Brain damage after intrathecal methotrexate

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SYNOPSIS Ten brains from leukaemic patients given intrathecal methotrexate and 10 from leukaemic patients without intrathecal therapy have been examined. Three of the methotrexate treated patients appear to have died from their therapy. The histological changes consisted of destruction of oligodendrocytes, sometimes complete over large areas, and sometimes relatively slight. All the patients who survived long enough after treatment showed severe astrocytosis.

Lymphatic leukaemias and some malignant lymphomas which have been treated apparently successfully with antineoplastic drugs sometimes recur in the meninges where presumably the cells have been protected from the chemotherapeutic agents by the blood-brain barrier. In some centres it has become the practice to use prophylactic cranial radiotherapy and intrathecal methotrexate to try to prevent this. This paper

describes the neuropathological findings in 10 patients given this regime, five of whom had malignant meningitis, and in the other five it was given as prophylactic treatment. In order to eliminate changes due either to the leukaemia itself or to systemic chemotherapy, 10 control brains were examined in the same detail from patients who had leukaemia treated with antineoplastic drugs but without any intrathecal drugs or radiotherapy.

(Accepted 16 April 1975.)

TABLE
DETAILS OF 20 PATIENTS

	Sex	Age (yr)	Diagnosis		IT dose MX (mg)	IT dose CA (mg)	Dose radiation (rads)	Loss of oligodendrocytes	Gliosis	Malignant cells in brain post mortem
1	M	15	NHL	P	62.5	—	2400	+++	+	—
2	F	27	NHL	P	60	50	200	+++	—	—
3	M	19	ALL	T	120	—	2400	+	+++	—
4	M	8	NHL	P	50	90	2000	+	++	+
5	M	25	ALL	T	106½	500	400	+	+++	—
6	M	2	ALL	T	50	—	2400	+	+	+
7	M	43	NHL	T	60	—	2400	+	+++	—
8	M	54	NHL	P	60	—	2400	+	+++	—
9	M	17	NHL	P	62.5	—	2400	+	+++	—
10	M	55	ALL	T	25	250	—	—	—	—
Controls										
11	M	27	AML		—	—	—	—	—	—
12	M	13	AML		—	—	—	—	—	+
13	F	24	AML		—	—	—	—	—	+
14	M	6/12	ALL		—	—	—	—	—	—
15	M	60	AML		—	—	—	—	—	—
16	M	64	AML		—	—	—	—	—	—
17	M	67	AML		—	—	—	—	+	—
18	M	17	AML		—	—	—	—	—	—
19	M	4	AML		—	—	—	—	—	—
20	M	43	HCL		—	—	—	—	—	—

ALL: acute lymphatic leukaemia. AML: acute myeloid leukaemia. NHL: non-Hodgkin lymphoma. HCL: hairy cell leukaemia. T: treatment. P: prophylaxis. MX: methotrexate. CA: cytosine arabinoside. IT: intrathecal.

METHODS

The 20 patients studied are shown in the Table. All received a number of systemic drugs, usually in several courses. These drugs included prednisolone, vincristine, cytosine arabinoside, adriamycin, daunorubicin, mercaptopurine, methotrexate, hydroxyurea, thioguanine, asparaginase, azathioprine, and cyclophosphamide.

Radiotherapy was delivered to the midplane of the cranium, the usual dose being 2400 rads in 20 fractions over 28 days delivered by a radiocobalt unit or a 4MEV linear accelerator. Methotrexate was given intrathecally in doses of 10, 12 or 12½ mg at weekly intervals. Cytosine arabinoside was given in doses of 50 mg at weekly intervals. The total dosage is given in the Table.

The brains were suspended in formol saline for three weeks, and sliced coronally. Ten standard paraffin blocks and two frozen blocks were taken from each case. The paraffin sections were stained with

haematoxylin and eosin, periodic acid/Schiff, Holzer's method for astroglia, Holmes/Luxol fast blue, azure A, phosphotungstic acid haematoxylin, and Foot's reticulin. The frozen blocks were stained with Sudan 1V, Weil Davenport's method for microglia, and Cajal's gold sublimate method for astrocytes.

RESULTS

Three of the patients appear to have died from brain damage related to their treatment, two from malignant cerebral involvement, and the other five from complications related to marrow failure. The general postmortem findings showed the changes of the primary disease and, in every case, the bone marrow was hypoplastic due to drug therapy. In cases 4 and 6 the liver showed leukaemic infiltration, but no case showed evidence of drug-induced liver or kidney damage.

CASE 1

A boy of 15 years presented with abdominal pain six months before death. Laparotomy showed a tumour in the terminal ileum and mesenteric lymph nodes. Histology showed a lymphoblastic lymphoma. He became jaundiced and developed a large abdominal mass. He was treated with systemic chemotherapy and three months later he was clinically clear of the disease. He was given prophylactic cranial irradiation and intrathecal methotrexate. His CSF was normal at all times. The disease recurred a month later in the abdomen and the bone marrow also became involved. A few days before his death, his mental state deteriorated and he went into coma.

