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Neurotoxic Potential of Gadodiamide after Injection into the Lateral Cerebral Ventricle of Rats

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PURPOSE: Results of a previous report showed that, if administered by intraventricular injection to access tissue normally protected by the blood-brain barrier, gadopentetate dimeglumine produced acute excitation, persistent ataxia, and widespread brain lesions in rats at 5- $\mu\text{mol/g}$ brain but not at 3.8- $\mu\text{mol/g}$ brain. The present study using gadodiamide was undertaken to see whether the effects were agent-specific.

METHODS: Rats, surgically prepared with a lateral ventricular cannula, were administered a slow injection at 2 $\mu\text{L}/\text{min}$ of gadodiamide into the lateral ventricle, and behavioral and neuropathologic changes were noted.

RESULTS: Both gadodiamide and gadopentetate dimeglumine produced focal and generalized myoclonus over several hours. Gadodiamide did not produce the medium-term tremor or persistent ataxia seen after treatment with gadopentetate dimeglumine. Neuropathologic changes developed over 1 to 3 days and took three distinct forms: vacuolated thalamic lesions closely resembling those produced by gadopentetate dimeglumine; small but similar vacuolated symmetrical caudate lesions not produced by gadopentetate dimeglumine; and severe swelling and astrocytic hypertrophy and hyperplasia in the cerebellar vermis, again not produced by gadopentetate dimeglumine. Unlike gadopentetate dimeglumine, gadodiamide produced no spinal cord lesions. The cerebellar changes were seen at 1.25- $\mu\text{mol/g}$ brain and above, behavioral changes at 2.5- $\mu\text{mol/g}$ brain and above, and thalamic and caudate lesions at 10- $\mu\text{mol/g}$ brain, the maximal dose used. Markedly reducing the rate of injecting the same volume over 28 hours prevented the acute excitation but did not reduce the severity of the morphologic effects.

CONCLUSION: The acute excitatory effects of high intraventricular doses of gadopentetate dimeglumine and gadodiamide are similar and appear to be attributable to local action at the infusion site, but differences exist between the two agents in the character and topography of the distant morphologic changes. The cerebellum was the brain area most sensitive to gadodiamide in this experimental model. It is unlikely that gadodiamide would gain access to the brain at these tissue doses when used intravenously for conventional clinical imaging, but our experimental model suggested that it had some unexpectedly specific neuropathologic potential.

A number of pharmacologic compounds are used clinically in conditions in which the permeability of

the blood-brain barrier is increased, notably agents for brain imaging and for antibiotic and anticancer purposes. These agents, therefore, gain access to brain tissue to an extent not possible either in healthy individuals or in healthy experimental animals used for toxicity testing. Consequently, several experimental methods have been used in attempts to assess the direct neurotoxic potential of such agents, notably osmotic opening of the blood-brain barrier (1), intrathecal dosing (2, 3), and in vitro systems using brain sections (4) or cell cultures (5). Extensive clinical use has shown modern MR imaging agents to be well tolerated in their many applications (6, 7), including use in a large number of patients with a compromised blood-brain barrier. Previous investigations, however, have reported that gadopentetate dimeglumine may

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cause neurotoxic effects at high doses in some animal test systems (1–3, 8). In addition, the limited dose range of MR imaging agents in clinical use provides little scope for estimating a safety margin and, because many patients have preexisting neurologic diseases, adverse effects of mild severity may be difficult to recognize. The purpose of this study was to extend previous observations in animal test systems by looking at a second agent, gadodiamide, to determine whether the previously reported neurotoxic effects were agent-specific.

As our experimental model we chose intrathecal administration to rats by the lateral ventricle. This system has the advantages of minimizing traumatic artifacts, causing no vascular pathologic changes, and potentially allowing access of the compound to the entire intact CNS (8). Disadvantages, however, include the difficulty of estimating true tissue dose and the high transient concentrations reached in the CSF during dosing. We compared both functional and morphologic indexes in our animals after injection of comparable doses of two formulations of gadodiamide (Omniscan, Nycomed, Norway), gadopentetate dimeglumine (Magnevist, Schering, Germany), or isoosmotic control solution. In addition, we attempted to control for potential artifacts that might have been induced by high transient concentrations in CSF by the use of very low infusion rates of gadodiamide over extended periods.

Methods

Intraventricular Injections

The experimental protocol used was essentially the same as that previously described (8), the agents being injected directly into the lateral ventricle of adult male F334 strain rats via a 30-gauge needle temporarily inserted into the ventricle using a guide tube implanted 7 to 10 days previously under isoflurane anesthesia and firmly fixed to the skull.

