Alterations in Liver Blood Flow and Reticuloendothelial Function in Progressive Cirrhosis in the Rat

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Cirrhosis of the liver was induced in rats by twice weekly inhalation of carbon tetrachloride in conjunction with sodium phenobarbitone administration. At sequential time intervals during induction, liver blood flow and extraction efficiency of colloid were assessed in order to elucidate changes in these parameters which occur with cirrhosis. Liver samples were also taken for histologic examination and graded for extent of disease. Initially there was a fall in extraction efficiency (and thus reticuloendothelial function), associated with early histologic change. Subsequently extraction efficiency recovered, as regeneration was observed on histologic specimens. From 4 wk and onward, blood flow gradually fell, as did extraction efficiency. These changes were associated with increasing severity of disease as demonstrated by histologic sections.

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Complex hemodynamic changes occur in cirrhosis (*1*) and alterations in the vascular bed have been postulated as major determinants of the disease. Previous studies show impaired colloid clearance rates in cirrhosis as compared with normal (*2*). This may be due to a reduction in both the blood flow and colloid extraction efficiency (*3*). This investigation was undertaken to measure separately the detailed changes in liver blood flow and reticuloendothelial function assessed by colloid extraction in progressive cirrhosis induced in the rat by carbon tetrachloride inhalation. Previously described noninvasive techniques for assessing liver blood flow by dynamic scintigraphy (*4–6*) were used in this study, in combination with other invasive and histological methods.

MATERIALS AND METHODS

Two hundred male Cob Wistar rats were studied, with a weight range between 200 g at the start of treatment and 300 g at the end. Cirrhosis was induced in 120 animals using the method described by McClean (*7*). Following 1 wk pretreatment with sodium phenobarbitone (0.5 g/l) in drinking water, animals received twice weekly inhalations of carbon tetrachloride (CCl_4) in a 37 l box, concentration 1% in oxygen at a flow rate of 1 l/min, for between 4 and 10 min for 8 wk. Sodium phenobarbitone treatment continued throughout induction of cirrhosis. Control animals continued to receive sodium phenobarbitone alone, to exclude the possibility that any effects which might be observed are due to sodium phenobarbitone. This substance acts as an inducer of liver enzymes and stimulates the development of cirrhosis when given in conjunction with CCl_4 inhalation.

Isotope Imaging

Radioisotope imaging was performed at 1, 2, 3, 4, 6 and 8 wk after the initial carbon tetrachloride treatment. At each time interval an assessment was made of both colloid clearance in ten animals and efficiency of hepatic extraction of colloid in an additional ten animals. Furthermore, uptake and efficiency measurements were carried out in normal animals (ten animals for each time point), without either sodium phenobarbitone or carbon tetrachloride, as a quality control for the isotope used. For these studies, anesthesia was induced with ether which was followed by intraperitoneal pentobarbital (50 mg/kg body weight).

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Colloid clearance. Anesthetized animals were taped in position on a stand and positioned under a gamma camera fitted with a 1.5-mm pinhole collimator so that the heart, liver, and spleen were included in the field-of-view. Approximately 1mCi (40 MBq) technetium-99m (^{99m}Tc) sulfur colloid* were injected as a bolus into the tail vein and sequential digital images stored at 5-sec intervals for a period of 8 min on an on-line digital computer system. At the end of the study, the animal was killed and the liver removed. Static images of the removed liver and the remainder of the animal were acquired using a parallel hole collimator to ensure uniform counting sensitivity.

The colloid clearance rate K was determined as follows. Using an integrated digital image of the last ten frames of the study the liver region was defined and a time-activity curve of this region for the whole study was obtained. Each point on the curve between 0.5 and 1.5 min was subtracted from a plateau that was taken as the mean value of the curve between 7 and 8 min. The value of K was obtained using least squares regression on the logarithm of the subtracted curve. This gave the sum of the effective blood flows to all reticuloendothelial cells, expressed as a percentage of blood volume [Eq. (1), section B].

