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Progress Report

"Radiopharmaceuticals for Diagnosis and Treatment"

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David E. Kuhl, M.D., Principal Investigator DE92 004065

Michael R. Kilbourn, Ph.D , Project Director

Summary

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

1. Development of new methods for fluorine-18 labeling. A new synthetic sequence, consisting of no-carrier-added fluorine-18 labeling of substituted benzaldehydes followed by reductive decarbonylation, has been developed. This new methodology can be applied to the fluorine-18 labeling of a wide variety of drugs not previously accessible through existing fluorine-18 labeling methods.

2. Predictive abilities in nucleophilic aromatic substitution with [18F]fluoride. Following up on a literature report that the ability to radiolabel aromatic rings can be predicted by 13C-NMR chemical shifts, we have examined the generality of this correlation in aromatic rings bearing a variety of substituents. Although the original correlation holds for nitro substituted anisaldehydes, it cannot be extended to other rings substitution patterns.

3. Quantitative Structure-Activity relationships for in vivo radioligands. We have examined the relationship of in vivo localization of various fluorine-18 labeled dopamine uptake inhibitors to thier in vitro binding affinities and lipophilicities. We have found that remarkably small decreases in binding affinity result in dramatic losses of in vivo binding to the desired high affinity binding sites.

4. Study of in vivo pharmacology. In order to probe the effects of endogenous neurotransmitter on the in vivo binding of radiolabeled dopamine uptake inhibitors, we have examined the in vivo regional localization of [18F]GBR 13119 after acute and chronic drug treatments which alter the endogenous levels of dopamine. We have found that acute changes in dopamine levels do not affect the binding of this radioligand, but chronic depletion of neurotransmitter results in down-regulation of the in vivo binding sites.

Details of this work are contained in the following sections. Portions of this work have been reported at two Department of Energy co-sponsored symposia, In Vivo Imaging of Neurotransmitter Functions in Brain, Heart and Tumors, (Montreal, CN, August 1990) and Research in PET: International and Institutional Perspectives (Washington D.C. , Oct. 1991).

PART I. New Methods for Fluorine-18 Labeling.

Aromatic nucleophilic substitution has emerged as the best method for high specific activity fluorine-18 labeling of PET radiopharmaceuticals. This reaction requires an elctron-activating substituent on the aromatic ring; this substituent must subsequently form a part of the intended radiopharmaceutical, be altered to a more desireable substituent, or be completely removed. As part of this research we have previously developed methods for changing such activating groups to phenols, and have reported methods for synthesis of NCA fluorine-18 labeled fluorophenols and fluorocatechols. In the last year, we have developed a new method which allows the removal of aldehyde activating groups, yielding simple fluorine-18 substituted ring systems. The reaction sequence uses decarbonylation with Pd-C to convert intermediate [18F]fluorobenzaldehydes to the corresponding aryl [18F]fluorides:

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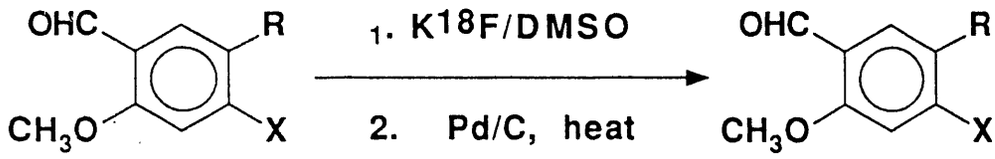

X = NO_2 OR F
R = CH_3 or H

R = CH_3 or H

This has been applied to the synthesis of 3-[18F]fluoro-4-methylphenol and 3-[18F]fluorophenol. This work is reported in publications 8 and 12.

PART 2. Predicting Yields in Nucleophilic Aromatic Substitution Reactions.

Following a published report showing a correlation between 13C-NMR chemical shift and radiochemical yields in nucleophilic aromatic substitution reactions with [18F]fluoride, we have examined the general applicability of this correlation, particularly in regards to different ring substituents and different leaving groups on the aromatic ring. A number of appropriately substituted benzaldehydes and benzonitriles were either purchased or synthesized, and subjected to reaction with no carrier added [18F]fluoride ion. Yields were determined under controlled reaction conditions and reaction times. These yields were then correlated with 13C-NMR chemical shifts, using latter values either measured in our laboratories, reported in the literature, or calculated de novo. We have found that there is a good correlation beteween 13C-NMR chemical shift for a leaving groups and the radiochemical yield within a group of methoxy or dimethoxy substitued benzaldehydes. However, two types of compounds clearly did not fit the correlation line: a trimethylammonium substituted benzaldehyde, and two methyl-substituted benzaldehydes. We have preliminary evidence that the reasons for this is that the kinetics of [18F]fluorinationof these derivatives is completely different: the trimethylammonium substituent is displaced far more rapidly the nitro or fluoro leaving groups, and the methyl groups are slowing down the nucleophilic aromatic substitution reaction. The faster kinetics of the trimethylammonium group is precedented in the literature, but the explantion for the deleterious effects of the methyl group is not clear. These studies have import for researchers trying to predict the yields obtainable in fluorine-18 labeling of complex drug structures.

