MAGNETIC RESONANCE IMAGING IN THE EVALUATION OF NIMODIPINE-TREATED ACUTE EXPERIMENTAL FOCAL CEREBRAL ISCHEMIA

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Abstract

We evaluated the sensitivity of magnetic resonance (MR) imaging in documenting effects of nimodipine in experimental focal cerebral ischemia. Twenty-five Sprague Dawley rats underwent unilateral occlusion of the middle cerebral artery and were imaged at different intervals thereafter. Neuropathologic and neurologic data were correlated with MR imaging results. Compared with controls, nimodipine-treated rats showed a significantly smaller infarct size ($p < 0.001$), as documented by MR imaging and confirmed by neuropathologic evaluations. A less intense signal on the T2 weighted sequence was found in nimodipine-treated rats in basal ganglia ($p < 0.001$) and cortex ($p < 0.05$). MR imaging may afford unprecedented diagnostic sensitivity in assessing pharmacologic efficacy in cerebral ischemia.

Although cerebral ischemia is one of the most common neurologic disorders, its treatment is still a matter of debate among neurologists and neurosurgeons. An evaluation of the effects of various forms of therapy for stroke is of great importance to both experimental studies and clinical follow-up review. A method for accurate detection of therapeutic efficacy in vivo would be of particular value.

Calcium channel antagonists recently have been evaluated to determine their effectiveness as therapeutic agents for a variety of acute ischemic insults to the brain (11). Nimodipine, one of the most potent calcium channel blockers with a specific action on the brain, has been shown to improve neurologic outcome and reduce infarct size in experimental focal cerebral ischemia (9).

The high sensitivity of MR imaging to cerebral edema provides exquisite visualization of pathology in the central nervous system (1, 6). As major alteration of brain water content occurs within the first few hours after ischemic insult, MR imaging has important implications for the experimental and clinical evaluation of acute cerebral ischemia (3). Recent evidence suggests a greater sensitivity of MR imaging in the detection of acute stroke than have other traditional methods (4). We undertook this study to assess the effect of nimodipine on infarct size in rats and to quantify the efficacy of proton magnetic resonance (MR) imaging in detecting evidence of ischemia and changes caused by therapy in vivo.

Material and Methods

Twenty-five Sprague Dawley rats (350–400 g), anesthetized with chloral hydrate (35 mg/100 g body weight, intraperitoneally) underwent a left parietal craniectomy. The middle cerebral artery (MCA) was exposed using microsurgical technique and was occluded by bipolar coagulation from its origin at the carotid artery to the point where it is crossed by the inferior cerebral veins. At the end of the procedure, rats were placed on a heating pad and allowed to recover from anesthesia.

Shortly before MR imaging, rats underwent a careful neurologic examination and their status was graded on the basis of a 3-grade scale (0 = no observable deficit, 1 = forelimb flexion, 2 = decreased resistance to lateral push and forelimb flexion). Blood pressure and body temperature were monitored continuously during the surgical and MR imaging procedures.

MR imaging was performed at 4 h ($n=4$), 6–8 h ($n=3$), 12 h ($n=12$), 24 h ($n=6$) after the ischemic insult. Five rats imaged at 12 h after ischemia were treated with nimodipine (20 μg/kg over 10 min, intravenously) at 1 h after MCA occlusion, and subsequently were compared with the untreated rats (controls) imaged at the same time after ischemic insult.

Rats were positioned prone into a custom-made distributed-capacitance balance-matched coil (5 cm diameter) tuned to the proton resonance frequency of 85.552 MHz. Images were obtained at 2.0 tesla using a wide-bore (25 cm diameter) imager/spectrometer (General Electric-CSI II, General Electric Co., Fremont, California). Coronal images were bisecting hemispheres at the level of the trigeminal nerve. T1 and T2 weighted images were obtained using the following parameters: spin-echo with TR=300 ms TE=15 ms, and TR=3 000 ms TE=80 ms with four and two
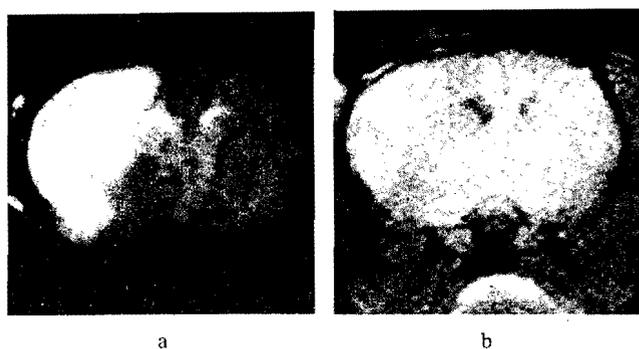
Fig. 1. MR coronal images of a control rat at 24 h after ischemic insult. a) T2 weighted image. SE TR = 3 000 ms, TE = 80 ms; field of view 4 cm × 4 cm; matrix 128 × 256; slice thickness 3 mm; 2 acquisitions. Well defined edema involving the MCA vascular territory in (a) and mass effect with left-to-right shift in (b).
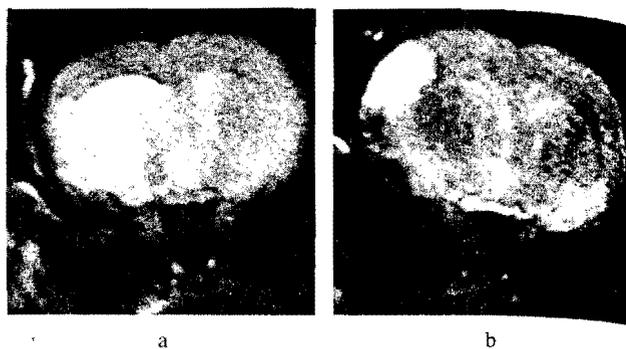

