Endothelial Cell Injury and Fibrin Deposition in Rat Liver after Monocrotaline Exposure

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Monocrotaline (MCT) is a pyrrolizidine alkaloid (PA) plant toxin that produces hepatotoxicity in people and animals. Human exposure to PAs occurs through consumption of contaminated grains and herbal remedies. Injection (ip) of MCT in rats produced dose-dependent hepatic parenchymal cell injury that was significant at 200 mg/kg. Injection of 300 mg/kg MCT produced time-dependent hepatotoxicity with significant injury beginning by 12 h after treatment. Histopathologic examination of liver sections revealed coagulative hepatocellular necrosis, widening of sinusoids and hemorrhage in centrilobular regions. MCT-induced damage to central venular endothelial cells (CVECs) and sinusoidal endothelial cells (SECs) in the liver was quantified using immunohistochemistry. Extensive endothelial cell injury was restricted to centrilobular regions. To determine if damage to endothelial cells in the liver stimulated activation of the coagulation system, fibrin deposition was quantified using immunohistochemistry and by increased plasma hyaluronic acid concentration. MCT damaged CVECs and SECs in the liver by 8 h after treatment. Extensive endothelial cell injury was restricted to centrilobular regions. To determine if damage to endothelial cells in the liver stimulated activation of the coagulation system, fibrin deposition was quantified using immunohistochemistry. Extensive fibrin deposition occurred in the liver after MCT treatment and was restricted to centrilobular regions. Interestingly, both endothelial cell damage and fibrin deposition preceded the onset of hepatic parenchymal cell injury. These results suggest that endothelial cell damage and fibrin deposition in centrilobular regions of the liver are prominent features of MCT-induced liver injury.

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ences might be related to differences in dose, route of administration, and the time at which livers were examined. Previous studies in which fibrin deposition in the liver has been observed after MCT treatment were qualitative, and they did not evaluate the extent or zonal distribution. In addition, the temporal relationship between fibrin deposition and the onset of hepatic parenchymal cell injury has not been reported. This may be relevant to the mechanism of injury because in a number of models of liver injury, coagulation system activation is required for hepatic parenchymal cell injury (Araki et al., 1996; Fujiiwara et al., 1988; Hewett and Roth, 1995; Yamada et al., 1989).

The studies presented herein tested the hypothesis that MCT produces zone-specific damage to endothelial cells and fibrin deposition in the liver. To this end, morphometric analysis of endothelial cells and fibrin in livers from MCT-treated rats was conducted using immunohistochemical techniques. The results of these studies show that treatment of rats with MCT produces time-dependent injury to SECs and CVECs that precedes parenchymal cell injury and only occurs in centrilobular regions of the liver lobule, i.e., regions that develop parenchymal cell necrosis. Similarly, fibrin deposition occurred only in centrilobular regions after MCT treatment and preceded the onset of hepatic parenchymal cell injury.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Crl: CD BR[SD] VAF/Plus, Charles River, Portage, MI) weighing 100–130 g were used in all studies. Animals were allowed food (Rodent Chow/Tek 8640, Harlan Teklad, Madison, WI) and water ad libitum. They were housed no more than 3 to a cage on Aspen chip bedding (Northeastern Products Company, Warrenburg, NY). The animals were maintained on a 12-h light/dark cycle in a controlled temperature (18–21°C) and humidity (55 ± 5%) environment for a period of 1 week before use. All procedures on animals followed the guidelines for humane treatment set by the American Association of Laboratory Animal Sciences and the University Laboratory Animal Research unit at Michigan State University.

Treatment protocol. Rats were fasted for 24 h prior to treatment with MCT (Trans World Chemicals, Inc., Rockville, MD). They received MCT by ip injection at doses indicated in the text and figures or an equivalent volume of sterile saline vehicle. Food was returned to the rats after MCT treatment. MCT was dissolved in sterile saline, minimally acidified by 2M HCl. The pH was brought to 6.7 by addition of 4M NaOH, and the volume was adjusted with sterile saline to the appropriate final concentration.

