL of 2-mercaptoethanol. After homogenization, 0.15 mL of 10% sodium lauryl sarcosine was added to the homogenates, and the resulting mixture was layered on a 5.7 M cesium chloride cushion. The cesium chloride plus sample was then spun at 50,000 *g* for 24 hr. This spin deposited the RNA at the bottom of the tube. The RNA was resuspended in 250 μL of 10 mM Tris (pH 7.6), 1 mM EDTA, and 0.1% sodium dodecyl sulfate (SDS), extracted with 125 μL of chloroform:isoamyl alcohol:butanol (96:4:25, by vol.), and precipitated from the aqueous phase by adjusting the salt concentration to 100 mM with sodium chloride, and adding 2.5 vol. of 100% ethanol, followed by freezing the samples on dry ice. Following the freezing, the samples were centrifuged, which deposited the purified RNA on the bottom of the tube. The supernatant was removed and the RNA was washed with 70% ethanol. After washing, the RNA was dried and suspended in 10 mM Tris (pH 7.6), 1 mM EDTA, and the RNA concentration was determined by measuring the absorbance at 260 nm. Each RNA sample was then separated by electrophoresis by loading 20 μg of RNA from each sample on a 1% agarose/formaldehyde denaturing gel. The separated RNA was then transferred from the denaturing gel to a nitrocellulose filter, and allowed to air dry on the filter at 68° for 3 hr.

RNA hybridization. The partial cDNA for ICAM-1 that had been cloned [31] into pBluescript SK- (Stratagene, La Jolla, CA) was used to generate radioactive anti-sense transcripts to ICAM-1 messenger RNA using T3 RNA polymerase (Promega Corp., Madison, WI) and nucleotide triphosphates with ^{32}P-labeled uridine triphosphate. Anti-sense transcripts were verified to be full length by analysis on a 1% TAE agarose gel by electrophoresing the transcripts and then exposing the gel to X-ray film, which generated an autoradiogram. The autoradiogram demonstrated a single band of approximately 1.9 kb when the transcripts were full length. Additionally a cDNA to murine histone-4 previously cloned into the vector PGEM 3 (Promega Corp.) was used to generate radioactive anti-sense transcripts to histone-4 messenger RNA using SP6 RNA polymerase (Promega Corp.) and nucleotide triphosphates with ^{32}P-labeled uridine triphosphate. The nitrocellulose filters containing separated RNA

were prehybridized for approximately 3 hr at 55° in 50% formamide, 50 mM Na_2PO_4, 0.8 M NaCl, 1.0 mM EDTA, 0.5 × Denhardt's reagent [32], and 250 μg/mL of heat-denatured herring sperm DNA. After prehybridization, the solutions were discarded and the filters hybridized for approximately 16 hr at 55° with approximately 20 × 10^6 counts of anti-sense transcripts to ICAM-1 and histone-4 in solutions otherwise identical to the prehybridization solutions. We included the anti-sense transcripts to murine histone-4 as an internal control to standardize the analysis. After hybridization, the filters were washed three times for 20 min with 0.1 × saline sodium citrate (SSC) (1 × SSC: 150 mM NaCl, 15 mM sodium citrate, pH 7.4) [32] and 0.1% SDS at 68° to decrease nonspecific binding of radioactive transcripts to the filters. Following these washes, the hybridized filters were exposed to X-ray film to generate autoradiograms. If the background radioactivity on an autoradiogram was too intense, the hybridized nitrocellulose filter that generated the autoradiogram was washed three more times for 5 min with 2 × SSC at room temperature, then one time for 10 min in 2 × SSC with 10 μg/mL of RNAse B (Worthington Biochemical Corp., Freehold, NJ) at room temperature followed by a wash with 0.1% SDS and 0.1 × SSC for 10 min at 55°. The filter was then re-exposed to X-ray film to produce an autoradiogram.

Measurements. All plasma samples were obtained by heart puncture and were stored at 4°; ALT activities were determined with a kit purchased from Sigma. Liver weights were divided by mouse weights and liver weight per 100 g mouse was calculated as an estimate of liver swelling. Livers were also analyzed by routine light microscopy after staining paraffin-embedded samples with hematoxylin and eosin.

