Massive Liver Cell Necrosis Induced in the Pig with Carbon Tetrachloride

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SUMMARY

An attempt was made to induce massive liver necrosis in the pig by the injection of varying doses of carbon tetrachloride into the afferent vessels of the liver. The optimal route and dose of injection were assessed as well as the effect of prior phenobarbitone administration. Survival, and biochemical and histological changes were noted.

A preliminary trial of exchange transfusion showed that the model could be used, but a number of variables complicates evaluation in this biological system. A large number of animals would be needed under strictly paired conditions in order to draw significant conclusions.

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Massive liver necrosis remains an important complication of infectious or drug-induced hepatitis and has a mortality rate of 80 - 90%.[1] This relates partly to inadequate methods of treatment resulting from inability to perform adequate clinical trials. An animal model of acute liver failure is urgently needed for the controlled assessment of procedures such as exchange transfusion, cross-circulation and hepatic assist with the isolated perfused liver.

For the purposes of this study, the following ideal model was defined, with the requirements of: (*i*) reversibility—such that the animals might respond and recover with treatment; (*ii*) reproducibility—with near uniform mortality, since evidence of the effectiveness of treatment would rest almost exclusively upon survival and reversal of biochemical changes; and (*iii*) death from liver failure with preceding disturbance in the level of consciousness commencing 6 - 12 hours after injection, with death of the animal within 36 - 48 hours.

Some previous attempts to cause massive liver necrosis in animals have included surgical manoeuvres which are generally irreversible,[2-4] with the exception of partial devascularisation.[5] Others have used drug administration. Galactosamine injected intraperitoneally into the rat causes a histological picture similar to that of massive necrosis in humans,[6,7] but use in large animals would be prohibitively expensive. Dimethylnitrosamine,[8] while successful, requires strict laboratory control because of the hazards of carcinogenesis. Carbon tetrachloride has been used with some success in the rat,[9,10] and more recently in the monkey.[11] Injection into a mesenteric radicle of the portal vein induced acute liver necrosis with coma within 6 hours.

This study investigated the pig as a readily available laboratory animal, and attempts were made to determine the optimal dose of carbon tetrachloride to be used, the optimal route of injection, and the value of administration of phenobarbitone to enhance the hepatotoxic effect of carbon tetrachloride.[12]

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MATERIALS AND METHODS

Pigs of Landrace X Large White stock of either sex and weighing between 12 and 30 kg were used. Their ages ranged from 25 to 60 days and they were fed commercial 'Creep' meal[13] until pre-operative starvation. For injection of carbon tetrachloride, light anaesthesia was induced with halothane, nitrous oxide and oxygen given via an endotracheal tube with intermittent positive pressure ventilation. Carbon tetrachloride was injected directly into a branch of the hepatic artery or into a catheter inserted via the splenic vein to lie at the confluence of the splenic and superior mesenteric veins.

Phenobarbitone powder was given daily for 5 days to one group of pigs. As no previous dose schedule could be found for the pig, an arbitrary amount of 1 g/litre was dissolved in the 5 litres of available drinking water. No measurement of induction by assay of cytochrome P450 or electron microscopy was made (see Discussion).

Biochemical tests of liver damage were chosen to align with clinical practice and sera were analysed for aspartate transaminase, bilirubin and alkaline phosphatase. Histological sections of the liver, kidneys and lungs were taken at death or sacrifice in all animals.

The experimental groups were:

I Injection of carbon tetrachloride 0.05 ml/kg into the hepatic artery—10 pigs.

II (a) Injection of carbon tetrachloride 0.05 ml/kg into the portal vein—2 pigs.

(b) Injection of carbon tetrachloride 0.14 ml/kg into the portal vein—25 pigs.

III Injection of increasing doses of carbon tetrachloride into the hepatic artery following administration of phenobarbitone—39 pigs.

RESULTS

Route of Injection

Injection of carbon tetrachloride 0,05 ml/kg into the hepatic artery of 10 pigs resulted in diffuse liver damage, and a mean survival of 10,1 ± 1 days. Injection of this volume into the portal vein in 2 animals resulted in protracted survival probably related to the localised damage which occurred as a result of streaming in the portal circulation. At a higher dose (0,14 ml/kg) in 25 animals, mean survival was 15 ± 2 hours (range 2 - 52 hours), with 2 animals sacrificed at 7 days.

