Methotrexate-Related Nonnecrotizing Multifocal Axonopathy Detected by β-Amyloid Precursor Protein Immunohistochemistry

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We describe a 64-year-old woman with biphenotypic leukemia involving the meninges who received 2 doses of intrathecal methotrexate. Soon after treatment, the patient developed postural rigidity and a marked decline in mental status. The patient died of respiratory failure 27 days after the initial dose of IT-MTX. Autopsy failed to reveal specific evidence of neurotoxicity until immunohistochemical staining for β-amyloid precursor protein, multifocal axonal injury was evident in the brain, spinal cord, and nerve roots. Our findings show that immunohistochemical staining for β-amyloid precursor protein can effectively demonstrate axonal injury associated with methotrexate neurotoxicity, even when conventional staining procedures are negative. This technique may therefore reveal a possible pathologic substrate for some of the neurologic complications seen in patients with methotrexate neurotoxicity.

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Intrathecal methotrexate (IT-MTX) has long been used as a chemotherapeutic agent in the treatment of leptomeningeal leukemia and carcinomatosis. Neurotoxicity in the form of disseminated necrotizing leukoencephalopathy, arachnoiditis, and multifocal axonopathy can result from intrathecal administration of the drug. More subtle neurologic side effects, such as intellectual decline or somnolence, may not have an obvious neuropathologic substrate. We describe a patient who developed seizures and a decline in mental status after receiving IT-MTX without irradiation. Autopsy failed to reveal specific evidence of neurotoxicity until immunohistochemical staining for β-amyloid precursor protein (β-APP) revealed multifocal axonal injury in the brain, spinal cord, and nerve roots.

REPORT OF A CASE

A 64-year-old woman with biphenotypic leukemia was admitted for administration of a mitoxantrone/etoposide regimen. Her history was significant for a left hemicolectomy 4 years previously, secondary to granulocytic sarcoma of the colon. The patient had previously received 2 cycles of vincristine and prednisone for her leukemia, but had persistent disease (20% blasts in bone marrow) after this induction therapy. Significant findings on admission included mildly clouded mentation, incontinence, and bilateral lower extremity weakness. A computed tomographic scan of the head, with and without contrast, showed no masses, bleeding, edema, or infarcts. Since a lumbar puncture revealed 20% blasts, consistent with a leukemic meningeal infiltrate, the patient was started on intrathecal preservative-free methotrexate. The first dose of methotrexate (12 mg) was administered via lumbar puncture. Four days later, a second 12-mg dose was administered intraoperatively upon placement of an Ommaya reservoir. Each dose was accompanied by a 50-mg dose of intrathecal hydrocortisone. At no time did the patient undergo cranial irradiation. A week after the second and final dose of IT-MTX, the patient was noted to have a generalized tonic-clonic seizure. A follow-up computed tomographic scan of the head was negative, and the patient was put on phenytoin. The patient became febrile and was found to be pancytopenic. She was then administered vancomycin and was given packed red blood cells and platelets intermittently. Throughout her hospital stay, the patient was vigorously wasting potassium and was treated with aggressive potassium replacement therapy. A week after her initial seizure episode, the patient developed postural rigidity and a marked decline in mental status. Computed tomographic scan of the head was unchanged, and electroencephalography showed diffuse θ/δ slowing, with no evidence of seizure activity. The patient died of respiratory failure 27 days after the initial dose of IT-MTX.

PATHOLOGIC FINDINGS

General autopsy findings were significant for candidal bronchopneumonia and splenitis, hepatic granulomas consistent with chronic transfusion therapy, and bilateral renal glomerulosclerosis. Neuropathologic examination grossly revealed an intrathecal catheter defect in the right frontal lobe with associated scant hemorrhage, patchy bilateral subdural hemorrhages, and a 0.2-cm meningioma in the right dura. No obvious necrotizing lesions, edema, or herniations were present. Microscopic examination of hematoxylin-eosin–stained slides revealed no meningeal or parenchymal leukemic infiltrates or evidence of infection anywhere in the brain, spinal cord, or nerve roots. Luxol fast blue stain showed no evidence of demyelination (Figures 1, A, and 2, A). Scattered chromatolytic anterior horn neurons were observed on hematoxylin-eosin–stained sections of the lumbar spinal cord. Immunostaining with glial fibrillary acidic protein (Dako Corporation, Carpinteria, Calif) showed diffuse subpial cortical and central white matter gliosis (Figure 1, B). An immunostain for phosphorylated

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Figure 1. Frontal lobe white matter. A, Myelination appears normal, and neither necrosis nor large axonal swellings are present (Luxol fast blue/ hematoxylin-eosin, original magnification ×50). B, Moderate reactive gliosis is present diffusely throughout the white matter (glial fibrillary acidic protein, original magnification ×50). C, Multifocal and confluent regions within the white matter are immunoreactive for β-amyloid precursor protein (β-APP) (original magnification ×10). D, Fragmented and beaded β-APP-positive axons are evident at higher power magnification (original magnification ×100).

