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EDITORIAL

Shades of Grey: Radiopharmaceutical Chemistry in the 1990s and Beyond

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Science, like the universe itself, is ever expanding. The fields of biochemistry, physiology and pharmacology are rapidly growing, and radiopharmaceutical chemistry is no exception. In a recent Editorial Dr William C. Eckelman briefly reviewed the current status of radiopharmaceutical development (Eckelman, 1991). The ballots are out and returns are mixed. Radiopharmaceutical chemists have made great strides in the development and refinement of methods for radiochemical syntheses, but have made little impact on clinical Nuclear Medicine. Dr Eckelman ends his Editorial with a plaintive plea for more validation of radiotracers.

Dr Eckelman did not go far enough; radiopharmaceutical chemists need to be educated as to what are the important questions to which they should be devoting their time and talents. We do not need radiotracers to tell us where certain high affinity binding sites are in animal or human tissues; the fields of autoradiography and molecular biology are doing that just fine, with far greater resolution than we can ever hope for. It is not sufficient that new radiotracers are "validated" by a lack of specific binding after pharmacological pretreatments with massive amounts of cold drugs, or after severe neurochemical lesions in animal models, or in end-stage human disease. The answers we seek are not so black and white, but rather *shades of grey*. We need to develop sensitive *in vivo* measures of biochemical parameters which impact on the prevention or therapeutic treatment of human diseases. Nothing less.

In his Editorial, Bill Eckelman outlines three steps in radiotracer development. First, "develop a radiotracer that binds preferentially to a specific site". Second, "determine the sensitivity of the radiotracer to a change in biochemistry". Finally, "find a biochemical change as a function of a specific disease that matches that sensitivity". Dr Eckelman may have put the cart before the horse. The first step in a radiopharmaceutical development program should be to identify the relevant disease and the needed sensitivity (the percent change in any given parameter) which will be the focus of the study. Fortunately, more and more diseases are now being defined biochemically, with definitions of both the site and extent of an abnormal biochemical process. These data should be our ticket to the fun-house and our guide through the unknown. Admittedly, in many disease states the needed sensitivity may not be known; a good example is Parkinson's disease, where it is not known exactly what degree of change in the striatal dopaminergic system results in progression from normal function to symptomatic disease (and will this imaginary boundary be the same for all subjects?). Even in these cases, however, it might be expected that the large scale changes present at end-stage disease will *not* be the primary target of *in vivo* studies, and development of radiopharmaceuticals with a matching all-or-none sensitivity might be of lesser interest.

One cannot disagree with Dr Eckelman that too little time, thought and effort is spent on the analysis of the sensitivity of a radiotracer measure, sometimes erroneously termed "validation". But just what is "validation"? *It depends upon the question being asked.* If one wishes to know the rate of loss of a specific binding site during the course of a neurodegenerative disease, a sensitivity to 5–10% changes in biochemistry might be required for medical utility. If one wishes, on the other hand, to differentiate between pathologies involving two optional but large scale changes in biochemistry, or simply the location of a large biochemical change, far less sensitivity might be perfectly acceptable.

So what kinds of sensitivity testing should be pursued? A favorite of many is the correlation between *in vivo* values, such as tissue ratios, B_{max}, binding potential, volume of distribution or other parameter and *in vitro* measures of the same binding site or enzyme site densities as measured in different

regions of an organ. Unfortunately, good *in vitro–in vivo* correlations do not necessarily equate with successful clinical radiopharmaceuticals. We have, in the past few years prepared numerous potential PET radioligands for which adequate to quite good *in vivo–in vitro* correlations can be made (Haka and Kilbourn, 1989; Kilbourn *et al.*, 1990a,b, 1991; Lee *et al.*, 1991; Mulholland *et al.*, 1992; Wieland *et al.*, 1990; Rosenspire *et al.*, 1990). However, in most cases the dynamic range of the *in vivo* measured values are only a fraction of the dynamic range for the *in vitro* values, and the sensitivity of such radiotracers to clinically relevant changes of *in vivo* biochemistry remains unknown. But are these radiotracers "validated" by the *in vivo–in vitro* correlation, and if so, what are they useful for? Do the *in vitro* values even represent the functional range of such binding sites in the intact organism, and do the *in vitro* values represent the optimal "image" one might obtain from an *in vivo* study resplendent with secondary binding sites and generalized non-specific binding?

