Applications of Nitroimidazole In Vivo Hypoxia Imaging in Ischemic Stroke

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Background and Purpose—Nitroimidazole imaging is a promising contender for noninvasive in vivo mapping of brain hypoxia after stroke. However, there is a dearth of knowledge about the behavior of these compounds in the various pathophysiologic situations encountered in ischemic stroke. In this article we report the findings from a systematic review of the literature on the use of the nitroimidazoles to map hypoxia after stroke.

Summary of Review—We describe the characteristics of nitroimidazoles as imaging tracers, their pharmacology, and results of both animal and clinical studies during and after focal cerebral ischemia. Findings in brain tumors are also presented to the extent that they enlighten results in stroke. Early results from application of kinetic modeling for quantitative measurement of tracer binding are briefly discussed.

Conclusions—Based on this literature review, nitroimidazole hypoxia imaging agents are of considerable interest in stroke because they appear, both in animal models and in humans, to specifically detect the severely hypoxic viable tissue, but not the reperfused nor the necrotic tissue. To fully realize this potential in stroke, however, formal validation by concurrent measurement of tissue oxygen tension, together with development of novel ligands with faster distribution kinetics, faster clearance from normal tissue, and well-defined trapping mechanisms, are important goals for future investigations. (Stroke. 2008;39:1629-1637.)

Key Words: imaging ■ ischemia ■ PET ■ stroke ■ hypoxia ■ nitroimidazole

The present management of acute stroke is guided by the identification and ultimately rescue of the ischemic penumbra. CBF thresholds defining the penumbra and core have been determined using multi-tracer 15O PET and were thereafter extended to other imaging modalities. Nonetheless, CBF alone does not indicate the actual penumbral topography because the infarction threshold depends on time since stroke onset. Furthermore, concurrent estimation of the oxygen extraction fraction (OEF) is necessary to define the penumbra in conditions of partial reperfusion and secondary hypoperfusion.

The penumbra can be defined as hypoxic tissue whose function is reversibly affected by the reduction of tissue partial pressure of oxygen (tPO2), itself caused by the scarcity of blood supply relative to oxygen consumption. Experimental and clinical studies measuring tPO2 directly in acute stroke indicate that it falls from normal values around 30 mm Hg to below 10 mm Hg and may even approach zero (see supplemental Appendix for further detail, available online at http://stroke.ahajournals.org). Mapping of the tPO2 noninvasively would provide a direct way to depict salvageable tissue, either alone or in conjunction with CBF. Although the 15O-PET can provide an estimate of end-capillary PO2, comparison with direct tPO2 measurement has shown discrepancies, implying that end-capillary PO2 reflects a different, though partly overlapping, entity. A group of oxygen-sensitive compounds known as 2-nitroimidazoles, originally developed as antibacterial agents, have now been adapted as imaging agents that can be radio-labeled and potentially map the viable hypoxic tissue in the live brain of animals and humans (see supplemental Appendix for a historical note).

An ideal hypoxia imaging agent for use in stroke should: (1) freely cross the blood brain barrier (BBB) and rapidly localize to the target viable hypoxic tissue with high specificity; (2) have a known relationship to true tPO2 allowing its indirect estimation; (3) have suitable tissue kinetics to allow sufficient tissue contrast within a timeframe permitting its use in the acute setting and compatible with known time-course...
of infarct growth; and (4) have favorable dosing and safety profiles for use in humans.

Although, as will be shown below, such an ideal hypoxia imaging agent remains to be found, some currently available nitroimidazoles satisfy several of these prerequisites.

**Pharmacological Properties of Nitroimidazoles**

**Biochemical Pathways**

Several nitroimidazole derivatives have been developed for imaging use (see supplemental Figure I, available online at http://stroke.ahajournals.org). Nitroimidazoles undergo selective bioreduction in hypoxic cells to form reactive products that irreversibly bind to cell components (Figure 1), a property exploited for their antibacterial action. The process is initiated by an enzyme-mediated single electron reduction to form a free radical. Under hypoxic conditions, this reduction is catalyzed by pyruvate (specific enzyme for this step not completely identified).8,10,11 Inside cells, the free radical anion is rapidly reversed to its original compound by intracellular oxygen, which has a higher electron affinity than the nitro group. The rate of oxidation is dependent on the intracellular concentration of oxygen. If tPO2 is low, subsequent steps ensure the irreversible retention of nitroimidazoles in the cells—a prerequisite to differentiating normoxic from hypoxic tissue. The cellular components that covalently bind the free radical-type nitroimidazoles have not been clearly identified, but might be intracellular macromolecules such as proteins or DNA.8 (See the supplemental Appendix for a note on the pharmacokinetics of nitroimidazoles).

