LOCALISATION AND PATHOLOGY OF MORTIERELLA WOLFII TOXIN IN MICE

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PLATES XXII AND XXIII

Mucormycosis is an opportunistic fungal infection which usually occurs in the presence of lowered or altered host resistance. The fungi are saprophytic phycomycetes of which Mortierella wolfii appears to be of considerable importance. Since 1967 this species has been isolated with increasing frequency from cases of bovine mycotic abortion and pneumonia in New Zealand (Carter et al., 1973). A purified toxin isolated from this fungus caused death in mice 18–24 hr following intravenous injection (Davey, Smith and Kalmakoff, 1973). At autopsy the only consistent gross abnormalities noted were enlarged pale greyish kidneys. In a preliminary report, using a radioactively labelled toxin we have shown that the kidney was the site of action (Davey and Kalmakoff, 1973). In the present study the pathology of the kidney was studied using histological and radiological techniques.

MATERIALS AND METHODS

Toxin purification

The strain of Mortierella wolfii used and the methods of purification have been reported in a previous communication (Davey et al.). Briefly this involved gel filtration on Sephadex G.200 and G.50 and the final toxin preparation gave a single protein band when electrophoresed on polyacrylamide gels containing sodium dodecyl sulphate.

125I labelling of toxin

One mg of toxin was labelled with 125I (1 mCi; 14 mCi/μg Radiochemical Centre, Amersham) according to the chloramine T method (Hunter and Greenwood, 1962), and free 125I was removed by gel filtration through a Sephadex G.50 column.

Localisation of 125I labelled toxin mice

Mice 16 wk old were injected intravenously via a lateral tail vein with 1 toxic unit of the labelled toxin. One toxic unit represents 10 μg of protein and is equivalent to about 3LD50s of toxin (Davey et al.). At 2-hourly intervals pairs of mice were killed by disarticulation and the liver, kidneys, lungs, heart, spleen and brain dissected out. After excess blood was removed the organs were weighed and the 125I level counted in a Packard gamma counter. In a control experiment 125I treated by the chloramine T method was injected followed by an injection of unlabelled toxin, the conditions otherwise being the same as for the labelled toxin. Results were expressed as a percentage of the total radioactivity in the organs monitored.

Received 3 Aug. 1974; accepted 26 Sept. 1974.
Autoradiography

Autoradiographic studies were carried out on the mouse liver and kidney from the labelling experiment. Tissues were fixed in 10 per cent. neutral buffered formalin for 10–12 days and 3-μm sections were cut. Deparaffinised sections were coated with Ilford L4 liquid emulsion according to the method of Caro and van Tubergen (1962). The air dried slides were stored in a sealed container at 4°C for 12–16 days. Development was carried out with Kodak D-19 for 5 min., washed in 1 per cent. acetic acid and fixed in hypo solution for 10 min. The sections were then stained with haematoxylin and eosin. Included as controls were sections containing unlabelled toxin.

Histology

Pairs of mice were injected intravenously with 1 toxic unit and with 4 toxic units. Shortly before death at approximately 18 hr the mice were killed by chloroform inhalation and full necropsy was performed. Organs required for histology were fixed in Helly's fixative for 24 hr, embedded in Waterman's wax, cut at 3 μm and stained with haematoxylin and eosin.

Chemical pathology

Blood obtained by cardiac puncture was subjected to a variety of examinations. Haematocrit (PCV) was estimated by a standard micro-haematocrit method. A blood film was made and blood urea levels were estimated by a phenite-hypochlorite technique (Searcy et al., 1961). Protein estimation on urine samples were carried out using Labstix reagent strips (Ames Company).

RESULTS

Localisation of labelled toxin

In the control experiment using chloramine T-treated $^{125}$I, followed by injection of toxin there was only minimal and short-term localisation of label after 16–24 hr (fig. 1A). When $^{125}$I-labelled toxin was used (fig. 1B) localisation occurred in the liver and kidney, while the lungs, brain, heart and spleen showed no concentration of the toxin. The labelled toxin was localised in the kidney and liver as early as 2 hr following intravenous injection. At 4 hr labelled toxin in the kidney was demonstrable by autoradiography. Considerable localisation of label in the proximal tubules of the kidney could be demonstrated by autoradiography at 6 hr (figs. 2 and 3). In contrast similar sections of the liver showed the label to be present only in very limited numbers of Küpffer cells (fig. 4).

Histology

Before autopsy all animals had the external appearance of good health. The livers were pale with weights ranging from 1369 to 1582 mg and were possibly slightly heavier than normal. Spleen weights ranged from 104 to 186 mg, which suggests general enlargement of the organ. The kidneys were pale, with weights ranging from 371 to 679 mg. The thoracic viscera and blood films were essentially normal. Urine albumin levels were abnormally high from 100 to 300 mg per 100 ml, and blood urea levels were markedly elevated between the limits of 145 and 287 mg per 100 ml.