As an example, we have considerable experience with [^{18}F]GBR 12909, which exhibits *in vivo* striatum/cerebellum ratios (str/cer, a crude measure of specific binding) which do not even approach the values that might be calculated from the *in vitro* concentrations of dopamine uptake sites in these tissues (given that it is difficult to calculate a precise ratio, as there are no DA uptake sites in cerebellum). Some investigators are willing to explain these results through secondary binding to sigma receptors or a cytochrome P450IID1 enzyme site (Niznik *et al.*, 1990). Surprisingly, the cocaine analog CFT (WIN 35,428), also a dopamine uptake site radioligand, exhibits the *exact* same behavior: relatively poor *in vivo* str/cer ratios (< 5) for [^{11}C]CFT (Madras *et al.*, 1991) despite exceptionally high values from the *in vitro* [^{3}H]CFT autoradiography in the same species (Kaufman *et al.*, 1991). Some of this difference can be attributed to the problems of partial volume averaging in imaging of monkeys, but should the rest be attributed to CFT binding to other high affinity sites such as the P450IID1 sites, for which cocaine has been reported to have a high (74 nM) affinity (Tyndale *et al.*, 1991), or possibly also the sigma receptor? Given the long list of compound types which show affinity for the sigma receptor (Koe *et al.*, 1991), maybe the question should be reversed; what does not bind to the sigma receptor? And, really, does it matter? Even more to the point, if we are to utilize a radiotracer to evaluate a specific binding site in specific tissue regions, should we even be concerned with the behavior of that radioligand in tissues or regions uninvolved in the physiology or pathology in question? As one begins to ponder these questions, "validation" by comparison of *in vivo* and *in vitro* regional organ distributions seems incomplete.

The second standard method of "validation" is determining the dose–response curve of radioligand binding in animals, using competition for sites by doses of the identical or pharmacologically equivalent but structurally different drugs. Is this sufficient? Such studies demonstrate that measured values of *in vivo* binding sites can be reduced by competing drugs, but are such pharmacological challenges the equivalent of the functional changes evidenced during a disease process? Probably not; biochemistry is very complex, and it is almost inconceivable that changes in one aspect such as a number of binding sites or enzyme molecules are not accompanied by other changes in biochemistry. Proving that a drug treatment "mimics" a disease is challenging, and one must question whether a dose–response curve is a "validation" of the usefulness of the radiotracer in the intended disease.

Should tracers be "validated" in animal models of human diseases, or even better, through clinical trials? The last is a poor alternative; the costs and ethics of radiotracer evaluation in humans can be prohibitive. What about animal models? This may be an under-utilized methodology in radiopharmaceutical development, as there are certainly a plethora of lesion (Conn, 1991), behavioral and genetic animal models for human diseases, even for such diseases as senile dementia (Lal and Forster, 1991). In the same issue which held Dr Eckelman's Editorial, we published one of our attempts in this area, utilizing the degeneration and subsequent regeneration of dopaminergic terminals in MPTP-treated mice as what might be envisioned as a "reverse" model of Parkinson's disease (Kilbourn *et al.*, 1991). Relatively crude measures of *in vivo* specific binding of [^{18}F]GBR 13119 (a dopamine uptake blocker and putative neuronal marker) in mouse striatum increased with recovery of the animals. Remarkable correlations with published recoveries of tissue dopamine ($r^2 = 0.98$) and tyrosine hydroxylase enzymatic activity (TH: $r^2 = 0.99$) measured *in vitro* for the same animal model could be made (Fig. 1.) (Donnan *et al.*, 1987; Nishi *et al.*, 1989), although such were not reported in that publication. Is this a more proper way of "validation"? The discerning reader will note that the dynamic range for [^{18}F]GBR 13119 binding is larger than the dynamic range for TH enzymatic activity, but less than the dynamic range for endogenous dopamine levels. Which are the proper *in vitro* data to use in such correlations, and which correlation is correct for modeling Parkinson's disease in humans? Which *in vitro* measure—tyrosine hydroxylase, which is perhaps a measure of surviving neurons, or dopamine, which might be a better measure of the functional status of the surviving neurons—would be important in the development (and perhaps evaluation through *in vivo* PET studies) of therapeutic drug strategies for prevention or amelioration of Parkinsonian symptoms? As Dr Eckelman points out, even such encouraging results in animals can be hard to translate to clinical efficacy, but perhaps more such efforts should complement current efforts at radiotracer "validation".

