

Imaging of Apoptosis: The Need to Distinguish Tracer Uptake Rate from Regional Contribution of Blood Flow

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In oncology, PET is known to contribute substantially to localization of tumors and metastases and to control of therapy. One particularly attractive application is the assay of apoptosis induced by chemotherapy or radiation. Various tracers, such as ^{99m}Tc -annexin-V and ^{18}F -ML-10, are under discussion and in application. At Stanford University, the group of Jianghong Rao reported the unique concept of a radiotracer that reaches its final form within the apoptotic cells (*1*). This happens in a series of 3 reaction steps: the ^{18}F -labeled precursor, ^{18}F -C-SNAT, is selectively cleaved by caspase-3/7 between the D and X of the DEVD-X peptide sequence (where X = any amino acid and DEVD = Asp-Glu-Val-Asp) and the disulfide is reduced to a thiol by intracellular glutathione; the released amino and thiol groups react with the cyano group on the hydroxyquinoline at the other end of the molecule via an intramolecular reaction; and the resulting cyclized compound (*cyclic* ^{18}F -C-SNAT) is highly lipophilic and thus remains trapped within the apoptotic cell and apoptotic bodies.

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It is noteworthy that in organic chemistry, intramolecular reactions are known to be up to 10^4 times faster than the corresponding intermolecular ones. Rao's group reported a half-life of 119.8 s for that type of reaction. Therefore, the intermediates formed after cleavage are unlikely to react with other molecules within the cellular environment, and *cyclic* ^{18}F -C-SNAT can be assumed to be the only ^{18}F product.

Biologic evaluations validated, in a proof of concept, that ^{18}F -C-SNAT is a tracer for the assessment of apoptosis. A parallel study with a fluorescence scaffold, C-SNAF, demonstrated the same concept on a molecular level (*2*). Overall, the concept is well suited to tracing apoptotic cells resulting from chemotherapy.

In this issue of *The Journal of Nuclear Medicine*, Rao's group continues its studies of ^{18}F -C-SNAT in tumor-bearing mice with and without doxorubicin treatment and applies a pharmacokinetic analysis using a 2-tissue-compartment model (*3*). As the input function, blood activity was acquired from the images by placing

a region of interest over the heart, as can conveniently be done in small animals. The compartment modeling allowed the investigators to distinguish tracer accumulation in apoptotic tissue from blood activity within the same region of interest. K_1 and k_3 are the velocity constants for tracer delivery from blood and accumulation in tissue, respectively. In tumors after treatment with doxorubicin, k_3 changed from 0.03 ± 0.01 to $0.12 \pm 0.02 \text{ min}^{-1}$. The velocity constants increased by a factor of 4, whereas the direct calculations (based on injected dose of activity) predicted a factor of 1.4 (1.17 ± 0.17 vs. 0.81 ± 0.28 percentage injected dose [%ID]/g) (*1*). In muscle, k_3 is close to zero, as is expected because of the biochemical concept of ^{18}F -C-SNAT. If uptake is related to injected dose, $0.7 \pm 0.1 \text{ %ID/g}$ is found in muscle of untreated mice, and a corresponding $1.6 \pm 0.2 \text{ %ID/g}$ is found in tumor tissue. Thus, determination of the accumulation rate k_3 clearly estimates the treatment outcome.

Independently of tracer accumulation in tissue, the 2-tissue-compartment model allows for the determination of K_1 , which describes tracer delivery from blood. After treatment with doxorubicin, K_1 increased from 0.31 ± 0.05 to $1.03 \pm 0.14 \text{ g/mL} \times \text{min}$. In muscle, the delivery constant also increased, from 0.17 ± 0.03 to $1.03 \pm 0.1 \text{ g/mL} \times \text{min}$. In tumor and muscle tissue, increases in K_1 are thought to result from a systemic effect originating with doxorubicin, as has been described for ^{18}F -FDG (*4–6*).

In experimental tracer applications, data are usually calculated as %ID/g. Because such uptake values represent primarily blood activity, reference regions are applied so that blood activity can be subtracted. The investigators' work illustrates that tracer accumulation and blood activity are parameters independent of each other and may change independently. In their work, k_3 is zero in muscle, but a high K_1 would feign tracer accumulation if uptake were determined as %ID/g.

The pharmacokinetic modeling allows for discrimination between tracer accumulation in tissue and tracer activity in blood. The study presented in this issue of *JNM* (*3*) clearly shows ^{18}F -C-SNAT to be a tracer very well suited to clinical application. It is particularly noteworthy that the applied modeling can be used directly in clinical studies. For the radiosynthesis of ^{18}F -C-SNAT, Rao's group applied the tracer principle. ^{18}F -C-SNAT was prepared with high efficiency, labeling precursor was removed, and the amount of peptide could be kept low (*1*). Therefore, clinical translation can easily be realized by the microdosing concept ($<100 \mu\text{g}$), for which the necessary toxicology data can be determined quickly and inexpensively.

Over the last 10 years, pharmacokinetic analyses have somewhat fallen away from the main focus of the community, but the work of Rao's group impressively shows the usefulness of applying—and even more, the need to apply—2-tissue-compartment modeling

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to PET data. Today, PET systems are fast enough to perform dynamic registrations, and a subroutine can analyze data resulting in parametric imaging of regional k_3 values so that modeling can be used even in daily PET work. PET/MR imaging opens the technical possibility of having blood flow recorded simultaneously with tracer registration. In February 2015, the 4th Tübingen PET/MR Workshop revealed the need for simultaneous blood flow measurements, for which a technical implementation into clinical PET/MR imaging systems was discussed to soon be forthcoming.

In summary, the work of Rao's group brings ^{18}F -C-SNAT close to direct translation on the clinical level—a perspective that is truly promising. The tracer is well suited to PET assessment of apoptosis caused by chemotherapy. Even more, the work exhibits an importance far beyond the development of ^{18}F -C-SNAT, as the value of pharmacokinetic modeling lies in its ability to determine tracer accumulation in tissue independently of blood flow. This work clearly illustrates how the way has been paved for precisely performed PET follow-up studies of therapies and disease progression.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

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