The characteristic feature of the nephrotoxic damage is that severe injury is inflicted on the proximal convoluted tubules. All the proximal tubules are
devoid of epithelial cells, and the cell contents are reduced to an eosinophilic granular mass (fig. 5) which distends the tubules. The integrity of the distal convoluted tubules, the loop of Henle and the collecting tubules is maintained although many contain eosinophilic amorphous casts. In some casts there are basophilic droplets which are possibly lipidic and represent nuclear breakdown residues (fig. 6). Because of the acute action of the Mortierella toxin, there is no apparent involvement of inflammatory cells. The histology of the liver appeared essentially normal.

DISCUSSION

In the present study the apparent sole site of action of *M. wolfii* toxin is the proximal convoluted tubules of the kidney. This is concluded from the localisation of the radioactive label and histology of kidney sections. A possible
explanation of the lack of demonstrable radioactive label in the liver is that it is more diffuse in this organ giving the Kupffer cells little time to ingest and concentrate the label before death of the animal.

Perhaps the most frequently quoted cause of nephrotoxic damage is mercuric chloride, ingested either accidentally or with suicidal intent by human beings. As most cases do not die until 4 to 30 days after taking the poison, post-mortem histology from such cases lacks the acuteness of the changes induced by mortierella toxin.

Treatment of mice with 1 and 4 units of mortierella toxin produced similar results. It was not possible to find an index of damage which consistently indicated that 4 toxic units of the toxin were more damaging than a single toxic unit. It would seem likely therefore that 1 toxic unit is sufficient to destroy all the proximal convoluted tubules and the recorded changes such as anaemia, azotemia and albuminuria are the sequela of such damage. Markedly elevated urea levels would suggest renal failure as the probable cause of death, although the exact mechanism remains obscure.

Toxic substances have been demonstrated in fungi pathogenic to animals and have been incriminated in the pathogenesis of a few mycoses. Crude endotoxins from the hyphae of Aspergillus fumigatus and Aspergillus flavus have been studied by Tilden et al. (1961) and the pathological effects of these toxins in mice show a similar picture for that of M. wolfii toxin. Such toxic substances liberated from degenerating fungal elements and diffusing into the tissues surrounding a mycelial mass, have been suggested as capable of producing the type of tissue response seen in aspergillosis (Gowing and Hamlin, 1960). It has also been suggested (Austwick, 1972) that under natural conditions in cattle, M. wolfii releases toxic and antigenic products during lysis of the hyphae in uterine and pulmonary lesions. We have also observed that following intravenous inoculation of M. wolfii spores in mice (unpublished results) a similar lysis of hyphae occurs in brain and kidney with death of mice in 4 days. In bovine mycotic placentitis caused by this fungus, release of the toxin could possibly contribute to necrosis of the placental tissue.

Apart from any economic significance of the infection with M. wolfii, the toxin appears to be an excellent model for studying renal damage.

**SUMMARY**

Using $^{125}$I labelled M. wolfii toxin the site of action in mice was shown to be the kidney. Autoradiographic studies revealed the label to be localised in the proximal convoluted tubules of the kidney, where there was a marked necrosis and degeneration of the epithelium causing the tubules to become considerably distended. Although the distal and collecting tubules maintained their integrity, many contained amorphous casts. An injected dose of 1 toxic unit (10 μg protein) was sufficient to produce damage to the kidney with subsequent anaemia, azotemia and albuminuria. Other organs appeared to be essentially normal and renal failure was the probable cause of death of mice.
FIG. 2.—A high-power photomicrograph of the kidney cortex of a mouse injected with 1 toxic unit of $^{125}$I labelled mortierella toxin and killed 6 hr after injection. The label is concentrated in an amorphous eosinophilic mass resulting from the destruction of the cells of the proximal convoluted tubules. Note the abnormal appearance of the nuclei adjacent to the eosinophilic material. Haematoxylin and eosin (HE). $\times 756$.

FIG. 3.—(As for fig. 2.) The label is concentrated in the amorphous eosinophilic cast-like material filling the distal proximal tubules. HE. $\times 756$.

FIG. 4.—A high-power photomicrograph of the liver of a mouse injected with 1 toxic unit of $^{125}$I labelled mortierella toxin and killed for examination 6 hr later. Note the presence of label in the Kupffer cells (arrows) and the absence of the labelled iodine from the liver parenchyma cells. HE. $\times 1000$. 
Fig. 5.—A lower-power photomicrograph of the kidney cortex of a mouse injected with 1 toxic unit of unlabelled mortierella toxin and killed for examination 18 hr later. Note the destruction of the proximal convoluted tubules and the presence of cast-like structures in the distal convoluted tubules. HE. ×248.

Fig. 6.—A view of the medulla of the kidney of a mouse injected with 1 toxic unit of unlabelled mortierella toxin and killed 18 hr later. Note the aggregation into droplets of haematoxyphilic material (arrows) which possibly represents nuclear breakdown products. HE. ×360.
REFERENCES


