LOCALIZATION OF ^{113m}In-CHELATES COMPARED WITH ^{99m}Tc-SODIUM PERTECHNETATE IN EXPERIMENTAL CEREBRAL LESIONS

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The ^{113m}In-tagged DTPA and EDTA chelates were first introduced for imaging the brain in February, 1967 (*1*). Little information exists, however, concerning the merits of these substances compared with other available radiopharmaceutical agents. Our investigation was designed to evaluate several ^{113m}In compounds in relation to ^{99m}Tc-sodium pertechnetate using experimental lesions of the cerebral cortex.

MATERIALS AND METHODS

Two methods were used to produce local disruption of the blood-brain barrier:

1. **Thermocoagulation trauma (Fig. 1).** Under nembutal anesthesia the cerebral cortex was exposed by craniotomy in a series of dogs. One lead of a resistance-controlled radiofrequency generator (Radionics, Inc., Burlington, Mass., Model RFG-2A) was grounded to the temporalis muscle while the other was connected to a needle electrode inserted 3 mm into the cortex. A single application of current (75–100 ma for 3–5 sec) resulted in a blanched area 3 mm in dia. To obtain a lesion size convenient for counting, contiguous areas were blanched until the total injured area was approximately 1 cm^2.
2. **Tumor (Figs. 2A, B).** Simian adenovirus #7* was injected intracerebrally into weaning hamsters using a transethmoid approach (*3*). Approximately 50% of the animals that survived weaning developed primary brain tumors (averaging 0.8 cm dia) within 4–6 weeks after injection. The histology of the tumors indicated that they were primarily of glial cell origin.

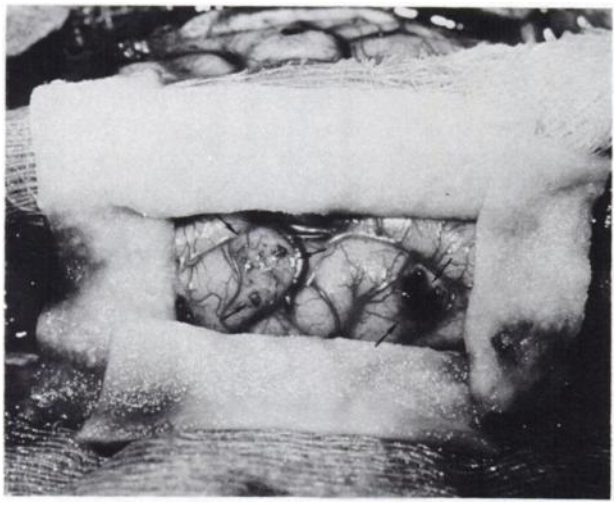

FIG. 1. 1-cm^2 lesions produced by thermocoagulation with radiofrequency generator electrode.

^{113m}In was obtained from a ^{113}Sn–^{113m}In generator (Neisler) by elution of the silica (SiO_2) column with 0.1 *N* HCl. Tin breakthrough was checked colorimetrically at a sensitivity of 0.1 ppm using hematoxylin.

The indium-tagged compounds that were tested consisted of unmodified indium chloride and the chelates ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), n-hydroxyethylethylenediaminetriacetic acid (HEDTA), nitrilotriacetic acid (NTA) and tetracycline. The injection material was prepared by adding 1 mg of ferric chloride in 0.1 *N* hydrochloric acid to 5–6 cc of the generator eluate followed by 2–4 mg of the chelate (*1*). Sodium chloride was then added to make the mixture isotonic, and the pH was adjusted to a point consistent with the maximum binding potential of the respective chelate. Finally, the material was autoclaved at 250°F and 15-psi pressure for 30 min. No macroagglutination was observed.

Received July 5, 1968; revision accepted Nov. 21, 1968.

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* An oncogenic strain isolated from the Vervet monkey (Cercopithecus aethiops) and known to produce subcutaneous tumors in hamsters (*2*).

The indium compounds were mixed with an aliquot of pertechnetate and injected intravenously so that comparative localization could be studied simultaneously in the same animal. The backscatter contribution by indium to technetium counts was corrected and minimized by giving approximately five times as much technetium as indium activity to each animal. Doses were selected so that photon yield would facilitate counting but would not introduce significant coincidence loss.

The radiofrequency lesions were made 15–30 min before radioisotope injection. It was found that uptake did not differ in lesions made between 5 and 120 min before injection. Both hamsters and dogs were sacrificed 30 min after injection, and samples of lesion and normal brain were taken for counting. The activity per gram of sample was determined and used to compute lesion-to-brain ratios. The fraction of injected dose per gram of lesion was also calculated from a standard in some of the dogs. A minimum of five dogs and five hamsters were studied with each agent listed.

RESULTS

In the trauma category indium-DTPA, EDTA and HEDTA resulted in lesion-to-brain ratios that were greater than those for pertechnetate in the same animals (Fig. 3). Somewhat contrary to previous reports (*4*), indium-EDTA and DTPA had essentially identical lesion-to-brain ratios, both averaging 1.7 times the ratios obtained with pertechnetate. Indium-NTA, tetracycline and unmodified indium chloride resulted in lesion-to-brain ratios that were less than pertechnetate.