Assessment of hepatic injury, plasma fibrinogen, and plasma hyaluronic acid. At 4, 8, 12, or 18 h after treatment with MCT or its vehicle, rats were anesthetized with sodium pentobarbital (50 mg/kg ip). A midline abdominal incision was made, and blood was collected from the descending aorta into a syringe containing sodium citrate (final concentration, 0.38%). Hepatic parenchymal cell injury was evaluated by measuring the activity of alanine amino-transferase (ALT) in the plasma using Sigma kit 59-UV (Sigma Chemical Co., St. Louis, MO). One transverse section from the middle of the left lateral liver lobe and one from the right lateral lobe were processed for light microscopy. Paraffin-embedded sections were cut at 5 mm, stained with hematoxylin and eosin (H & E), and evaluated using a light microscope. Another portion of the liver was frozen for immunohistochemical staining as described below. The remaining liver was snap frozen in liquid nitrogen for evaluation of tissue hemoglobin as described below. Plasma fibrinogen concentration was determined from the thrombin clotting time of diluted samples by using a fibrinometer and a commercially available kit (Sigma Kit 886-A). Plasma hyaluronic acid was measured using a commercially available, enzyme-linked immunosorbent assay (ELISA; Chugai Diagnostics Science Co., Tokyo, Japan).

Liver hemoglobin. The concentration of liver hemoglobin was used as a biomarker of hemorrhage and was estimated using a commercially available kit (Total Hemoglobin Kit; Sigma Chemical Co.) as described (Jaeschke et al., 2000). A 20% homogenate was made from samples of frozen liver in 50 mM sodium phosphate buffer (120 mM NaCl, 10 mM EDTA). The samples were centrifuged at 16,000 × g for 10 min at 4°C, and 200 μl of supernatant was diluted in Drabkin’s solution. After a 15-min incubation, the absorbance was measured at 540 nm, and the concentration of hemoglobin was calculated from a standard curve.

Immunohistochemistry. A 1 cm² block of liver cut from the middle of the left lateral liver lobe was frozen for 8 min in isopentane immersed in liquid nitrogen. For liver endothelial cell immunostaining, 8 μm-thick sections of frozen liver were fixed in acetone (4°C) for 5 min. Next, they were incubated with PBS containing 10% goat serum (i.e., blocking solution; Vector Laboratories, Burlingame, CA) for 30 min, then with mouse antirat RECA-1 (rat endothelial cell antigen-I, Serotec, Inc., Raleigh, NC), diluted (1:20) in blocking solution overnight at 4°C. The RECA-1 antibody binds to rat endothelium but not other cell types (Duijvestijn et al., 1992). In the liver, this antibody stains both SECs and endothelial cells of larger vessels. After incubation with the RECA-1 antibody, sections were incubated for 3 h with goat antimouse secondary antibody conjugated to Alexa 594 (1:1000, Molecular Probes, Eugene, OR) in blocking solution containing 2% rat serum. Sections were washed 3 times, 5 min each, with PBS and visualized using a fluorescein microscope.

For fibrin immunostaining, 8 μm-thick sections of frozen liver were fixed in 10% buffered formalin containing 2% acetic acid for 30 min at room temperature. This fixation protocol solubilizes all fibrinogen and fibrin species except for cross-linked fibrin; therefore, only cross-linked fibrin stains in sections of liver (Schnitt et al., 1993). Sections were blocked with PBS containing 10% horse serum (i.e., blocking solution; Vector Laboratories) for 30 min, and this was followed by incubation overnight at 4°C with goat antirat fibrinogen diluted (1:1000, ICN Pharmaceuticals, Aurora, OH) in blocking solution. Next, sections were incubated for 3 h with donkey antigoat secondary antibody conjugated to Alexa 594 (1:1000, Molecular Probes) in blocking solution for 3 h. Sections were washed 3 times, 5 min each, with PBS and visualized using a fluorescein microscope.

For both protocols, no staining was observed in controls in which the primary or secondary antibody was eliminated from the staining protocol. All treatment groups that were compared morphometrically were immunohistochemically stained at the same time.