Statistics. Results were expressed as means ± SEM, and measurements of ALT activities were log transformed to obtain a normal distribution within groups and equal variances between groups. Liver weights and ALT activities were analyzed with a one way ANOVA and a subsequent Neuman–Keuls if the ANOVA found a significant difference between groups when comparing more than two groups, and an unpaired Student's *t*-test when comparing two groups. $P < 0.05$ was used to determine statistical significance.

RESULTS

Mice treated with 400 mg/kg of AAP developed acute hepatic necrosis which was readily observable histologically (Fig. 1). The initial lesion appeared in the centrilobular area (Fig. 1A), and more extensive damage appeared to expand from the central vein in a manner not inconsistent with some degree of contribution of regional ischemia (Fig. 1, B and C). This was in contrast to the little or no injury observed in the livers of mice treated with 2 mg/kg of LPS despite the increase in neutrophil accumulation 6 hr after LPS treatment (Fig. 1D).

Northern analysis of hepatic RNA showed little expression of ICAM-1 messenger RNA in control animals and no increase in this messenger RNA at any time point after administration of 400 mg/kg of AAP (Fig. 2), regardless of pretreatment (PTX vs saline). In contrast, 2 mg/kg of LPS in mice initiated a rapid and profound increase in ICAM-1 messenger RNA (the increase was unaffected by PTX pretreatment), thus demonstrating the inherent capacity of mouse liver to upregulate ICAM-1 expression. Treatment with LPS also caused a marked increase in neutrophil infiltration, but caused no significant increase in plasma transaminase activities (data not shown), and only occasional single cell necrosis observable histologically (Fig. 1D). We included in our Northern analyses the use of a probe for histone-4 mRNA as an internal control, but as can be seen from Fig. 2, histone-4 messenger RNA decreased markedly following hepatotoxic doses of AAP. It is interesting to note that the animal given AAP that did not show a loss of histone messenger RNA at 6 hr also did not show an elevation in plasma ALT activities. Additional studies on the observed decrease in histone message caused by AAP have been conducted [33] and these results will be discussed separately.*

Figure 3 shows plasma ALT activities in mice 6 hr after treatment with either 400 mg/kg of AAP or saline, following pretreatment with either 50 mg/kg of PTX or saline. There was a significant increase in ALT activities in AAP-treated mice regardless of pretreatment, and there was no evidence of protection with pentoxifylline pretreatment.

Figure 4 shows mouse liver weights 6 hr after treatment with AAP or saline, in mice pretreated with either 50 mg/kg of PTX or saline. In this figure we see that liver weights were increased significantly in mice treated with AAP relative to mice not given AAP. At the same time there was a suggestion (not statistically significant) of an amelioration of the AAP-induced hepatic swelling in the mice pretreated with PTX compared with mice given AAP after saline (5.24 vs 5.90 g/100 g animal). This trend toward decreased liver swelling afforded by PTX pretreatment led us to further examine this possibility.

Figure 5 shows data from mice treated with either 250 or 400 mg/kg of AAP following pretreatment with either 100 mg/kg of PTX or saline (data from mice not treated with AAP not shown, but are similar to the data seen in Figs. 3 and 4). Plasma ALT activities were elevated by 6 hr after either dose of AAP (Fig. 5A). Pretreatment with PTX showed no effect on the animals given 250 mg/kg of AAP, but appeared to elevate the transaminase activities in the animals given 400 mg/kg, although this difference was not statistically significant ($P = 0.09$). Hepatic swelling was observed in all groups and was unaffected by PTX pretreatment (Fig. 5B; note control animals in Fig. 4). Additional mice were similarly treated, refed 6 hr after AAP, and plasma ALT activities were measured at 24 hr (Fig. 5C). There were no differences in the ALT activities 24 hr after AAP doses in either the lower or higher dose of AAP; however, 1 of 5 animals died in the PTX-pretreated group given 250 mg/kg of AAP and 3 of 5 animals died in the PTX-pretreated group given 400 mg/kg of AAP. No deaths were observed

* Manuscript in preparation.

