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Tactics for preclinical validation of receptor-binding radiotracers☆,☆☆

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ABSTRACT

Introduction: Aspects of radiopharmaceutical development are illustrated through preclinical studies of [^{125}I]-(*E*)-1-(2-(2,3-dihydrobenzofuran-5-yl)ethyl)-4-(iodoallyl)piperazine ([^{125}I]-*E*-IA-BF-PE-PIPZE), a radioligand for sigma-1 (σ_1) receptors, coupled with examples from the recent literature. Findings are compared to those previously observed for [^{125}I]-(*E*)-1-(2-(2,3-dimethoxy-5-yl)ethyl)-4-(iodoallyl)piperazine ([^{125}I]-*E*-IA-DM-PE-PIPZE).

Methods: Syntheses of *E*-IA-BF-PE-PIPZE and [^{125}I]-*E*-IA-BF-PE-PIPZE were accomplished by standard methods. In vitro receptor binding studies and autoradiography were performed, and binding potential was predicted. Measurements of lipophilicity and protein binding were obtained. In vivo studies were conducted in mice to evaluate radioligand stability, as well as specific binding to σ_1 sites in brain, brain regions and peripheral organs in the presence and absence of potential blockers.

Results: *E*-IA-BF-PE-PIPZE exhibited high affinity and selectivity for σ_1 receptors ($K_i = 0.43 \pm 0.03$ nM, $\sigma_2/\sigma_1 = 173$). [^{125}I]-*E*-IA-BF-PE-PIPZE was prepared in good yield and purity, with high specific activity. Radioligand binding provided dissociation (k_{off}) and association (k_{on}) rate constants, along with a measured K_d of 0.24 ± 0.01 nM and B_{max} of 472 ± 13 fmol/mg protein. The radioligand proved suitable for quantitative autoradiography in vitro using brain sections. Moderate lipophilicity, Log $D_{7.4}$ 2.69 ± 0.28, was determined, and protein binding was 71 ± 0.3%. In vivo, high initial whole brain uptake, >6% injected dose/g, cleared slowly over 24 h. Specific binding represented 75% to 93% of total binding from 15 min to 24 h. Findings were confirmed and extended by regional brain biodistribution. Radiometabolites were not observed in brain (1%).

Conclusions: Substitution of dihydrobenzofuranylethyl for dimethoxyphenethyl increased radioligand affinity for σ_1 receptors by 16-fold. While high specific binding to σ_1 receptors was observed for both radioligands in vivo, [^{125}I]-*E*-IA-BF-PE-PIPZE displayed much slower clearance kinetics than [^{125}I]-*E*-IA-DM-PE-PIPZE. Thus, minor structural modifications of σ_1 receptor radioligands lead to major differences in binding properties in vitro and in vivo.

1. Introduction

Radiotracers serve as scientific tools for biomedical research in nearly all fields, including neuroscience, neurology, psychiatry, oncology and cardiology. The diagnostic radiopharmaceutical counterparts are invaluable for answering clinical research questions concerning human health and disease by the non-invasive nuclear imaging techniques of positron emission tomography (PET) and single photon emission computed tomography (SPECT). Radiopharmaceuticals can interrogate enzyme function, neurotransmitter function and receptor status, define organ physiology and pathophysiology, and can visualize and quantitate the abnormal brain deposits (β-amyloid, τ protein) associated with Alzheimer's disease. Nuclear imaging studies can also reflect the competition between a radiopharmaceutical and a non-radioactive ligand at specific biological recognitions sites. Such "occupancy" studies inform on mechanisms and dosing protocols, and require the radioligand to be sensitive to this molecular interplay. Validated radiotracers are now indispensable aids for reducing the time and lowering the cost of developing new drug entities within the pharmaceutical industry [1–3].

Nuclear imaging can also elucidate modes of drug action. An enlightening example is SPECT studies of D_2/D_3 receptors in human beings using [^{123}I]-IBZM, which showed occupancy of the striatal sites by EMD 59983, an active metabolite of the "selective" sigma (σ) receptor ligand panamesine (EMD 57445). The observation suggests that the

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atypical antipsychotic profile of the drug is due, at least in part, to dopaminergic activity of the metabolite [4]. The role of imaging in drug development for neurodegeneration has been discussed [5] and a recent review, focused on the role of PET in the development of new drugs to treat heart failure [6], exemplifies the power of molecular imaging in this regard. The extensive biomedical advances made possible by radiopharmaceuticals and nuclear imaging are exemplified by over 150 books and review articles within the last three years. These scientific works can be general [7,8], focused on specific radionuclides [9] or on particular organ systems [10–12].

Questions to be answered through the use of radiopharmaceuticals change over time. In the 1970's, pioneering work on visualization of neuroreceptors by autoradiography [13,14] prompted the question of whether or not neuroreceptors might also be imaged in living human beings. The question was answered in the early 1980's by PET and SPECT brain imaging of dopamine D_2 and muscarinic receptors in human beings using [^{11}C]-*N*-methylspiperone ([^{11}C]-NMSP) [15] and [^{123}I]-quinuclidinyl benzilate ([^{123}I]-QNB) [16], respectively. In the ensuing years, the questions have become more complex, and dependent on clinical disease state [11,17]. When advances in instrumentation, radioligand synthesis and mathematical modeling are combined [18], imaging is likely to provide answers. A challenge for the field is the following conundrum: as the specificity of a radiopharmaceutical increases, applicability for truly "personalized medicine" increases [17]; however, the overall impact on patient care decreases if the indications for use are too narrow [19,20].

There is a reticence of governmental organizations such as the United States FDA to approve radiopharmaceuticals for broad indications without compelling proof of diagnostic accuracy and clinical utility. A recent success is the 2011 approval of [^{123}I]-ioflupane (DaTSCAN) to assist physicians in evaluation of patients presenting with neurodegenerative disorders [21]. At this time, however, the β-amyloid imaging compounds Amyvid™ and Vizamyl™ have limited approvals in the United States for a scan to *exclude* a diagnosis of Alzheimer's disease (AD) [22]. Additional clinical trials will determine if the indication can be broadened to include a diagnosis of AD [23]. On the horizon, PET imaging of human histone deacetylase with [^{11}C]-Martinostat [24] should yield valuable information for understanding the emerging field of neuroepigenetics [25].

The development of new radiopharmaceuticals is guided by the radiotracer principle, for which George de Hevesy won the 1943 Nobel Prize in Chemistry [26]. The validation process can be referred to as the radiopharmaceutical paradigm (Chart 1). Proposals to devise a new radiopharmaceutical often originate from reviewing the published literature and patent applications, from data presented at scientific meetings, or from discussions with colleagues. Inherent in these readings and conversations is relevance for the biological target, which must exist in sufficient density for imaging. Moreover, the synthesis and radiosynthesis of the chemical target must be feasible, with choice of radionuclide and physical decay characteristics being key components. Programs seeking new radiopharmaceuticals require significant capital investment, so the justification to embark on the journey is paramount [27,28]. The unmet need, or questions that could be answered, should be vetted prior to project initiation. Plans for synthesis, radiosynthesis and preclinical biological evaluation are then established. The "ADME" concept (Absorption, Distribution, Metabolism, Excretion) helps guide new drug development, and is also applied to radiopharmaceuticals. Although it may be tempting to fast track a new radiotracer into the clinic without extensive evaluation, this approach may be a failure from both scientific and ethical perspectives. Guidelines for "success" are quite stringent for radiopharmaceuticals. An extremely small mass of radioactive compound must survive chemically in vivo, reach the intended biological target, yield a target to non-target count rate sufficient for external imaging, and also be safely administered to the subject without adverse effects. When the physical half-life is short, as for [^{11}C]-labeled ligands ($t_{1/2}$ 20.4 min; 511 keV, β^+), these obstacles are formidable.