The major difference was the more extensive use of anesthetic to control the acute excitation seen shortly after injection of gadodiamide. Early in this study, acute excitatory signs were noted to be possibly fatal unless controlled in this way. With the animal placed in a restraining hammock, isoflurane anesthetic was administered via a face mask at a concentration of 0.5% to 2% in a 1:2 mixture of oxygen and nitrous oxide. The concentration of isoflurane used was the minimum needed to prevent generalized motor signs. The time course of excitation was followed during anesthesia by noting minor signs, such as head movements and jerky respiration. Careful note was made of the anesthetic concentration/time profile, so that this could be reproduced in control animals (which did not themselves need anesthetic to control hyperexcitation). The longest duration of anesthesia was 160 minutes, and at no time was its depth enough to impair ventilation by CNS depression.

Solutions

Gadodiamide/caldiamide clinical injection formulation (287 mg/mL gadodiamide [0.5 mol/L] + 12 mg/mL caldiamide [0.026 mol/L]) (Nycomed AS, Oslo, Norway) was injected at a concentration of 0.5 mol/L. Caldiamide (the calcium chelate) is present in this formulation to provide a sufficient excess of chelator to remove any free gadolinium that may be present. In the experimental groups IV through VI (see below), we studied the effect of variation in injectate composition in several ways. The original clinical gadodiamide/caldiamide formulation

was diluted to be isotonic with plasma; the caldiamide and gadodiamide components were tested separately (each brought up to the same osmotic strength as in the mixed formulation by adding sucrose); and, finally, osmotic control solutions of sucrose only were composed to match both the gadodiamide/caldiamide and gadopentetate dimeglumine formulations.

Dosages are presented here as $\mu\text{mol/g}$ brain. Previous work has indicated that brain weight in adult male F334 rats is close to 2 g, and this weight has been assumed for all animals. As the agents were injected as 0.5-mol/L solutions (or equivalent), injection volumes were all 2 $\mu\text{L}/\mu\text{mol}$, except in the three formulation studies in which isotonic gadodiamide was injected.

Animals and Injection Protocols

Eighty-one F334 male rats were used in the groups described below, and all intraventricular injections were administered at the rate of 2 $\mu\text{L}/\text{min}$ except in the prolonged slow infusion study (group IV). Specific pathogen-free rats (Harlan Olac, Bicester, UK) were acclimatized for 2 weeks before first use at 6 to 12 weeks old (180–250 g). All animals were housed singly in plastic boxes with white softwood sawdust bedding (Betabed "Research Grade"; Datesand Ltd, Manchester, UK) and maintained on a 12-hour light/dark cycle at an ambient temperature of $21^\circ\text{C} \pm 2$ and a relative humidity of $55\% \pm 10$. Animals were allowed free access to R&M No. 1 maintenance diet (SDS Ltd, Witham, Essex, UK) and filtered tap water. All procedures conformed with the ethical guidelines of the United Kingdom Home Office Regulations for Experimentation on Laboratory Animals.

Animals were divided into the following eight experimental groups (see summary in Table 1): *Group I*, dose-response study. *Group II*, lesion time course study. *Group III*, gadodiamide tonicity study (the gadodiamide/caldiamide formulation diluted to isotonicity, 300 mOsm/kg, administering an injection volume of 13.2 μL rather than 5 μL). *Group IV*, slow infusion studies; the gadodiamide/caldiamide formulation, diluted to isotonicity as above, was administered as a slow infusion to conscious rats over either 28 hours at a rate of 0.94 $\mu\text{L}/\text{hour}$ (total volume, 26 μL) or over 112 hours (total volume, 105 μL), in both cases using a subcutaneously implanted osmotic minipump (2ML1, Alza Corp, Palo Alto, CA) connected to an implanted indwelling lateral ventricle cannula similar to that used for the acute infusion studies. At the end of the infusion, the cannula was closed, the infusion pump removed under isoflurane anesthesia, and the rats allowed to survive a further 14 days before they were killed and processed for light microscopy. *Group V*, pure gadodiamide study; 286.9 mg of gadodiamide (WIN50678) and 16.74 mg of sucrose were dissolved per milliliter of water. *Group VI*, caldiamide study; 12 mg of caldiamide (WIN 59087, 99.5% purity [Nycomed AS]) and 206.1 mg of sucrose were dissolved per milliliter of water. *Group VII*, sucrose osmotic control study with sucrose at 12.65- $\mu\text{mol/g}$ brain in 40 μL (isoosmotic to gadodiamide/caldiamide) or at 24.4- $\mu\text{mol/g}$ brain in 40 μL (isoosmotic to gadopentetate dimeglumine). *Group VIII*, gadopentetate dimeglumine; each solution was filtered through a sterile 0.2- μm Minisart SM16534 cartridge (Sartorius, UK) just before injection.