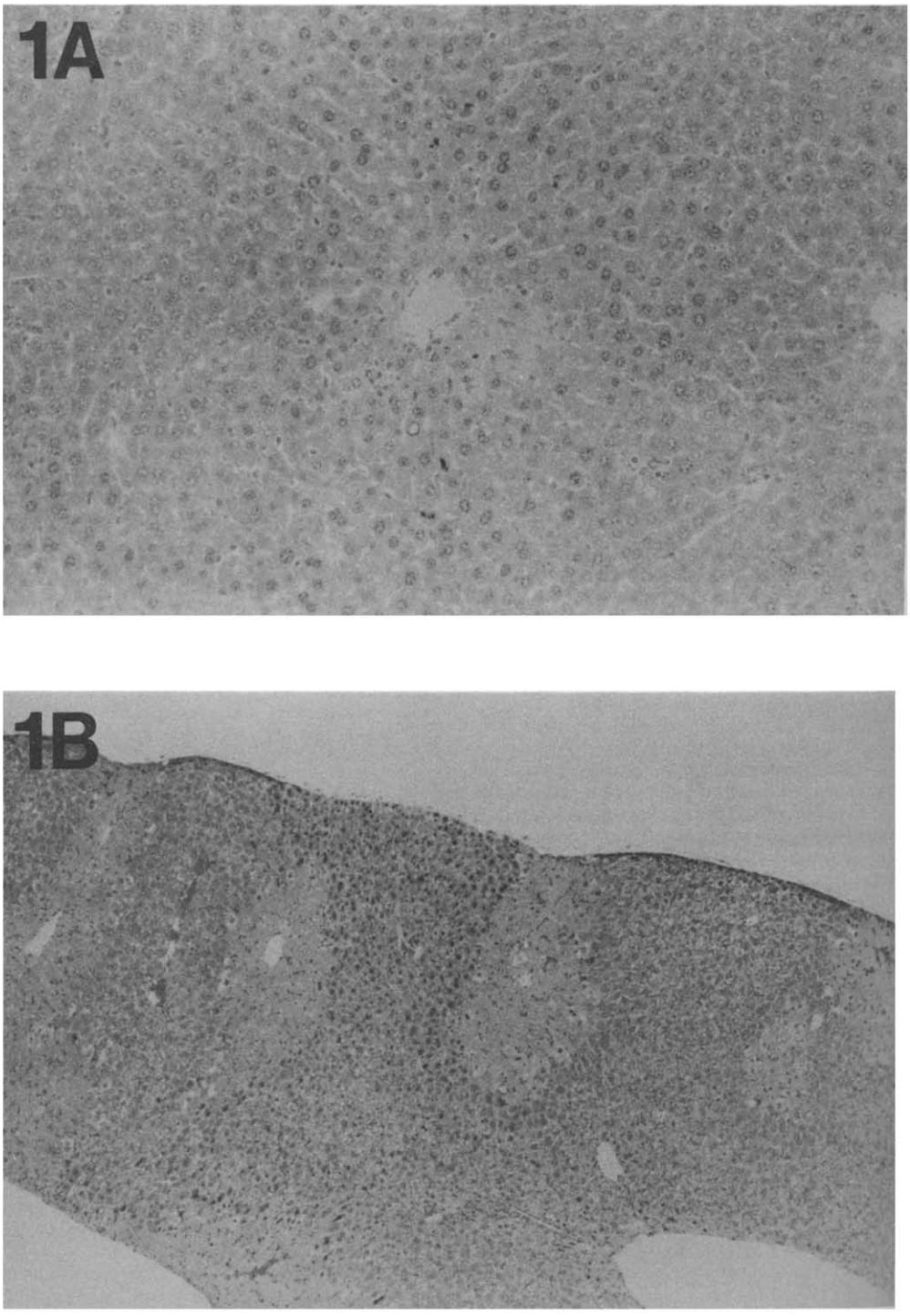
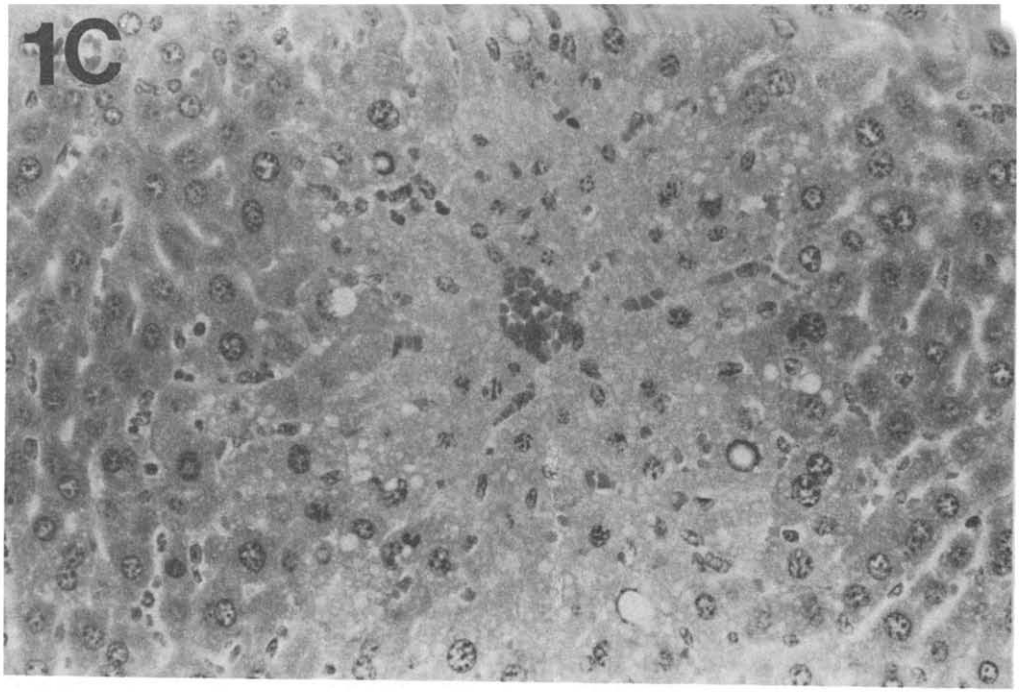