Effect of Administration of Phenobarbitone

Since hepatic arterial injection resulted in more diffuse damage, this route was chosen to assess the effect of administration of phenobarbitone. Comparison of the results of survival in groups I and III(d) shows that the administration of phenobarbitone reduced mean survival time from days to hours (Table I). The pattern of elevation of aspartate transaminase and alkaline phosphatase, shown in Fig. 1, was apparent earlier after administration of phenobarbitone but no greater rise was observed. Bilirubin levels were not elevated above 2 mg/100 ml in any animal.

TABLE I. EXPERIMENTAL DESIGN SHOWING THE SCHEDULE AND ROUTE OF INJECTION, THE NUMBER OF ANIMALS, THE DOSE OF CARBON TETRACHLORIDE AND THE MEAN SURVIVAL TIME WITH THE STANDARD ERROR OF THE MEAN

Group	No.	CCl4 dose ml/kg	Survival
I	10	0,05	10 ± 1 days
II (a)	2	0,05	Sacrificed 3 months
(b)	25	0,14	15 ± 2 hours* 2 sacrificed
III (a)	4	0,0125	32 ± 2 hours* 2 sacrificed
(b)	9	0,025	36 ± 11 hours* 2 sacrificed
(c)	4	0,035 - 0,045	37 ± 17 hours
(d)	16	0,05	25 ± 3 hours
(e)	6	0,1	40 ± 10 hours

Where not otherwise indicated in the table, animals were sacrificed at 7 days.
Group I — injection of carbon tetrachloride into the hepatic artery;
II — injection of carbon tetrachloride into the portal vein;
III — injection of carbon tetrachloride into the hepatic artery after administration of phenobarbitone.
* Calculations of the mean survival time have been made excluding data from the sacrificed animals.

Dose Response

In 39 animals in group III, increasing doses of carbon tetrachloride were given into the hepatic artery after phenobarbitone administration. No specific dose response was noted from the survival data, although animals given smaller doses were more likely to survive 7 days (Table I). A wide range of aspartate transaminase levels was found, and no dose response was noted. Alkaline phosphatase seemed to show a progressive rise with increasing doses (Fig. 2).

