Tissue Pertechnetate and Iodinated Contrast Material in Ischemic Stroke

DAVID C. ANDERSON, M.D., DAVID T. COSS, B.A., ROBERT L. JACOBSON, PH.D., AND MAURICE W. MEYER, D.D.S., PH.D.

SUMMARY Isotope uptake during static radionuclide scanning and contrast enhancement during CT scanning, which may result from similar pathophysiologic mechanisms after ischemic infarction, were investigated in an animal model. Infarction was produced by transorbital occlusion of the middle cerebral artery in cats killed one, 2, 4, 8, or 16 days later. Sodium pertechnetate containing technetium-99m and 30% methylglucaraine iothalamate labeled with I-125 were administered intravenously 60 and 15 min respectively prior to sacrifice. A coronal section through the infarct was parceled into 30 portions which were assayed for concentration of each isotope. Adjacent brain was prepared for histopathologic correlation. Concentrations of the 2 materials were highest in infarcted brain at 4 and 8 days. Strong positive correlation was found between tissue concentrations of the 2 materials in all brain samples. Elevated tissue levels correlated with necrosis, macrophage infiltration, and vascular hyperplasia. The results support the probability that radionuclide scan positivity and CT contrast enhancement reflect the same pathophysiologic development, probably extravasation of the respective labels, after ischemic stroke.

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access catheters were inserted. Through the foreleg intravenous line, sodium pertechnetate containing technetium-99m (approximately 2 millicuries per animal) and 5 cc/kg of 30% methylglucamine iothalamate (Conray 30, Mallinckrodt Inc., St. Louis, MO) containing a small amount of sodium iothalamate (Mallinckrodt Inc.) labeled (New England Nuclear) with 1-125 (20 to 60 microcuries per animal) were administered 60 and 15 minutes respectively prior to sacrifice. Just before the animals were killed by administration of a saturated solution of potassium chloride, a sample of venous blood was obtained from the femoral intravenous catheter for determination of pertechnetate and iodine content.

Following sacrifice the skull was rapidly rongeured and the neuraxis divided at the lower medullary level allowing for removal of the intact brain. A coronal slice 2 to 3 millimeters thick was taken from the area of cerebral infarction by making 2 coronal sections, the rostral-most at the level of the temporal tips. The fresh coronal slice was immediately parceled into 30 numbered specimens following a uniform scheme so that later histopathologic correlation could be made by knowledge of the relative location of the numbered specimens in the slice (fig. 1). Specimens one to 15 from the left hemisphere, containing ischemic and infarcted brain, and 16 to 30 from the nonischemic right hemisphere were then placed in pre-weighed vials. The brain remaining after removal of the coronal slice was put in formalin for histopathologic examination. The vials containing brain tissue were weighed, and wet weight was calculated for each specimen. The radioactivity due to the presence of pertechnetate labeled with technetium-99m and that due to iothalamate labeled with I-125 were then determined in each specimen using a gamma scintillation counter. Preliminary studies demonstrated that the radioactivity of the 2 isotopes could be separated by pulse-height analysis. The venous blood sample obtained just prior to sacrifice was treated in the same way. Brain:blood ratios for pertechnetate labeled with technetium-99m and for iothalamate labeled with I-125 were calculated for each specimen from the tissue and blood concentrations.

The brain remaining after removal of the coronal slice was fixed in formalin and subsequently embedded in paraffin. Thin sections of the 2 surfaces adjoining those of the removed slice were prepared, mounted on slides, and stained with hematoxylin and eosin. The uniformity with which the fresh coronal slice had been parceled into 30 numbered specimens was approximated on the slides by drawing a grid defining the area corresponding to each of the 30 specimens. The slides were then examined under a microscope, and each of the 30 areas of the 2 slides was characterized histopathologically. Thereafter, by inference, left-sided numbered specimens of the fresh coronal slice which were bordered by an infarcted area, either anteriorly or posteriorly, were considered "infarcted." Left-sided specimens not bordered by an infarcted area were designated "ischemic," and right hemispheral specimens were called "non-ischemic."

