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Calcium 45 Autoradiography and Dual-Isotope Single-Photon Emission CT in a Canine Model of Cerebral Ischemia and Middle Cerebral Artery Occlusion

Phillip D. Purdy, Michael B. Horowitz, Dana Mathews, Brandy S. Walker, George J. Carstens III, Michael D. Devous, Charles L. White III, Padmakar Kulkarni, Anca Constantinescu, and H. Hunt Batjer

PURPOSE: To determine whether transient ischemia can be separated from permanent ischemia via calcium 45 autoradiography and to assess the applicability of dual isotope single-photon emission CT (SPECT) in the evaluation of cerebral blood flow. **METHODS:** We examined calcium influx in 12 dogs (group A) by using whole-brain calcium 45 autoradiography: Animals received 250 μCi/kg 24 hours after 30-minute (n = 6) or permanent (n = 6) middle cerebral artery (MCA) occlusion. Forty-eight hours after MCA occlusion, 5-mm coronal brain sections were fixed for either autoradiography or pathologic examination. In a separate study, 9 mongrel dogs (group B) were given 250 μCi/kg calcium 45 and a mean dose of 700 μCi/kg technetium Tc 99m hexamethylpropyleneamine oxime intravenously. A silicone plug was then injected into the internal carotid artery and angiography was performed to verify MCA occlusion. A 10th (control) animal did not undergo occlusion. In an 11th animal, placement of the plug could not be achieved and a slurry of microfibrillar collagen was injected into the carotid artery. No angiography was performed in animals 10 and 11. After occlusion, each animal was injected with a mean dose of 126 μCi/kg ^{123}I-iodoamphetamine. The control animal was also injected. SPECT was performed using a simultaneous acquisition for technetium 99m and ^{123}I-iodoamphetamine. **RESULTS:** In group A, all animals who had permanent MCA occlusion showed infarction and increased calcium 45 uptake in infarcted territories. None of the animals who had 30-minute occlusion had either increased calcium 45 uptake or infarction at 48 hours. In group B, 7 of 10 dogs had SPECT findings that were consistent with the calcium autoradiographic marker for ischemia. One animal died during the procedure and 1 dog served as a control. **CONCLUSION:** Calcium 45 autoradiography allowed distinction between areas of temporary and permanent occlusion. Iodoamphetamine imaging was not consistently sensitive to that level of ischemia. Timing of calcium influx may lead to insight that could impact timing of pharmacologic or endovascular intervention.

Index terms: Animal studies; Arteries, stenosis and occlusion; Brain, ischemia; Radioautography; Single-photon emission computed tomography

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Though exact mechanisms of neuronal death as a consequence of ischemic insults are incompletely understood, cellular calcium influx has been hypothesized by many authors to play a critical role in this process. Calcium has been linked to activation of lipases, proteases, and endonucleases, which function in cellular autolysis. Excitatory neurotransmitters that increase with ischemia have been linked to calcium influx by way of calcium channels in the cell membrane.

The importance of calcium as a cellular toxin has been reviewed (1). This linkage between calcium rise and cell death has led to significant interest in the use of drugs to block calcium channels as a means of tissue preservation during cerebral ischemia. However, the relative

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From the Departments of Radiology (P.D.P., M.B.H., D.M., B.S.W., G.J.C., M.D.D., P.K., A.C.), Neurological Surgery (P.D.P., M.B.H., H.H.B.), and Pathology (C.L.W.), University of Texas Southwestern Medical Center.

Address reprint requests to Phillip D. Purdy, MD, Department of Radiology, Division of Neuroradiology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75235-8896.

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roles of ischemia and reperfusion and the effectiveness of these drugs in those settings require further elucidation (2, 3).

The technique of calcium autoradiography has been described by several authors as a means to examine calcium shifts during ischemia. Increases in calcium observed with autoradiography have been shown to be 83% intracellular (4). Previous work with whole-brain calcium autoradiography has been centered on small animal species, owing in part to the technical difficulties of whole-brain autoradiography in species with larger brains. However, application of these techniques in species with larger brains would potentially allow observation of larger areas of cortex surrounding infarcted areas (the ischemic penumbra) and would allow application of imaging techniques that are commonly used in clinical practice.

Using our nonneurosurgical canine model for cerebral ischemia (5), we sought to assess whether a temporary ischemic insult could be differentiated autoradiographically from a permanent one. If short and long ischemic insults could be distinguished by calcium autoradiography in this model, further investigations of calcium changes during ischemia could be conducted. Once we established the ability to assess ischemia with the use of autoradiography, we went on to study cerebral blood flow (CBF) before and after middle cerebral artery (MCA) occlusion. Accurate determination of CBF is essential in determining the level of ischemia induced by various diagnostic, therapeutic, or experimental techniques. While the standard of reference for measuring CBF in an experimental model is the use of radioactive microspheres, this technique is not feasible in a clinical setting. Instead, CBF is measured by using chemical microspheres, radioactively labeled agents that travel to the brain in proportion to the CBF at the time of injection and then are trapped there, either by binding to receptors (eg, iodine 123 iodoamphetamine [^{123}I-IMP]) or by undergoing a chemical transformation (eg, technetium Tc 99m hexamethylpropyleneamine oxime [^{99m}Tc-HMPAO]). Either of these agents may be used alone to assess CBF with single-photon emission computed tomography (SPECT). We have described a dual-isotope acquisition technique whereby one agent may be injected at baseline and the other after some CBF manipulation. This method allows simultaneous acquisition of both studies (6). While this technique has been shown to be clinically useful in assessing CBF in an acetazolamide test of vascular reserve or in trial balloon occlusion of a carotid or intracerebral artery, we have not tested its sensitivity and specificity in an experimental model of reversible ischemia.

