EFFECT OF EHRLICH ASCITES TUMOUR ON THE CLINICAL COURSE AND PATHOLOGY OF MICE HAVING CARBON TETRACHLORIDE-INDUCED HEPATO-RENAL NECROSIS

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Ehrlich ascites tumour, administered intraperitoneally 18 hr following a dose of CCl₄ by stomach tube, produces an irreversible illness characterised by failure to resolve extensive hepato-renal necrosis. Equally extensive hepato-renal necrosis occurs in animals given CCl₄ and saline; such animals, however, remain clinically well and show rapid histological regeneration of both organs. Carbon tetrachloride given to mice on day 5 of tumour growth produces hepatic necrosis only, the kidney of such animals being immune to the necrotising effect of CCl₄. Such animals remain well, and histological recovery of the liver is rapid. It is proposed that the Ehrlich tumour produces a “regeneration-inhibiting” toxin, active against the damaged liver and kidney; the CCl₄-damaged kidney fails to excrète this toxin, hence the irreversibility of hepato-renal damage and a fatal outcome.

A marked reduction of ascites volume as compared with controls was observed in those animals given Ehrlich tumour 18 hr after CCl₄, but not in animals given CCl₄ on day 5 of Ehrlich ascites tumour growth.

Hepatocellular mitosis occurring in Ehrlich ascites tumour-bearing mice has two highly characteristic features. First, it is confined to centrilobular, CCl₄-sensitive areas, and, second, it can further be distinguished from regenerative hepatocyte mitosis by virtue of its characteristic response to microtubule inhibitors, colchicine and vincristine sulphate.

Such peculiarities clearly indicate that tumour-associated hepatocellular mitosis is mediated in a manner different from that of a “demand” mitotic response stimulated by an imbalance between hepatocytes and non-hepatocytic cells, caused by increasing tumour bulk. Indeed, if the latter mechanism operates, one would expect the ensuing hepatocyte mitotic response to have the characteristics of regeneration, since, in one sense, post-necrotic or post-partial heptectomy regeneration may be regarded as a consequence of the imbalance between hepatocytes (reduced) and non-hepatocytic cells (remaining constant).

This highly individual nature of the Ehrlich tumour-associated hepatocyte mitotic activity prompted the present investigation. It was hoped to assign to this response a role in the tumour-host economy by studying the tumour during a period when the centrilobular zone was absent. Centrilobular zone ablation was carried out using CCl₄, and a variety of experimental protocols were used in the presence of this basic liver lesion. The initial aim, namely to elucidate the role, if any, of the centrilobular hepatic cells in terms of a measurable effect on the tumour has not been achieved. The results reported below, however, obtained during the course of these experiments, indicate that both liver and kidney play a role in maintaining the integrity of the tumour-bearing mouse.

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MATERIALS AND METHODS

All experiments were carried out in male inbred CBA-strain mice, 4–5 months old (20–30 g initial body weight). Experiments were carried out under identical environmental conditions, and animals had free access to food and water throughout.

Carbon tetrachloride (Analar Grade, B.D.H. Ltd., U.K.) was mixed with an equal volume of olive oil (B.P.), and appropriate animals given a dose of 0.15 ml by stomach tube. Control mice were given 0.15 ml of olive oil (B.P.) by stomach tube.

Two experimental situations, using appropriate controls, were explored. First, mice given CCl₄/olive oil were challenged 18 hr later with 1 × 10⁸ Ehrlich ascites tumour cells intraperitoneally (10 mice). Control mice were given olive oil only followed by 1 × 10⁸ Ehrlich cells (10 mice), or CCl₄/olive oil followed by the appropriate volume of saline intraperitoneally (8 mice).

Ehrlich cells used were pooled from several donors (between day 8 and day 10 of tumour growth) into an approximately equal volume of heparinized saline (10 U heparin/ml), then washed three times in large volumes of heparin-free saline. The final concentration was adjusted so that 1 × 10⁸ viable cells were contained in 1.0 ml.

In the second category of experiment, 0.4 ml of Ehrlich cells, treated as above, were injected intraperitoneally. On day 5 of tumour growth (day 0 being the day of transplantation), 8 mice were challenged with 0.15 ml of CCl₄/olive oil intragastrically, and 8 control tumour-bearing mice challenged with olive oil by stomach tube.

Animals were killed by ether anaesthesia at times indicated in the Results. Liver and kidney were fixed in Bouin’s fluid, embedded in paraffin, sectioned at 5.0 μm, and stained with Haematoxylin and Eosin.

RESULTS

Effect of 1 × 10⁸ Ehrlich Cells on Animals Pretreated with CCl₄/Olive Oil Two experiments were carried out, and the results of both were in agreement.

