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Cerebral Blood Volume in a Rat Model of Ischemia by MR Imaging at 4.7 T

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Perturbation of the cerebral circulation by occlusion of the vertebral arteries and a carotid artery can be visualized by using MR imaging and the intravascular contrast agent Gd-DTPA complexed to albumin. This tracer consistently reduced the T1 relaxation time in the brain and blood. The difference between hemispheres was revealed by less T1 reduction in the occluded hemisphere and by an adjustment in the display contrast of images that revealed the territory of decreased perfusion. These results were confirmed by comparing them with cerebral blood flow using radioactive microspheres and the intravascular blood volume tracer ^{51}Cr-EDTA. This method, combined with high-resolution MR imaging, can be applied to serial noninvasive studies of cerebral blood volume in ischemia and other conditions.

Paramagnetic agents, such as Gd-DTPA, enhance proton relaxation times and have been used to visualize blood-brain barrier disturbances during MR imaging. When complexed with albumin (alb), Gd-DTPA remains in the intravascular space, as recently described by Schmiedel et al. [1]. Our specific interest [2, 3] has been to develop techniques to visualize regional cerebral blood volume (CBV) by using Gd-alb. We report here that experimentally induced, modestly (about 30%) decreased hemispheric brain blood flow in rats can be visualized by using Gd-alb. This method may have utility in defining alterations in cerebral perfusion caused by cerebrovascular occlusive disease, which would be particularly advantageous when combined with the high-resolution imaging capability of MR. Moreover, because CBV can be estimated by using in vitro calibration curves [2], the possibility exists that this method could be used to follow the clinical course or response to surgical treatment of cerebrovascular disease.

Materials and Methods

Animal Preparation

Seven male Sprague-Dawley rats (250–350 g) were anesthetized with pentobarbital (50–100 mg/kg IP). The vertebral arteries were located and cauterized as described by Pulsinelli et al. [4]. A carotid artery was then ligated. The following day, the animal was anesthetized with chloral hydrate (400 mg/kg IP) and a femoral artery and vein were cannulated with PE50 tubing filled with heparinized saline. Approximately 1 ml of arterial blood was removed. The animal was placed on a circulating heated water bath (Hamilton) in the imaging coil.

Experimental Design

A baseline MR scan and an arterial sample (0.3 ml) were obtained. To document the effects of the contrast agent on T1, T1 of the arterial blood was determined by using a 10-MHz spectrometer (RADX) [5]. The Gd-alb was then injected intrave-

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nously. A repeat MR scan and arterial sample were obtained beginning approximately 5 min later. In three cases, the animal was then injected with ^{51}Cr-EDTA while still anesthetized. After 2 min, the animal was decapitated and the brain dissected into right and left cortex and diencephalon and counted in a gamma well counter to determine CBV as previously described [6] by the following equation:

$$CBV = {}^{51}Cr/g\ brain \div dpm\ {}^{51}Cr/ml\ blood \times 0.72 \times 100$$

In four animals cerebral blood flow (CBF) was determined by using the radioactive microsphere technique with modification as previously described [7]. For these studies ^{85}Sr-labeled microspheres (15μ ± 3) were utilized.

MR Techniques

All imaging experiments were performed on a 4.7-T spectroscopy and imaging unit* equipped with a 6-in. proton imaging coil operating at a proton frequency of 200.1 MHz. All images were 4 mm thick with a field of view of 100 × 100 mm.

In vivo T1 relaxation times were measured by using signal intensities for regions of interest in a series of spin-echo (SE) images with repetition times (TRs) of 235, 635, 1135, 2135, and 6135. Intensities were fit to the curve described by the equation:

$$I(SE) = I_0(1 - e^{-[TR/T1]})$$

where I is the observed intensity of the region of interest for a given TR and I_0 is the intensity in the condition in which TR $\ll$ T1. T1 relaxation times have been measured in this way by other investigators [8].

To minimize the error in T1 measurements, all other experimental parameters, including receiver gain and rat position, were held constant over the entire series of images. In addition, an intensity standard, a plastic ball filled with olive oil, was placed next to the animal's head. The total acquisition time for the progressive saturation series of images was 22 min. In vivo T1 measurements were made just prior to and 5 min after administration of Gd-alb. The regions of interest encompassed six pixels (0.8 × 1.2 mm) and were positioned in the gray matter of the anterior cortical regions in either the right or left hemisphere by an investigator not informed about the side of the carotid occlusion.