PATHOLOGY Examination of the brain showed some white matter swelling but no other abnormality. The histology of the white matter showed large areas of myelin pallor mainly deep in the white matter, sparing the U fibres, the corpus callosum, and the corticospinal fibres. In the pale areas most of the oligodendrocytes had disappeared, a number of ghosts remaining (Fig. 1). There was a gradation of change from areas where there was a reduction in the number of oligodendrocytes to areas where nearly all the nuclei were missing although blood vessels were intact. The myelin sheaths, though pale, could be seen to be intact, and there were no fat granule cells. There was a moderate astrocytosis away from the severely damaged areas but Weil Davenport stains showed no increase of microglia. The cortex showed no significant abnormality, the blood vessels were normal and there was no thrombosis. No malignant cells were seen.

FIG. 1 Case 1. A section of cerebral white matter. A few remaining oligodendrocytes can be seen, but most are represented only by ghosts. Azure A, $\times 500$.

FIG. 2 (left) *Case 2. A section of cerebral white matter, showing an area of localized oedema. Holmes/Luxol fast blue, $\times 200$.* FIG. 3 (right) *Case 2. A section of cerebral white matter. Some oligodendrocyte nuclei show fragmentation. Azure A, $\times 5\,000$.*

CASE 2

A woman aged 27 years presented with an influenza-like illness and had a neutropenia. A bone marrow aspiration showed malignant lymphoma. She was started on quadruple chemotherapy. Three days after admission she had a major epileptic fit and exudates were seen in the retina. Five specimens of CSF showed no malignant cells. A course of intrathecal methotrexate and cytosine arabinoside was given. Three days after the end of the course she became stuporose. At 6 pm the night before her death she had one dose of radiotherapy.

PATHOLOGY Examination of the brain showed some swelling of the white matter and pin-point haemorrhages, including one in the pyramids. Histology confirmed the presence of small haemorrhages in the

white matter and there were other small areas of localized oedema (Fig. 2) without evidence of myelin breakdown or fat granule cells. These were not seen to be perivascular. Large areas showed myelin pallor and in these areas nearly all the oligodendrocytes were lost as in case 1, and some showed fragmentation (Fig. 3). The remaining cells and blood vessels within the area were normal, so that it was a specific injury to the oligodendrocyte and not a general tissue necrosis. The cortex showed only non-specific abnormalities. There was no fibrinoid necrosis of blood vessels and no malignant meningitis.

CASE 3

A man aged 19 years had had acute lymphatic leukaemia for 18 months. A year after onset he developed malignant meningitis and received a course

FIG. 4 Case 3. A section of cerebral white matter showing an area of coagulative necrosis. Periodic acid/Schiff, $\times 200$.

FIG. 5 Case 5. A section of cerebral white matter, showing an increase in astrocytes. Cajal, $\times 200$.

of intrathecal methotrexate and cranial radiotherapy. Four months later it recurred and a further course of methotrexate was given into the cerebral ventricle through an Ommaya reservoir. Six weeks later he was readmitted with deteriorating consciousness and died in a few days. There were no malignant cells in the CSF.

PATHOLOGY Examination of the brain showed white matter oedema and some areas of discolouration. Histologically, there was no leukaemic meningitis and the cortex showed no significant abnormality. There were areas of coagulative necrosis of varying size in the white matter of both cerebral and cerebellar hemispheres (Fig. 4) and also areas of localized oedema as seen in case 2. There were a large number of these foci and none were seen to be perivascular. The brain away from the lesions showed severe diffuse astrocytosis with little glial fibre formation, but the lesions themselves showed no gliosis. The lesions may have been stages in the same process. There were

a few blood vessels showing fibrinoid necrosis but elsewhere they were normal.

CASES 4–9

These showed similar lesions and the pathology will be considered together. There was patchy loss of oligodendrocytes leaving relatively large areas of white matter devoid of nuclei, but no dying or abnormal nuclei were seen. The most striking feature was the severe astrocytosis (Fig. 5) with comparatively little fibre formation. This was somewhat variable in distribution. In case 9 it was mainly beneath the cortex, but in most of the others it could be found in varying severity throughout the central white matter of the cerebrum and cerebellum. There was sparing of the corpus callosum, the cortico-spinal pathway and the brain stem. Case 4 showed malignant invasion of the hypothalamus, but no malignant meningitis. Case 6 showed leukaemic meningitis with some extension in along the Virchow-Robin spaces.

Case 10, who had only two doses of methotrexate

just before death and no radiotherapy, showed very little abnormality.

DISCUSSION

The damage seen in these patients was confined to the white matter of the cerebrum and cerebellum. It consisted of a patchy destruction of oligodendroglial cells, which in two fatal cases was complete in some areas, and in seven others was much slighter but nevertheless enough to initiate a severe astrocytosis. Cases 2 and 10 did not show this astrocytosis, but both died within days of treatment and there may not have been sufficient time for this to occur. The mechanism of this astrocytosis is not clear, but it does not seem to be the usual repair process resulting from tissue damage as there is only slight fibre formation. If one accepts the unitary theory of neuroglia, it could be argued that these astrocytes were originally damaged oligodendrocytes.