We have previously shown by autoradiography that when radioactive calcium is administered before occlusion, the calcium will accumulate in the MCA territory in proportion to the duration of the ischemia (5). Although calcium accumulation indicates that ischemia has occurred, it does not give information as to the degree of CBF change induced by the MCA plug. The purpose of the second part of this study was to evaluate the utility of dual-isotope SPECT scanning in measuring changes in CBF in a canine model of MCA occlusion.

Materials and Methods

Group A

Twelve mongrel dogs, selected with disregard to age or sex, were prepared as described previously (5). Under general anesthesia, the cervical internal carotid artery (ICA) was exposed. A silicone plug measuring approximately 1 mm in diameter and 7 mm in length with a silk suture embedded in it was introduced into the ICA via a 16-gauge intravenous catheter. The silk suture was suspended in a saline-filled syringe. On injection, the plug embolized into the MCA and was either permanently left in situ or withdrawn after 30 minutes. In this study, several plugs were modified by placing a thin piece of platinum wire near their tips, which made the plug radiographically visible and allowed verification of placement by means of vertebral angiography. The common carotid arteries were clamped bilaterally during angiography to assure complete intracerebral filling.

Animals were divided into two groups. In group A (n = 6), the plug was placed in the MCA for 30 minutes and withdrawn. In group B (n = 6), the MCA was permanently occluded. The cervical incision was sutured and the animal was allowed to awaken from anesthesia and to return to the Animal Resources Center.

Twenty-four hours after plug placement, all animals were given intravenous injections of 250 μCi/kg calcium 45 (^{45}Ca). Forty-eight hours after plug placement, all animals were killed via pentobarbital sodium overdose (100 mg/kg) and their brains were removed immediately for processing. Difficulties usually encountered in slicing fresh brain were significantly ameliorated by the addition of a partial freezing step. For this, isopentane was cooled to -30° C by the addition of dry ice. The fresh brain was immersed in that solution for 1 minute, removed, and immediately sliced. Alternating slices were processed for histologic (infarct staining with 2,3,5 triphenyl, 2-H tetra-

zolium chloride [TTC]) or autoradiographic evaluation. Slices for autoradiographic imaging were immediately immersed in the isopentane solution and frozen. Slices for histologic examination were stained immediately with TTC and placed in formalin.

To verify concordance between the infarction seen with TTC staining and conventional histologic examination, brain slices from four animals that had shown decreased uptake with TTC staining were imbedded in paraffin, sliced, and stained with hematoxylin-eosin.

Slices frozen for autoradiography were stored in a −40° C freezer. They were subsequently fixed singly on a large (2 × 3-inch) cryomicrotome mounting base and sliced to a 40-μm thickness at −15° C. Slices were heat-fixed to glass slides. After they were labeled, slices were placed on a cardboard backing, loaded into film cassettes, and the film (Kodak SB-5 X-ray film) was exposed for 14 days. After 14 days of exposure, the film was removed and developed for autoradiographic images. Regions with poor uptake seen with TTC staining were compared with areas of increased ^{45}Ca uptake as observed on the autoradiographs.

Group B

Twelve mongrel dogs, selected with disregard to age or sex and weighing 15 to 32 kg, were endotracheally intubated and anesthetized using pentobarbital sodium induction and halothane maintenance. Cardiac rate and rhythm and arterial blood gases were monitored throughout the procedure. The animals' right femoral arteries, peripheral veins, and left femoral veins were catheterized. Both common carotid arteries were surgically exposed under sterile conditions and the ICA was isolated. Before plug placement, 250 μCi/kg ^{45}Ca (administered as calcium chloride) and ^{99m}Tc HMPAO (mean dose, 17.4 mCi; range, 7.6 to 26.4 mCi; Amersham Corp, Arlington Heights, Ill) were injected via the femoral venous line.

After administration of the baseline radionuclide doses, the ICA was selectively catheterized via direct puncture of the common carotid artery. The catheter was prepared beforehand with a 7-mm silicone plug with a 5–0 silk suture imbedded within it and a platinum marker at its tip. The details of plug construction and delivery have been described previously (5). After introducing the plug-loaded catheter into the ICA, the plug was injected and allowed to flow into the intracranial compartment. Angiography was then performed using selective left vertebral artery catheterization during bilateral common carotid occlusion. Transient occlusion of the common carotid arteries during vertebral angiography in dogs produces complete intracranial arterial opacification and is thus a good means for obtaining a total cerebral arteriogram without excessive contrast load from an injection in the aortic arch.

After verification of plug placement in the MCA, the suture with the plug attached was detached from the injecting syringe and the plug was left in place. The incisions in the neck that were used for carotid exposure were loosely oversewn, and the animal was transported to the SPECT scanner. Scans were acquired as previously described for the dual-isotope technique (6). Briefly, SPECT imaging of the brain was performed using a three-headed rotating gamma camera with fan-beam collimators (Toshiba, Tustin, Calif: GCA 9300 A camera with a GMS 550U computer). Because of the relatively small size of the dogs and the presence of an endotracheal tube, the animals were placed prone on the imaging table rather than in the usual supine position. The dogs were then injected with approximately 3 mCi of iodine 123, which was allowed to circulate for approximately 15 minutes. The dogs were then scanned for 30 minutes with 10% windows opened for the technetium 99m energy peak at 140 keV and the iodine 123 energy peak, which was offset slightly at 161 keV. Pentobarbital infusion was continued throughout the imaging.