Images in the series were acquired with 64 phase-encode steps of 512-point echo acquisitions and processed with zero-filling in the phase-encode dimension to afford 256 × 256 pixel images. High-resolution images were made by using 256 phase-encode steps. The echo time (TE) was uniformly set at 14 for all images.

Gd-alb Preparation

Gd-alb was prepared according to the method described by Hnatowich et al. [9] and modified by Ogan et al. [10]. The concentration was 40 mg alb/ml with 15 molecules of Gd-DTPA per albumin molecule. A dose of 0.1–0.2 ml/kg IV was injected slowly.

* CSI-II, General Electric, Fremont, CA.

Results

To determine whether there were changes in brain T1s not related to Gd-DTPA but caused by the relatively long experiment time, seven animals, prepared identically to our experimental animals, were tested. In these experiments, we tested for changes over time with no injection and with injection of nonlabeled albumin solution of the same concentration as that in the Gd-labeled injectate. These results are summarized in Table 1. In both groups we observed very small changes, which were within the error of the T1 measurement. We observed larger T1 changes in the normal hemisphere of all seven experimental animals, whereas changes greater than observed in control animals occurred on the side of the carotid occlusion in five of seven animals.

CBF on the side with the occluded hemisphere was decreased by a mean of 28% (unoccluded vs occluded, 1.87 ± 0.35 vs 1.34 ± 0.58 ml $min^{-1}g^{-1}$; × ± SD). CBV, measured by using ^{51}Cr-EDTA, paralleled the CBF results, and decreased overall by 25% (2.05 ± 0.30 vs 1.5 ± 0.22% w/w).

A high-resolution image of the representative slice in a rat with both vertebral arteries and the left carotid artery occluded is shown in Fig. 1A. In only one animal was a lesion visible on the baseline image. Fig. 1B is an example of a lower-resolution slice used for T1 determination at TR = 1135. In Fig. 1C, the windows for display contrast have been adjusted to two contrast levels, clearly demonstrating the territory of diminished perfusion of the left hemisphere. In this case, ^{51}Cr-EDTA counts confirmed a 40% decrease in CBV in the left hemisphere.

The overall results are shown in Table 2. There was no consistent side-to-side difference in baseline T1. In all cases, there was T1 shortening after contrast injection ($p < .05$, Wilcoxin sign rank test). T1 shortened by an average of 10.8% on the unoccluded side and 4.9% on the occluded side; and in all cases there was less change on the occluded side than on the unoccluded side ($p < .05$).

Blood Gd-alb concentration was estimated from the degree of T1 shortening as measured on a 10-MHz tabletop spin analyzer (RADX) or on the 4.7-T unit. For this purpose, blood samples were withdrawn before and after administration of the Gd-alb. The relationship between the concentration of Gd-alb and T1 shortening at 10 MHz is demonstrated by the curve in Figure 2. This curve was constructed by measuring the T1 shortening in blood samples containing known amounts of Gd-alb. Our 10-MHz spin analyzer provided a convenient means of determining in vitro T1s on blood samples immediately after withdrawal from the artery.

In vivo Gd-alb concentration was estimated from the degree of T1 shortening in brain tissue as measured from image intensities from various regions of interest. Brain tissue T1 relaxation times decreased by a range of 0 to 300 msec, corresponding to Gd-alb concentrations of 0 to 0.7 mmol. The

TABLE 1: Mean Cerebral T1 Changes in Animals with No Contrast Agent

Injectate	No.	Mean T1 Changes ± SD (msec)	
		Open Side	Occluded Side
Nothing	3	0 ± 30	13 ± 40
Albumin	4	−13 ± 60	−25 ± 50