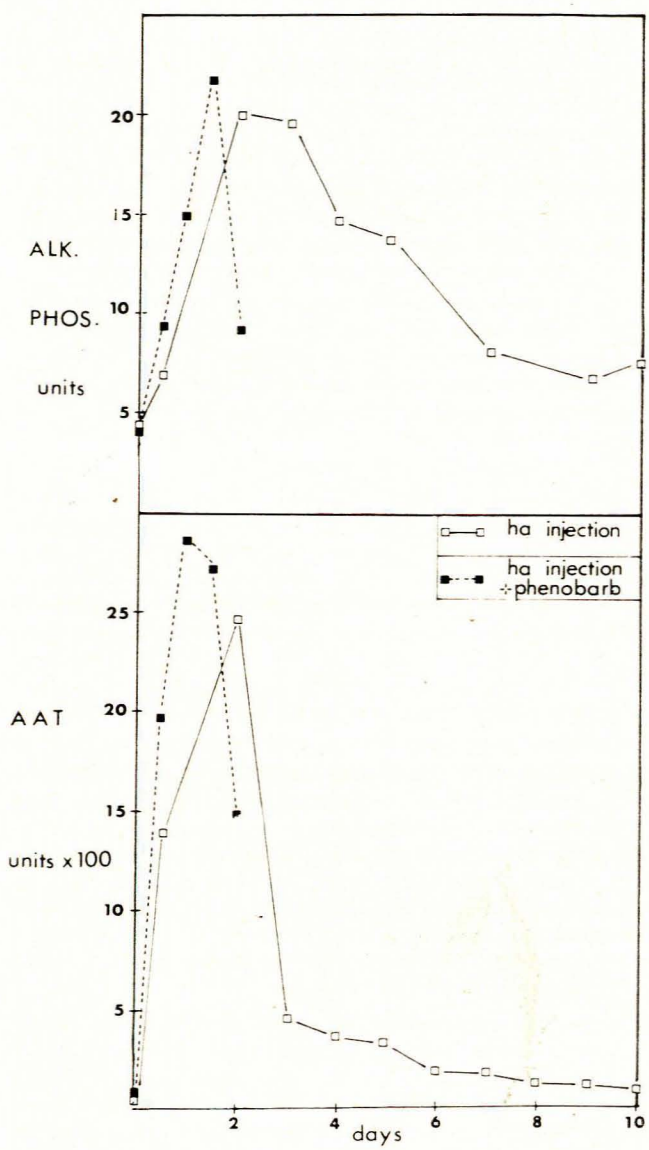

Fig. 1. The tempo of enzyme increases resulting from injection of 0,05 ml/kg carbon tetrachloride into the hepatic artery, with or without prior phenobarbitone administration.

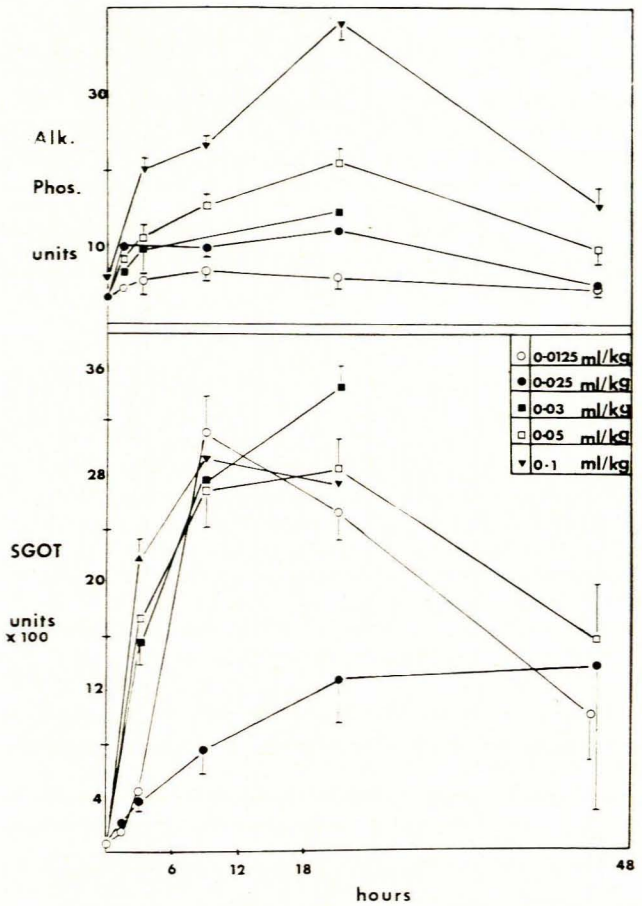

Fig. 2. The enzyme changes resulting from increasing doses of carbon tetrachloride injected into the hepatic artery following phenobarbitone administration.

Histology

Histological evidence of liver damage (Fig. 3) was more apparent with increasing doses. Initial centrilobular damage (shown on left in Fig. 3) gradually extended to involve the whole lobule (Fig. 3, right) as the dose was increased from 0,025 ml/kg to 0,1 ml/kg.

Complications

Hypoglycaemia was a marked feature requiring monitoring of blood sugar every two hours and continuous administration of intravenous dextrose. Large doses of carbon tetrachloride given into the hepatic artery without prior phenobarbitone administration caused death from pulmonary oedema and convulsions before hepatic necrosis was evident.

Coma developed suddenly preterminally in the pig and there was little evidence of deterioration until just before death. In 12 animals, which were given phenobarbitone, cerebrospinal fluid glutamine levels rose significantly, from a normal mean of 18,0 mg/100 ml to 94,2 mg/100 ml 8 hours after injection of 0,05 ml/kg carbon tetrachloride