Animals were kept under continuous active observation for 6 hours after dosing and thereafter checked daily. All signs of poisoning were noted and assessed by two experienced observers and scored as previously described (8). When transient excitatory effects became life-threatening or distressful, isoflurane was used at a concentration and duration sufficient to control them (as indicated in Table 1).

Histologic Processing

Of the 81 rats used in this study, 74 underwent pathologic examination. Of the remainder, three were lost from the higher-dose gadodiamide groups because of acute toxicity (two

TABLE 1: Experimental treatment groups

Group	Treatment	Dose ($\mu\text{mol/g}$ Brain)	Survival (days)	No.	Anesthesia	
I	Gadodiamide/caldiamide dose/response	0.25	14	4	None	
		0.5	14	4	None	
		1.25	14	4	None	
		2.5	14	6	None	
		3.75	14	4	None	
		5.0	14	4	1/5	
		10.0	14	7*	All	
II	Gadodiamide/caldiamide time course	Light and electron microscope	10.0	1	2† and 2	All
			10.0	3	2 and 2	All
			10.0	7	2 and 2	All
			10.0	14	2	All
III	Gadodiamide/caldiamide diluted to 300 mOsm/kg	1.25	14	4	None	
IV	Gadodiamide/caldiamide slow infusion	Over 28 h	2.5	14	2	None
			10.0	14	2	None
			10.0	14	4	All
VI	Caldiamide (pure agent)	0.5‡	14	4	All	
VI	Caldiamide (pure agent)	0.5‡	14	4	None	
			14	4	None	
VII	Sucrose osmotic controls	12.7§	14	4	None	
			24.4	14	2	None
VIII	Gadopentetate dimeglumine	10.0	14	4	All	
		10.0	14	4	None	

* Two animals lost because of acute toxicity.

† One animal lost because of acute toxicity.

‡ Equivalent to the caldiamide content of 10 $\mu\text{mol/g}$ brain gadodiamide/caldiamide formulation.

§ Isoosmotic to gadodiamide formulation.

|| Isoosmotic to gadopentetate dimeglumine formulation.

from group I and one from group II). Brains from four of the caldiamide group (group VI) were retained but not sectioned, because of an absence of lesions in the other four animals.

For light microscopy, the rats were killed by perfusion of 10% formalin, 2% acetic acid fixative via the ascending aorta under deep halothane anesthesia, as previously described (8). Step-serial 10- μm sections of paraffin-embedded brain contained in five coronal blocks from all animals were stained with hematoxylin-eosin (H and E). Sections from the 48 gadodiamide and caldiamide animals were stained immunocytochemically for glial fibrillary acid protein (GFAP) and, in some cases, with ED1 antibody for activated microglial cells.

The sections were examined by two observers, neither of whom was aware of the coding system. A 16-point scoring system was devised to indicate the severity of the cerebellar changes, as follows: 0–1 for granule cell layer edema, 0–2 for white matter edema, 0–4 for GFAP-positive reactive astrocytes, 0–4 for H and E-stained reactive astrocytes, 0–3 for astrocytic mitotic frequency, and 0–2 for granule cell layer thinning and nuclear pyknosis. The individual scores were then totaled.

For electron microscopy, after perfusion fixation with 6.5% glutaraldehyde in 0.14-mol/L cacodylate buffer (pH 7.2) and embedding in plastic (Epon-araldite), semithin (1 μm) sections were stained with 0.1% toluidine blue. Thin sections were stained with lead citrate and uranyl acetate and examined with a JEOL (UK) electron microscope with an EDAX X-ray analysis facility.

Results

Functional Changes (Table 2)

Episodic focal or generalized myoclonus was seen in all rats administered gadodiamide or gadodiamide/

caldiamide at 2.5- $\mu\text{mol/g}$ brain or greater. Episodes of myoclonus began 8 to 18 minutes after the onset of injection, and lasted for 1 to 153 minutes. Some incoordination in walking was seen for several hours after injection, but no persisting ataxia was seen at any dose. Caldiamide produced no motor effects. No animals administered slow infusions of gadodiamide (group IV) showed signs of hyperexcitation at any time, although, with 2.5- $\mu\text{mol/g}$ brain, both rats became minimally ataxic on day 15, whereas in both rats administered 10- $\mu\text{mol/g}$ brain, ataxia developed on day 4. In the latter case, this developed into a high-stepping gait by day 6, with truncal ataxia by day 13.

Gadopentetate dimeglumine caused both focal and generalized seizures, transient circling, and also produced marked ataxia at 24 hours exactly, as previously described (8). Those that were not anesthetized showed only moderate degrees of ataxia. However, in the four rats who were maintained under anesthesia for 2 hours after the onset of injection, so as to match the gadodiamide/caldiamide group, a motor impairment developed that became so severe that they were unable to walk. These animals were killed for humane reasons after only 24 hours.