Development of a new radiotracer, with a view toward use as a radiopharmaceutical, is an iterative process (Chart 2). Synthesis and evaluation of multiple compounds is often warranted, since gaining a better understanding of the relationships between structure and activity focus the efforts toward an entity that is likely to be successful. The present article illustrates general aspects of the early preclinical validation of receptor-binding radiotracers through selected literature examples, coupled with a synopsis of our recent studies of [^{125}I]-(*E*)-1-(2-(2,3-dihydrobenzofuran-5-yl)ethyl)-4-(iodoallyl)piperazine ([^{125}I]-*E*-IA-BF-PE-PIPZE), a radioligand for σ_1 receptors [29]. Our objective is to provide a road map for basic tactics that might prove of interest to those beginning studies in the field.

2. Sigma receptors and ligand design

2.1. Sigma receptors

Investigations of the sigma-1 (σ_1, S1R)/sigma-2 (σ_2, S2R) receptor pair are of current interest from a host of perspectives. This system was initially misclassified as a member of the opioid family [30], and the existence of two discrete subtypes was not recognized until the early 1990's [31]. These receptors have been implicated in psychostimulant and alcohol abuse, depression and anxiety, sequalae of stroke and pain, amyotrophic lateral sclerosis (ALS) and multiple sclerosis, as well as cancer. The reader is referred to recent reviews describing their known and suspected roles [32–40].

Of the potential radiopharmaceuticals developed for imaging σ_1 and σ_2 receptors [41–43] only a few have progressed to human imaging studies. Two of these are the σ_1 selective ligands TPCNE [44] and SA4503 [45] (Fig. 1). These compounds represent different chemical classes, with TPCNE having higher apparent affinity (0.67 nM) [44] than SA4503 (4.6 nM) [46] for the sites in vitro. In vivo, the pharmacokinetics of these radiotracers proved to be quite different. As a radioligand for SPECT, [^{123}I]-TPCNE exhibited good specific binding to cerebral σ_1 receptors, with no clearance from human brain over 30 h [47]. This SPECT imaging protocol exemplifies the flexibility of using I-123, which has a long physical half-life ($t_{1/2}$ 13.2 h; 159 keV, γ). A relentless binder such as [^{123}I]-TPCNE should give clear images and be useful for certain applications, but may not be sensitive enough to competition for use in occupancy studies [47–49]. By contrast, dynamic PET imaging of σ_1 receptors using [^{11}C]-SA4503 in human brain is usually conducted over a 90 min period [50], which is in keeping with the 20.4 min half-life of carbon-11. [^{11}C]-SA4503 is useful for occupancy studies, allowing PET confirmation that the clinically significant drugs donepezil (Aricept ®), [51,52], and fluvoxamine [53,54] interact with the σ_1 receptor in human brain. These findings suggest a σ_1 receptor contribution to the primary modes of action for these drugs. One drawback to [^{11}C]-SA4503 PET is the requirement for production using an on-site cyclotron.

We hypothesized that an iodinated TPCNE/SA4503 structural hybrid might give a ligand that retained high affinity and selectivity for σ_1 receptors, and also be readily reversible in vivo. For radiolabeling, we chose to use I-125, a radioisotope of iodine having a long half-life (~ 60 d) and soft (35 keV) γ emission. Thus, laboratory studies would be convenient for characterization of receptor binding and physicochemical parameters in vitro, and for establishing pharmacokinetics and pharmacology in small animals. Our target ligand, [$^{125/127}$I]-*E*-IA-DM-PE-PIPZE (Fig. 1), did exhibit high affinity and selectivity for σ_1 receptors in vitro, labeled the sites in mouse brain and periphery in vivo, and proved sensitive to in vivo competition by strong ligands [55–57] and by weak ligands, such as cocaine [58]. Extension to imaging by SPECT using I-123 or by PET using I-124 ($t_{1/2}$ 4.2 d; 511 keV, β^+) [59] may be possible.

For the most part, equivalent preclinical studies can be done using ligands labeled with In-111 ($t_{1/2}$ 2.8 d; 171, 245 keV, γ), Tc-99m ($t_{1/2}$ 6.0 h; 140 keV, γ), F-18 ($t_{1/2}$ 110 min; 511 keV, β^+), and even C-11, as long