Dear Editor,

We thank Drs. Koch and Evans for their insightful comments on our recent article on the hypoxia imaging agent \(^{18}\text{F-EF5}\) (1). In their letter, Koch and Evans have suggested possible reasons for the significant retention of unbound \(^{18}\text{F-EF5}\) in H460 tumor xenografts in rats compared to that in tumors grown in mice as described in our article. We agree that the differences in drug half-life between rats and mice could be the major factor contributing to higher retention of unbound \(^{18}\text{F-EF5}\) in rat tumors, especially when the radiotracer is co-administered with its nonradioactive analog for immunohistochemical (IHC) analysis of bound EF5-adducts on tumor sections. We used 2.5 hr for single time point imaging (3 hr post-injection for tumor collection and autoradiography) in order to enable direct comparison among the tumor models, and based on the literature reports suggesting that 2 - 3 hr is generally an optimal time window for imaging after \(^{18}\text{F-EF5}\) injection (2-4). For comparison of autoradiography and IHC images of \(^{18}\text{F-EF5}/\text{EF5}\) binding in tumors, we agree that fixation of tumor sections may remove unbound \(^{18}\text{F}\) activity and yield autoradiography images that may closely match the EF5-IHC images. In our studies, we used a standard method of comparing images derived from whole tumor sections (untreated) with the hypoxia profile determined from EF5-bound adducts in IHC images because the purpose of this analysis was to study the distribution (intratumoral) of the radiotracer and corroborate the microPET image findings at the selected time point (2.5 hr) (5-6).

In our article, we did not intend to make any suggestions on the metabolism of EF5 or \(^{18}\text{F-EF5}\), including nonhypoxic metabolism in vivo (7). We think that the observed effect of lower intratumoral contrast in H460 tumors at 2.5 hr post-injection of \(^{18}\text{F-EF5}\) in our study could be due to the presence of excess drug and/or due to slower clearance of the radiotracer from non-hypoxic tumor regions (areas not positive for EF5-adducts) when the radiotracer was co-administered with unlabeled EF5 at a 30 mg/kg dose. We note that this is in line with the Koch and Evans suggestion that the 10-fold difference in drug-concentration between the two groups of animals injected with radiotracer alone and co-injection with EF5 (30 mg/kg) could have caused changes in drug half-life and possibly affect the pharmacokinetic loss of unbound drug (\(^{18}\text{F-EF5}\)) in H460 tumors in rats. Given the longer half-life of EF5 in rats, imaging at later time points (e.g. \(>3\) hr) may allow better clearance of the unbound radiotracer and further improve the contrast between hypoxic and non-hypoxic tumor regions in tumors grown in rats and at the 30 mg/kg dose (100 \(\mu\)M).
With regard to the statement “visible binding in IHC selects for tissue with pO2 less than 10 mm of Hg”, again, we would like to clarify that we only used “partial pressure of oxygen < 10 mm Hg” in the introduction section (in parenthesis to a sentence) in order to provide general information that tumor retention of 2-nitroimidazole-based hypoxia tracers typically reflects pO2 values < 10 mm Hg, as the binding rate of 2-nitroimidazole hypoxia markers increases sharply at pO2 values < 10 mm Hg (8-10). The full sentence reads as follows: “With the exception of 64Cu-diacetyl-bis(N4-methylthiosemicarbazone), current small-molecule PET hypoxia tracers consist of a 2-nitroimidazole moiety that forms the basis for their selective uptake in hypoxic tumor cells (partial pressure of oxygen < 10 mm Hg).” In our studies, of the three tumor models, PC3 tumors displayed a distinctive pattern of hypoxia as indicated by large regions of EF5 binding in IHC images. In some tumors, the intensity of EF5 binding increased from the center to the outer margin of hypoxic regions. This binding pattern of EF5 in PC3 tumors appears consistent with the macroscopic regions of hypoxia reported by the Koch group in rat 9L gliosarcoma tumors (11).

Satish K. Chitneni, Gerald T. Bida, Michael R. Zalutsky, Mark W. Dewhirst
Duke University Medical Center,
Durham, North Carolina

References:


