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DISCLAIMER

QUANTITATIVE AUTORADIOGRAPHIC MICROIMAGING IN THE DEVELOPMENT AND EVALUATION OF RADIOPHARMACEUTICALS

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SUMMARY: Autoradiographic (ARG) microimaging is the method for depicting biodistribution of radiocompounds with highest spatial resolution. ARG is applicable to gamma, positron and negatron emitting radiotracers. Dual or multiple-isotope studies can be performed using half-lives and energies for discrimination of isotopes. Quantitation can be performed by digital videodensitometry and by newer filmless technologies. ARG's obtained at different time intervals provide the time dimension for determination of kinetics.

INTRODUCTION

ARG microimaging can be performed on organs or on whole body of small animals from mice to small monkeys, using large-block cryomicrotomes. Radiolabeled compounds and their metabolites can be studied in normal animals and in disease models. If patients are pre-injected, tissues obtained at surgery or biopsy can also be studied. Animals sacrificed at various time intervals after injection provide data with time dimension, and temporo-spatial distribution and biokinetic pathways of radiocompounds can be determined.

AUTORADIOGRAPHIC METHODS

ARG microimaging involves the administration of radiolabeled compounds to normal animals or to animals with models of disease. Pharmacological interventions can be performed to investigate the effect on receptor binding, blocking, or to study compounds that alter normal distribution. Mice, rats and small monkeys up to 4 lbs. in weight can be

studied at predetermined intervals. Animals are quickly frozen in hexane/solid CO2 or in liquid nitrogen and embedded in carboxymethylcellulose. Sections of parts, organs or whole body are made with large-blade cryomicrotome, mounted on scotch-tape, and exposed on X-ray film along with step-wedge dilution standards. Compounds labeled with negatron (ß-), positron (ß+), or gamma (γ) emitters can be used. Long lived radioisotopes in common use are I-125, C-14, H-3, Fe-59. Short-lived isotopes such as F-18 and C-11 as well as Tc-99m, In-111, Ga-67, and I-123 can also be used. For short-lived isotopes rapid drying of sections for immediate exposure on X-ray film is needed. High-energy and very low-energy radionuclides require special X-ray film. Quantitation of ARG involves image capture by digital scanner or by high-resolution TV camera, image digitization, and comparison of ROI's to an array of stepwise dilutions of standards. Quantitation of data from animals sacrificed at various time-intervals, enables determination of temporo-spatial distribution of radiotracers. Systems currently available commercially are simple to operate and enable image enhancement, normalization, pseudo-coloring, and separation of isotopes, as well as three-dimensional reconstructions.

ARG microimaging can be performed with single, dual or multiple tracers (1,2). Separation of tracers is based on the different energies and different half lives of isotopes.

ARG Microimaging Applications - Examples

a. Dual Isotope ARG: Effects of Chlorpromazine on Glucose Metabolism

The effect of chlorpromazine was studied (Fig. 1) using two glucose analogs (3). C-14 labeled 2-deoxy-D-glucose (C-14-2DG), followed by chlorpromazine and F-18 fluorodeoxyglucose (FDG). Using a digital videodensitometric method (4) a 2x2 cm field

Figure 1. The effect of chlorpromazine (CPZ) on glucose uptake using the dual isotope technique. C-14-2DG depicts the baseline state and F-18-FDG depicts the effect of CPZ as compared to control (saline).

of view (film) was digitized to a 128x128 array, each pixel representing 156x156 μm. This high spatial resolution enabled quantitation in structures as small as the basal ganglia of mice.

b. Bone Studies with Sn-117m (4+) DTPA

Tin-117m diethylene triamine pentaacetic acid (DTPA) was shown to bind to normal bone and to transplanted osteosarcoma (6). This isotope has suitable imaging properties (158.6 KeV gamma rays 13.6-day half-life) and the Auger electron emissions make it is suitable for therapy of bone malignancies. ARG studies defined these characteristics pictorially.

c. Triple Isotope Studies: Myocardial Perfusion and Energy Substrate Utilization in Hypertensive Heart Disease

Perfusion, glucose and fatty acid utilization was studied in salt-sensitive hypertensive rats compared to genetically identical normotensive controls. Tl-201 5-min distribution was used as a perfusion marker, C-14-2DG, as a glucose analog, and C-14 beta methyl heptadecanoic acid (BMHDA), a synthetic fatty acid analog (5). In control rats, the distribution of the three compounds was homogeneous. In hypertensive hearts Tl-201 distribution remained uniform, whereas fatty acid decreased in the endocardium and in the free wall of the LV with a concordant increase in glucose utilization in same regions. This shift from preferential use of fatty acid to glucose is typical in myocardial hypoxia. The hypoxia resulting in shift from aerobic to anaerobic substrate utilization was not detectable by Tl-201, which remained unchanged (5).

d. Pharmacological Interventions: Somatic and Cerebral Distribution of Iodoamphetamine (IMP)

I-131 or I-125 labeled IMP whole-body distribution, and the effect of pharmacological manoeuvres were studied in several species (7). In the marmoset, high IMP uptake was seen in the chorio-retinal and ciliary body of the eye, cortical gray matter and the adrenal cortex. In the lungs, IMP uptake was patchy. Pretreatment with potassium iodide did not significantly alter the distribution of IMP. In mice, 125I-IMP concentrated in a band at the juxtamedullary region. This discrete finding would have surely been missed by conventional tissue distribution or scintigraphy. A coronal ARG and diagram of a rat head 60 min post IMP is shown in Fig. 2.

Figure 2. Coronal section of rat brain ARG after I-131-IMP. Note the exquisite resolution showing structures less than 1 mm in size (retina, fibers of olfactory bulbs).

e. ARG Studies with Monoclonal Anti-Tumor Antibody

Radiolabeled anti-colon/ovarian tumor antigen (COTA) antibody was studied in athymic nude rats bearing human colon cancer xenografts (8). Whole body ARG complemented the information obtained by tissue distribution. Intense accumulation of radioiodinated antibody was demonstrated in tumors.

f. ARG Studies of Addictive Substances

The time-course distribution of H-3-phencyclidine (PCP, "angel dust") was studied in mice (9). Immediately after injection, H-3-PCP accumulated in the pituitary, thyroid, lung, and stomach. At 2-min post-injection, there was uptake in the adrenals and in the gastrointestinal tract.

Previous studies on cocaine focused mainly on the brain. Using C-14 cocaine we showed the kinetics of cocaine in the whole body (10-12). Within 2-3 min intensive C-14 cocaine uptake was seen in the brain. At the same time, intense accumulation of cocaine was noted in the heart and adrenals concurring with clinical symptoms after cocaine intake, i.e., the initial feeling of "high", the euphoric state, tachycardia and elevation of blood pressure, and perspiration. Later, there was gradual washout of cocaine from the "primary target organs" with progressive accumulation in kidneys, liver, and G.I. tract probably representing the excretory pathways. Pretreatment with GBR 12909, a dopamine transporter blocker, caused a decrease of C-14-cocaine uptake in the brain (striatum, cortex), heart and adrenals. Pretreatment with desipramine, a norepinephrine/serotonin reuptake transporter blocker decreased C-14-cocaine uptake in the spinal cord, cerebellum, and to a greater extent in the kidneys (Fig. 3).

Figure 3. Sagittal sections of rat ARG after [C-14]cocaine. Note the intense uptake in brain, spinal cord and heart (A). Pretreatment with desipramine (B) resulted in small changes in [C-14]cocaine uptake in the cerebellum and spinal cord, but significantly reduced activity in heart and adrenals. Pretreatment with GBR 12909 (C) reduced uptake of [C-14]cocaine in brain and heart with most of the activity accumulating in the renal collecting system.

g. Effects of Therapeutic Intervention: Myocardial Flow and Metabolic Studies in Cardiomyopathy

The Syrian golden hamster (B53.58), a model of congestive cardiomyopathy was studied using Tl-201, C-14-2DG, and I-131-dimethyliodophenylpentadecanoic acid (DMIPP), a fatty acid analog (13). Animals were followed from an early age when cardiomyopathy is not evident clinically or histologically, through the midstage and the full end-state of disease. ARG microimaging revealed that perfusion (Tl-201) became abnormal only in end stage of cardiomyopathy. Glucose uptake abnormalities were initially very small, while fatty acid

uptake abnormalities were much more pronounced. Verapamil-treated hamsters did not exhibit perfusion or metabolic abnormalities and did not develop cardiomyopathy as confirmed histologically. Autoradiographic microimaging studies indicated the potential for developing a clinical test for cardiomyopathy using radiolabeled fatty acid scintigraphy. This procedure may also enable to monitor the disease and the effects of treatment.

h. The Effect of Oral Prussian Blue on Tl-201 Distribution

It has been shown that immediately after i.v. injection Tl-201 is excreted into the gastrointestinal tract and reabsorbed through the enterohepatic circulation. Oral Prussian Blue traps Tl-201 in the G.I. tract preventing reabsorption and increasing gastrointestinal excretion thus reducing the radiation burden, as shown by ARG studies in mice (14).

i. ARG Studies of Abscess and Tumor with Tc-99m-labeled Human Immunoglobulin

Intracerebral glioma and turpentine abscess in small rodents were clearly delineated 2 hrs after the injection of Tc-99m IgG. This information complemented the data obtained by tissue distribution, blood clearance, and scintigraphy (15).

j. Placental Transfer ARG Studies

Preliminary studies on placental transfer of cocaine in early pregnant animals showed only minimal transfer to the embryo in early gestation (16) as opposed to later periods when transfer is obvious.

l. Chemical Analysis of Samples from ARG Sections

ARG technique can be also be used to identify the species of radioactive compounds (metabolites) in various organs on same section by punching out pieces of tissue and using chromatographic separation (17).

CONCLUSIONS

Compared to conventional tissue-sampling methods ARG microimaging has the following advantages:

a. ARG microimaging provides a high resolution map of tracer localization in organs and whole body of small animals.

b. ARG microimages can be digitized, quantitated and 3-D computer reconstructions can be performed with commercially available systems.

c. The high resolution of ARG (less than 1 mm), enables detection and localization of very small areas that are well below the size that can be dissected and weighed accurately.

Thus ARG may provide unique information leading to findings that could not have been otherwise anticipated.

d. Long exposure times can be used for ARG microimaging resulting in increased sensitivity compared to scintillation counting. The photographic emulsion on the film integrates the incident radiation emitted over long periods of time (days to weeks) beyond what is practical in tissue-counting methods or scintigraphy.

e. The assay of ß-emitting compounds in tissues is complex and time consuming. For scintillation counting samples have to be totally dissolved, colorless and preferably clear. On the other hand, ARG microimaging of ß-emitting compounds is not different from gamma emitters.

f. Tissue distribution studies can rarely encompass all parts of the body while whole body ARG microimaging will depict the global, as well as discrete, minute sites of tracer accumulation which can be missed by non-pictorial sampling methods.

The aforementioned examples demonstrate that wholebody microimaging, is a powerful tool for evaluation of new SPECT and PET radiopharmaceuticals, for simultaneous measurements of different physiological parameters, for studying the effects of therapeutic interventions, for determining receptor binding sites as well as showing placental transfer of drugs.

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REFERENCES

1. Conference on Autoradiography in Pharmacology and Toxicology. In: Acta Pharmacol. Toxicol. 41, suppl. 1. Ullberg S, Applegren LE, Dencker L, Hammarstrom L, Hansson E, Tjalve H, editors. Munksgaard, Copenhagen, Scandinavian Pharmacol Soc, 1977.

2. Som P, Oster ZH. Quantitative wholebody autoradiographic microimaging for pharmaceutical research . In: Nuclear Imaging in Drug Discovery, Development and Approval. Burns HD, Gibson RE, Dannals RF, Sigel PKS, editors. Boston, Basel, Berlin: Birkhèuser, 1992: 149-166.

3. Som P. Yonekura Y, Oster ZH, et al. Quantitative autoradiography with radiopharmaceuticals. Part II: Applications in radiopharmaceutical research. J Nucl Med 1983; 24: 238-244.

4. Yonekura Y, Brill AB, Som P, et al. Quantitative autoradiography with radiopharmaceuticals. Part I: Digital film analysis system by videodensitometry. J Nucl Med 1983; 24:231-237.

5. Yonekura Y, Brill AB, Som P, et al. Quantitative autoradiographic measurement of regional myocardial substrate utilization in hypertensive rats. Science 1985; 227: 1494-1446.

6. Oster ZH, Som P, Srivastava SC, et al. The development and in vivo behavior of tin-containing radiopharmaceuticals. II. Autographic and scintigraphic studies in normal animals and in animal models of bone disease. J Labelled Comp and Radiopharmaceuticals. 1984; 21:1042-1044.

7. Som P, Oster ZH, Yamamoto K, et al. Some factors affecting the cerebral and extracerebral accumulation of N-isopropyl-p-iodo-amphetamine (IAMP). Int. J Nucl Med Biol 1985; 12:185-196.

9. Zamora PO, Pant KD, Shah VO, et al. Anti-Colon/ovarian tumor antigen: Localization of colon cancer Xenograft in athymic rats. Int J Nucl Med Biol. 1988; 15:261-270.

10. Fand I, McNally WP, Koul O, et al. Whole-body autoradiographic localization of {3H}Phenylcycline and its metabolites in mice. Toxicol Appl Pharmacol 1988; 93:421-432.

11. Som P, Oster ZH, Volkow ND, et al. Studies on wholebody distribution and kinetics of cocaine J Nucl Med 1989; 30:831.