**Oxygen Dependency of Nitroimidazole Binding**

The sensitivity of nitroimidazole imaging for detecting hypoxic tissue is determined by: (1) the amount of tracer delivered to the target site via CBF and the BBB; (2) the fraction that gets past the initial reversible enzyme-dependant reaction; (3) the washout from normal cells; (4) the rate of trapping in hypoxic tissue, which in turn determines the duration of the experiment sufficient for reaching adequate contrast between normoxic and hypoxic tissue; and (5) the presence of the intracellular macromolecular components necessary for binding.8 Much of the research done on the oxygen dependency of nitroimidazole binding concerned the radiosensitizer misonidazole.8 Studies using multi-cellular spheroids have shown that very little accumulation of misonidazole occurs in the tPO2 range 20 to 60 mm Hg but a steep rise is seen as this approaches 10 mm Hg.12 In healthy brain tissue in the mouse, the Km of misonidazole in vitro is 3.04 mm Hg,13 whereas in animal and human tumor cell lines, it varies between 0.76 and 4.56 mm Hg8,14,15—tPO2 levels relevant to stroke. However, the retention of nitroimidazoles varies widely with the type of tissue and pathology under study.16 Thus it may not be possible to generalize data to other nitroimidazoles, particularly in acute stroke, and investigation of the individual ligands under various conditions in stroke models is therefore necessary to establish their fitness for this application.

In the gerbil, misonidazole binding positively correlates with the severity of stroke symptoms17 6 to 10 hours after carotid ligation. In rodent and canine tumors,18,19 fluormisonidazole (FMISO) appears to bind to tissue at similar tPO2 levels as misonidazole. In the ischemic porcine liver, Piert et al20 demonstrated a precise nonlinear inverse relationship between 18F-FMISO standardized uptake value (SUV) and tPO2 below 15 mm Hg (Figure 2) and were able to directly estimate the tPO2 from FMISO SUV. However, in murine and human soft tissue tumors, others did not replicate this relationship,21,22 possibly because of the tumoral nature of the tissue, tissue heterogeneities and necrosis in their selected tumor types, or the use of the polarographic microelectrode. Combining 18F-FMISO PET, deoxyglucose autoradiography, histological analysis and tPO2 measurements in murine tumors, high binding of FMISO was found (tumor-reference ratio of 11) in tumors with a median tPO2 of 7 mm Hg, in agreement with histological and autoradiographic patterns documenting cellular viability.16 Increased FMISO binding was also seen in tumors with a median tPO2 of 1 mm Hg, but the tumor-to-reference ratio was only 2, possibly reflecting cell death.

These results altogether indicate a close relationship between nitroimidazole binding and tPO2 in normal liver tissue and most tumors, but also point to a role for factors other than hypoxia in tracer uptake and binding, such as the type of

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**Figure 1.** Schematic of the proposed mechanism underlying the binding of nitroimidazole tracers.
tissue (e.g., normal versus tumor), cell viability, and local metabolic and enzymatic patterns. These considerations are critical when considering extending the use of nitroimidazoles to stroke.

Applications of Nitroimidazoles in Hypoxia Imaging

Experimental Stroke Studies

Considerable heterogeneity exists among experimental nitroimidazole stroke studies published to date with regard to stroke model, species, and hypoxia tracer used; time of assessment of tracer uptake after administration and arterial occlusion; method to assess and express tracer uptake; and use of ancillary techniques such as CBF. Furthermore, no experimental stroke study so far has validated nitroimidazole tissue uptake against concurrently obtained tPO2. This heterogeneity precludes any normalization, and hence combined analysis, across studies, and consequently a descriptive approach will be adopted here (see supplemental Table I for details).