Apart from the cases in both groups with malignant meningitis *post mortem* there were no cases of encephalitis, haemorrhage, or other known causes of cerebral pathology in leukaemia. Controls were used from leukaemic patients who had had anti-neoplastic drugs, but no intrathecal treatment and no cranial irradiation to try to eliminate changes which might be due to parenteral therapy by drugs, some of which are known to be neurotoxic or to non-metastatic effects of leukaemia. Malignant meningitis itself might have been a factor in cases 4 and 6, but there were no similar changes in cases 12, 14, and 19.

The patients examined all received intrathecal methotrexate with or without cranial radiotherapy or cytosine arabinoside. The latter was given to four patients but the changes were not significantly different from the others. Since no patient received cytosine arabinoside without methotrexate, it was not possible to assess the effects of this alone. The possible synergistic effect of radiation must be considered.

Freeman *et al.* (1973) have described a syndrome of somnolence occurring about six weeks after completion of cranial irradiation in children receiving prophylactic radiotherapy for acute lymphoblastic leukaemia. Some of their patients had intrathecal methotrexate as well, but the incidence of the syndrome was the same in the

two groups. Jones (1964) has also described transient radiation myelopathy after spinal cord irradiation. Acute destruction of some oligodendroglial cells with swelling might certainly account for temporary white matter disturbance.

Cranial radiotherapy has been in use for many years for treating brain tumours and doses many times that received by these patients have been given. The severe lesions involving complete loss of oligodendrocytes in large areas are unlikely to have been missed even if the clinical picture were confused by the primary lesion. When damage has been reported it has usually been delayed, occurring many years after conclusion of the treatment. This appears to be an entirely different lesion and may have a vascular basis.

There have been a number of reports of brain damage after methotrexate injection into the CSF pathways (Wilson and Norrell, 1969; Brennan *et al.*, 1972; Shapiro *et al.*, 1973; Norrell *et al.*, 1974). The drug has been given for brain tumours, often intraventricularly and in many cases there has been obstruction in the ventricular system. The main histological changes seen were areas of coagulative necrosis in the white matter as in case 3, although there was no ventricular obstruction in this case. If the drug is given intraventricularly it places it close to the central white matter. In case 3 the damage was severe, but it was also present in the white matter of the cerebellum. Rubin *et al.* (1968) have shown that, in dogs, tritiated methotrexate diffuses through the brain in a similar manner to inulin, and that *in vitro* it can enter the choroid plexus against the concentration gradient. Although the damage may be dose-dependent, what evidence there is suggests that it spreads diffusely throughout the nervous tissue even when given into the lumbar theca. In fact, if it did not do so, it would be ineffective as foci of perivascular malignancy deep in the brain are often seen *post mortem*.

Kay *et al.* (1972) described seven cases of encephalopathy after intrathecal methotrexate presenting mainly as confusion, dementia, and unsteadiness with EEG changes. One brain was examined histologically and showed lesions described as infarcts, and fibrinoid necrosis of blood vessel walls. They do not say whether there were fat granule cells or myelin breakdown. These lesions may be of a similar nature to those seen

in case 2, although there was no fibrinoid necrosis here.

It seems that methotrexate itself has a direct toxic effect on the oligodendrocyte. This cell does have a rather different metabolic make-up to the neurone which is unaffected. It is dependent largely on the pentose shunt for its energy and is low in Krebs cycle enzymes and cytochrome oxidase (Meyer and Meyer, 1964). This does not, however, explain why it should be more sensitive to folate antagonists. It is possible that the methotrexate remains bound to the cell, inhibiting further nucleic acid synthesis (Berlin, 1963). If such a cell sustained a chromosome fracture during radiotherapy, it might not be able to repair it as the repair process requires DNA-dependent RNA synthesis (Zeman, 1966). In this way, radiotherapy might exacerbate the damage, although it would not affect significantly the normal cell.

Intrathecal methotrexate may have two possible sequelae. One is severe brain damage, possibly fatal. If this occurs after a normal single course of treatment, it must be assumed that the patient has some metabolic abnormality which makes him unduly sensitive to folate deficiency. The second probably occurs in most cases and consists of patchy oligodendroglial loss with severe consecutive astrocytosis. This has no obvious clinical expression and in the context of a fatal disease, such as leukaemia, could be considered irrelevant. However, some of these children and young adults are surviving long periods and are possibly cured, therefore any brain damage is undesirable. Four of the patients described here received cytosine arabinoside intrathecally in addition to methotrexate. There is, so far, no evidence that this drug produces additional damage, but no brain was examined which had been in contact with this drug alone. Consideration might be given to using it instead of methotrexate for a trial period.

It is important that brains of patients who die and who have had intrathecal anti-neoplastic drugs should be examined in detail so that we can learn as quickly as possible how common and how extensive brain damage is.

I am grateful to Professor G. Hamilton Fairley for permission to quote from his case histories, and to Dr John Freeman for helpful criticism.

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