Morphometric quantitation of endothelial cells and fibrin in the liver. Endothelial cells and fibrin deposition in the liver were quantified morphometrically by analyzing the area of immunohistochemical staining in a section of liver. An increase in the area of staining for endothelial cells suggests a loss of these cells in the liver. An increase in the area of staining for fibrin in the liver indicates fibrin deposition. These were quantified in 2 ways. First, morphometric analysis was performed to quantify the area of endothelial cell or fibrin staining in randomly chosen, low-power fields irrespective of the region of the liver (i.e., centrilobular, perportal, etc.). This was done to determine if the total area of endothelial cells or fibrin deposition in sections of liver changed after MCT treatment. Secondly, the area of endothelial cells or fibrin deposition was determined in randomly chosen, centrilobular and perportal regions separately. This was done to determine if zonal differences in staining occurred after MCT treatment.

Fluorescent staining in sections of liver was visualized on an Olympus AX-80T microscope (Olympus, Lake Success, NY). For morphometric analysis of the total area of endothelial cells or fibrin deposition in a liver section, digital images of 10, randomly chosen, low power (magnification ×100) fields per tissue section were captured using a SPOT II camera and SPOT advanced software (Diagnostic Instruments, Sterling Heights, MI). Samples were coded such that the evaluator was not aware of the treatment, and the same exposure...
The criterion for significance was the fraction were transformed by arc sine square root prior to analysis. Compari-
sances in which variances were not homogeneous. Data expressed as a random ANOVA. ANOVAs were performed on log-transformed data in in-
centrilobular regions were analyzed using a 2
from studies comparing RECA-1 staining and fibrin staining in periportal and
among group means were made using the Student-Newman-Keuls test. Data

ing, liver fibrin staining, and liver hemoglobin concentration. Comparisons
plasma ALT, plasma fibrinogen, plasma hyaluronic acid, liver RECA-1 stain-
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extra-experimental variability was small and few deaths oc-
curred with this regimen.

Liver Injury Produced by MCT in Rats

MCT produced dose-dependent, hepatic parenchymal cell injury in rats 12 h after injection as measured by release of ALT into the plasma (Fig. 1A). A dose of 100 mg/kg produced no injury, whereas 200 mg/kg resulted in a statistically signif-
icant elevation in plasma ALT. No deaths were observed at any
of the doses examined up to and including 300 mg/kg. In
preliminary experiments, doses of MCT above 300 mg/kg
produced several deaths within 18 h after treatment. MCT (300
mg/kg) produced time-dependent hepatic parenchymal cell in-
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cally (Fig. 2A). Typical in livers isolated from rats treated 18 h
earlier with 300 mg/kg MCT was centrilobular, parenchymal
cell necrosis (Fig. 2B). Lesions consisted of areas of coagula-
tive and single cell necrosis. Individual, necrotic parenchymal
cells were present in periportal regions; however, these were
infrequent. Hemorrhage and widening of the sinusoids was
evident and occurred predominately in the centrilobular re-
gions; however, these areas occasionally extended into peri-
portal regions. Damage to CVECs also occurred after MCT
treatment (Fig. 2C) and frequently progressed to complete loss
of the vascular intima. Occasionally, cells with morphological
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RESULTS

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FIG. 1. Dose- and time-dependence of MCT toxicity. (A) Rats were
treated with 0–300 mg/kg MCT (ip). Plasma ALT activity was assessed 12 h
after MCT administration as a marker of hepatic parenchymal cell injury. (B)
Rats were treated with 300 mg/kg MCT or saline vehicle (ip), and plasma ALT
activity was assessed 4, 8, 12, or 18 h later. Data are expressed as means ±
STD; n = 4–8 rats. *Significantly different (p < 0.05) from saline-treated
rats.
consisting of neutrophils was present predominately within the centrilobular lesions. The acute lesions observed with this treatment protocol were very similar to lesions described previously using other MCT treatment protocols (Butler et al., 1970; DeLeve et al., 1999; Schoental and Head, 1955).

**Sinusoidal Endothelial Cell Damage**

Plasma hyaluronic acid was used as a marker of injury to SECs. Under normal conditions, approximately 90% of the hyaluronic acid circulating in the blood is removed and degraded by SECs in the liver (Kobayashi et al., 1999). Damage to SECs impairs the ability of these cells to clear hyaluronic acid from the circulation, leading to an increase in plasma concentration. This method has been used to monitor SEC damage *in vivo* after exposure to toxicants (Deaciuc et al., 1994, 1993). Figure 3 shows that treatment of rats with 300 mg/kg MCT produced a time-dependent increase in the plasma concentration of hyaluronic acid that became significant by 8 h after treatment.