12. Som P, Oster ZH, Volkow ND, et al. Organ variations in cocaine receptors. J Nucl Med 1990; 31:886.

13. Som P, Oster ZH, Wang G-J et al. Wholebody autoradiographic microimaging studies of the spatial and temporal distribution of cocaine. Synapse. Submitted, 1993

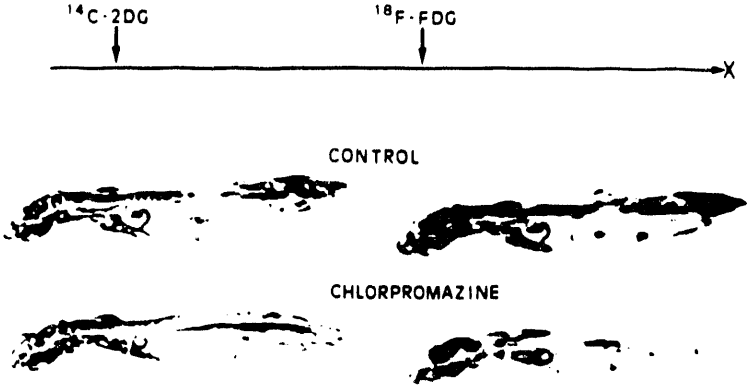
14. Kubota K, Som P, Oster ZH, et al. Detection of cardiomyopathy in an animal mode using quantitative autoradiography. J Nucl Med 1988; 29:1697-1703.

15. Oster ZH, Som P, Brill AB, et al. Enhancement of radiopharmaceutical excretion by chemical interventions In: Proc 3rd World Cong Nucl. Med. Biol. Raynaud C, editor. Paris: Pergamon Press, 1982: 3257-3261.

16. Oster ZH, Som P, Rhodes BA, et al. A one-step kit for 99mTc-labeling of non-specific immunoglobulin (HIG): Imaging of inflammation and tumor J Nucl Med 1992; 33:1028.

17 Som P. Localization of cocaine in fetal tissue. Unpublished data, 1991.

18. d'Argy R. Tissue pieces from wholebody sections used for chromatographic separation of metabolites. Acta Pharmacol Toxicol 1977; 41(Suppl. 1):16-17.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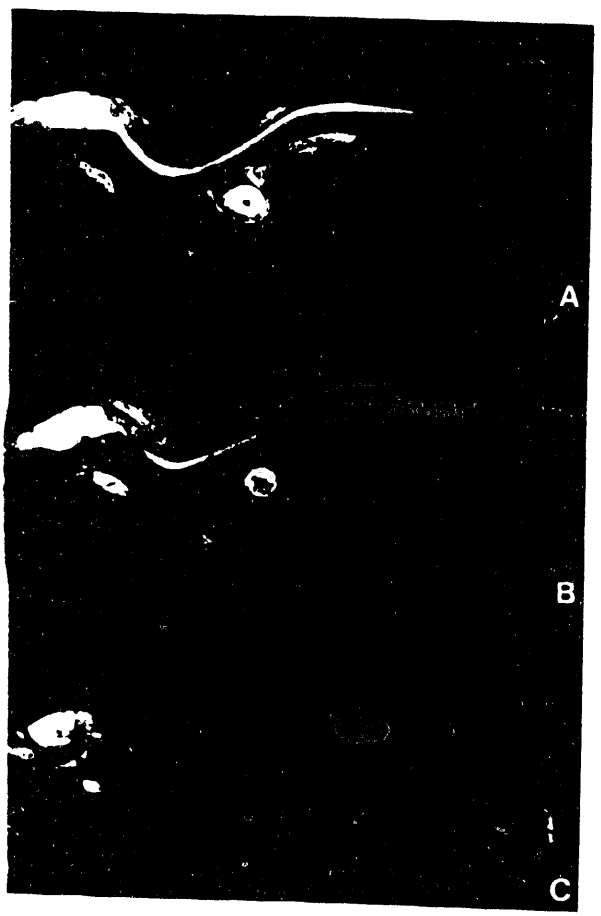
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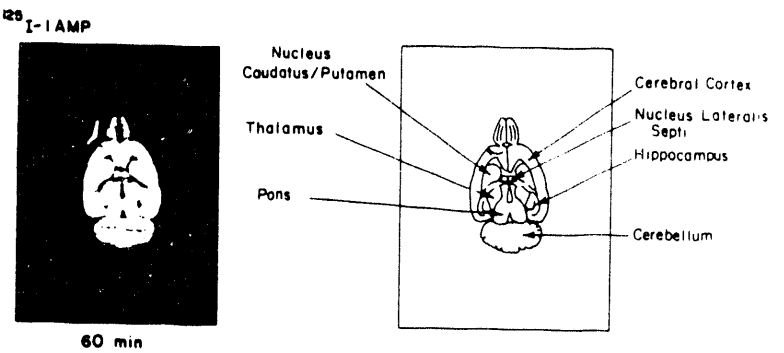
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