Fig. 1. Histopathologic analysis of AAP- and LPS-treated mouse livers. Fasted male ICR mice were treated with 400 mg/kg AAP, saline or 2 mg/kg LPS as described in Materials and Methods, and were killed; liver sections were then prepared and stained with hematoxylin and eosin. Figure 1A (10×) illustrates the AAP-induced necrosis of the parenchymal hepatocytes immediately surrounding the central vein, a result characteristic of a minimally toxic dose or of the early effects of a more hepatotoxic treatment. More extensive damage, expanding radially from the central vein in a manner not unlike an infarct, is shown in panel B (4×). At higher magnification (20×), the mass of necrotic sludge is seen (C). In contrast, LPS treatment caused little necrosis despite a substantial influx of polymorphonuclear neutrophils (D) (20×).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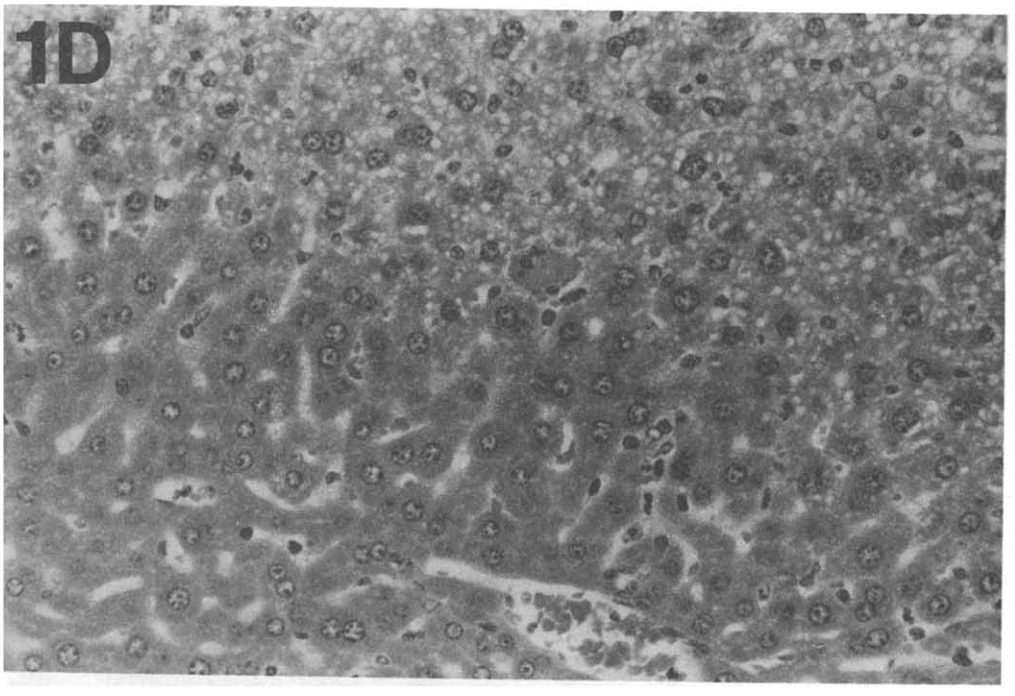
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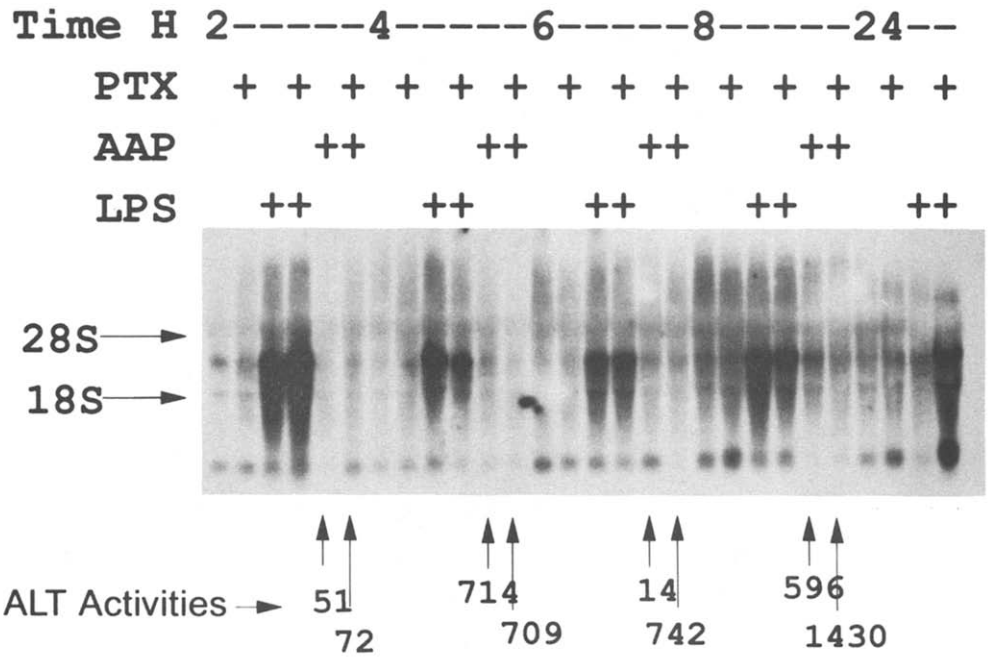