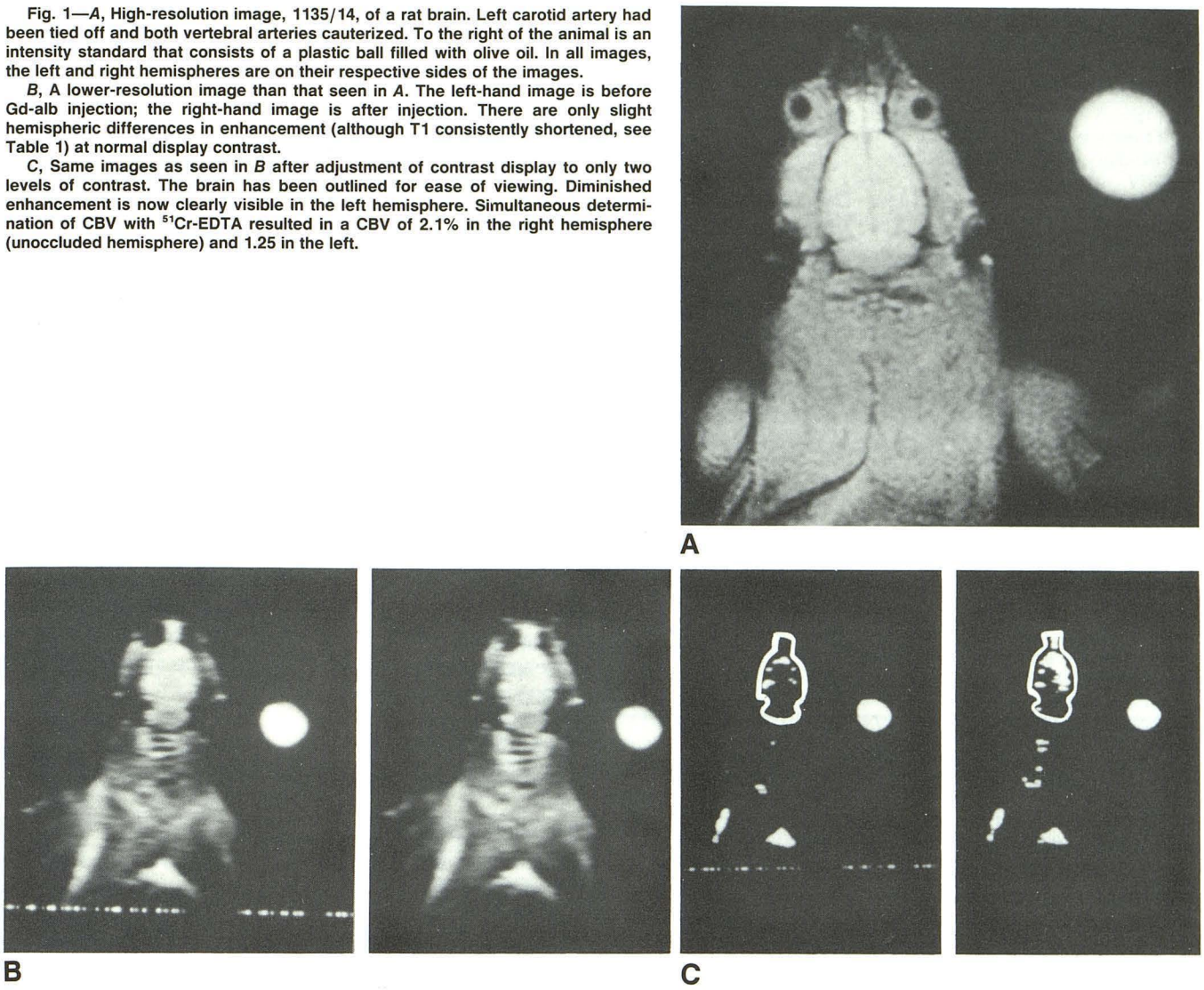

Fig. 1—*A*, High-resolution image, 1135/14, of a rat brain. Left carotid artery had been tied off and both vertebral arteries cauterized. To the right of the animal is an intensity standard that consists of a plastic ball filled with olive oil. In all images, the left and right hemispheres are on their respective sides of the images.

***B*, A lower-resolution image than that seen in *A*. The left-hand image is before Gd-alb injection; the right-hand image is after injection. There are only slight hemispheric differences in enhancement (although T1 consistently shortened, see Table 1) at normal display contrast.**

***C*, Same images as seen in *B* after adjustment of contrast display to only two levels of contrast. The brain has been outlined for ease of viewing. Diminished enhancement is now clearly visible in the left hemisphere. Simultaneous determination of CBV with ^{51}Cr-EDTA resulted in a CBV of 2.1% in the right hemisphere (unoccluded hemisphere) and 1.25 in the left.**

dependence of T1 shortening on the Gd-alb concentration at 4.7 T is shown by the curve in Figure 3. Using in vitro calibration curves and dividing derived Gd-alb concentrations in brain by blood, we estimated the mean CBV as 1.1% ± 0.8 on the occluded side and 3.4% ± 1.4 w/w on the contralateral side.

Discussion

In our analyses, we are assuming fast exchange of water between intra- and extravascular compartments. This assumption is consistent with previous studies of blood-brain barrier permeability, which have shown that 85% of ^{3}HOH is extracted from the capillaries on the first pass [11]. While gross changes in blood-brain barrier permeability may affect our measurement, we do not expect any such changes in our model of mild ischemia.

