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"Radiopharmaceuticals for Diagnosis and Treatment"

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Summary

In this grant period we have continued our efforts in the areas of PET basic radiochemistry, radiopharmaceutical synthesis, and preclinical radiopharmaceutical evaluation.

1. Predictive abilities in nucleophilic aromatic substitution with [18F]fluoride. We have completed a study of the predictive abilities for [18F]fluorination of substituted aromatic rings. Although radiochemical yields can be predicted within a very similar group of compounds with similar leaving groups and substituent patterns, generalization to all nucleophilic substitutions with [18F]fluoride is not warranted. Kinetic studies indicate significantly different rates of reactions, depending on ring substituents.

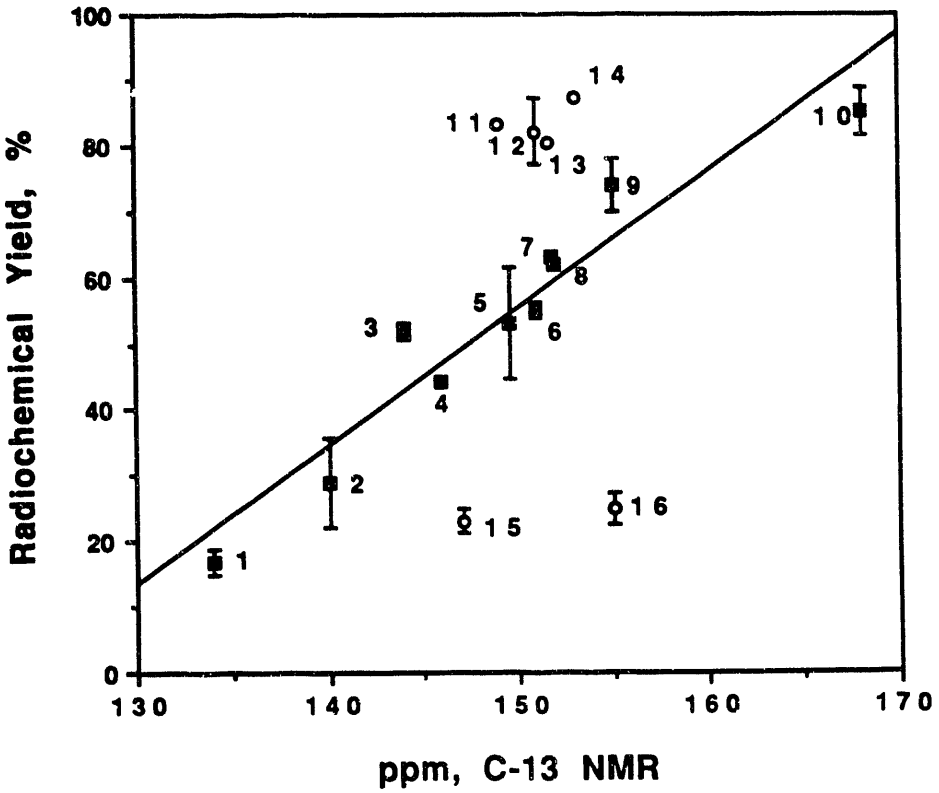
2. Preclinical evaluation of new radiopharmaceuticals. We have synthesized and begun the preclinical evaluation of [11C]tetrabenazine, a new radioligand based on the vesicular monoamine transport system.

3. Our work on [18F]fluorination/decarbonylation reactions, structure-activity relationships in dopamine uptake inhibitors and effects of chronic drugs on radioligand binding have been published.

Details of this work are contained in the following sections. Portions of this work have been reported several meetings in the last year (Society of Nuclear Medicine, Ninth International Symposium on Radiopharmaceutical Chemistry). Manuscripts published, in press or submitted can be found in Appendix I.

PART 1. Predicting Yields in Nucleophilic Aromatic Substitution Reactions.

The correlation between the ^{13}C-NMR chemical shift of the aromatic ring carbon bearing the leaving group and the yield of nucleophilic aromatic displacement with no-carrier-added [18F]fluoride ion was evaluated. In comparison of structurally analogous compounds (fluoro, nitro and trimethylammonium substituted benzaldehydes, benzophenones and benzonitriles), the ^{13}C-NMR chemical shift of the reactive aryl ring carbon correlated quite well with the [18F]fluorination yield ($r^2 = 0.91$) for most but not all ring structures (Fig. 1). Compounds with trimethylammonium leaving groups or methyl ring substituents were found to not fit the proposed correlation. Kinetic studies indicated clearly different rates of reaction for these compounds, with much higher than expected reactivity for the compounds with the cationic leaving group. Competition experiments suggest that low reactivity of methyl-substituted rings may be due to conversion of [18F[fluoride to an unreactive form. Our results indicate that the correlation between [18F]fluorination yields for nucleophilic aromatic substitution reactions and the ^{13}C NMR chemical shift of the aryl ring carbon bearing the leaving group is applicable to numerous structurally analogous compounds, but cannot be simply generalized to aromatic rings with different leaving groups or ring substituents.



1	3-methoxy-2-nitrobenzaldehyde	16.8 ± 1.9
2	3-methoxy-4-nitrobenzaldehyde	29 ± 6.9
3	6-nitroveratraldehyde	52 ± 1.2
4	6-nitropiperonaldehyde	44 ± .35
5	2-nitrobenzaldehyde	53 ± 8.6
6	4-nitrobenzaldehyde	55 ± 1.2
7	4-nitrobenzophenone	63[a]
8	4-nitrobenzonitrile	62
9	2-methoxy-4-nitrobenzaldehyde	74 ± 4.1
10	4-fluorobenzaldehyde	85 ± 3.6
11	4-trimethylammoniobenzaldehyde	82 ± 5.0
12	4-trimethylammoniumbenzophenone	80 ± 4.6[a]
13	4-trimethylammoniumbenzonitrile	83
14	4-trimethylammoniumnitrobenzene	87
15	3-methyl-4-nitrobenzaldehyde	25 ± 2.8
16	2-nitro-5-methylbenzaldehyde	23 ± 4.8

[a] Data from reference 6

Fig. 1. Correlation of radiochemical yields with carbon-13 NMR chemical shift values for various substituted benzaldehydes. Compounds 1-10 were used for the construction of the correlation line shown, with an r^2 value of 0.91.

PART 2 Preclinical evaluation of new radiopharmaceuticals: [11C]tetrabenazine

We have developed inhibitors of vesicular monoamine uptake as a new approach to the quantification of presynaptic neuron densities.

Chemistry

We have recently developed syntheses of [11C]tetrabenazine ([11C]TBZ) and [11C]methyl-dihydrotetrabenazine (TBZOMe), two high affinity radioligands for vesicular uptake sites. These radiotracers are easily prepared in one-step syntheses, in radiochemical yields of >30% and very high specific activities (>1000 Ci/mmol, end-of-synthesis).

In Vivo Pharmacology

We have begun the characterization of the pharmacokinetics and pharmacological specificity of these radioligands in rodents. Both [11C]TBZ and [11C]TBZOMe show rapid uptake and egress from brain tissues, with specific retention of the radioligands in the tissues most heavily innervated with monoaminergic neurons, striatum (STR) and hypothalamus (HYPO). From the kinetic curves it was determined that the best target/nontarget ratios, operationally defined as STR/CER and HYPO/CER (CER = cerebellum), were obtained at 10 and 15 minutes after i.v. injection for [11C]TBZ and [11C]TBZOMe, respectively. It was recognized that the cerebellum is not devoid of monoaminergic neurons (Slotkin et al, 1978), but from in vitro studies (Henry and Scherman, 1989) there is a much small er fraction present relative to the other brain regions.

Our studies have shown some interesting characteristics of the binding of these radioligands, and are summarized below:

(a) Specific binding can be significantly reduced by pretreatment with reserpine; unexpectedly, complete blocking of striatal binding was not observed. We have repeated these studies, and each time find that reserpine pretreatment does not completely block [11C]TBZ or [11C]TBZOMe binding in the striatum, although it is completely effective in the hypothalamus. This raises interesting questions of the relative affinities of these two drugs for the purported two binding sites on the monoamine transporter. Studies with reserpine also point out the difficulties in simple analysis of tissue ratios when delivery of radioligand is uniformly disturbed, as can be the case with reserpine.

(b) Specific binding is completely blocked by co-injection of unlabeled TBZ. Even though our results and the literature suggest that TBZ is essentially completely out of the brain after a couple of hours, we find that in vivo TBZ binding is still reduced (about 20%) even 4.5 hours after injection of a large dose of cold TBZ .

(c) Specific binding is reduced by co-injection of ketanserin, a low affinity inhibitor of the TBZ binding site. The amount of TBZ blocking is dose dependent, but high doses of ketanserin were fatal to 50% of the test animals.

(d) Specific binding is not blocked by haldol, a D2 receptor antagonist. Our observation that haldol does not block [11C]TBZ binding, together with the loss of specific TBZ binding upon a presynaptic lesion (unilateral MPTP-treated monkey, (see below), and 6-hydroxydopamine-treated rats (Masuo et al 1990) provides evidence that [11C]TBZ does not bind to dopamine receptors, even though it can be shown to have a low affinity (5 mM) in vitro (Login et al, 1982; Reches et al, 1983).

(e) Specific binding is unaffected by pretreatment with GBR 12935, the neuronal reuptake inhibitor.

(f) Specific binding is blocked by i.v. injection of possible metabolites of TBZ, including a-TBZOH, b-TBZOH, and 9-desmethylTBZ (OH-TBZ, the phenol).

Representative distributions of [11C]TBZ and [11C]TBZOMe in control animals are shown in Table 1. These preliminary results support the conclusion that in vivo [11C]TBZ and [11C]TBZOMe bind to the vesicular transport site for monoamines. Our preliminary experiments also demonstrate that drug treatments can alter radioligand delivery (as for reserpine, haldol and ketanserin by changes

in radiotracer levels in cerebellum) and such phenomena need to be understood and accounted for in any many vivo studies using radioligands.

Table 1. Regional mouse brain distributions of [11C]TBZ (10 min post inj) and [11C]TBZOMe (15 min post inj). Data are given as mean ± S.D., for N = 4 animals.

region	[11C]TBZ	[11C]TBZOMe
striatum	6.02 ±1.24	6.09 ± 0.69
cortex	1.96 ±0.31	2.36 ± 0.25
hippocampus	2.13 ± 0.30	2.37 ± 0.18
hypothal	3.14 ± 0.51	3.82 ± 0.32
thalamus	2.02 ± 0.37	---
cerebellum	1.77 ± 0.26	1.77 ± 0.24
blood	1.47 ± 0.16	1.71 ±0.31
STR/CER	3.39 ±0.30	3.48 ±0.37
HYPO/CER	1.78 ± 0.11	2.18 ± 0.22

In Vivo Imaging

We have completed the first successful in vivo imaging of monoamine vesicular transporters in living primate brain is described, using [11C]tetrabenazine ([11C]TBZ) and Positron Emission Tomography (PET). Radioligand uptake into brain is rapid, and at short time periods (10-20 minutes) the higher uptake and retention of the radiotracer in the more densely dopaminergic innervated striatum is clearly visualized. Ratios of striatum to nontarget tissues were around 2; however, it should be recognized that there is not a good non-innervated region to use as a "blank", particularly in a small monkey brain. Specific binding in striatum can be entirely blocked with co-administration of a pharmacological dose (1 mg/kg i.v.) of tetrabenazine. In a unilaterally MPTP-lesioned monkey, specific binding of radioligand was absent in the striatum on the affected side, with no effect on radiotracer distribution in the contralateral striatum.

At the time of writing of this application, we had received permissions (Radioactive Drug Research Committee and Institutional Review Board) for human studies of the biodistribution of [11C]tetrabenazine in normal human subjects.

Kinetic modeling

In preliminary experiments, we have applied a 2-compartment model to the mouse brain distribution of [11C]TBZ. This analysis gave distribution values ranging from 1.7 (cerebellum) to 4.36 (striatum), and these values correlate well (r = 0.97) with the in vitro distribution of [3H]TBZOH binding sites (Henry and Scherman, 1989).

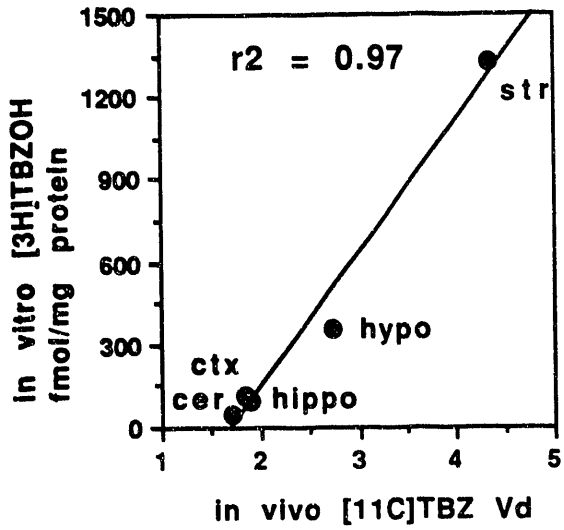


Fig. Correlation between in vitro and in vivo binding of radioligands for vesicular transport sites.

PART 3 Development of Radioactive Gases Containment System

Radioactive Gas containment system. In response to concerns about radioactive gas emissions from our Cyclotron/PET facility, we have undertaken the design, construction and implementation of a unique radioactive gas containment system. The design is a proactive system to remove, compress and store all air passing through our shielded hot cells during a radiochemical synthesis. Compressed gases are stored in high pressure cylinders located in our cyclotron vault, and kept there for an appropriate length of time until they can be released to the environment (complete decay of radionuclides). The system was built with sufficient capacity that a new synthesis, using up to 2.5 Curies of carbon-11, can be initiated at every 40 minute period. The stack exhaust is now permanently monitored, and no radioactive gases now escape out of our facility through the hot cell exhaust stacks. The entire system is self-controlled, using a Programmable Logic Controller with computer access for monitoring/troubleshooting.

This system exceeds all requirements meeting regulatory requirements (federal or state regulatory bodies). The costs of this system were largely met through University funds. Installation of this system did, however, result in 12 weeks of down-time for the cyclotron and radiochemistry laboratories.

Published Papers:

1. Chakraborty PK and Kilbourn MR: [^{18}F]Fluorination/decarbonylation: A new route to aryl[^{18}F]fluorides. Appl Radiat Isot 1991:42;1209.

2. Van Dort ME, Kilbourn MR, Chakraborty PK, Richfield E, Gildersleeve DL, Wieland DM: Iodine-125 and fluorine-18 aryl-1,4-dialkylpiperazines: potential radiopharmaceuticals for in vivo study of the dopamine uptake system. Appl Radiat Isot 1992:43;671-680.

3. DaSilva JN and Kilbourn MR, In vivo binding of [^{11}C]tetrabenazine to vesicular monoamine transporters in mouse brain, Life Sciences 1992:51;593-600.

4. Kilbourn MR, Sherman PS and Pisani T: Repeated reserpine administration reduces in vivo [^{18}F]GBR 13119 binding to the dopamine uptake site, Eur. J. Pharm. 1992:216;109-112.

Papers Submitted:

5. Rengan R., Chakraborty P.K. and Kilbourn M.R.: Can we predict reactivity for aromatic nucleophilic substitution with [^{18}F]fluoride ion?, J. Labeled Compds. Radiopharm., submitted

6. DaSilva JN, Kilbourn MR, and Domino EF: In vivo imaging of monoaminergic nerve terminals in normal and MPTP-lesioned primate brain using positron emission tomography (PET) and [11C]tetrabenazine. Synapse (submitted).

7. DaSilva JN, Kilbourn MR, and Mangner T: Synthesis of [11C]tetrabenazine. Appl. Radiat, Isot. (submitted)

Abstracts :

8. DaSilva JN, Kilbourn MR, Mangner TJ, and Toorongian SA, Synthesis of [C-11]tetrabenazine and a [C-11]methoxy derivative of a-dihydrotetrabenazine for PET imaging of monoamine terminal. J Nucl Med 1992:33;983.

9. DaSilva JN, Kilbourn MR, Koeppe RA, Sherman PS, Pisani T, and Mangner TJ, In vivo mouse brain biodistribution and monkey PET imaging of [C-11]tetrabenazine, a new PET marker for monoaminergic neurons. J Nucl Med 1992:33;870.

10. Kilbourn MR and DaSilva JN, In vivo imaging of dopaminergic processes: studies of neuronal and vesicular monoamine transporters using positron emission tomography, 7th International Catecholamine Symposium, Amsterdam, 1992.

11. Kilbourn MR, PET radiotracers for neuronal and vesicular transport sites of neurotransmitters, 145th Annual Meeting of the American Psychiatric Association, May, 1992, Washington, DC

12. DaSilva JN, Kilbourn MR, Mangner TJ and Toorongian SA: Synthesis of a [11C]methoxy derivative of [3H]dihydrotetrabenazine for PET imaging of monamine terminals. J Labeled Compds Radiopharm 1992 (in press).

13. Rengan R and Kilbourn MR: Can we predict reactivity for aromatic nucleophilic substitution with [18F]fluoride? J Labeled Compds Radiopharm 1992 (in press).

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