The rats administered sucrose osmotic control solution (group VII) showed no behavioral changes at any time.

TABLE 2: Prevalence of functional disturbances after intraventricular injection of gadodiamide, caldiameter, and gadopentetate dimeglumine

Agent	$\mu\text{mol/g Brain}$	Focal Seizures	Generalized Seizures	Ataxia at 24 h
Gadodiamide/caldiameter (groups I and II)	1.25	0/20	0/20	0/20
	2.5	0/6	1/6	0/6
	3.75	4/4	1/4	0/4
	5	4/4	4/4	0/4
	10	13/21	7/21	0/21
Gadodiamide/caldiameter infusion (group IV)	2.5	0/2	0/2	0/2
	10	0/2	0/2	0/2
Gadodiamide (group V)	10	4/4	4/4	0/4
Caldiameter (group VI)	10*	0/8	0/8	0/8
Gadopentetate dimeglumine (group VIII)	10	7/8	3/8	8/8

* Dose equivalent to that contained in 10 $\mu\text{mol/g}$ brain gadodiamide/caldiameter formulation.

Neuropathologic Changes

The lesions seen in the animals administered gadopentetate dimeglumine (group VIII) were similar to those previously described (8), with focal glial necrosis and demyelination within the thalamus, brain stem, and spinal cord, and associated neuronal loss in the more severe lesions. However, both the brain stem and spinal lesions were markedly more severe in the four animals in the anesthesia subgroup than in those in the conscious animals used for the present study or in those in previously reported studies (8). Three types of morphologic changes were seen after injection of gadodiamide:

1. Small symmetrical vacuolated foci of acute neuronal degeneration and increased cellularity mainly of microglia in the ventrolateral and sometimes ventromedial nuclei of the thalamus. These were somewhat smaller than those seen with gadopentetate dimeglumine (group VIII). At 14 days' survival, these lesions (Fig 1) appeared to be more recent than those in the caudate nuclei (see below) but were associated with considerable astroglial hypertrophy as shown by GFAP immunostaining. This change closely resem-

bled the thalamic lesions produced by gadopentetate dimeglumine (8).

2. Small cellular symmetrical lesions in the caudate/putamen (Fig 2) also closely similar in nature to the thalamic lesions of gadopentetate dimeglumine, with astroglial hypertrophy, incursion of microglia, and abundant fine eosinophilic PAS-positive-staining granules in the neuropil (8). These lesions primarily affected the areas of gray matter, sparing the fiber tracts characteristic of the corpus striatum.

3. Severe astrocytic hypertrophy in the granule cell layer of the cerebellum that spread into the underlying white matter. This was contained within the vermis and paravermal regions of the cerebellum (Fig 3), so that the extreme lateral parts and the floccular-nodular lobes were generally not affected. With routine stains, the granule cells were separated by swollen astroglial processes not visible in control animals (Fig 4). There was no indication in the early phases of this lesion that neurons were undergoing degenerative changes, which was confirmed by electron microscopy. Some granule cell loss, as shown by scattered pyknotic nuclei, was seen at later times in the more

FIG 1. Lesion produced by gadodiamide in the ventrolateral thalamus, stained with H and E at 14 days' survival. The lesion (*center field*) is characterized by prominent vacuolation, microglial infiltration, and many fine eosinophilic granules. Gadodiamide was administered at 10- $\mu\text{mol/g}$ brain (*calibration bar*, 200 μm).

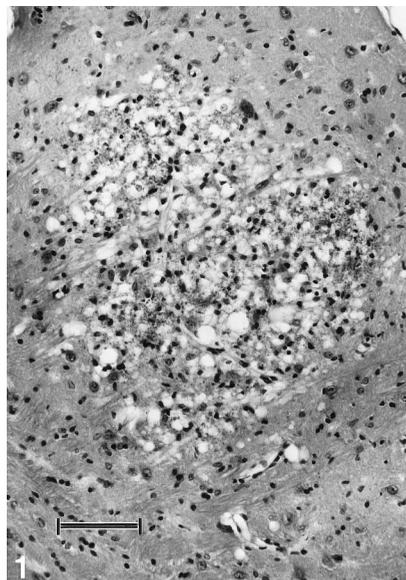


FIG 2. Lesion produced by gadodiamide in the caudate nucleus, stained with H and E at 14 days' survival. The area of pallor in the caudate/putamen is indicated by *arrows*. Gadodiamide was administered at 10- $\mu\text{mol/g}$ brain (*calibration bar*, 1 mm).



