Metronidazole neuropathy

Metronidazole is one of the 5-nitroimidazole group of compounds, several of which have found a wide range of applications in therapeutics. Metronidazole is well established as a protozoacide in trichomoniasis,[1] giardiasis, and amoebiasis; as a bactericidal agent in anaerobic infections[2-5]; and as a potentiating agent in radiotherapy.[6-7] Its use has been suggested for various other conditions including alcoholism,[8] Crohn's disease,[9] endocrine exophthalmos,[10] rheumatoid arthritis,[11] rosacea,[12] and acne.[13] Tanga *et al*[14] have evaluated metronidazole as an anti-inflammatory agent. Despite the fears prompted by animal experiments that metronidazole might be mutagenic or teratogenic,[1 15-17] it has proved remarkably safe in clinical practice.[1 17] Nevertheless, six cases of peripheral neuropathy with metronidazole have been reported,[2 9 18 19] and we describe here a further patient with peripheral neuropathy due to metronidazole. We also describe animal studies performed to investigate the mechanism of neurotoxicity.

Case report

This 33-year-old man had a 17-year history of histologically confirmed Crohn's disease. He had undergone three bowel resections (right hemicolectomy, resection of the ileocolonic anastomosis and part of the ileum with repair of a duodenocolic fistula, and a further ileocolic anastomosis resection). He had been treated intermittently with sulphasalazine and oral and rectal prednisone. Because of progressive weight loss and increasingly frequent bowel actions treatment was started with metronidazole 800 mg three times daily for five days, and thereafter 400 mg three times daily, in May 1975. At this time he was receiving Lomotil (diphenoxylate and atropine) 2–4 tablets/day, ferrous sulphate, and vitamin C and kaolin mixture. He received maintenance vitamin B_{12} injections every six weeks. His symptoms improved within six weeks, but after eight weeks he noticed that his feet felt hot. Six months after starting metronidazole he developed peripheral paraesthesiae starting in the feet and later spreading to the hands. Examination nine months after starting metronidazole showed a distal glove and stocking hypoalgesia and hypoaesthesia, with mild hyperalgesia above the sensory impairment. Other modalities of sensation, muscle power, and tendon reflexes were preserved. At this time the red cell folate (280 mg/l) and vitamin B_{12} (>1000 ng/l) concentrations were normal.

Electrophysiological investigations showed normal motor maximum conduction velocity in the median, ulnar, and lateral popliteal nerves (47·5, 50, and 39·5 m/s respectively), with terminal latencies at the upper limit of normal or slightly prolonged (3·8, 3·1, and 7·1 m/s). Sensory nerve action potentials in the median, ulnar, and sural nerves had normal conduction velocities (43·5, 42·0, and 53·0 m/s respectively), though the amplitudes were reduced (2·5, 2·5, and $<1\ \mu V$). These findings indicated a distal, mainly sensory, axonal neuropathy.

Sural nerve biopsy showed a loss of many myelinated fibres and axonal degeneration of some of the remaining fibres of all diameters. The total number of myelinated fibres was reduced to 2230/mm^2 (normal >6000/mm^2), though the fibre diameter histogram showed the normal bimodal distribution. A few clusters of myelinated fibres indicated axonal regeneration. Electron microscopy confirmed the axonal degeneration both of myelinated and unmyelinated fibres without special features. The density of unmyelinated fibres was at the lower limit of normal (22 500/mm^2), though the unmyelinated fibre diameter spectrum was shifted to the left, indicating regeneration after degeneration. The changes were those of chronic active axonal degeneration affecting all fibre sizes.

Metronidazole was stopped and seven months later all symptoms and signs of peripheral neuropathy had disappeared.