Endothelial cells in sections of liver were stained immunohistochemically with RECA-1 antibody. This antibody selectively stains rat endothelial cells (Duijvestijn et al., 1992). In liver sections from rats treated with saline 18 h earlier (Fig. 4A), RECA-1 staining was present along the sinusoids and lined the major vessels of the liver. This indicated that the RECA-1 antibody recognized both CVECs and SECs within the liver. In livers from rats treated 18 h earlier with 300 mg/kg MCT, RECA-1 staining within the sinusoids was discontinuous or completely absent in centrilobular regions (Fig. 4B). RECA-1 staining of the sinusoids within periportal regions of livers from MCT-treated rats appeared similar to that seen in liver sections from saline-treated rats. A comparison of high

**FIG. 2.** Photomicrographs of liver sections from rats treated with MCT or saline. Rats were treated with either MCT or its saline vehicle, and livers were removed 18 h later and processed as described in the Materials and Methods. (A) Liver of a rat treated with saline vehicle. (B) Liver from a rat treated with 300 mg/kg MCT. Both regions of coagulative (wide closed arrow) and single cell (closed curved arrow) hepatic parenchymal cell necrosis were present in the centrilobular region. (C) Photomicrograph of a centrilobular region of a liver section from a rat treated with 300 mg/kg MCT. Loss of endothelial cells lining central veins occurred frequently (wide closed arrow). CV, central vein. PP, periportal. Bar in (A) and (B) = 50 μm. Bar in (C) = 30 μm.

**FIG. 3.** Effect of MCT on plasma hyaluronic acid concentration. Rats were treated with saline vehicle or 300 mg/kg MCT (ip), and plasma hyaluronic acid was evaluated 4, 8, 12, and 18 h later using a commercially available ELISA. Plasma samples from saline-treated rats evaluated at different times were combined into 1 group because no differences were observed among them. Data are expressed as means ± SEM; *n* = 3 rats/group. *Significantly different (*p* < 0.05) from saline-treated rats.
power fields of centrilobular regions from rats treated with either saline (Fig. 4C) or MCT (Fig. 4D) revealed that RECA-1 staining within the sinusoids and along central veins was obvious and continuous in control animals, whereas discontinuities were observed within the sinusoids and around central veins in treated rats.

MCT caused a time-dependent decrease in the area of RECA-1 staining in the liver that was significant by 12 h after treatment (Fig. 5A). To determine if there were zonal differences in the MCT-induced decrease in RECA-1 staining, the area of RECA-1 staining was analyzed separately in centrilobular and periportal regions. MCT caused a time-dependent decrease in the area of RECA-1 staining in centrilobular regions that was significant by 12 h after MCT treatment (Fig. 5B). In contrast, MCT did not decrease the area of RECA-1 staining in periportal regions.

Accumulation of Red Blood Cells in the Liver

To quantify the degree of hemorrhage in the liver after MCT treatment, the concentration of hemoglobin in the liver was determined. Treatment of rats with 300 mg/kg MCT caused a time-dependent increase in the concentration of liver hemoglobin that was significant by 12 h after treatment (Fig. 6).

Activation of the Coagulation System

Activation of the coagulation system leads to the conversion of plasma fibrinogen into fibrin, resulting in a decrease in the circulating levels of fibrinogen. This change in plasma fibrinogen was used as an indicator of activation of the coagulation system. Treatment of rats with 300 mg/kg MCT caused a time-dependent decrease in plasma fibrinogen that was significant by 12 h after treatment (Fig. 7).
To determine if conversion of fibrinogen to fibrin in the liver contributed to the decrease in plasma fibrinogen, hepatic fibrin deposition was evaluated immunohistochemically. In liver sections from rats treated 18 h earlier with saline (Fig. 8A), no fibrin staining was observed in the sinusoids. Staining was observed along the intima of large vessels in the centrilobular and periportal regions. This staining could not be eliminated with increased blocking or washing of the tissue and may have resulted from fibrin deposition that occurred after sacrifice of the rat. In livers from rats treated 18 h earlier with 300 mg/kg MCT, fibrin staining was evident in the sinusoids within centrilobular regions of the liver (Fig. 8B). Staining in periportal regions of livers from MCT-treated rats, however, was absent and did not appear different from that in livers from saline-treated rats. Figure 8C shows a high power field of a central vein from a rat treated 18 h earlier with saline. No fibrin staining was observed in the sinusoids adjacent to the central sinusoid.