IMP was prepared using iodine 123 (sodium iodide) in 0.1 NaOH, pH 12 to 13 (Nordion Int, Kanata, Ontario, Canada). The material had a radioactivity concentration greater than 200 mCi/mL with a radiochemical purity greater than 95% and a low contamination of iodine 124. Radioiodinated (iodine 123) IMP was obtained by radioisotope exchange reaction. The reaction was carried out for 30 minutes in a closed system with acetic acid in the presence of Cu(II) sulfate as a catalyst at 150° to 155° C. The radiolabeled product (at pH >10) was extracted into ethyl ether with an efficiency of 80% to 90%. The radiochemical purity of the labeled compound at this stage was 95% as determined by thin-layer radiochromatography in two different solvent systems. The organic layer was washed and then ether was evaporated under a slow stream of nitrogen. The residue was dissolved in 0.3 mol/L HCl and the pH was adjusted to 6 to 7 with 0.3 mol/L NaOH. The final product was diluted to 5 mL with saline. The product had a specific activity of 2 to 3 mCi/mL.

After scan acquisition, animals were sacrificed via pentobarbital overdose (100 mg/kg). All brains were immediately removed and partially frozen to aid in cutting. The brains were cut into coronal slabs approximately 8 mm thick. In six animals, these slabs were returned to the nuclear medicine department for planar imaging. The slabs were imaged by placing them on a single camera face with energy windows set as above. At the conclusion of this imaging in these animals, and at the time of slicing in the others, the slabs were frozen until hard. All freezing was performed by dipping the slabs into an isopentane solution that had been cooled with dry ice. After being stored at −35° C for a minimum of 3 days to allow for decay of the technetium 99m and iodine 123, 40-μm sections were mounted on glass slides, heat-fixed, and loaded with film in autoradiography cassettes for subsequent exposure. Films were exposed for 14 days, after which they were removed and processed.

Angiograms were graded in a nonblinded manner by one investigator as to the degree of MCA occlusion. No occlusion was graded as 0; incomplete occlusion was graded as 1; and complete occlusion of the MCA was graded as 2.

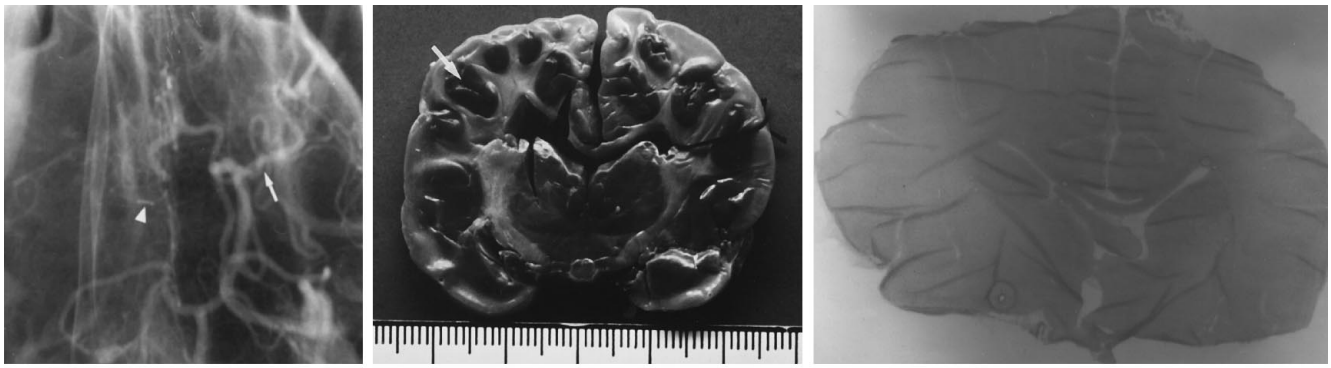


Fig 1. Example of normal brain in an animal undergoing 30 minutes of MCA occlusion 48 hours earlier.

A, Angiogram shows occlusion. Note filling of MCA on the nonembolized side (*arrow*) and plug in MCA with nonfilling of that artery on the embolized side (*arrowhead*).

B, TTC-stained (*arrow*) coronal slice of brain shows symmetry without evidence of infarction.

C, Coronally oriented ^{45}Ca autoradiograph shows hemispheric symmetry without areas of significantly increased uptake.

Increased ^{45}Ca uptake on autoradiography or decreased IMP uptake relative to the baseline HMPAO SPECT images was graded on a scale of 0 (none), 1 (subtle, questionable), 2 (definite, mild to moderate change), and 3 (marked change).

An adequate dose of calcium (250 μCi/kg) was established in previous studies by using a 2-week exposure and 40-μm-thick sections (P. D. Purdy, A. R. Peterson, H. H. Batjer, et al, "Ca^{45} Autoradiography Distinguishes Uptake between Temporary and Permanent Cerebral Artery Occlusion," *Stroke* 19;23:139, abstract). The doses of technetium 99m and iodine 123 were established in the first few animals studied and were similar to those given to humans.