Our results are among the first to demonstrate the ability to determine abnormal brain tissue perfusion by using MR and Gd-alb. The method of quantification appears to provide values proportional to those determined with radioactive indicators. Low values were underestimated, presumably because of a lack of sensitivity at low CBV. This might be overcome by a more potent formulation of Gd-alb.

The model chosen produced only a minimal decrease in CBF. Our results are consistent with the hemispheric decreases in CBF seen by Pannier et al. [12] in rats with right subclavian and right carotid artery occlusion. We selected this model to determine whether the method would allow visualization of subinfarction degrees of ischemia, such as might occur early in carotid stenosis. In other experiments with this model we found increases in CBF and CBV in some regions of the occluded hemisphere, although these increases did not occur in the animals presented here. These increases may be due to such factors as anomalous collaterization or differences in blood pressure, and have been observed in patients as well [13]. Our interest here was solely in acutely perturbing the cerebral circulation without causing infarction.

The changes detected in CBV in regions without apparent MR abnormalities seen on the image before injection of Gd-DTPA and without consistent differences in baseline T1 imply that differences in brain perfusion can reliably be detected before dramatic increases in brain water content such as would occur in significant ischemia. We have not observed lesions, even on heavily T2-weighted images or in histologic postmortem studies (data not shown).

When complexed with albumin [1], the relaxivity of Gd-DTPA increases, permitting low doses to be employed. We

TABLE 2: Brain T1 Before and After Gd-alb and Occlusion of Three Cerebral Vessels

Animal No.	Open[1]	Occluded	Blood
1. T1b[2]	1370 ± 50 (L)	1370 ± 50 (R)	634[5]
T1 decrease[3]	80	10	473
[Gd-alb][4]	0.08	0.01	2.7
CBV	3.0	0.4	
2. T1b	1670 ± 50 (L)	1780 ± 50 (R)	825[5]
T1 decrease	250	160	640
[Gd-alb]	0.33	0.16	10.5
CBV	3.1	1.5	
3. T1b	1440 ± 30 (R)	1370 ± 70 (L)	790[5]
T1 decrease	170	70	582
[Gd-alb]	0.17	0.07	5.2
CBV	3.2	1.3	
4. T1b	1780 ± 30 (R)	1700 ± 10 (L)	800[5]
T1 decrease	170	50	565
[Gd-alb]	0.17	0.05	5.0
CBV	3.4	1.0	
5. T1b	1480 ± 10 (L)	1540 ± 30 (R)	760[5]
T1 decrease	50	0	590
[Gd-alb]	0.05	0	5.4
CBV	0.9	0	
6. T1b	1480 ± 80 (L)	1420 ± 60 (R)	739[5]
T1 decrease	210	70	579
[Gd-alb]	0.27	0.07	5.2
CBV	5.2	1.3	
7. T1b	1900 ± 50 (L)	1610 ± 60 (R)	1800[6]
T1 decrease	320	200	1400
[Gd-alb]	0.56	0.26	11.2
CBV	5.0	2.1	

Note.—CBV = cerebral blood volume, L = left, R = right.

[1] Open = unoccluded carotid artery (hemisphere in parentheses).

[2] T1 before Gd-DTPA-alb ± SD (msec). All brain T1 measurements were made at 4.7 T.

[3] T1 decrease after Gd-DTPA-alb (msec).

[4] mM concentration of Gd-alb, estimated as in text.

[5] Measurements made at 0.24 T (msec).

[6] Measurements made at 4.7 T (msec).

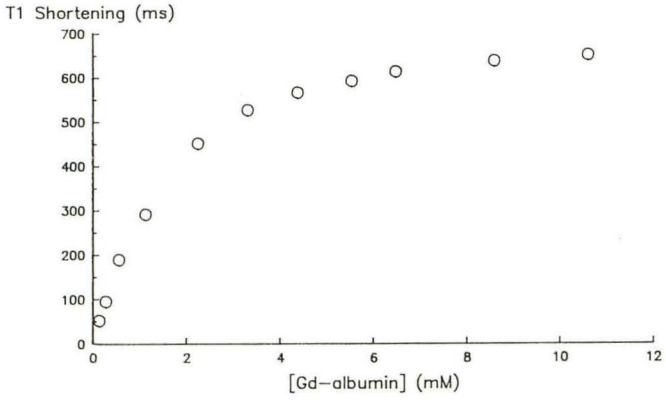

Fig. 2.—T1 shortening in blood samples containing known concentrations of Gd-alb. Measurements were made in vitro at 0.24 T.

have observed no untoward behavioral reactions and have followed animals for several days with serial scanning, although we have not performed complete toxicologic studies. This application of a potent intravascular agent is of potential value, since the effect of treatment and the course of cerebral ischemia can be followed. Moreover, utilization of the localized spectroscopic capabilities of our unit will permit correlation of tissue perfusion with alterations in phosphate metabolites and other metabolic parameters.