Fig. 2. T2 weighted MR coronal images. Images of a control rat (a) and a nimodipine-treated rat (b) at 12 h after ischemic insult. Parameters are the same as those noted in Fig. 1. Evidently smaller infarct size in nimodipine-treated rat (b).

Table 1
Mean infarct size as evaluated by MR imaging, TTC, and H & E staining

Rats	Mean size of infarct*		
	MR imaging	TTC	H & E
Control			
4 h	32F1	30F6	31F2
6−8 h	34F7	32F1	33F3
12 h	37F8	35F5	36F4
24 h	41F2	39F4	39F5
Nimodipine			
12 h	25F2**	24F8**	23F4**

*Values are expressed as per cent of coronal section involved.
** $p < 0.001$.

Table 2
Mean values of T2 weighted signal intensity changes ($\Delta I\% = 100 [Ii - In]/In$)

Rats	Mean $\Delta I\%$*	
	Basal ganglia	Cortex
Control		
4 h	13F11	41F 8
6−8 h	5F 6	45F16
12 h	57F31	75F24
·24 h	49F19	73F24
Nimodipine		
12 h	8F 3**	57F31***

*Values are expressed as per cent variation compared with the contralateral side.
** $p < 0.001$.
*** $p < 0.05$.

acquisitions, respectively. The data matrix size was 128 phase encoded steps by 256 real frequency encoded points. The field of view was 4 cm×4 cm and the slice thickness, 3 mm. The intensity of a squared region of interest was measured in the injured and corresponding contralateral area, in cortex and basal ganglia, and compared with

$$\Delta I\% = 100 \ (Ii\text{-}In)/In,$$

in which: I is intensity; ΔI is change in intensity; Ii is intensity of injured cortex or basal ganglia; and In is intensity of normal cortex or basal ganglia. Size of MR regions of interest was measured using cutting and weighing techniques.

At the end of the MR imaging procedure, rats were anesthetized and infused with the vital stain 2, 3, 5 triphenyltetrazolium chloride (TTC) (10) for evaluation and quantification of infarct size. Infarct size was first evaluated on whole brain and then quantified on photographs of coronal sections using cutting and weighing techniques. After formalin-fixation and paraffin-embedding, the same sections were stained with hematoxylin and eosin (H & E).

Statistical analysis of the data obtained was by Student's t-test.

Results

In all rats, an infarcted area in the hemisphere subjected to MCA occlusion was shown by MR imaging and confirmed by TTC and H & E staining. The ischemic lesion was evidenced on MR imaging by an obvious increase in signal intensity on the T2 weighted sequence. The affected area showed the best definition from surrounding normal brain on the images made at 24 h (Fig. 1a). Mass effect asso-
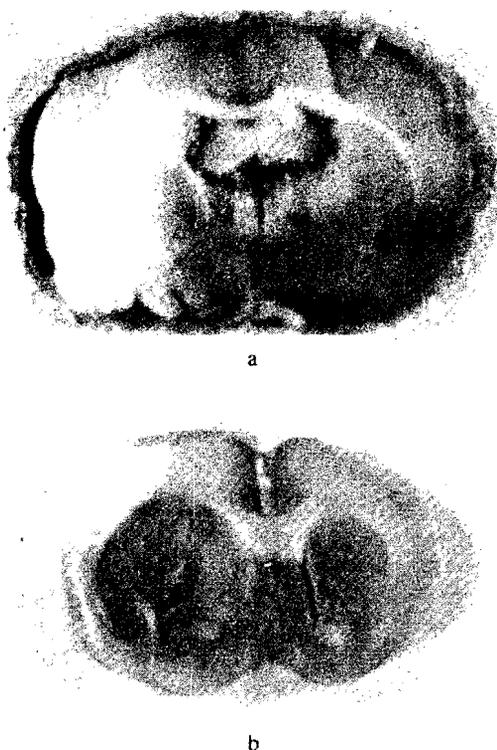
a

b

Fig. 3. TTC stains of brain in the same rats as are shown in Fig. 2. Excellent correspondence with the respective MR images.