The effect on brain:blood ratios of time elapsed since occlusion was investigated. In the initial analysis specimens were grouped by the histopathologic criteria described above. While the extent and distribution of infarction was variable among the 13 animals, 2 areas, corresponding to specimens 7 and 8, were infarcted in all 13 (fig. 1). It was possible, therefore, to assess evolving infarction in topographically and histologically identical regions of brain over time after occlusion. In that analysis, the brain:blood ratios of the homologous specimens 22 and 23 in the non-ischemic right hemisphere were compared. Correlations were determined between pertechnetate and iodine brain:blood ratios for each time interval in infarcted, ischemic, and non-ischemic tissue. Finally, the histopathologic features of infarcted tissue at various intervals following occlusion were correlated with brain:blood ratios of pertechnetate and iodine.

Table 1
Clinical Experience is Indicated by the Likelihood that Each Finding Will be Demonstrated by Static Radionuclide and CT Scanning at Various Intervals Following Cerebral Infarction. (Scale: 0 = unlikely; ++++ = Almost Certain)

<table>
<thead>
<tr>
<th>Time</th>
<th>1st week</th>
<th>2nd-4th weeks</th>
<th>2nd-6th months</th>
<th>≥ 7 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT Scan (Ref. 12-26)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low density lesion</td>
<td>+ + + +</td>
<td>+ + + + + + +</td>
<td>+ + + + + + +</td>
<td>+ + + + + + +</td>
</tr>
<tr>
<td>Mass effect</td>
<td>++</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Contrast enhancement</td>
<td>+</td>
<td>+ +</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Static RN Scan (Ref. 1-13)</td>
<td>+ - + +</td>
<td>+ + + + + + +</td>
<td>+ + + + + + +</td>
<td>+ + + + + + +</td>
</tr>
</tbody>
</table>

Figure 1. The study slice was parceled fresh into 30 specimens.
Results

Effect of Time and Histopathologic Grouping on Brain: Blood Ratios

The effect of time after occlusion on brain: blood ratios of pertechnetate and iodine in infarcted, ischemic, and non-ischemic tissue is shown in figure 2. The mean brain: blood ratios for all specimens at each time interval are connected by the lines, while the mean for each animal is indicated by an individual point. There is no apparent change with time in brain: blood ratios in the hemisphere contralateral to the occlusion (non-ischemic tissue). There is modest elevation of ratios in ischemic tissue and prominent uptake of the 2 materials in infarcted tissue. It can be seen that the brain: blood ratios are highest in infarcted tissue at 4 and 8 days after occlusion. Variability, reflected by distribution of the individual animal means for each ratio, is greatest when the ratios themselves are highest, at 4 and 8 days. Finally, the lines representing the changing brain: blood ratios with time in each of the tissue types are parallel for the 2 materials. The pertechnetate brain: blood ratio is consistently slightly greater than that for iodine, but changes in one ratio are faithfully mirrored by changes in the other.

The brain: blood ratios of specimens 7 and 8, which were adjoined by infarcted areas in all animals, were also graphed against time for each material (fig. 3). The ratios of the homologous areas 22 and 23 contralateral to the occlusion are shown for comparison. Again, brain: blood ratios in the infarcted areas are markedly elevated at 4 and 8 days but not at one, 2, or 16 days after occlusion. The changes are parallel for the 2 isotopes, and again the ratio for pertechnetate is slightly greater at each time interval.

Correlation of Pertechnetate and Iodine Brain: Blood Ratios

As expected, strong positive correlations were found between pertechnetate and iodine brain: blood ratios (table 2). Strong direct correlation was observed in each histopathologic category and at all time intervals after occlusion.

Histopathologic Correlates

The hematoxylin and eosin stained sections of brain adjoining the slice were examined to determine specific histopathologic correlations. Neuronal ischemia, histopathologic edema, inflammatory response, frank necrosis, macrophage infiltration, vessel hyperplasia, cyst formation, and gliosis were noted for each time period. The most prominent features of the specimens of 4 and 8-day-old infarcts were necrosis, macrophage infiltration, and hyperplastic vessels.