Results

Group A

All animals survived the 48-hour experimental procedure. No animal that underwent 30-minute temporary plug placement showed evidence of infarction with TTC staining or abnormal areas of increased ^{45}Ca uptake on autoradiographic examination (Fig 1). All animals that underwent permanent plug placement had infarction on TTC staining. In the four animals whose brains were stained with hematoxylin-eosin, histologic infarction was seen in areas stained with TTC. In addition, all animals in whom infarction was induced showed increased ^{45}Ca uptake in the infarcted region, although some variation in the size and location of the infarct was demonstrated (Figs 2–4). There appeared to be clear delineation between infarcted and normal brain with no visible elevations in ^{45}Ca influx in the normal surrounding regions (presumed penumbra region).

Group B

Overall results are summarized in the Table. Group B contained 12 study dogs. One animal (dog 3) served as a control (Fig 5). Dog 1 had its MCA territory embolized with microfibrillar collagen (7), and dogs 2, 4, and 6 through 11 underwent successful plug occlusion of the MCA. One plug failure occurred (dog 5) and one animal (dog 12) died during the procedure. In animal 10, a plug was placed on one side for 2 hours and then removed and repositioned on the contralateral side, because we believed that adequate occlusion had not been achieved in the first attempt. This animal nevertheless had bilaterally increased calcium uptake. This dog was given a SPECT score of 0 because both MCAs were sufficiently occluded and as a result no difference could be seen on the SPECT scans (HMPAO versus IMP), since SPECT is qualitative rather than quantitative and, as such, depicts asymmetries between regions.

Of the nine successfully embolized or plugged animals (dogs 1, 2, 4, and 6 through 11), two failed to show SPECT changes despite positive calcium autoradiographic changes (dogs 6 and 8). Dog 2 had SPECT changes on the left and calcium uptake on the right. This inconsistency is most likely the result of an error

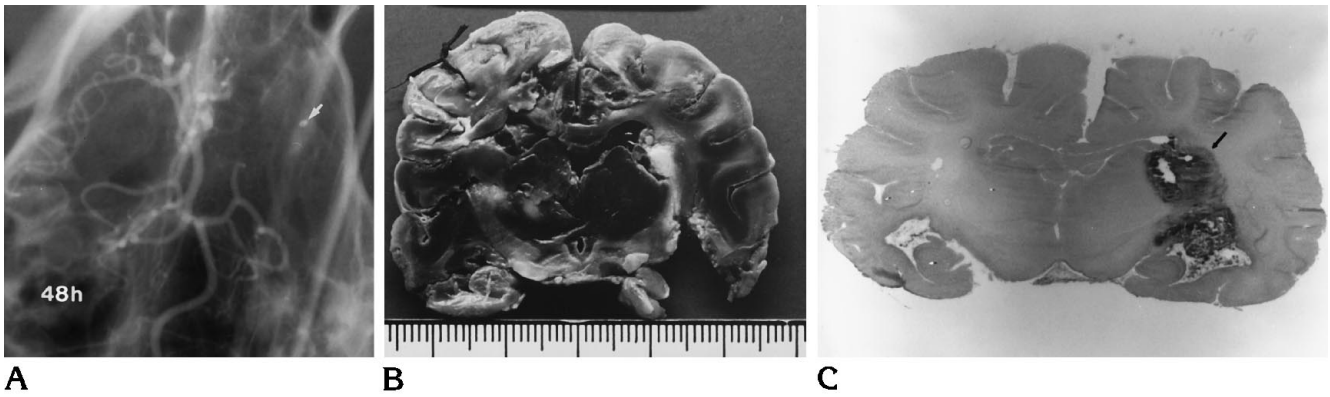


Fig 2. Corresponding arteriogram (*A*), photograph of specimen showing infarct staining (*B*), and autoradiograph (*C*) in a dog that underwent permanent arterial occlusion. Note variability in infarct size. *Arrow* in *A* indicates fine (0.008-cm) platinum wire embedded in the plug. Arteriogram was obtained 48 hours after plug placement. *Arrow* in *C* indicates calcium uptake at infarct site.

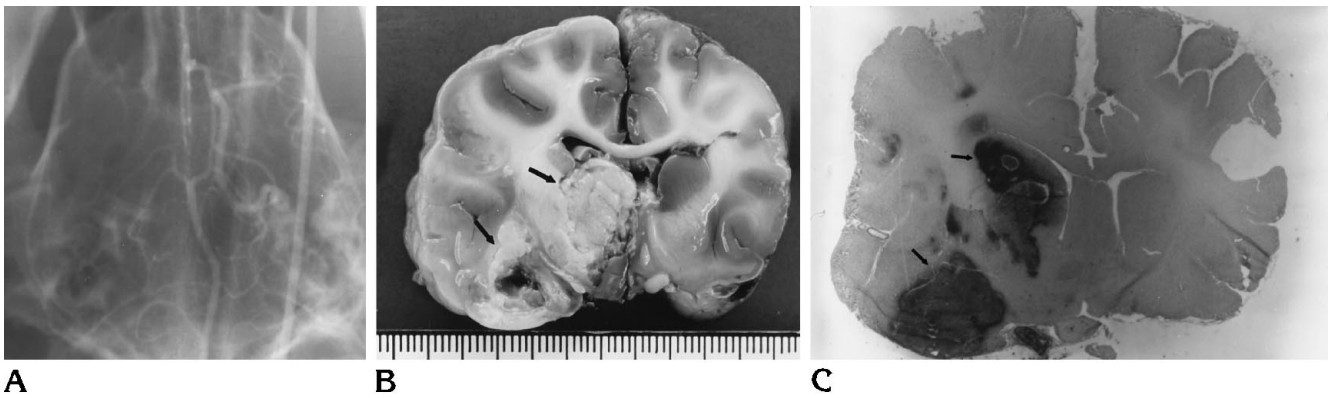


Fig 3. Corresponding arteriogram (*A*), photograph of specimen showing infarct staining (*B*), and autoradiograph (*C*) in a dog that underwent permanent arterial occlusion. Note variability in infarct size. *Arrows* show areas of histologic infarct (*B*) and corresponding calcium uptake (*C*).