The relative plateau count ratio of liver and extrahepatic sites at the end of the dynamic study, L (∞) and E (∞) were estimated from the static images. This allowed calculation of the ratio of effective blood flows [Eq. (2)]. Using both Eqs. (1) and (2) effective liver blood flow was calculated.

Extraction efficiency measurements. These measurements were performed in separate animals. The pyloric vein was cannulated and, with the rat under the gamma camera as described above, ~0.5mCi (20 MBq) [^{99m}Tc] sulfur colloid were injected through the cannula directly into the portal vein over a period of 10 sec. Sequential digital images were stored at 0.5-sec intervals for a period of 50 sec. The final count rate in the liver was also measured after the plateau value had been reached. Extraction efficiency was obtained using Eq. (3). These measurements were repeated in a further group of normal animals, with injection into the hepatic artery.

Histologic Studies

After imaging, each animal was exsanguinated and the liver removed. Livers were fixed in formol saline for histologic studies. Paraffin embedded sections were stained with H&E, and by Sweets method for reticulin.

THEORY OF MATHEMATIC MODEL

Intravenously injected radioactively labeled colloid is removed by reticuloendothelial cells in the liver and other sites, particularly spleen and bone marrow. This clearance is described by a single exponential function, the rate of which (K) is given by the sum of the effective blood flows to the above organs (*2*). The effective blood flow is the product of the actual blood flow and the extraction efficiency of the organ for colloid. Thus

$$K = K_1 + K_2, \quad (1)$$

where K_1 and K_2 are the effective blood flows to liver and extrahepatic sites expressed as fractions the blood volume.

The final amounts of colloid in each site are proportional to their effective blood flows (*2*). Thus, the liver to extrahepatic activity ratio at the end of the study is given by

$$\frac{L(\infty)}{E(\infty)} = \frac{K_1}{K_2}. \quad (2)$$

L (t) and E (t) are the activity variations with time in the liver and extrahepatic sites respectively. Using Eqs. (1) and (2), the effective liver blood flow can be found.

Assessment of the extraction efficiency of colloid by the liver was achieved in a separate study in which radioactive labeled colloid was injected slowly into the portal vein through an indwelling catheter. The extraction efficiency is given by the equation

$$E = \frac{A_i x}{A_p - (1 - x)A_i}, \quad (3)$$

where $x = K_1/(K_1 + K_2)$. A_i is the count rate in the liver 5 sec after the end of the injection and A_P the final count rate (*2*).

The true blood flow to the liver Q_l is given by

$$Q_l = K_l/E. \quad (4)$$

Thus Q_l can be found from Eqs. (3) and (4).

RESULTS

Morphologic and Histologic Changes

After initiation of treatment the following changes were observed. Weight increase was less than that occurring in normal animals and treated animals appeared unhealthy. Their fur became dirty and crusting appeared around the eyes. Towards the end of treatment many animals had developed obvious ascites. Some animals also developed hydrothorax which necessitated more care being taken with inhalation anaesthesia. All animals were unable to tolerate normal dosages of barbiturate. During operative procedures tissues appeared fragile and considerable bleeding occurred.

Consistent histologic changes were seen from as early as 1 wk after the initial carbon tetrachloride inhalation.

By the end of the first week (Fig. 1A) focal hepatocytic fatty change and necrosis were present. There was increased mitotic activity and small areas of abnormal fibrosis. Kupffer cells were more prominent than in