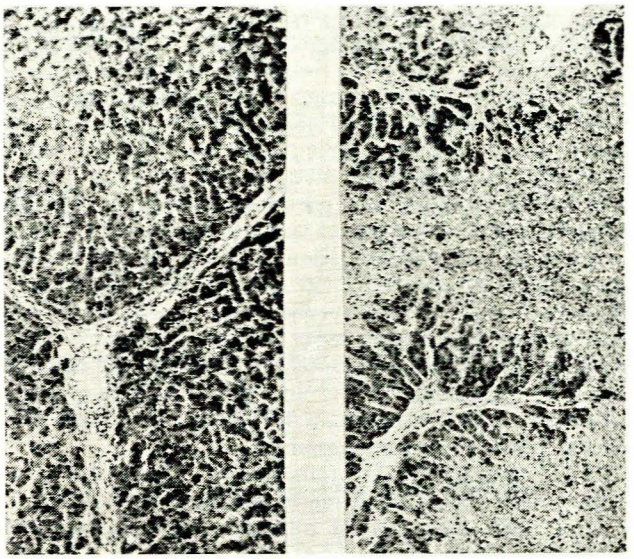

Fig. 3. The changes in liver histology resulting from injection of carbon tetrachloride—(left) low dose with relatively minor effects; (right) more severe damage caused by the larger dose (H. and E. × 40).

into the hepatic artery ($P<0{,}001$). Thus there was biochemical[1,14] but not clinical evidence of coma, and one aspect of the ideal model was not met.

Preliminary Trial

In a preliminary trial of the use of exchange transfusion, 25 control animals were given 0,14 ml/kg carbon tetrachloride into the portal vein, with a mean survival of 15 ± 2 hours, excluding 2 animals which survived 7 days. In 9 animals given the same dose of carbon tetrachloride, exchange transfusion of 2 litres of fresh heparinised pig blood was performed at 6 hours. Five animals survived a mean of 13 ± 2 hours, and 4 were sacrificed after 7 days.

DISCUSSION

This study aimed to establish a reproducible, reversible model of acute liver failure in the pig, with coma and death resulting within 36 - 48 hours of administration of carbon tetrachloride. This time interval was to be used to define the biochemical status of the animals, to note changes in level of consciousness and to initiate treatment on a controlled basis.

An unsuccessful attempt was made to define a dose-related response to injection of carbon tetrachloride. Administration of 0,05 ml/kg via the hepatic artery or the portal vein resulted in long survival. Increase of the portal venous dose to 0,14 ml/kg caused death in 40% of the 25 animals within the desired time, but survival ranged from 2 to 52 hours. Administration of phenobarbitone before injection of carbon tetrachloride into the hepatic artery resulted in variable survival which was not entirely

dependent upon the dose—volumes from 0,035 to 0,1 ml/kg resulted in similar survival times. Phenobarbitone was given in an attempt to render the carbon tetrachloride more specifically hepatotoxic.[12] Since no data were available for administration to the pig, the choice of dose and route was arbitrary. Oral administration was probably unreliable, but in a recent study in this laboratory, Miller and Saunders[15] found that phenobarbitone given by intramuscular injection for 5 days was also followed by variable response to paracetamol administration, despite fivefold elevation of cytochrome P450 (unpublished observations). Phenobarbitone reduced survival time in animals treated with carbon tetrachloride from days to hours, but did not result in any obvious dose-related response. It has subsequently been suggested that the drug should be given for 10 days to achieve optimum levels of cytochrome P450 (Davis; personal communication).

In a preliminary trial of the value of exchange transfusion in this model, 44% of treated animals survived for 7 days, while only 8% of untreated animals were long survivors. There is a significant correlation between survival and treatment ($P<0,05$ using chi-squared analysis), but long survivors among untreated animals made this conclusion unsatisfactory.

It is concluded that this model may be of use with some modification to the original ideal design. Although the mean survival time was suitable, a wide range should be anticipated, and 25% of animals in this series died before 8 hours, i.e. when treatment would have been instituted. Coma was not as obvious as is described in the monkey,[11] but evidence for liver necrosis was drawn from elevation of aspartate transaminase and alkaline phosphatase, and histological features were similar to those reported in rats[9] and sheep.[16] Total massive necrosis as seen in rats given halothane[17] or fluoroxene[18] after phenobarbitone induction, was not seen.

The preliminary trial of treatment by exchange transfusion showed this to be a feasible procedure in the pig but emphasised the need to use large numbers of animals under strictly paired conditions to enable meaningful conclusions to be drawn.

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