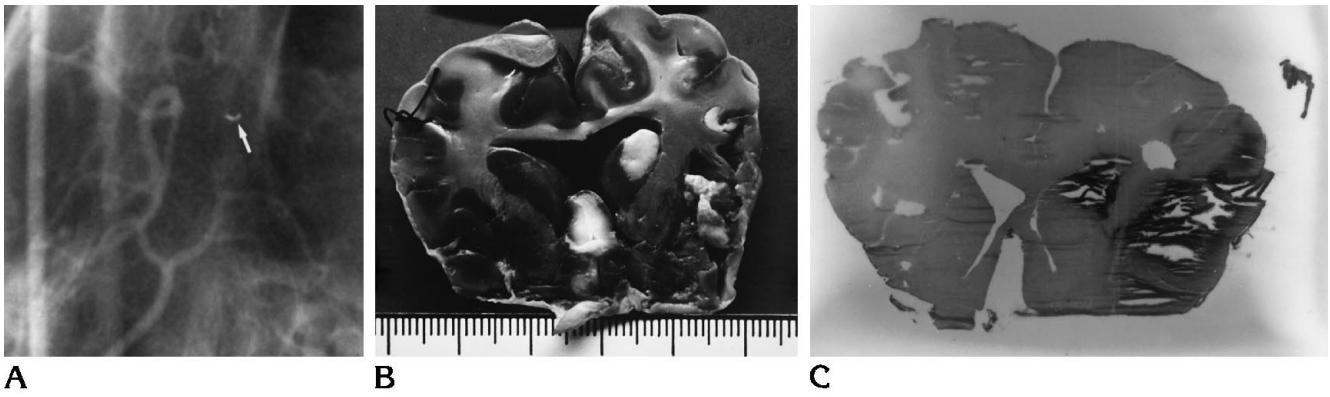


Fig 4. Corresponding arteriogram (*A*), photograph of specimen showing infarct staining (*B*), and autoradiograph (*C*) in a dog that underwent permanent arterial occlusion. Note variability in infarct size. *Arrow* in *A* indicates fine (0.008-cm) platinum wire embedded in the plug.

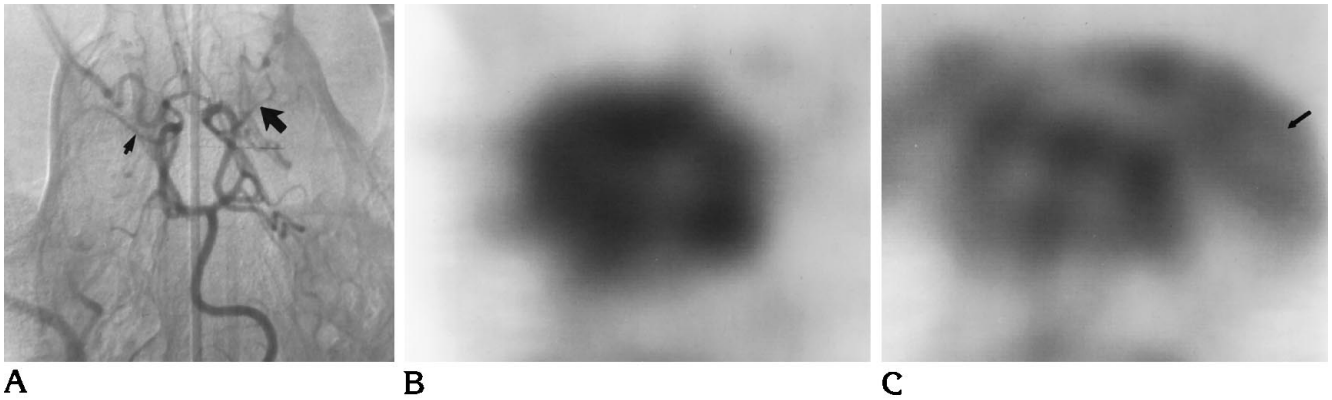
Results of calcium-45 autoradiographic and SPECT studies in a canine model of cerebral ischemia and MCA occlusion

Animal*	Weight, kg	Angiography Score†	Autoradiography Score‡	SPECT Score‡	Plug Side	HMPAO, mCi	IMP, mCi	Arterial Oxygen Pressure	Arterial pH
1	15	None	2 R	3 R	No plug; collagen	8	1	221	7.41
2	23	2 L	2 R	1 L	L	10	1	96.5	7.41
3	14	None	0	0	None	7.6	1	None	None
4	26	2 R	3 R	3 R	R	20	3	82	7.37
5	32	0	0	0	R	20	3.5	191	7.17
6	24	2 R	3 R	0	R	19.1	3	168	7.37
7	27	1 R	0	0	R	None	4	188	7.57
8	31	1 L	2 L	0	L	26.4	4.4	249	7.40
9	25	1 L	2 L	2 L	L	23	4.8	168	7.39
10	28	1 R	2 Both	0	R	18.7	4.6	84	7.35
11	28	2 R	2 R	2 R	R	20.9	4	154	7.39

* Dog 12 died during the procedure.