Animal experiments

Wistar white rats, 180 g body weight, were used to study the peripheral neurotoxic effects of metronidazole. Six rats received daily subcutaneous injections of metronidazole 180 mg/kg, and another six received 90 mg/kg. The metronidazole was administered as 0·5% solution in physiological saline. Four control animals received subcutaneous injections of similar volumes of saline. The administration was continued for up to 16 weeks. Histological sections were prepared from the cerebral hemispheres, the cerebellum, brain stem, cervical and lumbar spinal cord, dorsal root ganglia, sciatic nerve at thigh and ankle level, the extensor digitorum longus muscle, and the viscera. No degenerative changes were seen in the central or peripheral nervous system, including the intramuscular nerves.

Three rats receiving high doses of metronidazole, three receiving low doses, and three receiving saline were used to determine the rate of DNA and RNA synthesis in the brain, spinal cord, and dorsal root ganglia using one-hour pulse labelling with ^{3}H-thymidine and ^{14}C-uridine respectively. There was no significant difference between the uptake of radioactivity in metronidazole-treated and control animals. One previously untreated rat received an intraperitoneal injection of 500 μCi of ^{14}C-metronidazole (specific activity 27 mCi/g). RNA and DNA were separated from the brain, spinal cord, and dorsal root ganglia by standard biochemical techniques, and the total radioactivity in each fraction was determined by scintillation counting (see table).

Total ^{14}C bound to RNA and DNA of nervous tissue of rat after injection of ^{14}C-metronidazole. Results are expressed as disintegrations per minute (dpm) per g wet weight of tissue

	Brain	Spinal cord	Dorsal root ganglia
DNA	3 158	2 809	11 440
RNA	22 197	27 903	32 033

In the rat brain the concentrations of DNA and RNA are about equal.[20] If this relationship holds for other nervous tissue then the ratio between the uptake of radioactivity from metronidazole into RNA and that into DNA was about 7·0, 13·4, and 2·8 for brain, spinal cord, and dorsal root ganglia respectively.

Discussion

Metronidazole has been widely used to treat trichomonas vaginitis for many years without producing major side effects. Some of its new therapeutic indications, however, necessitate prolonged treatment with relatively high doses. Peripheral neuropathy is clearly one of the complications that can arise with such use. As argued by Coxon and Pallis,[19] it seems unlikely that the polyneuropathy in our patient was related to his underlying Crohn's disease. The condition is predominantly a distal sensory polyneuropathy, and investigation of our patient showed that the symptoms were due to a degeneration of the axons of fibres of all sizes. In animals some metronidazole binds to areas of the brain, though the major activity is in liver and kidney.[21] Metronidazole is to a large extent in its unchanged chemical state in the blood stream for the first eight hours after oral ingestion in animals.[22]

Rats fed metronidazole 600-800 mg/kg/day (about 25 times the maximum human dose) developed histological lesions in the cerebellum and brain stem accompanied by behavioural changes.[23] Our experiments with subcutaneous injections of doses approximating to those used in man showed no neurological degeneration or change in DNA or RNA synthesis. Significant amounts of radioactivity derived from metronidazole were, however, bound to RNA. This result is surprising since evidence indicates that the specific bactericidal and trichomonicidal effect of metronidazole, which is restricted to anaerobic micro-organisms, is probably due to reduction of the 5-nitro group on the molecule to a hydroxylamine group, which then binds to DNA, thus blocking cell division.[24] The highly reducing environment required for this conversion, though present in anaerobic organisms, is unlikely to be found in mammalian neurons. In this situation metronidazole or some metabolic product probably binds to RNA, inhibiting neuronal protein synthesis, and thus causing peripheral axonal degeneration.

The peripheral neuropathy is clinically relatively mild, and full recovery appears to occur on stopping the drug. It is important, however, that this side effect should be recognised, since pathological investigation shows a major degree of nerve degeneration occurring in such patients.