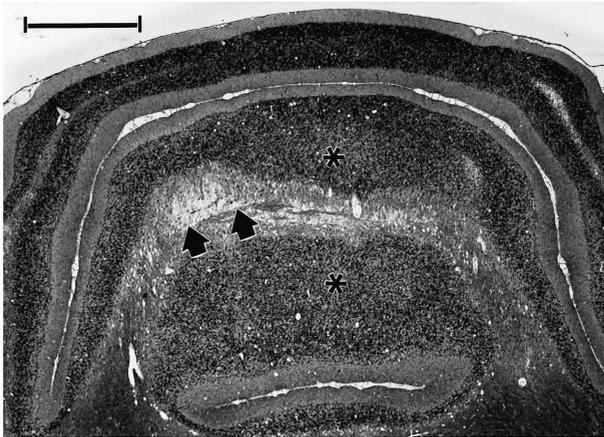


FIG 3. Lesion produced by gadodiamide in the cerebellar vermis, stained with H and E at 14 days' survival, showing decreased density of the granule cell layer (asterisk) and vacuolation of the adjacent white matter (arrow). Gadodiamide was administered at 10- μ mol/g brain (calibration bar, 1 mm).

severe lesions. Purkinje cells were not involved, although, in the more severe cases, the Bergmann glia showed hypertrophic changes (Fig 4B).

The evolution of the cerebellar lesion was best seen in semithin sections stained with toluidine blue. At 24 hours after intraventricular injection, the cerebellar cortex appeared to be almost normal except for a rare mitotic astrocyte, but, ultrastructurally, in the processes of Bergmann astrocytes, especially those close to the subpial surface, numerous small electron-dense multilamellar bodies were seen. These were not present deeper in the cortex at this time. By day 3, multilamellar bodies were more numerous in Bergmann glial processes and throughout the molecular layer, as well as within astrocytes in the granule cell layer (Fig 5). Considerably hypertrophic, and occasionally mitotic, astrocytes were now readily visible in both semithin and paraffin sections (Fig 6) with increased GFAP immunoreactivity. No apparent ultrastructural changes took place in the extracellular

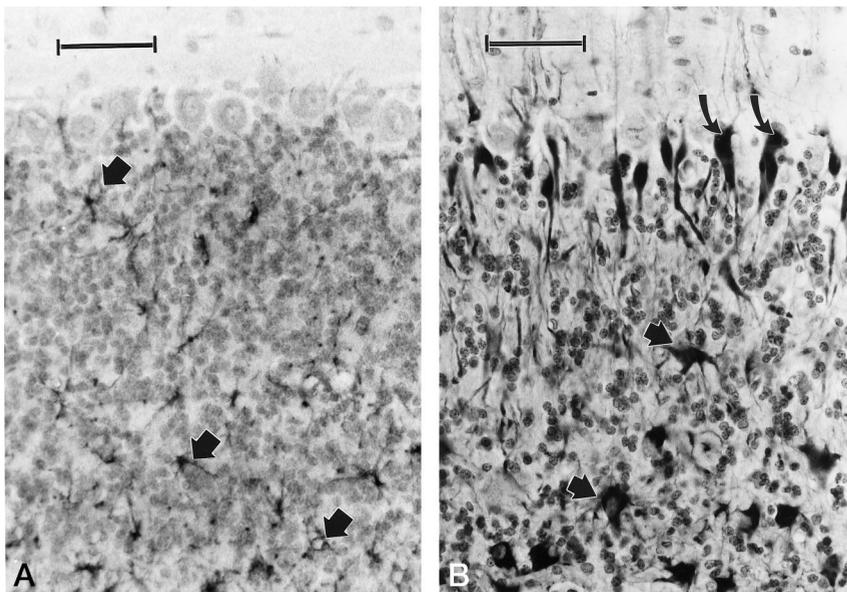


FIG 4. Glial activation in the cerebellar vermis. Both sections were stained with GFAP antibody and counterstained with Mayer's hematoxylin (calibration bars, 50 μ m).

A, Low basal level of GFAP expression in normal astrocytes (arrows) from a sucrose-injected control animal at 14 days' survival.

B, Bergmann glial (curved arrows) and astrocytic (straight arrows) hypertrophy and increased GFAP expression in a 10- μ mol/g gadodiamide-injected rat with 14 days' survival.

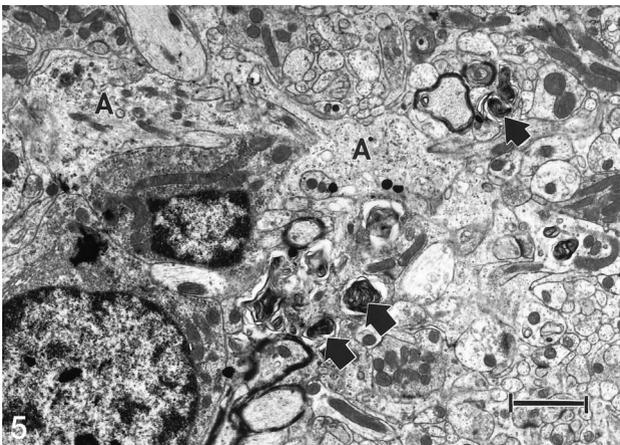


FIG 5. Electron micrograph shows a gadodiamide lesion in the Purkinje cell layer of the cerebellum. Expanded astrocytic processes (A) with multilamellar bodies (arrow). Gadodiamide at 10- μ mol/g brain with 3 days' survival (calibration bar, 2 μ m).