† Degree of MCA occlusion: 0 indicates no occlusion; 1, incomplete occlusion; 2, complete occlusion.

‡ Increased calcium-45 uptake on autoradiography or decreased IMP uptake relative to the baseline HMPAO SPECT images: 0 indicates none; 1, subtle or questionable; 2, definite, mild to moderate change; 3, marked change.



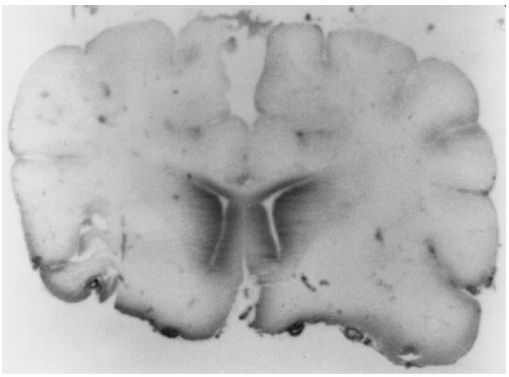


Fig 5. *A*, Canine cerebral angiogram in a control animal. The left MCA (*small arrow*) and right MCA (*large arrow*) are patent.

B, SPECT IMP scan in a control dog. IMP uptake is symmetric.

C, SPECT HMPAO scan in a control dog. HMPAO uptake is symmetric. The *arrow* points to scalp uptake of the radioisotope.

D, Coronally sectioned calcium autoradiograph of a control animal's brain. The middle and anterior cerebral territories show no abnormal calcium uptake.

in labeling the slab during autoradiography. Therefore, in summary, seven animals (dogs 1, 2, 4, 5, 7, 9, 11) had calcium and SPECT findings that were consistent (Fig 6). Six of these represented ischemic conditions (dogs 1, 2, 4, 7, 9, 11) and one represented a nonischemic condition (dog 5). One plugged animal (dog 5) had poor MCA occlusion, with calcium and SPECT findings that were consistent with no decrease in cerebral perfusion. One animal (dog 10) had calcium uptake bilaterally with no asymmetry on SPECT, reflecting reductions in blood flow that were bilaterally symmetric. Direct planar imaging of brain slices was not thought to increase sensitivity to hypoperfusion beyond that of the reconstructed images of the brain.