Fig. 2. Analysis of liver ICAM-1 mRNA in AAP-, LPS- or saline-treated mice. Fasted male ICR mice were treated with PTX (50 mg/kg) or an equal volume of saline i.p. 1 hr prior to administration of saline, LPS (2 mg/kg) or AAP (400 mg/kg) i.p. The mice were killed 2, 4, 6, 8, or 24 hr later as indicated. Hepatic RNA was isolated as described in Materials and Methods, separated by agarose gel electrophoresis, and probed with ^{32}P-labeled anti-sense transcripts to mRNA for murine ICAM-1 (band between 28S and 18S ribosomal RNA), and to histone-4 (band below 18S).

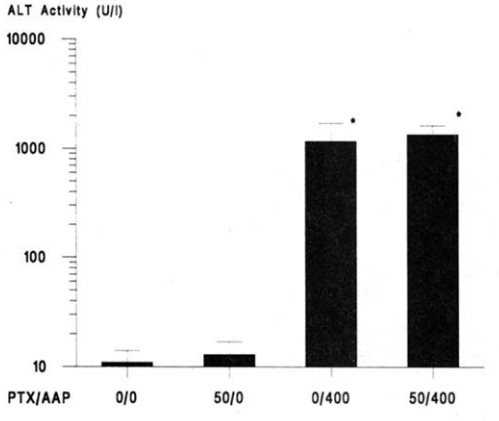

Fig. 3. ALT activities in AAP-treated mice and the effect of PTX (50 mg/kg) pretreatment. Male ICR mice were fasted for 18 hr, and then given 50 mg/kg of PTX or an equal volume of saline i.p.; 1 hr later the animals were given 400 mg/kg of AAP or saline i.p. as indicated. Six hours after the second injection blood was obtained by cardiac puncture, and plasma ALT activities were measured. Data are means ± SEM of six animals per group. Key: (*) $P < 0.05$ compared to saline-treated animals.