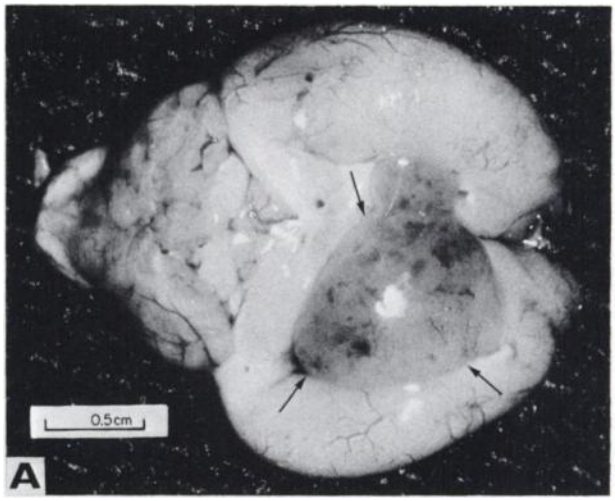
The hamster tumors were restricted to the study of indium-DTPA, EDTA and indium chloride. The results are quite similar to those obtained in the trauma system with the exception of indium chloride which localized somewhat better in the tumors than did technetium.

DISCUSSION

Almost all blood solutes are prevented from passing into normal brain by a phenomenon widely referred to as the blood-brain barrier (BBB) (*5,6*). Any lesion affecting the cerebral substance will alter this physiologic mechanism in some fashion, allowing certain material to pass in varying degrees from the blood to the abnormal area. The precise constitution of the BBB is still poorly understood but possibly contains some or all of the following factors:

1. Active brain-blood transport of the solute.
2. The rate of metabolic consumption of the solute by the brain cells.
3. The sink action of the cerebrospinal fluid.
4. The lipoid membrane formed by the capillary wall and the neuroglial foot processes covering it.
5. The effect of a small cerebral extracellular fluid space.

Permeability of the BBB to nonelectrolytes is primarily a function of their lipid solubility and to a lesser extent is related inversely to their molecular size. Since charged particles have negligible affinity



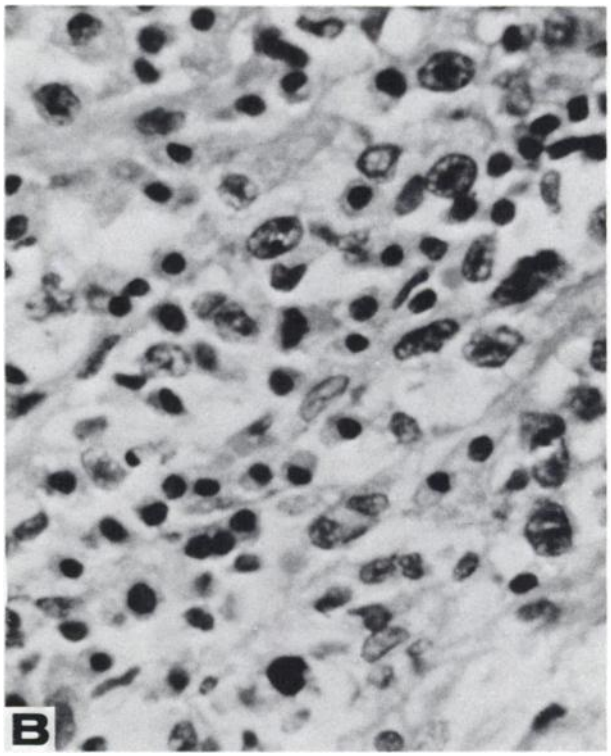


FIG. 2. A shows hamster brain with tumor produced by injection of SA-7 virus. B is microscopic section of tumor showing cells of glial origin.

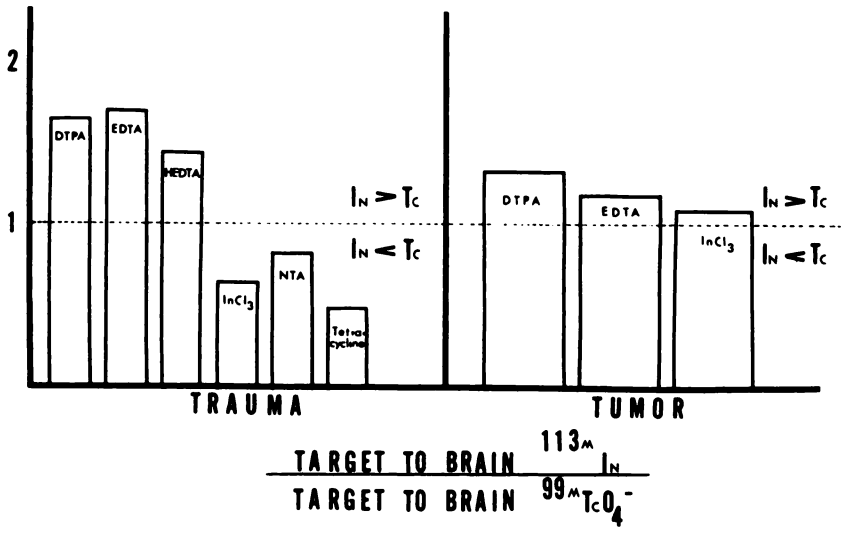


FIG. 3. Localization of ^{113m}In compounds in cerebral lesions compared with ^{99m}Tc-sodium pertechnetate. Values extending above dotted line indicate a higher ratio than that achieved with pertechnetate in same animals.