With only 2 exceptions,23,24 all of these studies have used ex vivo autoradiography. In gerbils,17 3H-misonidazole was shown to have good brain penetration and binding to hypoxic-ischemic tissue in correlation to stroke severity. Subsequently,25,26 a correlation to histological infarction was demonstrated, though direct comparisons could not be made. Trapping was consistently found in regions with reduced rCBF, yet a correlation to CBF was not ascertained. It was also shown that 3H-misonidazole trapping was not secondary to BBB leakage.26

One hour after permanent middle cerebral artery occlusion (pMCAO) in rats, the relative uptake of 99mTc-BMS-181321 in the most affected region was 6.4±2.2, corresponding to a mean 92% reduction of rCBF.24 However, comparison of uptake ratios to rCBF on a pixel-by-pixel basis along the edge of this region revealed a gradual increase of uptake from around 50 mL/100g/min (corresponding to the penumbra threshold in that model) and a rapid rise at lower rCBF levels near the infarction threshold (Figure 2). Importantly, when the tracer was administered 24 hours after pMCAO, no retention was seen in the area of infarction. These results imply that BMS-181321 only localized in areas of severe hypoxia, but not in areas of moderate hypoxia nor in irreversibly damaged tissue. In experiments on cats, BMS-181321 retention was detected by SPECT after 3 hours of MCAO24 and was further confirmed on ex vivo autoradiograms. Interestingly, early SPECT imaging (immediately postinjection), but not late imaging, appeared to closely approximate CBF.

125I-IAZA was subsequently used in rat pMCAO experiments.27,28 The first study involved visual inspection of double-label ex vivo autoradiograms of 125I-IAZA and the semiquantitative perfusion tracer 99mTc-HMPAO. In animals injected 2 hours after the start of occlusion and euthanized 7 hours later, uptake of IAZA was seen in regions of moderately decreased perfusion but was absent in regions of either slight or severe hypoperfusion, probably corresponding to oligemia and infarcted core, respectively, consistent with the above BMS-181321 studies. These findings were confirmed in their follow-up study using the same design plus diffusion-weighted imaging (DWI) at 2 hours after pMCAO.28 They reported four distinct regions: (1) “normal”; (2) “oligemic misery perfusion”, showing CBF down to 34% of normal, increased IAZA uptake (2.1), and low ADC values (<50% of normal); (3) “ischemic misery perfusion”, showing CBF down to 34% of normal, increased IAZA uptake (0.8), and further decreased ADC. The presumed “penumbra” indicated by increased IAZA uptake lay entirely within the area of restricted diffusion and was later found to be part of the...

Figure 2. A, The relationship between 18F–FMISO trapping (expressed in standardized uptake values [SUV]) and tPO2 in the pig liver at various levels of hypoxia (from reference20 with permission). A significant nonlinear relationship is seen (the different symbols represent different groups of experimental animals). B, The relationship between 99mTc-BMS18,1321 trapping, expressed as ischemic/nonischemic relative optical density (ROD) ratio, and CBF in different brain regions from three permanent MCAO rats (shown as filled squares, open triangles and crosses, respectively; from reference24 with permission). Tracer trapping increased gradually as CBF decreased below 50 mL/100g/min and dramatically for CBF values below 20 mL/100g/min (~8% of normal value).
infarct on histology. However, there was a region with very low CBF and ADC but no IAZA trapping, likely representing the core (ie, region 4; illustrated in their Figure 2), so the area of tracer trapping did not exactly match the DWI lesion. This lack of IAZA trapping despite probable ongoing hypoxia in Region 4 probably reflects damage to the key enzymes and proteins that underlie trapping in viable tissue and make the important point that hypoxia tracers, in conjunction with DWI, may allow the differentiation between the core (DWI lesion, no tracer trapping) and the penumbra (tracer trapping regardless of DWI). Although the finding that the area of IAZA trapping was embedded within the DWI lesion would agree with the notion that the DWI lesion includes areas of penumbra, it is surprising that IAZA uptake was not also seen in the surrounds, because in principle the DWI lesion does not contain the whole penumbra. However, IAZA was injected 2 hours after pMCAO, by which time the entire penumbra perhaps manifested high DWI signal. This later time of administration than in the BMS-181321 study probably accounts for the different behavior of the tracers at very low blood flows, ie, in the BMS study the core still demonstrated maintained cellular machinery for hypoxia tracer binding, whereas viability was probably lost in the IAZA study. Finally, both the BMS and the IAZA studies seem to concur that hypoxia tracers do not show trapping in mildly hypoperfused tissues (ie, the oligemia), although close inspection of the data in Figures 3 and 4 of the IAZA article suggests a mild but definite gradual tracer retention for perfusion values above the penumbra threshold. Nevertheless, both studies agree that nitroimidazoles will show substantial retention in the moderately to severely hypoperfused tissue, provided it is still viable.