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1 Robinson, S C, and Mirchandani, G, *American Journal of Obstetrics and Gynecology*, 1965, **93**, 502.
2 Ingham, H R, *et al*, *Journal of Antimicrobial Chemotherapy*, 1975, **1**, 355.
3 Willis, A T, *et al*, *British Medical Journal*, 1976, **1**, 318.
4 Study Group, *Lancet*, 1974, **2**, 1040.
5 Ralph, E D, and Kirby, W M M, *Antimicrobial Agents and Chemotherapy*, 1975, **8**, 409.
6 Sutherland, R M, *Cancer Research*, 1974, **34**, 3501.
7 Adams, G E, *et al*, *Lancet*, 1976, **1**, 186.
8 Goodwin, D W, and Reinhard, J, *Quarterly Journal of Studies of Alcohol*, 1972, **33**, 734.
9 Ursing, B, and Kamme, C, *Lancet*, 1975, **1**, 775.
10 Harden, R M, *et al*, *Metabolism*, 1967, **16**, 890.
11 Wyburn-Mason, R, *Lancet*, 1976, **1**, 489.
12 Pye, R J, and Burton, J L, *Lancet*, 1976, **1**, 1211.
13 Carne, S, *Lancet*, 1976, **2**, 367.

[14] Tanga, M R, *et al*, *International Surgery*, 1975, **60**, 75.
[15] Voogd, C E, *et al*, *Mutation Research*, 1974, **26**, 483.
[16] Rustia, M, and Shubik, P, *Journal of the National Cancer Research Institute*, 1972, **48**, 721.
[17] Berget, A, and Weber, T, *Ugeskrift for Laeger*, 1972, **40**, 2085.
[18] Ramsay, I D, *British Medical Journal*, 1968, **4**, 706.
[19] Coxon, A, and Pallis, C A, *Journal of Neurology, Neurosurgery and Psychiatry*, 1976, **39**, 403.
[20] Long, C, editor, *Biochemist's Handbook*. London, Spon, 1961.
[21] Placidi, G F, *et al*, *Archives Internationales de Pharmacodynamie et de Thérapie*, 1970, **188**, 168.
[22] Ings, R M J, *et al*, *Xenobiotica*, 1975, **5**, 223.
[23] Rogulja, P V, *et al*, *Acta Neuropathologica*, 1973, **25**, 36.
[24] Ings, R M J, *et al*, *Biochemical Pharmacology*, 1974, **23**, 1421.

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SHORT REPORTS

Excessive alpha-fetoprotein concentrations in hepatocellular carcinoma

We describe a patient with hepatocellular carcinoma, whose serum concentration of α-fetoprotein exceeded 4 800 000 μg/l and produced an extra band on protein electrophoresis.

Case report

A boy aged 16 was admitted to Frimley Park Hospital, Surrey, on 18 February 1976 complaining of severe right shoulder pain and abdominal distension of about two weeks' duration. He had a massively enlarged liver with considerable abdominal distension. Laboratory investigations gave the following results (normal ranges in parentheses): haemoglobin 12·1 g/dl (13-18 g/dl); total protein 76 g/l (60-74 g/l); albumin 43 g/l (36-47 g/l); total bilirubin 43 μmol/l (2·5 mg/100 ml) (2-19 μmol/l (0·12-1·1 mg/100 ml)); alkaline phosphatase 1866 U/l (80-300 U/l); aspartate aminotransferase 476 U/l (0-40 U/l); γ-glutamyltransferase 792 U/l (0-45 U/l).