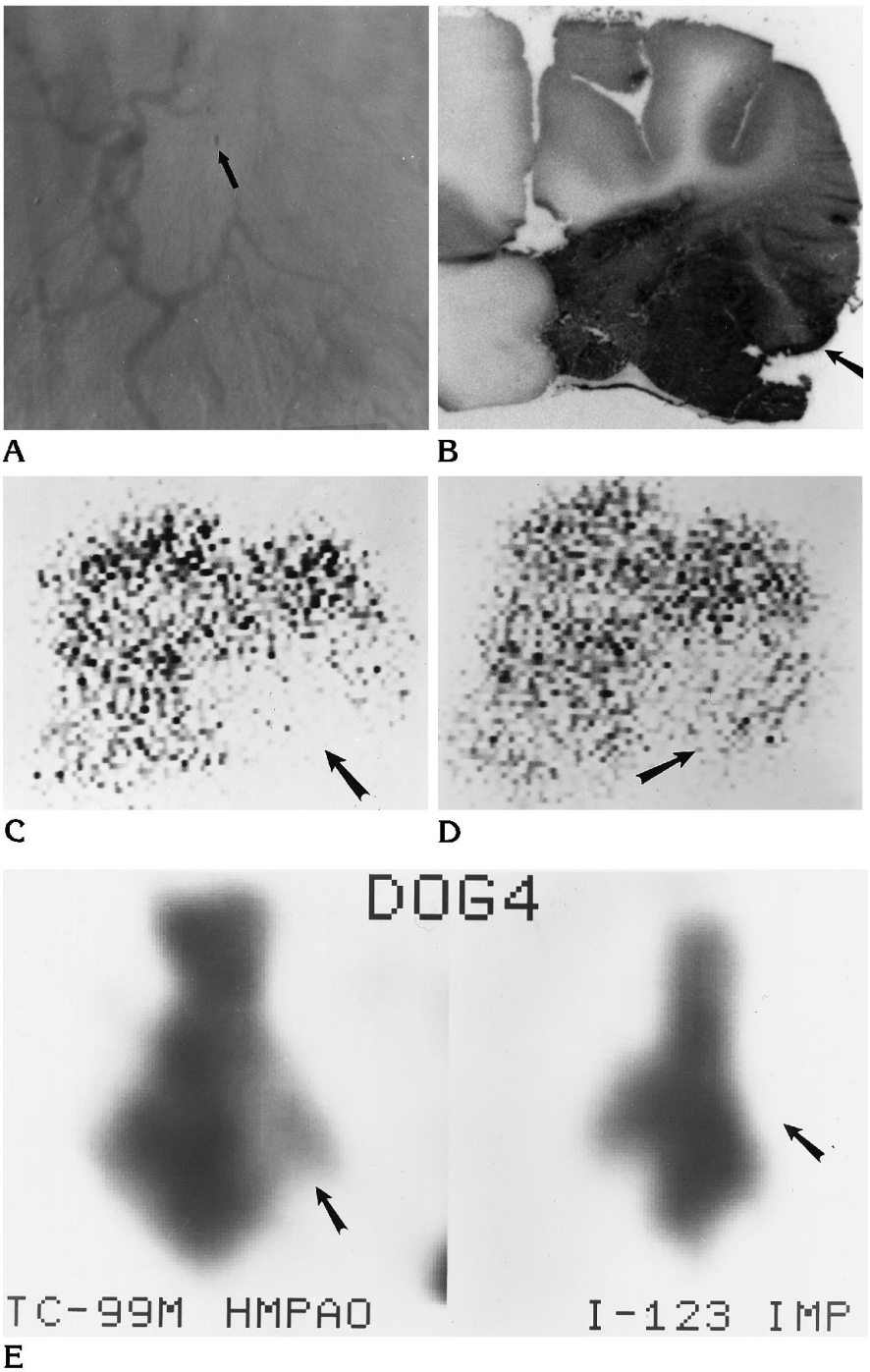


Fig 6. *A*, Cerebral angiogram shows a plug with marker in the left MCA (*arrow*). There is no opacification of the middle cerebral branches on the occluded side.

B, Calcium autoradiograph shows abnormal calcium uptake in the left middle cerebral territory (*arrow*) after left MCA plug occlusion.

C, SPECT IMP scan shows reduction in uptake in the left MCA territory (*arrow*), corresponding to the area of abnormal calcium uptake in *B*. The image was obtained by exposing film to a coronal section of brain.

D, SPECT HMPAO scan shows reduction in uptake in the left MCA territory (*arrow*), corresponding to the area of abnormal calcium uptake in *B* and decreased IMP uptake in *C*.

E, HMPAO and IMP uptake after MCA occlusion as recorded by using the gamma camera. The areas of reduced uptake are indicated by *arrows*. The areas of uptake at the top of the image represent the nasal mucosa.

Discussion

Calcium Autoradiography

Many authors have studied the role of calcium as an intracellular toxin (8–13). Early ischemic events include membrane depolarization followed by depletion of energy reserves (adenosine triphosphate [ATP], adenosine diphosphate [ADP], and adenosine monophosphate [AMP]) as membrane sodium Na-K ATPases act to maintain the transmembrane ion balance and potential difference. As intracellular sodium rises, calcium also moves from the extracellular space to the intracellular compartment. Mitochondrial calcium as well can be released within the intracellular space. As calcium rises, ATP-dependent processes in the cell membrane, endoplasmic reticulum, and synap-

tic vesicles would normally act to restore equilibrium but are disabled in the ATP-depleted state. Mitochondria are capable of exchanging calcium for hydrogen ions at the expense of oxidative phosphorylation, thus restoring some balance at the cost of additional energy utilization and lowered intracellular pH. Rising intracellular calcium also activates membrane phospholipases, contributing to further membrane breakdown. Release of excitatory neurotransmitters (eg, glutamate and aspartate) during ischemia open the so-called calcium channels, allowing the inflow of calcium ions from the extracellular fluid to the intracellular space. These processes have been summarized by Raichle (14) in greater detail.

With inflow of calcium and subsequent disruption of the cell membrane, large fluid shifts into the cell occur, and cell body integrity is lost. In the setting of ischemia, calcium therefore acts as an intracellular toxin (14). Investigators have shown that the time course of calcium accumulation predicts a tissue's fate (3, 15). Following reperfusion, areas with rising or persistently elevated calcium concentrations are histologically more severely damaged than are areas where calcium concentrations normalize (3, 16). NMDA-receptor-mediated channels may be responsible for these calcium fluxes, since injury is ameliorated by preischemic administration of MK-801 (17). The desire to regulate the inflow of calcium has resulted in trials of calcium channel blockers in ischemia with some success (18–23), although the need to administer these drugs early during ischemia, if not before ischemia, may limit their usefulness. Wong and Haley (24) reviewed the status of calcium channel blockers in stroke therapy.

One method with which to study intracellular calcium is via ^{45}Ca autoradiography, which involves the administration of a radioactive substrate followed by placement of the tissue specimen on a photo detecting device (film or detector). By standardizing the radioactivity, quantitation of calcium activity in injured versus normal brain becomes possible. The majority of work with this technique has involved smaller species. Gramsbergen et al (25) used kainic acid to create lesions in rat striatum and then performed ^{45}Ca autoradiography 1, 4, 14, 28, and 90 days after injury. By injecting 200 μCi 24 hours before they killed the animals, these researchers found that peak intracellular calcium influx occurred 4 to 14 days after injury, thus leading them to conclude that calcium accumulated in dying brain and not in already dead tissue. Dienel (26) used transient forebrain ischemia in rats to document an increase in intracellular calcium when calcium was administered 2.5 hours before ischemia or 0, 24, 48, and 72 hours after ischemia. Although some accumulation was seen as soon as 1 hour after injection, this accumulation continuously increased for at least 24 hours after injection. Of note, uptake in the caudate-putamen was greatest after 24 and 48 hours of ischemia. Therefore, by administering ^{45}Ca 24 hours after an ischemic insult and allowing it to accumulate for 24 hours, demonstration of uptake was maximized. This formed the basis for the timing chosen by us, although Dienel used intraoperative administration. Other investigators have used ^{45}Ca to study pathologic brain processes. Hovda et al (27) and Fineman et al (28) used ^{45}Ca autoradiography to study neuronal injury after percussive trauma to the lateral part of the brain in a rat model. Araki et al (29, 30), Nakamura and Hatakeyama (18), and Shirotani et al (31) used these techniques to investigate postischemic calcium uptake and the effects of NMDA-receptor antagonists and calcium channel blockers in modifying calcium uptake and neuronal damage.