A manuscript on this work is currently being prepared for submission.

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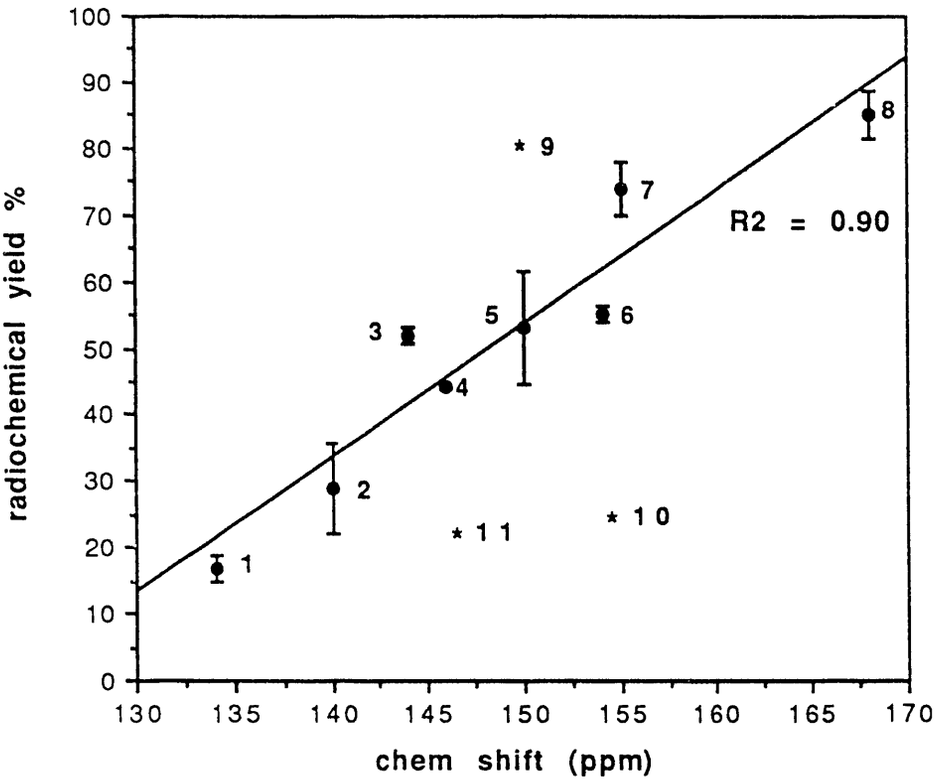

1	3-methoxy-2-nitrobenzaldehyde
2	3-methoxy-4-nitrobenzaldehyde
3	6-nitroveratraldehyde
4	6-nitropiperonaldehyde
5	2-nitrobenzaldehyde
6	4-nitrobenzaldehyde
7	2-methoxy-4-nitrobenzaldehyde
8	4-fluorobenzaldehyde

Derivatives which do not fit correlation:

9	4-trimethylammonium benzaldehyde
10	3-methyl-4-nitrobenzaldehyde
11	2-nitro-5-methylbenzaldehyde

PART 3 In Vivo Structure-Activity relationships

As part of our development of radiopharmaceuticals for imaging the dopamine reuptake system in man, we have synthesized a number of fluorine-18 and radioiodine-labeled derivatives of the parent compound, [^{18}F]GBR 13119. For the fluorine-18 labeled derivatives, the changes in the structure were intended to reduce the lipophilicity of the drug; the effects of such changes in the structure had been previously examined in in vitro binding assays. The two new derivatives, lacking either one or two methylene groups, were synthesized using the methodology developed for preparation of [^{18}F]GBR 12909 and [^{18}F]GBR 13119. Regional brain distribution was determined in mice, according to previously developed protocols. Surprisingly, both new derivatives showed essentially no localization in the striatum, and hence no selective binding to the dopamine uptake carrier. This was despite reasonably good in vitro binding affinities to be expected for these compounds. It is thus clear

that structural modification of the GBR series of cmpounds will be limited in the search for an optimal in vivo imaging agent. This work is reported in publications 9 and 14.

PART 4. In Vivo Pharmacology

Recently, it has become evident that endogenous neurotransmitter levels may affect the in vivo biodistribution of radioligands, and thier binding to high affinity sites. As part of this project we have introduce [18F]GBR 12909, a dopamine uptake inhibitor, into clinical trials for brain imaging. The effects of endogenous transmitter on in vivo binding have not been previously examined. We have therefor studied, in mice, the effects of acute and chronic treatment with dopaminergic drugs which should alter endogenous levels of dopamine. Treatment with acute or chronic pargyline, a monoamine oxidase (MAO) inhibitor, did not affect in vivo binding of [18F]GBR 13119, despite the known effects of this drug to raise extracellular dopamine levels. Acute amphetamine did decrease the in vivo binding of [18F]GBR 13119, but it is not possible to distinguish between the ability of amphetamine to release dopamine and its ability to block dopamine reuptake. Reserpine, a drug known to acutely and chronically deplete tissue levels of dopamine, had interesting effects on [18F]GBR 13119 binding. Acute reserpine had no effect on the binding of this radioligand, but chronically reserpinized animals showed a statistically significant decrease in [18F]GBR 13119 binding sites. This would be consistent with down-regulation of the dopamine uptake system, as a compensatory mechanism for the lower tissue dopamine levels. This has been previously seen in the norepinephrine and serotonin upptake systems. Down-regulation of dopamine uptake sites would have important ramifications for the interpretation of PET scans using dopamine reuptake inhibitors: whereas they have previously been considered as possible markers of neuronal loss, the in vivo decreases should now be considered as resulting from either decreased dopamine uptake densitiy per neuron, loss of neurons, or both. This work has been reported in publications 10,11 and 13.