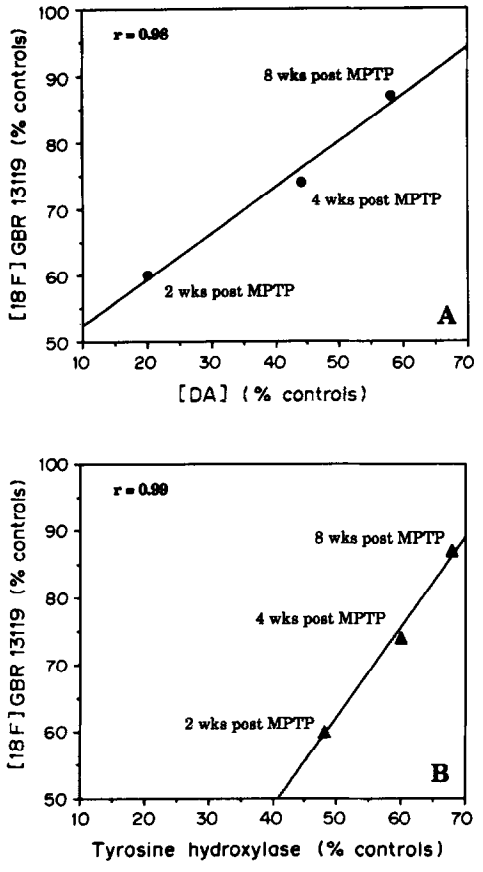

Fig. 1. Correlations of *in vivo* specific binding of [^{18}F]GBR 13119 with *in vitro* measures of endogenous dopamine concentrations (A) and tyrosine hydroxylase enzymatic activities (B) in the striatum of mice at selected times following systemic MPTP treatment. *In vivo* data from Kilbourn *et al.* (1991) and *in vitro* data from Donnan *et al.* (1987) and Nishi *et al.* (1989).

Development of new radiopharmaceuticals has become, if anything, even more complicated and challenging: neurochemistry, pharmacology and molecular biology have spawned a whole new and sometimes bewildering neurochemical landscape. There are five muscarinic receptors, and at least five dopamine receptors; who knows how many subtypes of subtypes of serotonin receptor may eventually surface. Glutamate pharmacology, once neatly wrapped up as NMDA, kainate and quisqualate receptors, is exploding: there is at least one new receptor type not linked to an ion channel (metabotropic receptor), and possibly multiple subtypes of each of these receptor types (Aizawa *et al.*, 1991; Vecil *et al.*, 1991; Sakurai *et al.*, 1991) There may be multiple forms of tyrosine hydroxylase; the functional significance of this finding still eludes us (Melchitz *et al.*, 1991). Armed only with a radiochemical, an imaging device and a pharmacokinetic model (Carson, 1991), are we too presumptuous to think we can ever figure out nature's complexities?

Do we make too many new radiochemicals with too little evaluation of their applicability? Perhaps; certainly a disproportionate share of the resources is devoted to synthesis of new variants of radiopharmaceuticals that have not been fully evaluated in the first place. But we may also analyze ourselves into a corner, and suffer paralysis of both thought and action. The synthesis of new radiotracers and their evaluation through *in vivo–in vitro* correlations, dose–response analyses and animal models will continue as our route to new clinical radiopharmaceuticals. So until such time as we have all of the answers on a theoretical basis, much has been and will continue to be learned from the experimental approach.

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