Recently, Saita et al reported on 18F-FMISO ex vivo autoradiography in rats subjected to 2-hour MCAO. Rats were injected at varying time points from occlusion (0.5 to 22 hour) and euthanized 2 hour later, and tracer retention was compared to triphenyltetrazolium chloride (TTC) postmortem staining. Consistent with being selectively retained in ischemic tissue, FMISO uptake was seen in large areas within the MCA territory at prereperfusion time points. These became progressively smaller and more localized to the edges of the infarct over time. Only a thin rim of uptake surrounding the TTC-determined infarct was seen at the late time points. All animals showed reduced 18F-FMISO uptake at the core of the infarct. Nonetheless, the finding that 18F-FMISO was retained in periflaccid tissue up to 20 hours despite reversal of MCAO was intriguing. As possible explanations, the authors suggested periflaccid edema, small vessel occlusion, or “no-reflow” may have resulted in persistent tissue hypoxia. Furthermore, the thread-up MCAO method used in that study can induce vascular injury or rupture during withdrawal of the thread, and thus may have precluded effective reperfusion, which would also account for the TTC-determined infarct growing until the last time point. That retention occurred in the core also cannot be completely excluded because superimposing the autoradiograms and the TTC sections was not technically feasible. This study raised the disconcerting possibility that FMISO could be trapped in normoxic periflaccid or in hypoxic nonsalvageable tissue, in contrast to the above BMS and IAZA studies. However, further experiments dispelled these concerns. Using an improved thread-up technique and tracer injection either during the occlusion or 1 hour after 45 to 90 minutes MCAO, 3H-FMISO binding occurred only in ongoing tissue hypoxia and not if it was administered after reperfusion. Interestingly, the long half-life of 3H enabled direct comparison between early tissue hypoxia and histology done 24 hours later.

Overall, the above (mostly ex vivo) experimental studies support the notion that nitroimidazoles can specifically detect the viable severely hypoxic tissue in acute stroke, but not the mildly hypoperfused tissue nor the severely hypoperfused and necrotic core, although the latter point remains incom-
pletely solved. An important limitation is that direct measurement of tPO$_2$ was not performed in any of these studies. Furthermore, CBF was not systematically quantified in conjunction with the hypoxia tracer, so the CBF threshold for significant tracer uptake has not been fully clarified.

We have successfully used animal PET to study $^{18}$F-FMISO in the rat distal MCAO model.\textsuperscript{23} In pMCAO conditions, $^{18}$F-FMISO was injected around 30 minutes after occlusion and dynamic scanning performed for 3 hours; a follow-up PET study was performed 48 hours later (all the $^{18}$F from the first study would have decayed by then), at a time when pan-necrosis was expected. In the acute stage, there was continuous uptake of the tracer in the affected MCA cortical territory, reaching more than twice that of the unaffected side at 3 hours postinjection (Figure 3). No significant retention was found on the follow-up scan, which corresponded to frank tissue infarction at histology, nor when the tracer was administrated immediately after 45 or 120 minutes MCAO. These results were confirmed by modeling of the full plasma and tissue FMISO kinetics. The findings from this study using in vivo imaging are therefore largely consistent with the above ex vivo studies, further supporting the notion that $^{18}$F-FMISO is trapped only in hypoxic viable tissue, but not in necrotic or reperfused tissue, potentially making it a specific marker for the salvageable penumbra. Follow-up PET at shorter intervals after pMCAO are however needed to address whether early irreversibly damaged but still hypoxic tissue still binds F-MISO.

**Clinical Studies**

Nitroimidazoles have been used in the detection of hypoxia in humans in stroke as well as in a number of other fields, particularly in tumors (see supplemental Appendix for details).