ciated with the ischemic insult was evident at 24 h on T1 weighted images (Fig. 1b).

MR imaging showed a significant decrease in infarct size in all nimodipine-treated rats, as compared with controls (p <0.001). Control rats imaged at 12 h after ischemia showed a large injured zone that involved more than one-third of the coronal section (Table 1). The area involved was the complete vascular territory of the MCA, with infarction affecting both cortex and basal ganglia (Fig. 2a). At 12 h after ischemia, nimodipine-treated rats showed a smaller injured area (Fig. 2b).

MR imaging data were confirmed by the neuropathologic findings. Nimodipine-treated rats had a smaller infarct size relative to controls (p <0.001), as shown by postmortem macroscopic and microscopic brain evaluations (Fig. 3). Data related to infarct size as evaluated by TTC and H & E stains, expressed as per cent of the coronal section involved, are shown in Table 1. There was no significant difference between the infarct size as determined by the MR imaging, TTC, and H & E evaluations, despite a trend toward increasing infarct size portrayed by the MR imaging data. The infarct size data from the TTC and H & E evaluations showed an exact correlation.

The neurologic outcome in the nimodipine-treated rats was significantly better than that in controls (p <0.001), and had a linear correlation with the infarct size shown by MR imaging.

A statistically smaller increase in intensity of signal on T2 weighted images was found in nimodipine-treated rats than in controls (basal ganglia, p <0.001; cortex, p <0.05). Data expressed as per cent of intensity change compared with the contralateral side are shown in Table 2.

Discussion

The possibility of using a small, inexpensive animal model for experimental studies of cerebral ischemia is appealing. To date, the principal restrictions on such studies have been a lack of reliable models and difficulties in setting appropriate MR imaging parameters for very small volume of interest (rat brain, 2 cm maximum diameter). Our results support the reliability of the surgical model we used (2), and indicate the feasibility of using a rat model when optimal MR imaging parameters are set utilizing a high field system, small fields of view, and very long TR and TE.

The high sensitivity of MR imaging in detecting acute ischemia is established (3). However, its ability to quantify the area of infarction has not been fully investigated. Beneficial effects of nimodipine in experimental cerebral ischemia have been shown to correlate with a decreased infarct size, as documented by postmortem neuropathologic evaluation (9). The ability of MR imaging to quantify the area involved in acute ischemia is shown to be reliable by our data. Moreover, MR imaging consistently quantified the treatment-induced modification of infarct size. No statistical difference between the quantification of in vivo infarct size by MR imaging and that of postmortem infarct size by TTC or H & E stains was found. The trend toward increasing size noted in our MR imaging data is not surprising. Whereas TTC indeed evidences mitochondrial enzymatic activity (13) and H & E shows ischemic cell changes, MR imaging is sensitive to water content. Edema and cell death have been shown to have slightly different distribution (8), particularly of the infarcted area (5). In the ischemic 'penumbra', therefore, vasogenic edema may occur in the presence of still viable neurons.

The entry of calcium ions into neurons has been shown to be at the basis of edema formation (14). Through the activation of intracellular phospholipase, calcium ions lead to formation of polyunsaturated fatty acids, among them arachidonate, that increase blood-brain barrier permeability (12). Whether the calcium channel antagonist nimodipine is acting on cerebral vessels only, at the neuronal level through the calcium channels, or both is still controversial (7). Our results showing a less intense signal on T2 weighted images indicate a smaller component of edema formation in the injured area of nimodipine-treated rats as compared with controls. This corroborates previously reported results of a beneficial effect of nimodipine on cerebral ischemia (9). Further studies are necessary to better investigate the mechanism of action of nimodipine, however, as both increased cerebral blood flow and decreased production of arachidonic acid may be responsible for decreased water content in ischemic brain.

In conclusion, our study would appear to indicate an unprecedented diagnostic sensitivity of MR imaging in quantifying infarct size and assessing effects of pharmacologic therapy in experimental focal cerebral ischemia.

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