TABLE 1

Effects of chronic reserpine (2 mg/kg i.p., once daily for 3 days, last injection 24 h prior to injection of radiotracer) on the regional mouse brain accumulation of [18F]GBR 13119. Radiotracer was injected i.v. and animals sacrificed at one hour after injection. Values are mean ± S.D. (n = 3 to 6).

	control	chronic reserpine
	%ID/g	
tissue		
striatum	2.33 ± .63	1.77 ±.19 (-25%)*
cortex	0.64 ± .13	0.91 ± .14 (+42%)*
cerebellum	0.58 ± .13	0.75 ±.16 (+29%)*
blood	1.03 ± .11	1.55 ± .27 (+50%)*
STR - CER	1.66 ±.6	1.05 ± .39 (-58%)*
STR/CER	3.66 ± .71	2.25 ±.39 (-39%)*

* $p < 0.05$ vs. control.

Published Papers:

1. Haka MS and Kilbourn MR; Synthesis of [^{18}F]GBR 12909, a dopamine reuptake inhibitor. J Labeled Compds Radiopharm 1990; 28: 793-800.
2. Kilbourn MR, Pavia MR, and Gregor VE; Synthesis of fluorine-18 labelled GABA uptake inhibitors. Appl Radiat Isot 1990:41; 823-828.
3. Kilbourn MR and Subramanian R. Synthesis of fluorine-18 labeled 1,1,-difluoro-2,2,-dichloroethyl aryl ethers by ^{18}F-for-^{19}F exchange. J Labeled Compds Radiopharm 1990: 28; 1355-1361.
4. Kilbourn MR: Synthesis of [^{18}F]flunarizine. Nucl. Med. Biol 1991: 42; 109-111.
5. Kilbourn MR, Mulholland GK, Sherman PS, and Pisani T: In vivo binding of the dopamine uptake inhibitor [18F]GBR 13119 in MPTP-treated C57BL/6 mice. Nucl Med and Biol 1991:18;803-806.
6. Chakraborty PK and Kilbourn MR: Oxidation of substituted 4-fluorobenzaldehydes: Application to the no-carrier-added syntheses of 4-[^{18}F]fluoroguaiacol and 4-[^{18}F]fluorocatechol. Appl Radiat Isot 1991: 42;673-681.
7. Kilbourn MR: Radiotracers for PET studies of neurotransmitter binding sites: design considerations. In Vivo Imaging of Neurotransmitter Functions in Brain, Heart and Tumors, American College of Nuclear Physicians/Department of Energy, 1990, pp 47-65.

Papers in Press:

8. Chakraborty PK and Kilbourn MR: [^{18}F]Fluorination/decarbonylation: A new route to aryl[^{18}F]fluorides. Appl Radiat Isot (in press).
9. 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

Papers Submitted:

10. Kilbourn MR, Sherman PS, and Pisani T: Effects of reserpine or pargyline on in vivo radioligand binding to dopamine uptake sites. Submitted to European Journal of Pharmacology.

Abstracts :

11. Kilbourn MR, Sherman P, Pisani T: Drug effects on in vivo mouse brain binding of [F-18]GBR. J Nucl Med 1991:32;1097.
12. Chakraborty PK and Kilbourn MR: [F-18]Fluorination/decarbonylation: new route to aryl [F-18]fluorides. J Nucl Med 1991:32;1009.
13. Kilbourn MR: PET and drug development; examples from drugs affecting neurotransmitter reuptake systems. Int Symp Nuclear Imaging in Drug Discovery, Development, and Approval, Baltimore, MD, June 1991.
14. Kilbourn MR: Design and synthesis of radiolabeled inhibitors of neurotransmitter reuptake sites. 4th International Symposium on the Synthesis and Applications of Isotopically Labeled Compounds, Toronto, Canada, Sept 3-7, 1991, abstract 36.

Presentations, Department of Energy Sponsored Symposia:

15. Kilbourn MR: "Neuronal Function Imaging", DOE-ICP Symposium: Research in PET: International and Institutional Perspectives, Washington D.C. , Oct. 1991
16. Kilbourn MR: "Radiotracers for PET studies of neurotransmitter binding sites: design considerations", at American College of Nuclear Physicians/Department of Energy/University of Michigan Sponsored Symposia: In Vivo Imaging of Neurotransmitter Functions in Brain, Heart and Tumors, , Montreal, CN, August 1990

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