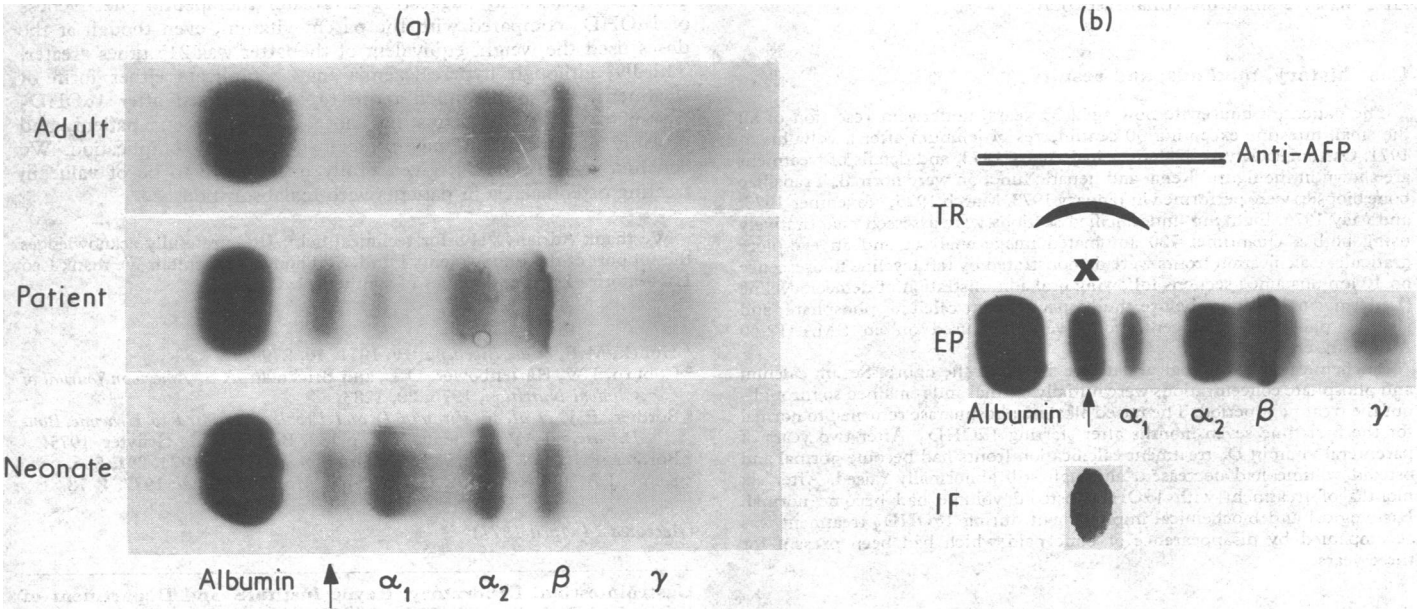
On 24 February laparoscopy showed blood-stained ascites and a grossly enlarged liver with multiple deposits in both lobes. Histological examination of a small biopsy specimen taken from a deposit confirmed the diagnosis of primary hepatocellular carcinoma. The serum concentration of α-fetoprotein (AFP) measured by radioimmunoassay was so abnormally high—4 800 000 μg/l (the normal range for healthy adults being < 25 μg/l (< 25 ng/ml))—that on serum protein electrophoresis on cellulose acetate it formed an extra band between albumin and α_1-globulin (see fig). The identification of the additional band was performed by the following techniques.

A transfer technique[1 2] in which the relevant band is cut out, placed on the surface of an agar plate, and left to diffuse against a specific antiserum applied to a strip of filter paper. The resulting precipitation arc may be visualised, stained, and localised.

A modification of an immunofixation technique using cellulose acetate for separation[3]—after electrophoresis a specific antiserum (in this case anti-AFP) is applied to the separation pattern on agar gel and left to diffuse. Then the agar gel is washed, the "unfixed proteins" removed, and the gel stained.

Both techniques confirmed that the additional band seen between the albumin and the α_1 fraction was α-fetoprotein.

Treatment with intravenous Aminosol and Intralipid was started on 27 February and continued until 6 March. The AFP concentration increased substantially during intravenous feeding, reaching a maximum value of 7 400 000 μg/l on 28 April. Despite treatment with *x*-irradiation to the liver area (300 rads), and subsequently a course of doxorubicin, he died on 7 July 1976.



Results of protein electrophoresis on cellulose acetate (*a*) showing presence of extra α-fetoprotein band (arrow) between albumin and α_1 band in patient's and neonate's serum, and absence of extra band in normal adult serum. Immunochemical confirmation of result (*b*) showing precipitation arc obtained with anti-α-fetoprotein (anti-AFP) serum using transfer technique (TR); electrophoretic pattern of patient's serum (EP); and precipitation band corresponding to additional fraction (X) obtained by immunofixation using anti-AFP serum (IF).