INVITED COMMENTARY

Enzyme Inhibition as an Aid to Simplify Pharmacokinetic Measurements?

The widely used chemotherapeutic agent 5-fluorouracil (5-FU) has been in the oncologist's armamentarium for the last 40 y. In spite of the widespread use of 5-FU the success rate varies between 10% and 30%; thus, any method of either predicting or rapidly monitoring the response of any particular tumor to this agent would clearly be welcome. This would allow the oncologist to rapidly adjust therapies either by changing the dosage or changing to another compound that might be effective in those cases in which the initial regime does not work. To this end, 5-[^{18}F]FU was one of the first tracers prepared with ^{18}F (*1*) and has been used, in conjunction with PET, in attempts to measure the pharmacokinetics of the compound in vivo in human cancer patients (*2*). Unfortunately, the compound is rapidly metabolized, which results in circulation of several labeled metabolites that can penetrate the tumor (*3*). In the PET study, these metabolites can be distinguished only by kinetic analysis of the uptake and washout data. The metabolism results in limited tumor uptake and requires complex multicompartment models with multiple kinetic parameters to evaluate this uptake. These parameters are difficult to evaluate with any confidence and, as a consequence, the ^{18}F-labeled compound has not been widely used (*4*) for the evaluation of 5-FU therapy. The rapid metabolism of the compound is also a confounding and limiting factor in the therapeutic use of 5-FU.

The first step in the primary metabolic pathway of 5-FU is through the enzyme dihydropyrimidine dehydrogenase (DPD). Several inhibitors of the enzyme are under investigation as adjuncts for 5-FU therapy (*5–11*). Conceptually, limiting the metabolism of the drug will improve tumor targeting and make lower doses effective in the tumor, reducing the normal tissue toxicity. The preliminary results of these studies appear encouraging. The same strategy has been applied by Bading et al. (*12*), who used 5-[^{18}F]FU imaging studies in an animal model. To investigate the effects of the inhibitor eniluracil (5-ethnyluracil), which is a potent inhibitor of DPD (*6*), it was administered to the animals before 5-[^{18}F]FU. Both the tumor uptake and the presence of metabolites in the tumor and the circulation were investigated. The results indicated that the tumor uptake was substantially increased and metabolism of the tracer was substantially reduced in both the tumor and the circulation. Tumors implanted on the animal's thigh were well imaged in the presence of the inhibitor but not in its absence. Clearly, the inhibitor made a substantial difference in the biodistribution of the tracer. If the same inhibition of tracer metabolism can be obtained in humans, then the use of eniluracil would provide substantial improvement in the quality of human 5-[^{18}F]FU images.

The same general strategy, inhibiting unwanted metabolism of a tracer and thereby simplifying the pharmacokinetics modeling, has been used before. 6-[^{18}F]fluoro-3,4-dihydroxyphenylalanine (fluoroDOPA), a tracer for cerebral dopamine metabolism, has been used in conjunction with carbidopa, an inhibitor of the enzyme catechol *O*-methyltransferase (COMT) (*13*). In the absence of the inhibitor the tracer is methylated in the liver. The methylated derivative passes into the brain but is not a substrate for the enzymes involved in dopamine synthesis. The radioactive signal coming from the methylated derivative has to be separated from that of the fluoroDOPA and fluorodopamine (*14*). This can be done by kinetic analysis, but the necessity of evaluating the additional parameters adds to the uncertainty of the parameters of interest (*15*). The use of carbidopa substantially reduces the signal from the methylated derivatives and so simplifies the kinetic analysis (*16*). However, in this case, the enzyme inhibitor does not affect the metabolism of the tracer in the organ of interest, the brain, but merely reduces the presence of labeled metabolites that confuse the interpretation of the data.

The improvement in the image quality brings into question the nature of the information that would be present in a 5-[^{18}F]FU–eniluracil image if the method is to be used on tumor patients. The first point that can be made with some confidence is that the results will provide real pharmacokinetic data for studies in which 5-FU is used in conjunction with eniluracil. If the qualities of the human data are comparable with those obtained in rats, the data will be of high quality and suitable for analysis. However, two points suggest that broader application of this method to interpreting the pharmacokinetics of 5-FU in the absence of DPD inhibition should be applied with some caution. Because the first step in the primary route of metabolism of pyrimidines is catalyzed by DPD, inhibition of this enzyme may result in a buildup, in the cell, of pyrimidines and other metabolites. Thus, the rate-determining steps that control the routes of pyrimidine metabolism and use—of both the endogenous species and the radioactive tracer, in the tumor and normal tissue—

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will change when DPD is inhibited. As a consequence, the flux of the tracer through the tumor will change. This flux will represent the behavior of the tracer in the absence of metabolism by DPD and will not be applicable in the presence of active DPD. As a second, and perhaps secondary, consideration, the dosage of 5-FU used in combination with eniluracil is significantly less than when the compound is used by itself. This is because, although the tumor-to-normal tissue toxicity is increased with eniluracil, the maximum tolerated dose of 5-FU is lower in the presence of the inhibitor (*17*). As a result, the full pharmacologic dose of 5-FU used as a single agent cannot be delivered safely in the presence of the inhibitor. Thus, the pharmacokinetic data can be obtained only with a reduced dose of 5-FU.

The more general conclusion from studies where a metabolic modifier is combined with an imaging agent to ''simplify'' the imaging data is that the ''simplification'' is better characterized as a symbiotic relationship than a simplification. Then the imaging data can be understood in terms of the behavior of both the tracer and the metabolic modifier. This adds the complication that we now have to understand the in vivo kinetics of 2 different compounds rather than just 1. With luck, or perhaps good management, the behavior of the modifier will be simple enough for the effects to be directly predictable. This certainly appears to be the case in the use of carbidopa and COMT inhibition when used with [^{18}F]fluoroDOPA. Here the site of action is remote from the area under study, the brain, and it is only the measured input function to the region of interest that is affected by the modifier. The use of eniluracil and 5-[^{18}F]FU, described by Bading et al. (*12*), may be somewhat more complicated in that the effect of the modifier is both remote to and involved in the region of interest—in this case, the tumor. This adds to the measurements necessary to identify the role that each component of the relationship plays in directing the observed behavior. It also adds an additional dimension in that it may be possible to measure the critical behavior of the (unlabeled) metabolic modifier by its effect on the pharmacokinetics of the labeled tracer, if these are well enough understood.

The primary role of nuclear medicine is to image the function of the organism rather than its form. Therefore, whenever a functional tracer is used in conjunction with a modifier of that function the results have to be understood as a change rather than a simplification or enhancement of that tracer. The effect on the images can be assessed only when the changes in function of the tracer produced by the modifier are understood.

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