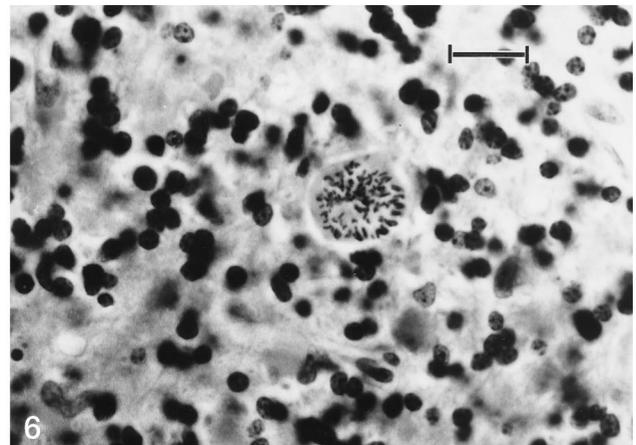


FIG 6. Abnormally expanded mitotic figure (center field) in an astrocyte from the granule cell layer of the cerebellar vermis. Gadodiamide was administered at 10- μ mol/g brain with 14 days' survival, stained with H and E (calibration bar, 20 μ m).

TABLE 3: Summary of pathologic changes in various brain regions after intraventricular injections of the agents administered at various doses

Agent	Dose ($\mu\text{mol/g}$ Brain)	Cerebellum*	Striatum	Thalamus	Brain Stem	Spinal Cord
Gadodiamide/caldiamide (groups I, II, and III)	0.25	0/4 (0)	NE	NE	NE	NE
	0.5	0/4 (0)	NE	NE	NE	NE
	1.25	7/8 (5.3)	NE	NE	NE	NE
	2.5	6/6 (11.3)	NE	NE	NE	NE
	3.75	4/4 (11.5)	NE	NE	NE	NE
	5	4/4 (13.5)	0/4	0/4	0/4	0/4
Gadodiamide/caldiamide slow infusion (group IV)	2.5	17/18 (15.4)	4/4	4/4	0/4	0/4
	10	2/2 (15.5)	0/2	2/2	0/2	0/2
Gadodiamide (group V)	10	2/2 (14)	0/2	2/2	0/2	0/2
Caldiamide (group VI)	10	4/4 (15.0)	0/4	0/4	0/4	0/4
Gadopentetate dimeglumine (group VIII)	$\equiv 10^\dagger$	0/4 (0)	0/4	0/4	0/4	0/4
Sucrose (group VII)	10	0/8 (0)	0/8	0/8	8/8	7/8
	$\equiv 10^\dagger$	0/6 (0)	0/6	0/6	0/6	0/6

Note.—NE indicates not examined.

* Prevalence and mean damage score, 16. For a description of cerebellar damage score, see text.

\dagger Dose equivalent to that contained in 10- $\mu\text{mol/g}$ brain gadodiamide/caldiamide formulation.

spaces, although astrocytes were already showing increased glycogen, mitochondria, and other organelles characteristic of cell hypertrophy. These also contained many small electron-dense multilamellar bodies (Fig 5).

By day 7, astroglial changes were more marked, and an occasional axon with swollen myelin sheaths was visible in the granule cell layer and the nearby underlying white matter fibers. By this time, some pyknosis of granule cells and an apparent thinning of the granule cell layer was seen, but this was clearly a late effect. The astrocytes were even more markedly swollen at this stage, with greatly enlarged nuclei and occasional mitoses with prominent widely spaced chromosomes. The glomeruli of the mossy fiber endings appeared to be normal, although surrounded by swollen astroglial processes. By day 14, astrocytic changes were even more pronounced, with edematous expansion of their processes between the other cells, including those in the Purkinje cell layer and numerous greatly enlarged nuclei and mitotic figures (Fig 6). Purkinje cell dendrites and Bergmann glia in semithin sections were substantially swollen and more pronounced than usual at 7 and 14 days' survival, but no Purkinje cell degeneration was seen. There appeared to be virtually no microglial reaction in the evolution of this lesion, most probably because of the largely intraastrocytic nature of this reactive process and the absence of neuronal death in all but the most severe lesions.