FIG. 5. Quantitative morphometry of RECA-1 staining in the liver after treatment of rats with MCT. Rats were treated with saline vehicle or 300 mg/kg MCT (ip). Livers were removed 8, 12, or 18 h after treatment and processed for immunohistochemical staining of RECA-1 as described in Materials and Methods. (A) The total area of RECA-1 staining in 10 randomly chosen, low power fields per tissue section was analyzed morphometrically as described in the Materials and Methods. Data are expressed as means ± SEM. *Significantly different (p < 0.05) from saline-treated rats. (B) The area of RECA-1 staining in 5 randomly chosen fields that contained a centrilobular region and 5 randomly chosen fields that contained a periportal region was analyzed morphometrically as described in Material and Methods. Data are expressed as means ± SEM. **Significantly different (p < 0.05) from periportal regions of MCT-treated rats at the same time point. For these studies, saline-treated rats at the various times were combined into 1 group because no differences occurred among saline-treated groups from different time-points.

FIG. 6. Effect of MCT on the concentration of hemoglobin in the liver. Rats were treated with saline vehicle or 300 mg/kg MCT (ip). Livers were removed 4, 8, 12, and 18 h after treatment and analyzed for hemoglobin as described in Materials and Methods. Liver samples from saline-treated rats at the various times were combined into 1 group because no differences were observed among saline-treated groups from different time-points. Data are expressed as means ± SEM; n = 3 rats/group. *Significantly different (p < 0.05) from saline-treated rats.

FIG. 7. Effect of MCT on plasma fibrinogen. Rats were treated with saline vehicle or 300 mg/kg MCT (ip). Plasma fibrinogen was evaluated 4, 8, 12, and 18 h after treatment using a commercially available kit, as outlined in Materials and Methods. Data are expressed as means ± SEM; n = 3 rats. *Significantly different (p < 0.05) from saline-treated rats.
Treatment of rats with 300 mg/kg MCT, however, resulted in extensive pericentral fibrin staining in the sinusoids (Fig. 8D).

Quantitative morphometry revealed that MCT caused a time-dependent increase in the area of fibrin staining in the liver that was significant by 8 h after MCT treatment and became progressively more pronounced with time (Fig. 9A).

To determine if there were zonal differences in MCT-induced fibrin deposition in the liver, the areas of fibrin staining in centrilobular and periportal regions were analyzed separately. MCT caused a time-dependent increase in the area of fibrin staining in centrilobular regions that was not evident in periportal regions (Fig. 9B).

DISCUSSION

Several studies have shown that MCT produces hepatic parenchymal cell injury in rats (Butler et al., 1970; DeLeve et al., 1999; Hayashi and Lalich, 1968; Rose et al., 1945; Roth et al., 1981; Schoental and Head, 1955). The treatment protocols used in these studies have varied widely in the doses of MCT used, the routes of administration, and the purity of the MCT preparations. Unfortunately, some protocols produce liver injury that is highly variable, which makes studying toxicological mechanisms difficult. To study the development of endothelial cell injury and fibrin deposition in livers after MCT treatment, a protocol was chosen that produced consistent, reproducible hepatic parenchymal cell injury with a predictable time-course and minimal lethality. A single ip treatment of rats with 300 mg/kg MCT met these criteria. This dose of MCT produced liver injury with low intra- and inter-experimental variability and a predictable time-course (Fig. 1). In addition, few deaths occurred with this dose within 24 h, and the histopathological characteristics of the liver lesions (Fig. 2) were similar to those described previously (Butler et al., 1970; DeLeve et al., 1999; Schoental and Head, 1955).
Previous studies have shown by light and electron microscopy that damage occurs to both CVECs and SECs within the liver after MCT treatment (Allen et al., 1969; Brooks et al., 1970; Butler et al., 1970; DeLeve et al., 1999). In addition, studies in vitro indicate that MCT directly damages SECs, suggesting that these cells may be a target for toxicity in the liver (DeLeve et al., 1996). It is unclear from these studies, however, whether MCT produces widespread endothelial cell damage in the liver or whether it is limited to zonal or focal lesions. In the studies presented here, morphometric quantitation of immunohistochemically stained liver sections revealed that endothelial cell damage was extensive. On average, approximately 40% of the endothelial cell staining in the liver was completely lost within 12 h after MCT treatment (Figs. 4 and 5). In many regions, the sinusoids and central veins were completely denuded of endothelial cells (Fig. 4). This is consistent with what has been described previously using electron microscopic analysis of liver tissue from rats, monkeys, and humans exposed to MCT; however, the studies presented here are the first to quantify endothelial cell damage after MCT treatment and to define its time of onset (Allen et al., 1969; Brooks et al., 1970; DeLeve et al., 1999).