Supplemental Table II tabulates all the nitroimidazole studies performed in acute stroke so far. In the first study, reported in abstract form,\textsuperscript{34} 3 of 6 patients presenting with “acute” ischemic stroke (no further detail provided) showed regions of intense $^{18}$F-FMISO retention surrounding the core of the infarction on PET. This presumed “penumbra” had disappeared when the patients were rescanned 1 month later. Also in a conference abstract, $^{99m}$Tc-BMS-181321 SPECT was used in 3 patients presenting with subacute stroke (11 to 38 hours).\textsuperscript{35} Only 1 patient, imaged within 12 hours, demonstrated a region of (moderately) increased uptake (lesion-to-contralateral ratio 1.33) located in close proximity to the infarct seen on CT at 36 hours, which could have represented penumbral tissue. The other 2 patients, scanned at 22.5 and 38.8 hours, had negative studies. Work with this tracer has not, to our knowledge, been pursued.

$^{99m}$Tc-ethylene dicysteine-metronidazole ($^{99m}$Tc-EC MN) SPECT has been tested in 8 patients scanned in the late subacute stage (6 to 14 days after onset).\textsuperscript{36} Surprisingly, increased periinfarct uptake (lesion-to-normal ratios 3.5±1.5) was reported in all the subjects. Because scanning was done so late, this tissue is unlikely to represent the penumbra,
though may have represented persistent oligemia if ongoing vascular occlusion was present at the time of imaging (information not reported). Validation against established PET techniques is needed to confirm these results.

The Melbourne group have extensively investigated the potential of 18F-FMISO for depicting the penumbra, using a similar methodology whereby PET scanning was performed at least 2 hours after tracer injection. They first reported definite FMISO retention in the first 48 hours after stroke onset, which subsequently disappeared on follow-up imaging. This tissue shared the topographical and temporal characteristics of the penumbra. FMISO retention was based on selecting voxels with uptake ≥3SD above contralateral hemisphere mean, a conservative threshold that may have excluded lesser but potentially important tracer trapping. Similar approaches were used in all subsequent studies.

In a second set of patients, the results further suggested that tissue defined by high FMISO uptake represented the ischemic penumbra because: (1) both the volume of hypoxic tissue and the frequency of a positive study declined with time after stroke onset; (2) these areas comprised part of the final infarct volume; and (3) the initial severity of stroke correlated with the “initially affected” tissue volume, defined as the sum of the final infarct and the hypoxic tissue eventually surviving infarction. However, they also reported that if a high proportion of the initially affected volume proceeded to infarction, neurological deterioration was likely. This is somewhat unexpected, because if the hypoxic tissue only represented the penumbra, then its progression to infarction would not in principle contribute to clinical worsening. A possible explanation is that the area defined by FMISO PET may have not been fully symptomatic at the time of imaging because it represented oligemic rather than penumbral tissue—which could also account for the observation of hypoxic tissue up to 43 hours after stroke onset.

In later studies, statistical parametric and nonparametric mapping was used to identify voxels with increased FMISO uptake relative to normal controls, allowing the 3D extent of hypoxic tissue (so-called penumbrogram) to be mapped. This tissue was found predominantly superior, mesial and posterior to the center of the final infarct. Furthermore, this tissue was preferentially seen in the center of the final infarct in patients studied 6 hours from onset, while it was mostly peripheral or external to it at later times. These findings were consistent with the known topography of the penumbra and the notion of peripheral infarct expansion. Although of clear interest, this method assumes that the geographic center of the infarct corresponds to the true core of infarction, which seems only true for MCA territory infarcts with a convex surface. Validation of the usefulness of the penumbrogram is necessary before it is accepted as a clinical or research tool.

A further study on an enlarged series of 27 patients with positive FMISO scans up to 48 hour after onset showed that ∼46% of the initially hypoxic tissue (∼7% of the total ischemic volume) survived infarction spontaneously. The chance of tissue survival was independent of time of onset, consistent with the concept of the penumbra. Furthermore, hypoxic tissue survival was associated with clinical improve-

ment, even for the group studied beyond 12 hours, suggesting that it contributed to the deficit. This satisfies an essential operational criterion defining the penumbra and further confirms the presence of potentially salvageable tissue >12 hours in a subset of anterior circulation stroke patients.