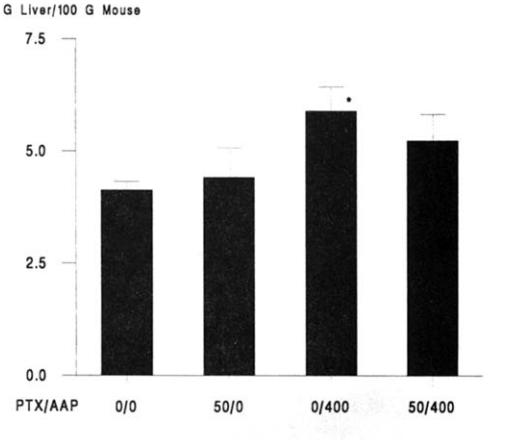

Fig. 4. Hepatic swelling in AAP-treated mice, and the effect of PTX (50 mg/kg) pretreatment. Liver weights were determined on the animals described in Fig. 3 and are expressed as g of liver per 100 g of animal body weight. Data are means ± SEM, $N = 6$. Key: (*) $P < 0.025$ compared to mice given only saline.

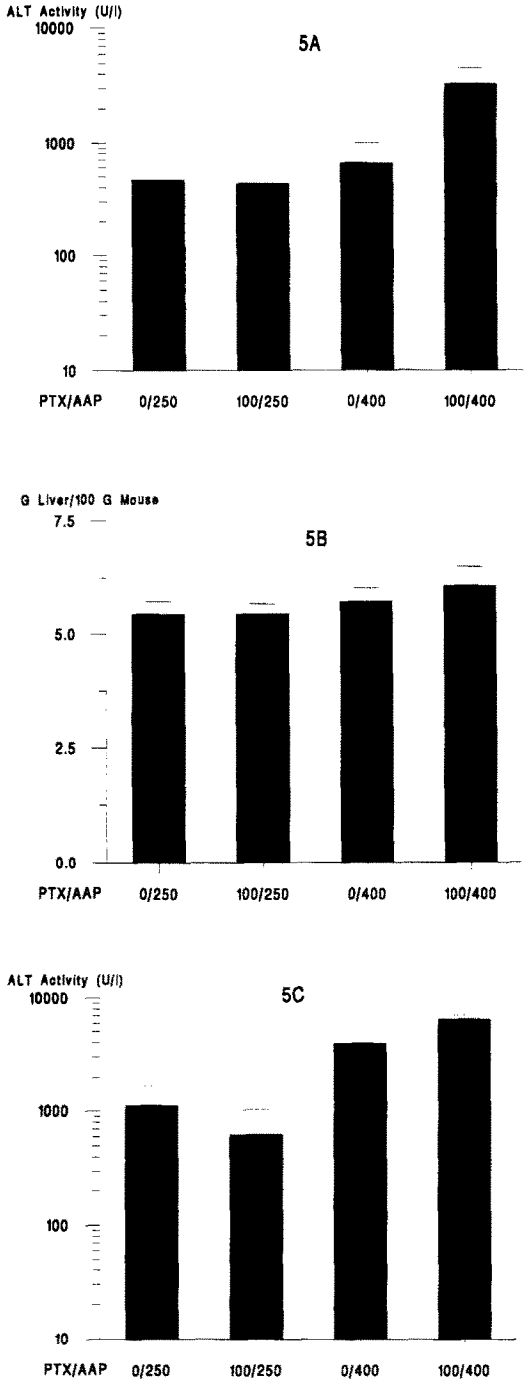

Fig. 5. ALT activities in AAP-treated mice and the effect of PTX (100 mg/kg) pretreatment. Male ICR mice were fasted for 18 hr, given saline or 100 mg/kg of PTX, and given 0, 250 or 400 mg/kg of AAP 1 hr later. Blood and tissue were obtained 6 hr after AAP for determination of plasma ALT activities (A) and liver weights (B). Additional animals were refed 6 hr after AAP and killed at 24 hr for measurement of plasma ALT activities (C). Data are means ± SEM of five animals per group. The values for animals not receiving AAP are not shown, but were similar to the values presented in Figs. 3 and 4. Values were compared between groups given the same dose of AAP (0/250 vs 100/250 and 0/400 vs 100/400).