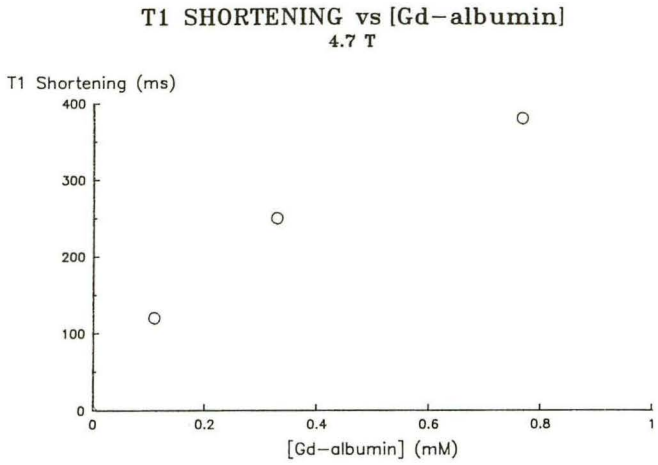

Fig. 3.—T1 shortening in blood samples with added Gd-alb measured in vitro at 4.7 T.

Application of this method to humans may not be as straightforward because of variability in CBF in cerebral ischemia of chronic origin [13]. The model developed here is an acute change in blood perfusion, and the method appears to be useful in imaging studies of this condition. Further research is needed to determine its value in detecting CBV changes in chronic ischemia.

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REFERENCES

1. Schmiedl U, Ogan M, Paajanen H, et al. Albumin labeled with Gd-DTPA as an intravascular blood pool-enhancing agent for MR imaging: biodistribution and imaging studies. *Radiology* **1987**;162:205–210
2. Kent TA, Amparo EG, Najafi A, Gevedon R. Functional cerebral blood volume and quantitative blood brain barrier determination by contrast enhanced MRI. *J Cereb Blood Flow Metab* **1987**;7:514 (abstr)
3. Amparo EG, Kent TA, Najafi A, et al. Quantitation of cerebral blood volume using albumin-gadolinium-DTPA and magnetic resonance imaging. *AJNR* **1987**;8:932 (abstr)
4. Pulsinelli WA, Brierly JB, Plum F. Temporal profile of neuronal damage in a model of transient forebrain ischemia. *Ann Neurol* **1982**;8:491–498
5. Burnett KR, Wolf GL, Goldstein EJ. NMR *in vitro* measurements: a quality control study of the RADX table-top spectrometer. *Physiol Chem Phys Med NMR* **1985**;17:123–126
6. Kent TA, Preskorn SH. A modified method for the simultaneous determination of cerebral blood flow and the single-transit extraction of diffusion-limited substances: utilization of non-invasive measure of transit time. *Brain Res* **1985**;335:251–255
7. DiPette D, Burris J, Rogers A, Waeber B, Brunner H. Propranolol vasodilation in adrenalectomized glucocorticoid hypertensive rats. *Am J Physiol* **1987**;252:H1243–H1248
8. Crooks LE, Hylton NM, Ortendahl DA, Osin JP, Kanfinan L. The value of relaxation times and density measurements in clinical MRI. *Invest Radiol* **1987**;22:158–169
9. Hnatowich DJ, Childs RL, Lanteigne D, Najafi AJ. The preparation of DTPA coupled with antibodies radiolabelled with metallic radionuclides: an improved method. *Immunol Meth* **1983**;65:147
10. Ogan MD, Grodd W, Paajanen H, et al. Approaches to the chemical synthesis of macromolecular MR imaging contrast media for perfusion-dependent enhancement. *Radiology* **1985**;157:100
11. Rapoport SI. *Blood brain barrier in physiology and medicine.* New York: Raven Press, **1976**:87–98
12. Pannier JL, Weyne J, Demeester G, Leusen I. Effect of nonrespiratory alkalosis on brain tissue and cerebral blood flow in rats with damaged blood-brain barrier. *Stroke* **1978**;9:354–359
13. Knapp WH, von Kummer R, Kubler W. Imaging of cerebral blood flow to volume distribution using SPECT. *J Nucl Med* **1986**;27:465–470