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Table 2: Correlation Coefficients Demonstrate the Strong Direct Relationship Between Brain/blood Ratios for Pertechnetate and Iodine in all Tissue Types and at all Time Intervals Following Occlusion

<table>
<thead>
<tr>
<th></th>
<th>Time (days) after occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Infarcted (left)</td>
<td>0.95</td>
</tr>
<tr>
<td>Ischemic (left)</td>
<td>0.90</td>
</tr>
<tr>
<td>Non-ischemic (right)</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Discussion

The study results indicate that the behaviors of pertechnetate and iodinated contrast material are similar during the pathologic evolution of cerebral infarction. Because the 2 materials become concentrated at the same time and in the same tissue, it seems likely that the pathogenetic mechanisms which determine the behavior of one material determine the behavior of the other as well. Several mechanisms have been proposed to explain the radioisotope uptake responsible for static RN scan positivity in cerebral infarction. Suggested as being pathogenetically important have been edema, the so-called "luxury perfusion," and several features of necrosis including overt extravasation of blood (hemorrhagic infarction), endothelial proliferation, and macrophage infiltration. Finally, the development of gliosis has been suggested as responsible for radionuclide uptake.

Several investigations have demonstrated that the temporal profile of edema following infarction is different from that of isotope tracer accumulation. Ischemic cerebral edema has characteristics of both the "cytotoxic" and "vasogenic" models which have been produced experimentally. The earliest and major fluid accumulation following ischemic injury is presumably the result of intracellular water accumulation due to metabolic failure of cellular membrane homeostasis. This cytotoxic edema is not accompanied by alteration of the blood-brain barrier to radioactive and other tracers. Subsequently, as the lesion "matures," alteration in barrier characteristics does occur, but water accumulation is then on the wane.

Luxury perfusion, an angiographic syndrome of local contrast "blush" with early draining vein, has been another proposed mechanism of static RN scan positivity. However, the temporal profiles of the angiographic phenomenon and static RN scan positivity are different. The angiographic finding correlates instead with increased local isotope activity in the rapid sequence radionuclide scan, the so-called "isotope flow study." In such infarctions or "hot strokes," the static RN scan is negative when the isotope flow study is positive, and the angiogram shows luxury perfusion. Later, at the expected time, the static RN scan becomes positive, presumably because of other developments.

There is little support for the idea that gliosis is responsible for RN scan positivity since the neuropathologic phenomenon persists, while the scan eventually becomes negative after infarction.

There is better support for the importance of several features of brain necrosis in static RN scan positivity. The development of altered barrier characteristics to various labels is typical of the maturing infarct. An extreme example of barrier alteration is hemorrhagic infarction with extravasation of red cells into brain parenchyma. The phenomenon is particularly common following cerebral embolism with recannalization or clot lysis exposing the compromised distal vascular bed to increased intraluminal pressure. Such a course of events occurs early in the process of infarct evolution and may be responsible for unexpectedly early static RN scan positivity. Less flagrant barrier disruption is reflected by accumulation of intravenously delivered, protein-bound labels as the process of necrosis continues. The alterations are probably important in static RN scan positivity in the second through fourth weeks. Label concentration occurs contemporaneous with the peak activity of mononuclear macrophages and neovascular capillary proliferation. There is experimental support that these latter 2 developments are causally related to isotope concentration.

The mononuclear macrophages may take up and trap the label after it gains entry to the extravascular space through defects in the walls of proliferating immature vessels. Radioactive mercury-labeled chloromerodrin, which is thought to behave similarly to technetium-99m, has been demonstrated by autoradiographic techniques in mononuclear cells, particularly in regions of capillary proliferation, after infarction. Employment of similar techniques to localize iodine accumulation more precisely would be of interest.

Investigations of the mechanism of contrast enhancement in CT scanning have suggested that it also depends on extravascular accumulation of the index material in most lesions. 10-12 It has been pointed out that following the intravenous administration of contrast material, enhancement follows a different time course than that of plasma iodine concentration. Enhancement is not limited to lesions which are vascular by other standards, and tissue iodine concentration occasionally exceeds that of blood, which would be impossible if the material were confined to the intravascular compartment.