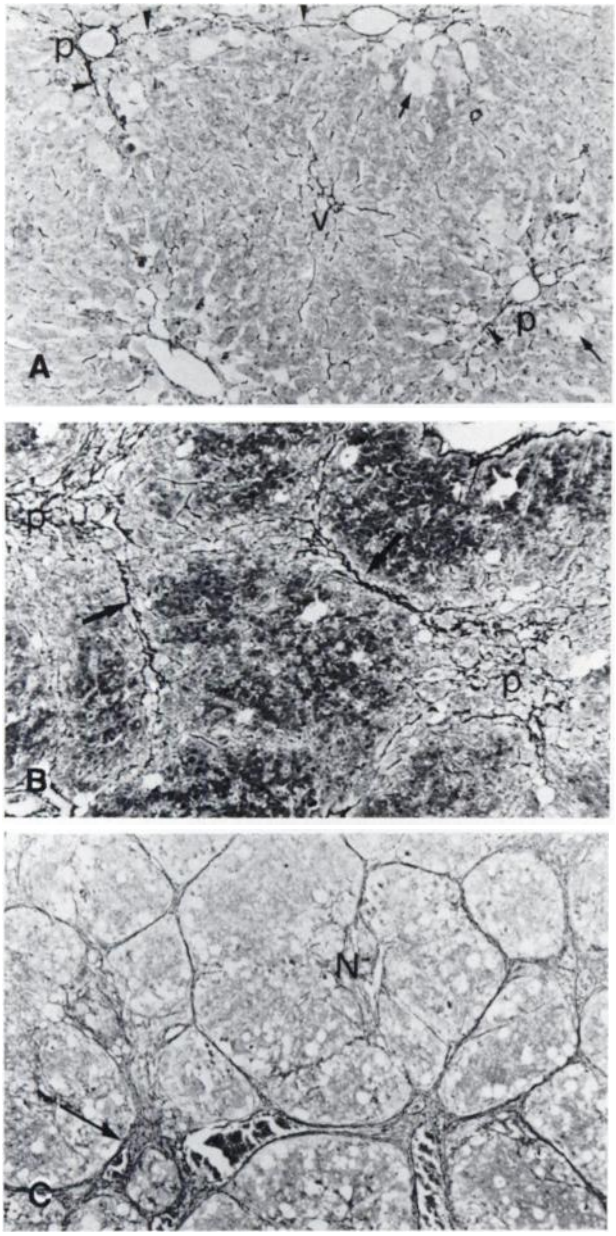

FIGURE 1
Histologic changes in liver after carbon tetrachloride. A: *Grade 1–1 wk.* Some areas of fatty change (arrows) and slight increase in periportal fibrous tissue (arrow heads). Portal tracts, p; central vein, v. (Gold toned reticulin × 85). B: *Grade 2—2 wk.* Increased fibrous tissue around portal tracts (p) and bridging of adjacent tracts by fibrous tissue (arrows) (Reticulin, neutral red counter stain × 110). C: *Grade 3—6 wk.* Advanced cirrhosis with numerous nodules (N). Some larger aggregates of fibrous tissue (arrow). (Gold toned reticulin × 50)

control animals (Grade 1 cirrhosis). After 2 wk both macroscopic and microscopic nodules were visible. Fibrosis and fatty change were more marked than in the first week and prominent mitotic activity and nuclear pleomorphism were seen in hepatocytes (Grade 2 cirrhosis—Fig. 1B).

Progressive changes were seen between the third to eighth week. Macroscopically the liver was pale and pitted and was often associated with ascites. Microscopically, increasing cellular necrosis and fibrosis was observed (Grade 3 cirrhosis, Fig. 1C) and finally, extensive necrosis was seen. Bridging necrosis between portal tracts and central veins was prominent and fibrosis was more extensive. The vascular pattern was grossly abnormal and in some animals prominent bile duct proliferation was seen.

Blood Flow and Colloid Extraction

Sodium phenobarbitone alone had no effect on total liver blood flow or extraction efficiency. Similarly, efficiency values were the same for normal animals, whether injection was into the portal vein or the hepatic artery. Mean extraction efficiency values were calculated for each stage of treatment. Substituting these values in Eq. (4) the total liver blood flow was calculated for each animal in the colloid clearance study. The mean value of this blood flow was calculated at each stage of treatment. The results of extraction efficiency and liver blood flow are shown in Fig. 2, which also illustrates the relationship between these parameters and histologic grade.

DISCUSSION

Previous workers have shown that liver enzyme induction facilitates induction of cirrhosis when animals are treated with CCl_4. While induction of cirrhosis without sodium phenobarbitone was not attempted, the method has already been validated (*7*), and gave consistent and reproducible cirrhosis in all animals.