for lipids, electrolytes are excluded partly on this basis and in some instances by an active transport mechanism out of the cerebrospinal fluid. It should be emphasized that the correlation of entry rate with lipid solubility is not a feature peculiar to the central nervous system but has also been shown in connection with the passage of nonelectrolytes into both plant and animal cells (*7–11*).

Selective BBB disruption has been reported; it implies that certain agents may enter abnormal areas while others are partially or completely excluded (*12,13*). The factors that determine the degree of entrance of an agent into an abnormal area have not been defined in relation to radiopharmaceutical localization. An optimal brain-scanning compound should concentrate radioactivity highly in the lesion while at the same time be low in concentration in areas contributing to background. Since background activity comes from normal brain, blood-filled spaces and possibly extracerebral tissue in the immediate area, all of these must be evaluated in comparing scanning agents so that an estimate of the true target-to-background activity can be determined.

The type of lesions used in this investigation deserves comment. While they contain glial elements, the tumors represent only one category of neoplasia. Whether the lesion-to-brain ratios recorded here will be similar to those established with tumors of different cellular morphology remains for future observation. The thermocoagulation trauma is also highly restrictive and the cause corresponds poorly to that of spontaneous human pathology. Lesions of this kind studied at an inappropriate time might be misleading because large and inconsistent gradients in isotope concentration could exist in the surrounding area, thus rendering selection of similar lesion samples difficult. Consequently, we selected a time for animal sacrifice (30 min) when the disrupted barrier had reverted from a transient and rapidly changing high-grade breach to a relatively stable and persistent low-grade abnormality (*14*). Other investigators using vital dyes have reported minimal concentration gradients in the first few hours after thermocoagulation injury (*15,16*). In addition if one compares ^{113m}In and ^{99m}Tc content simultaneously in each lesion and normal brain sample, the problem of isotope gradients is largely overcome. This contention is supported by the consistent ratio of indium-to-technetium activity which we found in lesion samples from a given dog. For these reasons, we believe that this type of trauma provides a valid basis for the initial comparison of radiopharmaceutical localization. Further studies using different modes of injury will be necessary before broad conclusions can be reached regarding clinical disease.

The higher lesion-to-brain ratios obtained with the indium-tagged compounds in our study compared with pertechnetate could be due to greater lesion concentration, less concentration in normal brain or a combination of both. In an attempt to better define these factors, the actual fraction of injected dose per gram of lesion and per gram of normal brain was calculated in five dogs (Table 1). The concentration of radioactivity in the lesions did not differ significantly for indium-EDTA or pertechnetate, indicating no obvious selective permeability. The higher lesion-to-brain ratio with indium-EDTA must therefore be attributed to a difference in passage into normal brain, an observation which was borne out by the low fraction of injected dose in that tissue. This information, coupled with the lower absolute blood levels of activity at the time of scanning compared with pertechnetate, indicates a better over-all target-to-background radioactivity gradient.

TABLE 1. THERMOCOAGULATION LESION AND NORMAL-BRAIN RADIOACTIVITY RELATIVE TO ADMINISTERED DOSE (FIVE DOGS)

	^{113m}In-EDTA	^{99m}Tc-sodium pertechnetate	P
Lesion-to-brain ratios	11.0 ± 2.3 s.d.	6.9 ± 1.3 s.d.	<0.005
Fraction of dose/gm lesion (× 10^5)	7.16 ± 1.5	7.37 ± 1.7	0.35
Fraction of dose/gm normal brain (× 10^5)	0.651 ± 0.12	1.11 ± 0.20	<0.005

SUMMARY AND CONCLUSION

A series of ^{113m}In-tagged chelates was compared with ^{99m}Tc-sodium pertechnetate for degree of localization in experimental brain lesions of dogs and hamsters. The lesion-to-brain ratios obtained when the two radionuclides were given simultaneously were consistently higher for indium-EDTA and DTPA. Because the rapid disappearance of these compounds from the blood reduces background contribution to total activity, they may prove to be the agents of choice for brain imaging.

ACKNOWLEDGMENT

The authors are grateful for those indium generators supplied by Dean B. Holzgraf of Union Carbide Corp.

This work was supported in part by The National Cancer Institute, NIH Grant CA-04600, and USPHS Grant FR-05425.

Fred Rapp is an American Cancer Society Professor of Virology.

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