Overall, therefore, the above seminal FMISO studies are consistent with the animal studies described earlier in that trapping after ischemic stroke occurs in severely hypoxic but viable tissue, whereas its disappearance within 48 hours of onset suggests that necrotic or reperfused tissues do not retain FMISO. Figure 4 illustrates some of these points in 2 patients, showing complete MCA occlusion with marked 18F-FMISO trapping overlapping the MR DWI lesion and extending beyond it to a lesser degree, whereas in the other there is no trapping in the region of the DWI lesion after partial recanalization of the MCA, with only mild trapping posterior to it.

Differences in vulnerability to ischemia between white and gray matter have also been addressed using 18F-FMISO. White matter appeared to have a higher proportion of hypoxic tissue than gray at the time of imaging, though the volumes of hypoxic tissue eventually surviving infarction were similar in both compartments. This may indirectly suggest that white matter was initially more resistant to ischemia and proceeded more slowly to infarction than gray matter. Several studies have recently echoed these observations using MR imaging, yet the results should be considered with the view that: (1) binding of 18F-FMISO was assumed to be independent of tissue type; (2) it was assumed that the observed differences in survival were not the manifestation of differential blood flow to each of those compartments; and (3) the classification of voxels as white or gray matter was based on probabilistic maps generated from healthy controls, rather than on the subjects’ own scans.

**Discussion**

Imaging the viable hypoxic tissue in acute stroke is a highly desirable goal. Nitroimidazoles are the prime contenders among imaging tracers as they fulfill the main pharmacological and clinical prerequisites, and have been successfully used in imaging hypoxia in animal stroke models and in humans. Animal studies, including one using PET, generally support the idea that nitroimidazoles have rather high sensitivity and specificity for viable hypoxic tissue. Imaging studies in humans using 18F-FMISO PET also indicate that tracer trapping occurs mostly in viable hypoxic tissue that satisfies the operational criteria for the penumbra and matches its spatial pattern and temporal evolution.

Despite these clear potentials, nitroimidazoles have several significant limitations as in vivo clinical imaging tracers. The main limitation is their slow tissue kinetics, requiring for instance at least 2-hour equilibrium for 18F-FMISO before scanning is commenced, which precludes their use for clinical decision-making. The long scanning time is also incongruous with the dynamics of cerebral ischemia such that the resulting images represent the entire time epoch since injection, during which the hemodynamic and metabolic state of the tissue may have evolved (eg, demise of the penumbra, reperfusion, etc). Furthermore, as FMISO is not readily metabolized in the
human body and remains available for tissue uptake for many hours after injection, areas with even mild hypoxia may over time show increased uptake. Development of novel ligands with faster distribution kinetics and clearance from normal tissue than 18F-FMISO would therefore be paramount. The sugar-coupled tracer 18F-FAZA has not been tested yet in stroke, but data in animals indicate it has fast clearance from normal tissues and might be suitable in providing good contrast in a reasonable time-frame. In this regard, copper-labeled compounds such as 111In-ATSM, 64Cu-ATSM have been investigated in tumor hypoxia and also appear promising.40–42 Nonnitroimidazole compounds with more than one bioreductive site may also prove useful, as again these would require less time to bind to the target tissue.43 In addition to improved tracers, shortening of scanning time could be achievable if quantitative modeling allowed rate constants to be estimated reliably with short data collection (see below).

Another limitation derives from the almost exclusive use of PET in acute stroke studies so far. PET is an expensive, logistically complex, and scarcely available tool, making the study of acute stroke patients challenging. SPECT hypoxia ligands would therefore be desirable. Iodinated derivatives labeled with125I, such as IAZA, appear promising.125I and perhaps also 99mTc-BMS-181321,24,35 appear promising. On the other hand, the increasingly wider availability of PET in general hospitals for oncology diagnosis may have unexpected implications for hypoxia imaging in stroke.

Formal validation of nitroimidazole trapping against direct tPO2 measurements in the ischemic brain is still lacking. The only formal validation against concurrently measured tPO2 to date refers to the liver only (Figure 2). Knowing the quantitative relationship between nitroimidazole trapping and tPO2 should in turn allow the mapping of tPO2 in stroke.

Another limitation of nitroimidazole imaging agents refers to their somewhat ill-defined trapping mechanisms, with major gaps remaining in our knowledge of the successive steps leading to intracellular retention in situations of hypoxia, particularly the nature of the macromolecules that covalently bind reduced nitroimidazoles. Enhanced knowledge of these mechanisms would lead to improved interpretation of findings and better compartmental kinetic modeling.