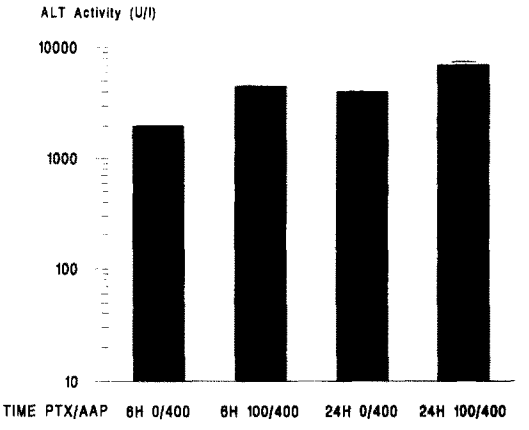

Fig. 6. ALT activities determined 6 and 24 hr after AAP treatment in mice and the effect of PTX (100 mg/kg) pretreatment. Male ICR mice were fasted for 18 hr, given saline or 100 mg/kg of PTX, and then given 400 mg/kg of AAP 1 hr later. Blood was obtained at 6 and 24 hr for determination of plasma ALT activities. Data are means ± SEM; N = 10 per group except for N = 9 in saline-pretreated group analyzed at 24 hr. Key: (*) P = 0.03.

in animals not given both PTX and AAP. Although no statistically significant differences in ALT activities were observed in these animals, the late mortality (between 6 and 24 hr) again suggested that pentoxifylline may potentiate the hepatotoxicity of AAP.

Figure 6 shows plasma ALT activities 6 and 24 hr after mice were treated with 400 mg/kg of AAP with either 100 mg/kg of PTX or saline pretreatment. Six hours after AAP treatment, the increase in the plasma ALT activities was significantly higher in the mice pretreated with pentoxifylline ($P = 0.03$). In contrast to the 6-hr results, in the mice examined 24 hr after AAP treatment no statistical difference in ALT activities between pretreatment groups was observed. However, in this experiment significant mortality was observed, with 1 of 9 animals dying in the saline-pretreated group versus 6 of 10 in the PTX-pretreated group. The difference in death rates between these two groups was statistically significant by chi-square analysis ($P < 0.05$).

DISCUSSION

Despite extensive research, the pathogenesis of AAP-induced hepatic necrosis is still controversial. AAP-induced hepatic necrosis appears to be initiated by alteration of critical cellular constituents by one or more chemically reactive metabolites. The marked hepatic swelling [13, 14, 21, 34], the zonal progression of the lesion [14], and the associated decrease in hepatic ATP concentrations observed by Tirmenstein and Nelson [10], by Jaeschke [11], and which we also saw at the lower doses used in this study (data not shown) suggest that ischemia may complicate the initial cellular injury and expand the metabolite-induced zone of damage. We therefore chose to examine some potentially important implications of

the hypothesis of ischemic expansion of AAP-induced hepatic necrosis.

Recently, Mitchell [12] proposed that the secondary expansion of injury in AAP-induced hepatic necrosis is mediated by neutrophils plugging the hepatic microvasculature, causing an ischemic infarct. Because neutrophil adherence is an important initial interaction that ultimately leads to neutrophil accumulation, activation, and neutrophil-mediated tissue injury, and because the interaction of endothelial ICAM-1 with the neutrophil β_2 integrins plays a pivotal role in the process of adherence, we examined the effects of hepatotoxic doses of AAP on the steady-state levels of messenger RNA for ICAM-1 in mouse livers. In other studies, the cell surface expression of ICAM-1 protein has been found to be regulated primarily at the level of transcription [18, 20], but we found no upregulation of ICAM-1 mRNA following administration of hepatotoxic doses of AAP. This lack of response of ICAM-1 mRNA is in contrast to the dramatic upregulation observed in the livers of mice treated with lipopolysaccharide, despite the fact that the dose of LPS used in these studies caused much less hepatic swelling than did AAP (data not shown), and caused only scattered single-cell necrosis, again in contrast to the marked hepatic necrosis caused by the doses of AAP studied. In addition, a much greater hepatic accumulation of neutrophils was caused by LPS. These experiments clearly do not support the conclusion that neutrophil adherence and accumulation represent significant contributions to AAP-induced hepatic swelling and necrosis. A similar conclusion also has been reported by Jaeschke *et al.* [35].

