HETEROGENEITY OF CYTOCHROME P-450 DISTRIBUTION IN THE HEPATIC LOBULE REVEALED BY CARBON TETRACHLORIDE

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UDC 612.351.11

The concentration of cytochrome P-450 was compared with the histological picture of development and elimination of necrotic foci in the liver of CBA mice after inhalation of CCl_4 vapor. After 2 h the cytochrome P-450 content was reduced by 49%, and after 17, 24, and 48 h by 84%; only about 40% of the area of the hepatic lobule underwent necrosis under these circumstances. It is suggested that the selectivity of injury by CCl_4 to the central lobular hepatocytes is connected with the sharp difference in cytochrome P-450 content in different zones of the lobule.

KEY WORDS: cytochrome P-450; hepatic lobule; carbon tetrachloride.

On the basis of information now available it is suggested that the system of enzymes of microsomal oxidation, and cytochrome P-450 as its specific marker, are not uniformly distributed in the parenchyma but are concentrated in the central regions of the hepatic lobules. For instance, damage produced by certain hepatotoxins including CCl_4 to the hepatocytes of the central zone of the hepatic lobule is known to be selective [6]. The hepatotoxicity of CCl_4 is explained by the formation, through the participation of cytochrome P-450, of intermediate toxic metabolites [2]. The insensitivity of the hepatocytes of the peripheral zones of the lobules to CCl_4 suggests differences in the concentration of cytochrome P-450 in the zones of the hepatic lobule. Damage to the peripheral cells cannot be produced by any known procedure, including reversing the direction of the blood flow [12]. Varying the dose of CCl_4, up to an increase of over 100 times, does not affect the area of necrotic foci [6]. After a single dose of CCl_4 the peripheral zones that remain outwardly undamaged are insensitive to repeated action of the toxin up to the complete elimination of necrotic foci [7, 8]. Finally, phenobarbital, which induces enzymes of microsomal oxidation, acts selectively: Proliferation of the smooth endoplasmic reticulum, which is regarded as a structural manifestation of induction, affects cells of the central zones of the lobules only [14].

Data on the distribution of cytochrome P-450 in the hepatic lobule are not available. In the investigation described below, in order to discover heterogeneity of the distribution of cytochrome P-450 in the hepatic lobule, the concentration of cytochrome P-450 was compared with the morphological picture of development and elimination of necrotic foci in the mouse liver after poisoning with CCl_4.

EXPERIMENTAL METHOD

Experiments were carried out on 90 male CBA mice aged 4 months. Liver damage was produced with CCl_4 vapor. For this purpose, the animals were placed six at a time for 15 min in a 3-liter exsiccator into which 0.1 ml CCl_4 was introduced. The mice were decapitated 2, 17, 24, 48, 72, and 93 h after poisoning. The liver was perfused in situ with cold 1.15% KCl. Pieces of tissue were fixed in 10% formalin in order to prepare 5-μ histological sections which were stained with hematoxylin and eosin. The remaining liver (from three animals in each sample) was used to obtain the microsomal fraction [1]. The concentration of cytochromes P-450 and b_5 were measured by the method of Omura and Sato [11] on a Shimadzu MPS-50L spectrophotometer. To estimate the cytochrome P-420 concentration the ratio of the optical density at 420 nm to the total absorption in the region of 420 and 450 nm was

Laboratory of Cytology, N. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR. Laboratory of Physical Chemistry of Biomembranes, M. V. Lomonosov Moscow State University. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 4, pp, 364-366, April, 1979. Original article submitted June 12, 1978.

 0007-4888/79/8704-0374$07.50

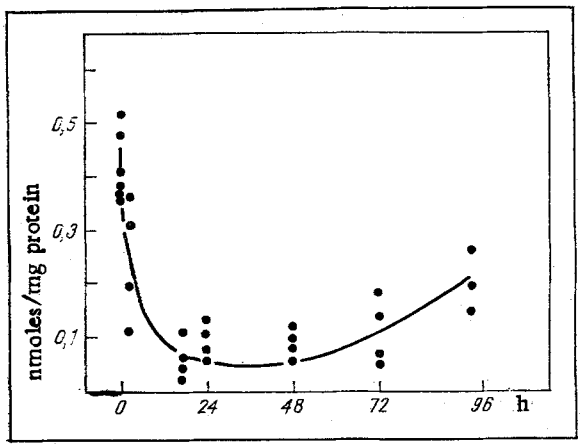

Fig. 1

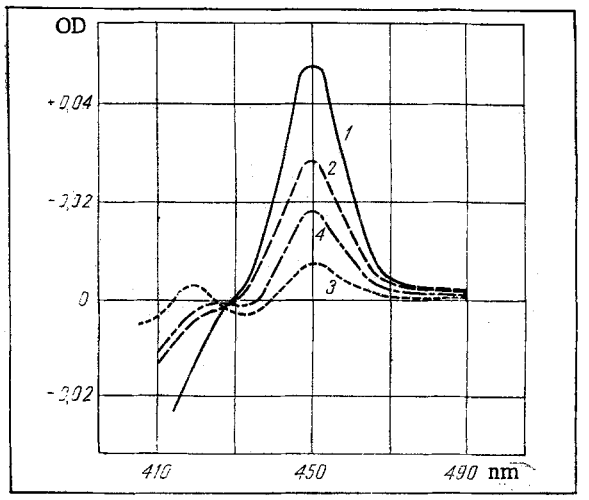

Fig. 2

Fig. 1. Concentration of cytochrome P-450 in microsomal fraction of liver in control and after inhalation of CCl_4. Abscissa, time after CCl_4 poisoning (in h); ordinate, concentration of cytochrome P-450 (in nmoles/mg protein). Each point is result of one determination in sample taken from three animals.