The antigen that the RECA-1 antibody recognizes has not been determined (Duijvestijn et al., 1992), and it is possible that expression of this antigen was modified by MCT. To confirm that the loss of RECA-1 staining was due to detachment and loss of endothelial cells and not due to down-regulation of the RECA-1 antigen, electron microscopy was performed on the same tissues that were analyzed by immunohistochemistry in Figure 4. Electron microscopy confirmed that SECs were absent in many sinusoidal regions 18 h after treatment with 300 mg/kg MCT (data not shown), which suggested that the decrease in RECA-1 staining was due to endothelial cell loss and not to down-regulation of the RECA-1 antigen. In addition, the increase in plasma hyaluronic acid observed after MCT treatment (Fig. 3) supports the immunohistochemical data suggesting endothelial cell injury.

Interestingly, severe endothelial cell injury leading to complete detachment of SECs and CVECs occurred only in centrilobular regions (Figs. 4 and 5). In livers of humans exposed to PAs, damage to sinusoidal endothelial cells occurred in all regions of the liver lobule (i.e., periportal, midzonal, centrilobular; Brooks et al., 1970). Occasional extravasation of erythrocytes into the space of Disse in the periportal region were observed by electron microscopy in the studies presented here (data not shown), suggesting that changes to the sinusoidal endothelium do occur in this region. However, these changes were much less than those observed in the centrilobular region and likely too subtle to be reflected in changes in RECA-1 staining. In addition, it is apparent from the RECA-1 immunohistochemical staining (Fig. 4) that loss of SECs occurred in the midzonal region, although this area was not quantified due to the difficulty of defining this region in tissue sections for morphometric analysis. Accordingly, SEC damage occurred to some extent in all of the regions of the liver lobule early after MCT exposure; however, damage to centrilobular endothelium was most severe, and damage in other regions was subtle.

The mechanisms contributing to greater endothelial cell toxicity in the centrilobular compared to the periportal region are not known, but they may be related to differences in the extent of metabolic activation or detoxification of MCT in...
these two zones. MCT requires bioactivation by cytochromes P450 to produce liver injury (Schultze and Roth, 1998; Wilson et al., 1992), and SECs express cytochromes P450 (Lester et al., 1993). Studies in vitro have suggested that SECs can bioactivate MCT to a toxic metabolite (DeLeve et al., 1996). The observation that greater SEC injury occurred in centrilobular regions suggests the possibility of zonal differences in expression of cytochromes P450 in SECs, similar to what has been observed in parenchymal cells (Oinonen and Lindros, 1998). Cytochromes P450 of the 3A family metabolize MCT to MCTP (Kasahara et al., 1997), and members of this P450 family are primarily expressed in centrilobular regions (Oinonen and Lindros, 1998). Accordingly, if SECs express these activity may be greater in centrilobular SECs. Differences in zonal distribution of SEC proteins have been reported, suggesting that gene regulation differences exist in SECs from different zones of the liver lobule (Scoazec et al., 1994). Alternatively, it is possible that metabolism of MCT to MCTP by parenchymal cells contributes to SEC toxicity in vivo. That is, injury to centrilobular SECs might be greater because cytochromes P450 of the 3A family are primarily expressed by parenchymal cells of this region. Finally, the difference in zonal toxicity observed in vivo might result from an inability of centrilobular SECs to detoxify MCTP. This is unlikely, because injection of MCTP preferentially damaged endothelium of perportal regions (Butler et al., 1970). This result is inconsistent with periportal SECs being more capable of detoxifying MCTP and suggests that zonation of endothelial cell toxicity observed after MCT treatment may be more related to the location in which the MCTP is produced.