Although neutrophil adherence and vascular plugging do not seem to contribute significantly to AAP-induced hepatic swelling and necrosis, the vascular congestion that develops during AAP-induced necrosis is unmistakable. The consequences of this dramatic alteration in hepatic blood flow are not understood completely, but the histological pattern of the expansion of the area of injury certainly would seem to be consistent with a contribution from tissue hypoxia or ischemia. We would not regard the moderate decrements in hepatic ATP content reported previously [10, 11], and seen by us to be likely to threaten cell viability. However, it is quite possible that the whole tissue measurements of ATP obscure much more severe depletion of ATP in a limited subset of cells at risk.

We therefore sought to determine whether administration of PTX would ameliorate the adverse effects of toxic doses of AAP on hepatic blood flow and thereby possibly limit this hypothetical ischemic expansion of the initial lesion. PTX is used clinically in humans to improve peripheral circulation, and a number of studies have revealed a variety of mechanisms by which PTX helps improve blood flow. PTX has been reported to increase the deformability of erythrocytes and neutrophils [22, 23], improve endothelium-dependent vasodilation [24, 25], inhibit neutrophil adhesion and release of primary and secondary granules [27, 36], and inhibit the release of superoxide [28, 29]. Studies in humans using ^{198}Au-colloid scintigraphy showed a 10–40% increase in hepatic blood flow following intravenous administration of PTX [37]. PTX also has been shown to increase the production of prostacyclin by vascular tissue [24], and Guarner *et al.* [15] have reported that administration of prostacyclin significantly decreases AAP-induced hepatic injury in mice *in vivo* [15].

We therefore reasoned that PTX might diminish the extent of AAP-induced hepatic necrosis by sustaining hepatic blood flow and tissue oxygenation, even if neutrophil adhesion and vascular plugging were not involved. Our initial studies were encouraging in that the extent of hepatic swelling caused by AAP appeared to be somewhat diminished in animals pretreated with PTX (Fig. 4). Additional experiments were conducted employing higher doses of PTX and lower doses of AAP, in an attempt to identify a range in the dose–response curves of these two drugs where a possible beneficial effect of PTX might be observable. To our surprise, PTX-pretreated animals proved to be more susceptible to AAP-induced hepatic injury and mortality than did control animals pretreated with saline, as demonstrated by the greater increase in plasma ALT activities shown in Fig. 6 and in the higher animal death rate (6/10 in PTX-pretreated animals vs 1/9 in saline-pretreated animals, $P < 0.05$) occurring between 6 and 24 hr after AAP. This outcome stands in direct opposition to our initial intent to attenuate hepatic necrosis through improvements in hepatic blood flow. However, PTX has been reported to increase the cytotoxicity of anti-tumor alkylating agents, apparently by preventing delays in cell cycle transit through the G2 phase [38]. It is thought that PTX decreases the time available for repair of DNA damage caused by the alkylating agents, resulting in a greater lethality to the affected cells [38]. Although most of the hypotheses dealing with the mechanisms of AAP-induced hepatotoxicity have centered upon protein alkylation versus protein oxidation or lipid peroxidation, a smaller number of studies have been published in which genotoxic actions, including covalent binding to DNA by metabolites of AAP, have been noted both in experimental animals and in human subjects [39–41]. The possible relationship between nuclear and genetic alterations and acute lethal cell injury by AAP has begun to receive attention [42], but little is known about this relationship at this time.

In summary, in mice treated with hepatotoxic doses of AAP we did not observe an upregulation of ICAM-1 mRNA. This apparent lack of involvement of ICAM-1 suggests that the vascular congestion observed in AAP-induced hepatotoxicity is better understood in terms of congestion of the space of Disse and collapse of the sinusoids as proposed by Walker *et al.* [34], rather than as a result of vascular plugging by neutrophils as proposed by Mitchell [12]. In addition, PTX treatment did not reduce AAP-induced hepatic necrosis; in fact, at higher doses PTX appeared to enhance AAP-induced hepatic necrosis. This apparent enhancement of necrosis and the marked loss of histone-4 mRNA in livers of mice treated with toxic doses of AAP suggest that there are genotoxic components of AAP-induced hepatic necrosis.

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