An intriguing issue regarding the use of nitroimidazole imaging in stroke refers to the relationship between trapping and the DWI lesion. In the rat IAZA study detailed above, tracer trapping was roughly similar in topography and size to the DWI lesion. If nitroimidazole trapping equated the DWI lesion, then MR-DWI would be sufficient to map the hypoxic area in acute stroke without recourse to complex and time-consuming radiotracer-based investigations. However, in that study there was a region (denoted Region 4) that demonstrated very low ADC but no IAZA trapping, likely representing the necrotic core. The area of tracer trapping therefore did not match the DWI lesion, and hypoxia tracers in conjunction with DWI may in fact allow the differentiation between the true core and penumbral tissue. Furthermore, the area of IAZA trapping was embedded within the DWI lesion, which conflicts with the established notion that the latter is not supposed to contain the whole of the penumbra, and this surprising finding was probably attributable to the late timing of the assessment after MCAO. More generally, the DWI lesion is a marker of cellular dysfunction and develops consequent to, but is not a direct marker of, hypoxia. Accordingly, after MCAO: (1) the DWI lesion takes several minutes to develop, whereas hypoxia is immediate; (2) it expands over time in the face of stable ischemia, reflecting progressive metabolic dysfunction; and (3) it generally persists after reperfusion. Figure 4 illustrates the clear disassociation between the DWI lesion and the presence of FMISO trapping.

A key issue that remains in the interpretation of nitroimidazole imaging in acute stroke is whether the increased uptake only reflects the viable hypoxic tissue, ie, the penumbra. As detailed in previous sections, the available experimental evidence supports this idea, yet a study raised doubts that trapping might also occur in the ischemic core. This is theoretically plausible if the tissue, although inevitably destined to proceed to infarction, still includes cells that are nonnecrotic and consume oxygen at levels higher than oxygen delivery.40 Thus possibly the very high tracer retention seen in Figure 4 (top row) overlying the DWI lesion may have represented core rather than penumbra. It would still need to be established whether tracer retention in such tissue is attributable to slower wash-in and wash-out of the tracer over the scanning period rather than true active uptake. Experimental and clinical studies combining FMISO and multitracer 15O PET are needed to elucidate these points—although implementing this sort of studies is challenging.

Likewise, whether the oligemic tissue may demonstrate nitroimidazole trapping is unclear. All the methods used thus far for depicting significant tracer trapping result in a binary classification into positive and negative voxels—often applying conservative thresholds—which differs from more conventional multiparameter classification. These dichotomous images lack information on the severity—as opposed to presence—of tracer trapping, and hence of hypoxia. It is possible that less marked, yet pathophysiologically meaningful, levels of tracer trapping exist surrounding areas of stringently significant trapping, beyond simple partial voluming effects. Consistent with this idea, both the BMS and the IAZA rat studies28 in fact suggest the presence of mild tracer retention at CBF values above the penumbra threshold (see Figure 2). Quantifying the irreversible binding rate constant k, using compartmental modeling might resolve this issue in the future. Again, directly comparing nitroimidazole binding to 15O-PET would help clarify this point.

As alluded to above, deriving quantitative rate constants for nitroimidazole binding, instead of currently used uptake ratios, may also allow for shortening of the required imaging time. Although there are some reports from oncology studies (see supplemental Appendix), there is still no complete model that takes into consideration all the chemical characteristics of nitroimidazoles, including any influence of their labeled metabolites. The optimum number of compartments and rate constants necessary to describe the observed tissue kinetics in normal and hypoxic conditions remains uncertain. Kinetic modeling may also allow generation of the initial entry of the tracer into tissue, which is proportional to perfusion if BBB passage is normal. Thus, maps of both relative perfusion and
tissue hypoxia may be obtained from a single tracer administration, which would be of considerable interest.

Conclusion
It is hoped that eventually better ligands, together with a better understanding and full quantification of hypoxia imaging and its translation to widely available technologies, will allow its wider application in clinical research and potentially also in clinical routine. Beyond identification of the penumbra, nitroimidazole imaging may also prove useful in monitoring treatments directed at tackling hypoxia, such as normobaric oxygen therapy.

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