In vitro, SECs are more sensitive to the toxic effects of MCT than parenchymal cells (DeLeve et al., 1996). Accordingly, it has been suggested that MCT-induced damage to SECs in vivo results in microcirculatory disturbances that lead to hypoperfusion of the liver and consequent parenchymal cell injury (DeLeve et al., 1996). Observations presented here support this hypothesis. First, the histopathological finding of coagulative, hepatocellular necrosis in centrilobular regions is consistent with ischemic injury (Fig. 2; DeLeve et al., 1999). Second, if destruction of SECs contributes causally to microcirculatory changes and parenchymal cell damage, then it should precede these changes. The plasma concentration of hyaluronic acid began to increase between 4 and 8 h after MCT treatment (Fig. 3), suggesting an early onset of endothelial cell injury. SEC injury was most severe in centrilobular regions (Fig. 5) and preceded evidence of hemorrhage (i.e., liver hemoglobin, Fig. 6) and parenchymal cell injury (Fig. 1). Both of these changes occurred primarily in centrilobular regions and began between 8 and 12 h after treatment (Figs. 1 and 6). Therefore, extensive SEC damage in centrilobular regions may lead to microcirculatory disturbances that promote ischemic, hepatic parenchymal cell injury in this region.

Another factor that may contribute to ischemic parenchymal injury after MCT treatment is fibrin deposition. Electron mi-

croscopic studies have shown that fibrin deposition occurs in the liver after MCT treatment (Allen et al., 1969; Butler et al., 1970; Schoental and Head, 1955). Fibrin deposition was not quantified in these early studies, and the temporal relationship between the appearance of fibrin and the onset of endothelial cell injury was not defined. Treatment of rats with 300 mg/kg MCT provoked activation of the coagulation system (Fig. 7) and fibrin deposition in the liver (Fig. 9). Fibrin deposition occurred simultaneously with endothelial cell injury and prior to hepatic parenchymal cell injury. In addition, it occurred only in centrilobular regions, where endothelial and hepatic parenchymal cell injury were extensive. This suggests the possibility of a causal link between fibrin deposition and hepatic parenchymal cell injury. Several hepatotoxicants promote coagulation system activation and fibrin deposition in the liver (Ahmed et al., 1987; Arai et al., 1996; Fujiwara et al., 1988; Pearson et al., 1996), and inhibition of coagulation system activation completely prevents hepatic parenchymal cell injury in these models (Arai et al., 1996; Fujiwara et al., 1988; Pearson et al., 1996). This suggests that the coagulation system may be a critical mediator of liver injury after exposure to some hepatotoxicants. Considering the extent of fibrin deposition in the liver and the nature and location of the hepatic lesions, it is reasonable to hypothesize that coagulation activation and fibrin deposition are required for MCT-induced liver injury.

Fibrin deposition in the liver after exposure of rats or humans to MCT has not been a consistent finding (Brooks et al., 1970; DeLeve et al., 1999). Electron microscopic analysis of liver samples from children exposed to PA-containing plants showed no evidence of fibrin deposition (Brooks et al., 1970). However, these samples were not taken until several weeks after the children were exposed to PAs, and it is possible that within this time the fibrinolytic system had been activated and removed fibrin from the liver. Fibrin deposition may not occur in all models of MCT-induced liver injury. In a detailed study by DeLeve et al. fibrin deposition in the rat liver was not observed at any time after MCT treatment (DeLeve et al., 1999). Clearly, further study is needed to understand the role of the coagulation system in MCT hepatotoxicity.

In summary, treatment of rats with MCT produced extensive endothelial cell injury and fibrin deposition in centrilobular regions of the liver. These events were followed by extensive hemorrhage and evidence of hepatic parenchymal cell injury. The results raise the possibility that MCT produces direct damage to centrilobular endothelial cells, which promotes hemorrhage and fibrin deposition in the liver. Such events might lead to local hypoperfusion that could contribute to parenchymal cell injury.

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