Sixty-six hr after CCl₄/olive oil and 48 hr after Ehrlich tumour challenge, the animals were well and indistinguishable clinically from controls. Animals given CCl₄/olive oil followed by intraperitoneal saline and animals given olive oil only followed by Ehrlich tumour remained well throughout the experimental period. By 90 hr after CCl₄/olive oil, however, all of the Ehrlich tumour-challenged animals were clearly ill, 3 were moribund and one died. All such treated animals became moribund during the ensuing 24 hr. Histologically, all animals given CCl₄/olive oil by stomach tube developed not only centrilobular hepatic necrosis but also extensive renal tubular necrosis with necrosis of both proximal and distal tubules, sloughing of the eosinophilic cells, and extensive hyaline cast formation in the medullary tubules. Tumour-bearing animals pretreated with olive oil did not show these lesions.

Clinically normal CCl₄/olive oil-treated mice given saline instead of Ehrlich tumour, showed the expected regeneration and reconstitution of the liver following CCl₄/olive oil. By 114 hr after CCl₄/olive oil in such tumour-free animals, hepatic healing was well advanced, and renal tubules, although containing eosinophilic necrotic debris, were all lined by new, flattened or low cuboidal epithelium (Photos 1, 2, and 3). That such recovery of hepato-renal integrity proceeds to completion is strongly suggested by the fact that a separate group of three mice given CCl₄/olive oil and saline did not suffer any obvious clinical illness, and are alive and well at the time of writing (6 weeks later).

The severely ill and dying mice given CCl₄/olive oil followed 18 hr later by intraperitoneal Ehrlich tumour, however, all showed a dramatic failure of hepato-renal regeneration. In these animals, mitotic activity of hepatocytes was low or absent, and the eosinophilic centrilobular necrotic zones showed no signs of being removed (Photo 4). The renal tubules were full of cellular debris and there was no evidence of new tubular epithelium formation (Photos 5 and 6).

Apart from the four animals (2 CCl₄/olive oil/Ehrlich tumour, and 2 olive oil only/Ehrlich tumour) collected at 66 hr, when no obvious difference in the volume of ascites was observed, there was, at all other times

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studied, a clearly discernible reduction in the volume of ascites in CCl₄-treated mice. In view of the dramatic differences in clinical state and in the progress of hepato-renal regeneration as between control (CCl₄/olive oil-treated non-tumour bearing) mice and CCl₄/olive oil-treated tumour-challenged mice, the tumour itself was not studied in any detail in the present work.

**Effect of a Challenge Dose of CCl₄/Olive Oil in Mice Bearing an Established Ehrlich Ascites Tumour**

A total of 14 animals from two experiments were finally studied. (Two experimental animals died less than 18 hr after stomach tubing, one from a massive gastrointestinal haemorrhage, the other from undiscovered causes.) The surviving mice, experimental and controls, all remained active and clinically well. Three experimental mice and three control mice were collected 114 hr following challenge; a further 3 experimental and 5 controls were collected 138 hr following challenge. In complete contrast with the previous experiment, CCl₄/olive oil-treated mice show no evidence of either resolving or of unresolved renal tubular necrosis (Photo 7); however, centrilobular hepatic necrosis has occurred and is healing well (Photo 8). In both experimental and control animals, there was voluminous ascites. At the first time point (114 hr), ascites weight, tumour cell viability, and total packed cell volume of tumour cells were determined. The results of these measurements are comparable as between experimental and control mice. At the second time point (138 hr post-challenge), only tumour cell viability and ascites weight were recorded; again there was no difference between experimental and control mice.

**DISCUSSION**

Centrilobular liver necrosis and extensive renal tubular necrosis follow a large dose of CCl₄ (approximately 3.0 ml/kg body weight) given intragastrically to normal male CBA-strain mice. At no time following such treatment alone is there any obvious adverse clinical reaction; there are no fatalities, and histologically both organs heal rapidly, the liver more rapidly than the kidney. In such CCl₄-treated animals, the intraperitoneal injection of $1 \times 10^8$ Ehrlich ascites tumour cells 18 hr after the halogenated hydrocarbon, radically alters the clinical picture, animals becoming severely ill, moribund, or dying over the time period studied. Histologically, all these animals show a persistence of unresolved hepato-renal necrosis. It is therefore clear that the cause both of the clinical illness and of the failure of hepato-renal regeneration is the presence of Ehrlich ascites tumour cells.