Our data confirm that static RN scan isotope and CT contrast material probably are concentrated in the same tissue at the same time following cerebral infarction. Their presence probably correlates with the histopathologic development of tissue necrosis with macrophage infiltration and vascular hyperplasia. Further, the magnitude of brain-blood ratio changes is similar, suggesting that characteristics which are important in extravasation and tissue uptake of the 2 materials — such as degree of plasma protein binding, the actual protein species to which the materials are bound and the size and charge of the resulting complex — resemble one another.
In the study of experimental cerebral infarction in the cat model, the materials in question are most avidly taken up at 4 and 8 days. Because the temporal profiles of tissue concentration of the 2 materials are similar, positivity of static RN scanning predicts that in the cat model, the materials in question are most avidly taken up at somewhat earlier than in the human. Further, conclusions must be qualified by the realization that the 2 imaging techniques may not be equally sensitive to the presence of increased tissue concentration of their index materials.

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Histochemical Changes of Brain Dopamine
In an Acute Stage of Cerebral Ischemia in Gerbils

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AND HAJIME HANDA, M.D.

SUMMARY The fluorescence histochemical method of Falck et al. was applied to 40 gerbil brains after ligation of a unilateral common carotid artery to investigate alterations of brain dopamine in the acute stage of cerebral ischemia. The distribution of dopaminergic terminals and cell bodies in gerbils is the same as in other mammals. On the ligated side after one hour of ischemia, diffuse green fluorescence of dopaminergic terminals showed only a slight decrease in intensity when compared to the nonligated side. But white matter and bundles of myelinated fibers adjacent to and in the dopamine-rich regions had an intense green fluorescence in contrast to the non-ligated side where they are normally non-fluorescent. This is considered to indicate the extraneuronal leakage and diffusion of dopamine. The intensity of extraneuronal green fluorescence was especially high in glial cells. Occasionally, there was also an unusual green fluorescence in the lumen of small vessels in dopamine-rich regions on the ligated side. Dopaminergic cell bodies in the substantia nigra on the ligated side revealed a conspicuous reduction in the fluorescence intensity in severely affected cases. After 2 or 3 hours of ischemia, there was a marked reduction or disappearance of the diffuse green fluorescence on the ligated side. This may be attributed in part to further diffusion of leaked dopamine.

CEREBRAL ISCHEMIA due to thrombi, emboli or vasospasm results in neural damage which may lead to alteration in synthesis, storage, release, receptor binding, re-uptake and degradation of neurotransmitters. Wurtman et al. suggested that monoamine neurotransmitters which escape from ischemic neurons may exacerbate the pathophysiological changes in ischemic brain. Recently, using Mongolian gerbils after unilateral common carotid artery ligation which consistently induces cerebral hemispheric ischemia in 30% to 60% of animals, changes in monoamine neurotransmitters such as dopamine, norepinephrine and serotonin have been reported. In this study, the fluorescence histochemical method of Falck et al. densely packed, very fine dopaminergic terminals are visualized by diffuse green fluorescence, mainly in nucleus caudoputamen, nucleus accumbens, tuberculum olfactorium, and median eminence. Dopaminergic cell bodies with green fluorescence are distributed in substantia nigra, area ventralis tegmenti and some hypothalamic regions. In this study, this histochemical method was applied to Mongolian gerbils with unilateral common carotid artery ligation to determine alterations in brain dopamine in cerebral ischemia.

Materials and Methods
Forty mature gerbils, each weighing between 60 and 80 gm, were anesthetized with ethyl ether. Using a stereoscopic microscope, we doubly ligated and transected the left common carotid artery. All animals were divided into a symptomatic and an asymptomatic group according to the neurological signs of cerebral ischemia described by Kahn. Three gerbils were sham-operated. Each animal was decapitated either 1, 2 or 3 hours after the ligation, and the brain was rapidly removed and processed by the monoamine fluorescence histochemical method of Falck et al. Sections of 10 μm thickness were made and observed with a fluorescence microscope, a Schott BG12 filter as an excitation filter and a Zeiss 50 filter as a barrier. Fluorescence differences were determined by comparing the cerebral hemispheres of gerbils with ligated carotid arteries and non-operated controls. One observer (M.I.) determined the change in fluorescence intensity without knowing which sections were taken from the brains of gerbils with or without neurologic abnormalities. Light microscopic observation was performed in some sections after Klüver-Barrera staining in order to identify anatomical structures and ischemic changes. The normal distribution of
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