An important finding was the lack of damage to the spinal cord, the superior olivary nuclei, and the other brain stem centers so conspicuously affected by gadopentetate dimeglumine (8). An additional effect was seen in just one animal in group I, which also showed bilateral damage with marked incursion of microglial cells in the medial part of the hippocampal H₁ sector. This change probably arose because this animal was the first in which we attempted to control seizures with isoflurane, and it had undergone six tonic seizures, lasting 1 to 15 seconds before control was

established. All subsequent animals experienced only focal seizures.

Dose-Response Relationships

The data in Table 3 show an abrupt dose-response effect, with a no-effect level below a dose of 1.25- $\mu\text{mol/g}$ brain, and an almost maximal effect at 10- $\mu\text{mol/g}$ brain. Even at this maximal dose, the purely astrocytic lesion seen in the cerebellum did not occur elsewhere in the brain. At the lowest neuropathic dose, obvious granule cell layer swelling was seen with reactive astrocytes, but minimal granule cell layer thinning or white matter edema was observed. Unambiguous granule cell loss was present only at the 10- $\mu\text{mol/g}$ brain dose and in the slow infusion study. The threshold for detection of astrocytic reactivity was not influenced by the use of GFAP staining, except to render it easier to identify.

Animals administered gadodiamide diluted to isotonicity (group III) showed the same cerebellar lesions as those administered the undiluted agent; the mean lesion score was 5.0, compared with 5.5. The scores of these two sets of four animals were therefore combined in Table 3.

Formulation Studies

Slow infusions (group IV).—Very similar cerebellar changes to acute injection of 10- $\mu\text{mol/g}$ brain was produced by 2.5- $\mu\text{mol/g}$ brain, but infusion of 10- $\mu\text{mol/g}$ brain caused the most severe cerebellar changes seen in any group. Despite a more obvious loss of granule cells and extension into the lateral lobes and paraflocculi, Purkinje, stellate, and basket cells were still spared, and minimal microglial activation was seen. These animals also had ventrolateral thalamic lesions closely similar to those seen in other groups, but no brain stem or spinal cord lesions.

Gadodiamide alone (group V).—Gadodiamide itself produced lesions in the central cerebellar regions

TABLE 4: Summary of lesion topography after gadodiamide and gadopentetate dimeglumine

Site	Corpus Striatum	Thalamus	Cerebellum	Brain Stem	Spinal Cord
Gadodiamide + caldiamide	+	+	++		
Gadopentetate dimeglumine		(+)		++	++

Note.—+ indicates small and focal; ++, larger and more severe; (+), only at ≥ 21 days.

resembling in character and severity those produced by the gadodiamide formulation, but not others.

Caldiamide alone (group VI) and sucrose (group VII).—These produced no significant changes in any animal. One rat had a small needle-track lesion in the corpus callosum.

Discussion

The main objective of this study was to compare the neurotoxic potential of gadodiamide with that of gadopentetate dimeglumine in an animal model applicable to clinical usage. The findings of the small study of gadopentetate dimeglumine, included in the present series, showed that the nature and prevalence of gadopentetate dimeglumine effects (transient hyperexcitation; focal lesions in the thalamus, brain stem and spinal cord; and persisting ataxia) were similar to those previously described (8), except that thalamic lesions previously seen only in animals surviving for 21 days or more (8) were absent in the present study. A new finding, however, was that anesthesia (essential to ensure survival in the gadodiamide series) enhanced the severity but not the frequency of both the lesions and the ataxia produced by gadopentetate dimeglumine. It is difficult to explain this effect of anesthesia, but as the tissue damage occurred predominantly in the lower regions of the CNS, an absence of the displacement mixing normally induced by active body movements may, perhaps, have led to the relatively dense agent being concentrated in the basal subarachnoid cisterns. The presence of lesions in the anesthetized group further served to establish that acute excitation played no part in their pathogenesis.

The acute functional effects of gadopentetate dimeglumine and gadodiamide were quite similar, except that the latter was approximately twice as potent as the former, hence the need for anesthesia. The lack of any excitatory signs during or after the slow infusions of gadodiamide suggests that the acute excitation was a result of a transiently high concentration of the agent near the injection site, and is therefore probably an artifact of intraventricular injection, with little direct clinical significance. Excitation may have been a transient disturbance of local zinc or calcium homeostasis, since we have found other chelators that produce similar effects (unpublished data, Ray, 1998), and other investigators have reported that the gadodiamide/caldiamide formulation has the potential to interact with zinc at clinical doses in humans (9) and also to reduce the activity of a zinc-dependent enzyme *in vitro* (7). We were able to

establish that this excitation was entirely attributable to the gadodiamide component.