The following general hypothesis is offered as an explanation of the observed phenomena. The Ehrlich tumour is potentially toxic to the damaged liver and kidney, preventing the normal operation of the regenerative process. The normal kidney plays an important role in excretion and/or inactivation of the responsible toxin(s); renal damage caused by CCl₄ may, however, reasonably be expected to so impair renal function as to substantially decrease toxin excretion. Impaired toxin excretion leads to high circulating toxin levels which inhibit hepatic and renal regeneration, possibly by a direct action on one or more of the complex processes whereby organ integrity is restored. The toxic effect on the damaged kidney may thus be envisaged as ensuring a vicious circle whereby tumour toxin levels will be maintained at a high and rising level, and indeed, high levels of toxin may well contribute to irreversible illness by as yet unknown systemic effects quite apart from the demonstrable "regeneration-inhibition" effect. Thus it is believed that the conditions of this experiment have unmasked a hitherto undescribed facet of the inimical effect of tumour on the host; a key role for the kidney in the elimination of toxic tumour products is also strongly suggested. At least one tumour toxin, toxohormone, is known to be
present in the urine of the tumour-bearing host. 1)

Further support for the above general hypothesis is provided by the results of the second category of experiment. Here, rapid recovery of liver necrosis follows the administration of CCl₄/olive oil on day 5 of Ehrlich ascites tumour growth. In such animals, the well-established tumour comprises a relatively massive number of cells (3 \times 4 \times 10⁸ cells; Parry, unpublished observation) at the time of challenge. It is clear that under these experimental conditions, there is no effective toxicity of the tumour towards the damaged liver. However, in all such animals, the key finding that there is no evidence of resolving or of unresolved renal damage on histological examination, enables this apparently paradoxical lack of effect of the tumour for the damaged liver to be explained in terms of the proposed general hypothesis. Thus it is clear that part of the host adaptation to Ehrlich tumour is the acquisition by the kidney of a striking resistance to the necrotising effect of CCl₄. Such resistance, by allowing presumably normal renal function to be maintained, allows for adequate excretion of “regeneration-inhibiting” tumour toxins, and hence liver recovery is rapid. Such animals remain well clinically.

Previous work may also be quoted in support of this hypothesis. Ehrlich ascites tumour-bearing mice given CCl₄ subcutaneously on day 5 of tumour growth, regularly show a high regenerative mitotic activity 66 hr later. ³) A similar experiment carried out on day 12 of tumour growth²) provided a comparable result. In both situations, animals developed centrilobular hepatic necrosis, but renal necrosis (although the kidney was not examined histologically) is very unlikely to have occurred in view of the present results, and of the fact that CCl₄ given by the subcutaneous route, even in normal animals, produces relatively little renal necrosis (Parry, unpublished observation). In both these quoted situations²,³) the (regenerative) mitotic index, although high in tumour-bearing animals, did not reach the levels attained by regenerating hepatocytes of non-tumour-bearing control mice. One explanation for this is that even in the presence of normal kidneys, low levels of the proposed tumour toxin are circulating, exerting a small but detectable effect on the hepatic regenerative process.

The other interesting and consistent finding was the reduction in the amount of ascites 90 hr and 114 hr following CCl₄/olive oil treatment and transplantation 18 hr later of 1 \times 10⁸ Ehrlich tumour cells, as compared with the amount of ascites in olive oil-treated controls. Visual assessment alone indicated the differences, and it is clearly of interest to study this phenomenon in detail in future experiments, bearing in mind that when CCl₄ is given on day 5 of tumour growth, no discernible differences in the amount of tumour is seen as between such animals and olive oil-treated controls.

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REFERENCES


EXPLANATION OF PLATES

Photo 1. Liver of mouse 114 hr after CCl₄/olive oil and 90 hr after intraperitoneal saline. Recovery of the liver is almost complete. \( \times 120 \).

Photo 2. Kidney of mouse 114 hr after CCl₄/olive oil and 90 hr after intraperitoneal saline. Recovery from extensive renal tubular necrosis is occurring. \( \times 120 \).

Photo 3. Same preparation as Photo 2. The new flattened epithelial cell lining of renal tubules is clearly shown; much eosinophilic necrotic debris is still present in the lumen of recovering tubules. Mitotic figures are present. \( \times 480 \).
Photo 4. Liver of mouse 114 hr after CCl₄/olive oil and 90 hr after $1 \times 10^8$ Ehrlich tumour cells intraperitoneally. The appearance is that of totally unresolved centrilobular necrosis. This illustration may be contrasted with Photo 1. ×120.

Photo 5. Kidney 114 hr following CCl₄/olive oil and 90 hr after Ehrlich cells intraperitoneally. Extensive unresolved renal tubular necrosis. For comparison with Photo 2. ×120.

Photo 6. Same preparation as Photo 5. Surviving tubules (bottom and left) contrast with totally necrotised, debris-filled tubules in which there is no evidence of regeneration. Compare with Photo 3. ×480.

Photo 7. Kidney of mouse 114 hr after CCl₄/olive oil given on day 5 of Ehrlich tumour growth. Here there is no evidence of resolving or of unresolved renal tubular necrosis — the appearances are normal. ×120.

Photo 8. Liver of mouse 114 hr after CCl₄/olive oil given on day 5 of Ehrlich tumour growth. As in Photo 1, healing of centrilobular necrosis is well advanced. ×120.