Fig. 2. Differential absorption spectra of cytochrome P-450 in microsomal fraction of liver in control and after inhalation of CCl_4. 1) Control; 2) 2 h, 3) 24 h, 4) 93 h after inhalation of CCl_4. Abscissa, wavelength (in nm); ordinate, optical density. Protein concentration in samples 1 mg/ml.

calculated and expressed as a percentage. The concentration of microsomal protein was determined by the biuret method.

EXPERIMENTAL RESULTS

No definite morphological signs of injury were found in the liver 2 h after inhalation of CCl_4 vapor. After 17 h pycnosis and karyolysis of the nuclei and eosinophilia and homogeneity of the cytoplasm were observed in the cells of the central zones. At this time the zones of necrosis were clearly outlined and the necrotic cells bordered directly on outwardly uninjured cells. About 24 h after poisoning cellular reactions leading to gradual elimination of the necrotic foci began to develop. Uninjured peripheral cells commenced a mitotic cycle, divided, and displaced the necrotic masses, which were ultimately eliminated through the action of immigrating inflammatory cells. After 17 and 24 h the area of necrosis was about 40% of the total area of the parenchyma, after 48 h it was very slightly reduced, after 72 h it was reduced by half, and after 93 h it was still clearly visible. The normal structure of the lobule was not restored until 7-8 days after poisoning.

The concentration of cytochrome P-450 in the liver was reduced on average by 49% 2 h after poisoning, and later by 84% ($P < 0.001$) (Figs. 1 and 2). Similar results were obtained previously in experiments on rats [3, 8, 9]. The decrease in the cytochrome P-450 content 2 h after poisoning reflected its destruction as a result of the action of CCl_4 [2, 13]. The subsequent sharp decrease in its concentration after 17, 24, and 48 h was due to death of about half the cells of the inner part of the lobules. The peripheral cells, which remained intact, were the source of regeneration of the parenchyma. Evidently cytochrome P-450 was synthesized in the newly formed cells migrating toward the center of the lobule, although the increase in its concentration after 72 and 93 h was not significant ($P < 0.2$).

The decrease in the cytochrome P-450 concentration was accompanied by the formation of its inactive form cytochrome P-420 (Fig. 2). The mean concentration of cytochrome P-420 was 16, 45, 40, 32, 31, and 30% after 2, 17, 24, 48, 72, and 93 h respectively. The results show that the accumulation of degradation products of cytochrome P-450 reached a maximum 17 and 24 h after CCl_4 poisoning, by the time of appearance of zones of necrosis. Attention is drawn to the absence of any sharp decrease in the concentration of cytochrome P-420. The reason was evidently that cytochrome P-420 was preserved in the necrotic masses. Cytochrome b_5 — a much more stable component of the microsomal membranes [2] — showed only a tendency to decrease in these experiments.

When these results are examined it is important to note the discrepancy between the degree of decrease of the cytochrome P-450 concentration (by 84%) and the mass of dying cells (40%), which could be attributed to the originally uneven distribution of this substance in the parenchyma, most of it being located in the cells of the central zones of the lobules. During the interpretation of the results it must also be remembered that not only the formation of trichloromethyl radicals and induction of peroxidation of lipids, mediated through cytochrome P-450 [2, 15], are important in the development of the necrotic lesion, but also disturbance of the systems of utilization of products of free-radical reactions. The toxic effect of CCl_4 is known to be substantially reduced or even abolished by inhibitors of these reactions [3-5, 10]. Possibly peripheral cells, which never undergo necrosis, contain a quantity of cytochrome P-450 which, although smaller, is nevertheless adequate for activation of CCl_4, but unlike the central cells they do not die because the subsequent stages of elimination of toxic metabolites proceed to completion.

Let us examine two models to explain the precise determination of the zones of CCl_4 damage: sharp differences between the hepatocytes of the central and peripheral zones of the lobules in their concentration of cytochrome P-450, presupposing differences in their differentiation; the gradient distribution of functions, corresponding to the structure of the lobule, a characteristic feature of the organization of the liver parenchyma. It can tentatively be suggested that the zone of sensitivity to CCl_4 is determined by interaction between two gradients. First, from the center of the lobule to the periphery, the concentration of cytochrome P-450 falls steadily. The gradient of substances serving to eliminate toxic metabolites and controlling the process of lipid peroxidation is in the opposite direction. A distinct boundary between cells sensitive and insensitive to CCl_4 arises at a point corresponding to the intersection of the two gradients. Direct evidence in support of one or the other hypothesis can be obtained either by cytochemical methods or by investigation of hepatocytes isolated from different zones of the lobule.

The authors are grateful to L. L. Prilipko and I. A. Eluashvili for help with the investigation and useful discussion of the results.

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