The two agents, however, differed markedly in their subsequent effects, and these were not local dose artifacts. Gadodiamide failed to produce the delayed onset tremor or the persisting ataxia that was seen after neuropathic doses of gadopentetate dimeglumine. This occurred despite the use of anesthetic for most of the gadodiamide animals, a procedure that enhanced ataxia with gadopentetate dimeglumine. This lack of persisting ataxia may be attributable to the absence of brain stem and spinal cord damage in the gadodiamide animals (Table 4), because all animals with persisting ataxia who were administered gadopentetate dimeglumine had lesions in these regions. The lesions produced by the gadodiamide/caldiamide mixture were either nonneuronal or did not involve motor regions, the only exceptions being in the animals administered the high-dose slow infusions who did develop ataxia, probably because of the severe loss of cerebellar granule cells. Hence, the patterns of motor dysfunction produced by the two chelates correlated well with lesion topography.

Despite the chemical similarities between gadopentetate dimeglumine and gadodiamide, the topography of the brain damage produced after intraventricular infusion was curiously different in each (Table 4). Gadopentetate dimeglumine produces major lesions in brain stem nuclei and the central spinal cord (8), neither of which was affected by gadodiamide. The major damage from gadodiamide was confined to the vermis of the cerebellum, with lesser damage to the striatum.

The lesions in the basal ganglia were all deeply situated and relatively distant from the ventricles, and, for these reasons, the underlying mechanisms are not understood. Although the caudate/putamen lesions were the closest yet seen at the site of the unilateral injection, they were still symmetrical. The dose threshold for producing these lesions was between 5- and 10- $\mu\text{mol/g}$ brain gadodiamide/caldiamide, a value similar to that obtained for gadopentetate dimeglumine. When administered as a prolonged infusion, gadodiamide/caldiamide was able to cause caudate putamen lesions at the lower dose level of 2.5- $\mu\text{mol/g}$ brain. This 28-hour exposure, however, is unlikely to be experienced clinically.

Interpretation of the significance of the cerebellar lesion is made difficult by the seemingly unique nature of the cellular response. The apparently reactive nature of the astrocytic lesion (10, 11) led us to look for primary damage elsewhere, but, with the excep-

tion of minor swelling of Purkinje cell dendrites and some late granule cell pyknosis seen at high doses, no evidence of such damage could be discerned. Lack of neuronal damage in the early stages is supported by the lack of any microglial reaction. The early appearance of electron-dense multilamellar bodies within the end-feet and processes of Bergmann glial cells may have resulted from the phagocytosis of material from the extracellular space, but we have been unable to detect gadolinium within the bodies by X-ray diffraction analysis. Restriction of the changes to the vermal and paravermal regions of the cerebellum is a feature that has been found with other agents that enter the CSF either in disease or by experimental design, such as alcoholic cerebellar disease (12, 13) and superficial siderosis of the CNS (14). It is also seen in experimental animals administered phencyclidine (15), L- β -methylaminoalanine (16), and the mycotoxin penitrem A (17), either parenterally or intraventricularly. The capacity of cerebellar cortical astrocytes and the dendrites of Purkinje cells to take up materials from the CSF, and the relatively large volume of CSF in the subarachnoid spaces overlying the vermis of the cerebellum, should both be considered as likely factors in this localization. It is possible that the astrocytes were reacting to an abnormal ionic disturbance in a similar way to their reaction to the hyperammonemia induced by portacaval anastomosis (18), although our control data showed that any disturbance would have to be more specific than a change in overall ionic strength or osmolarity. A similarly dramatic astrocytic response, resembling a colchicine effect (19) with enlarged nuclei and greatly expanded mitotic figures, is seen when the microtubular functions of the mitotic spindle are impaired, as in the brain swelling associated with hyperammonemia in the rat (20). We suggest, therefore, that the early astroglial response seen at 3 days in the present study, when multilamellar bodies were numerous in their cytoplasm, may have been the direct response to the incorporation of the gadolinium compound from the extracellular space.

Conclusion

The brain lesions produced after intraventricular infusion of gadodiamide are significantly different in pattern and character from those produced by gadopentetate dimeglumine. The nature of the individual lesions appears to be characteristic of the agent and of the route of administration and not related to injection rate or concentration. The cerebellar lesions occur at dose levels below those producing hyperexcitation and showed a clear dose dependency, with a no-effect level at 0.5- μ mol/g brain. This compares with a no-effect level of 3.8- μ mol/g brain for the different lesions produced by gadopentetate dimeglumine. The cerebellar lesions are attributable to the gadodiamide component of the clinical formulation but are of uncertain pathogenesis. The relationship of

the doses used in intraventricular injection studies to those used for clinical imaging have been discussed elsewhere (8), but the no-effect dose level established in this study and the good safety record of the gadodiamide/caldiamide formulation (6) support an acceptable margin of safety for all current clinical applications.

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