Stein and Vannucci (4) studied calcium fluxes during hypoxia-ischemia and concluded that significant alterations in cellular balance may be critically associated with overt tissue injury. Using ^{45}Ca autoradiography, they showed cerebral homogeneity of ^{45}Ca activity in ischemic regions up to 15 days after the insult. By sampling plasma and cerebrospinal fluid activity, they were also able to calculate that at least 83% of the calcium activity observed was from the intracellular compartment.

Dosages of ^{45}Ca and expected normal appearances in dogs have not been previously reported in detailed fashion. The size of the dog brain creates significant technical obstacles to whole-brain autoradiography owing to the need to produce thin tissue sections free of significant artifacts. Our experience with this technique has shown its reliability and feasibility even when ^{45}Ca is administered intravenously. While adequate for radionuclide autoradiography, the dose of ^{45}Ca used in this study produced no pharmacologic effects.

We confirm in the canine model that 48 hours after a 30-minute ischemic insult there is no

evidence of infarction or abnormal ^{45}Ca uptake when the ^{45}Ca is given 24 hours before the animal is killed. Conversely, if there is permanent occlusion, infarction occurs and the infarcted tissue takes up ^{45}Ca more readily than the normal tissue does. Although this finding seems simplistic and is redundant relative to prior studies with smaller species, it is an important step toward the validation of this technique in dogs. Since the same mechanism is used for both temporary and permanent ischemia (plug placement), the 30-minute ischemic mechanism would have resulted in infarction had the plug not been extracted.

Our experience with intravenous calcium administration in dogs shows accumulation of calcium in infarcted regions but no accumulation in areas that undergo a transient ischemic insult if calcium is administered 24 hours after permanent or temporary MCA occlusion (7). These studies show that calcium autoradiography can indicate ischemic change quite early after the onset of vascular occlusion. We have seen marked uptake of calcium in as little as 30 minutes after the onset of occlusion (P. D. Purdy, unpublished results). The combination of rapid uptake of calcium in tissue with a permanent ischemic insult but a lack of long-term calcium uptake in brain undergoing a reversible ischemic insult with subsequent reestablishment of flow before an infarction develops suggests that there are significant reversible calcium shifts during cerebral ischemia, thus confirming the findings of Uematsu et al (17). A disturbing finding is the presence of increased calcium in hemispheres with an occluded or near-occluded MCA despite a normal IMP SPECT study that failed to detect the blood flowchange that induced the increased calcium uptake.

SPECT

SPECT imaging of CBF has proved useful in a variety of clinical and diagnostic situations in which it is desirable to have blood flow information both before and after therapeutic or diagnostic intervention (6). For the most part, these scans represent qualitative rather than quantitative information. What is evaluated are changes in patterns of perfusion or development of hemispheric asymmetries rather than calculations of actual blood flow. There is also some question as to the reliability of this technique in detecting changes at the extremes of perfusion (less than 20 mL/100 mg per minute and greater than 60 to 80 mL/100 mg per minute). In the present study, SPECT imaging failed to detect asymmetries of perfusion in three animals (dogs 6, 8, and 10) despite adequate plug placement as demonstrated both by angiography and by deposition of calcium in the appropriate MCA distribution. In dog 10, this appearance may have been due to diminished perfusion in both hemispheres, as evidenced by bilateral calcium deposition, which resulted in a relatively symmetric SPECT appearance. The lack of asymmetry in the postplug scans is not easily explained in the two remaining animals (dogs 6 and 8), although technical limitations in the dual-isotope technique as well as resolution problems in adequately imaging the much smaller dog's brain on a system designed for clinical use may be explanatory.

Because of the much smaller dose of ^{123}I-IMP used relative to ^{99m}Tc-HMPAO (almost 1:6), count statistics in the IMP scans were much lower than for the baseline scan. Low count statistics inherently lower the image quality and resolution and while it would be ideal to have the situation reversed such that IMP was used for the baseline, the in vitro instability of HMPAO used in this study would not permit that. The lower counts in the postplug studies may have made subtle asymmetries of perfusion less perceptible. In addition, the small size of dogs' brains relative to skull size and thickness may have further reduced image quality because of attenuation of counts. Finally, delivery of either of these brain agents is highly dependent on overall CBF. It may have been that general cerebral perfusion was diminished in the two dogs such that delivery of the agent was reduced.

Conclusion

This study raises many questions. First, because ^{45}Ca was administered 24 hours after the ischemic insult in the dogs who underwent permanent occlusion, we do not know what calcium fluxes occurred during this initial 24-hour postischemic period. Clearly, there was ongoing uptake; however, this study failed by design to elucidate its time course. It would be of particular interest to study ^{45}Ca shifts very early during ischemia. With ^{45}Ca uptake as a marker of ischemic injury, this model can be used to study SPECT scans. Dual isotope SPECT can

show changes in cerebral perfusion after an experimental or diagnostic manipulation of CBF. Our study results point to the potential clinical applicability of dual-isotope scanning yet also indicate that detection of ischemia with IMP using current technologies and models is not yet ideal. Dual-isotope imaging cannot be considered to have reliable clinical applicability to humans until its sensitivity and specificity are further defined.

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