Figure 2. Basis pontis. A, The basis pontis shows normal myelination, with no necrosis or large axonal swellings identified (Luxol fast blue/ hematoxylin-eosin, original magnification ×25). B, Axons within the transverse fiber tracts show segmental swellings, irregularity, and beading with β-APP immunostaining (original magnification ×100).
neurofilament (Dako) showed well-preserved axons. Immunostaining for β-APP (Dako) revealed scattered β-APP-positive segmental axonal fragmentation and beading (Figures 1, C and D, and 2, B) consistent with axonal injury. These changes were observed in a multifocal distribution involving the white matter of the cerebrum, cerebellum, brain stem, and spinal cord. There was also evidence of axonal injury in the lumbar anterior nerve roots with β-APP-positive axonal fragmentation and some macrophage infiltration.

No β-APP-positive axons were detected in sections of frontal lobe, basal ganglia, cerebellum, pons, and spinal cord from 4 randomly selected, aged-matched control cases.

COMMENT

Neurological complications caused by IT-MTX are fairly common, with recent estimates of the incidence of transient motor paralysis or seizures ranging up to 10% of patients receiving this therapy. The pathologic substrate for these symptoms is usually not evident, since most patients recover from episodes of acute MTX toxicity. Pathologic documentation of MTX neurotoxicity is relatively uncommon, representing about 1% of patients treated with IT-MTX. A confounding issue in these cases is the fact that many of these patients have also received cranial radiation therapy.

The most common neuropathologic abnormality associated with MTX is disseminated or multifocal necrotizing leukoencephalopathy. Other less frequently reported pathologic findings include diffuse parenchymatous degeneration with gliosis and axonal dystrophy, diffuse and focal subpial gray matter necrosis, mineralizing microangiopathy, dystrophic calcification, and axonopathy. Unlike most of these cases, our patient showed no obvious or specific neuropathologic changes on routine gross or microscopic examination. The relatively short time interval between the development of neurologic symptoms and death most likely allowed us to capture a very early and possibly reversible pathologic effect of acute MTX neurotoxicity in our patient. Since the general autopsy established that the candidal pneumonia and sepsis was the likely cause of death, we do not believe our patient died directly of an unusually severe and idiosyncratic reaction to the administration of IT-MTX.

The principal neuropathologic abnormality in our patient was the presence of axonal injury, detected only by β-APP immunohistochemistry, which was diffusely and evenly distributed throughout all regions of the brain and spinal cord. Since the autopsy showed no evidence of other possible causes of axonal damage, including leukemic infiltration or other focal changes such as tumor, meningitis, trauma, or infarction, and given that the patient did not receive central nervous system irradiation, we conclude that methotrexate toxicity was the likely cause of the axonal injury in our patient.

Shibutani et al. reported a case of methotrexate-related axonopathy with pathologic findings of multifocal axonal hydropic swelling on routine hematoxylin-eosin–stained slides. However, in that case, the patient received IT-MTX over 6 months, amounting to a total dose of 550 mg. Additionally, the patient received 40.8 Gy of cranial irradiation fractionated over 1 month. Radiation appeared to have had a synergistic effect on axonal damage in the setting of IT-MTX. Our patient received a total of only 24 mg of methotrexate and no irradiation. Axonal damage was evident only with β-APP immunostaining, suggesting that axonal injury may be a progressive process that begins early in IT-MTX therapy.

Although the pathogenesis of methotrexate neurotoxicity is poorly understood, animal studies suggest that the drug, by inhibiting folic acid metabolism, may have a direct toxic effect on axons. Methotrexate-induced disruption of anterograde axoplasmic flow may cause axonal accumulation of β-APP, a constituent neuronal transmembrane glycoprotein. This accumulation of β-APP is readily detectable by immunohistochemistry and may be associated with small swellings, beading, and segmental fragmentation of affected axons. Such changes may be subtle and often cannot be recognized on routine hematoxylin-eosin–stained stains or even with more specific stains for axons (eg, silver or neurofilament).

The application of β-APP immunohistochemistry in the forensic setting to demonstrate axonal shear injury associated with craniocerebral trauma is well-established, but it has also been used to identify axonal injury in a variety of nontraumatic conditions, including cerebral infarction, multiple sclerosis, and human immunodeficiency virus encephalitis. Our case shows that β-APP can be used to identify axonal injury in a toxic-metabolic condition affecting the central nervous system. This technique permitted identification of a multifocal axonopathy in the setting of IT-MTX therapy, which was undetectable by conventional histology and provided a possible neuropathologic substrate for the neurologic complications seen in this patient.

References