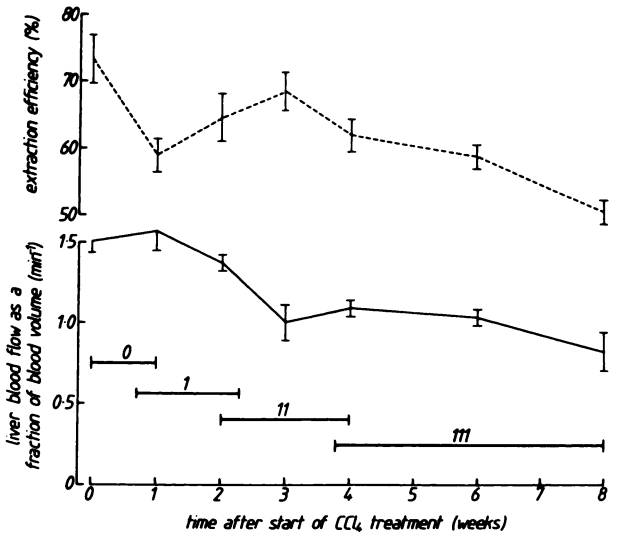

FIGURE 2
Relationship between extraction efficiency, liver blood flow, and progressively severe cirrhosis (shown by histologic grade). Results show mean of ten animals for each time point ± 1 s.e.m. Statistically significant changes are detailed in text

During the first week of induction of cirrhosis when early histologic changes were present, although no significant changes in liver blood flow were detectable, a significant fall in extraction efficiency occurred (Week 1 results compared with normal, $p < 0.01$). The Mann Whitney U-test was used to test significance throughout the study.

In the subsequent 2-wk period, hepatic blood flow started to fall (Week 1 was not significantly different from normal, Weeks 2 and 3 were significantly different from normal, $p < 0.02$ and $p < 0.01$, respectively), but this was associated with a significant recovery of extraction efficiency (Week 1 results compared with Week 3, $p < 0.05$). This may have resulted from regeneration which could be seen in the periportal region at this stage (Fig. 1B).

The small increase in liver blood flow occurring between Weeks 3 and 4 was not statistically significant, and from this time onwards a gradual reduction in blood flow occurred as has been previously documented (*8,9*). This was accompanied by a fall in extraction efficiency which, by the end of the study, had been reduced by 35%. The reduction in extraction efficiency, however, cannot be directly equated with deterioration in reticuloendothelial function, since it is well-established that intrahepatic shunting occurs as cirrhosis develops (*10,11*), and this is currently being investigated. The confluent loss of hepatocytes combined with fibrosis causes bridging of portal tracts to central veins. Blood flowing in these "shunts" may thus bypass the sinusoidal system completely and pass directly from both hepatic artery and portal vein to the central vein. Approximately 20% of both hepatic arterial and portal blood flow may thus be shunted even in moderate degrees of cirrhosis (*10,11*), and particulate matter will therefore not be adequately filtered by the Kupffer cells. Radiolabeled colloid will be no exception so that the use of this method will tend to overestimate any reduction occurring in cellular function.

SUMMARY

The use of an animal model has demonstrated the detailed changes to total liver blood flow and colloid extraction efficiency occurring in progressive cirrhosis. Specifically, it has been shown that the early histologic changes were accompanied by a fall in extraction efficiency of radiolabeled colloid. Extraction efficiency recovered significantly with regeneration, but subsequently deteriorated as cirrhosis progressed. Liver blood flow was maintained in the early stage of the disease but then fell progressively with increasingly severe cirrhosis.

Previous workers have shown reduced blood flow and colloid extraction efficiency (*3*), resulting in impaired colloid clearance rates (*2*) in cirrhosis. This new noninvasive technique, which can detect the deterioration in hepatic colloid extraction and the later fall in blood flow, may therefore be of value clinically for monitoring the progression of or recovery from cirrhosis.

FOOTNOTE

* Squibb Diagnostics (Tesuloid), New Brunswick, NJ.

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