Unexpected Keys in Cell Biochemistry Imaging: Some Lessons from Technetium-99m-Sestamibi

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Some radiopharmaceuticals originally developed as myocardial perfusion agents are emerging as powerful tools to explore the function of other tissues at the cellular level. Technetium-99m-sestamibi (hexakis-2-methoxy isobutyl isonitrile, MIBI) is the most widely evaluated representative of this class of new compounds (*1*), although evidence is raising the issue that other lipohilic cations share most of the pharmacokinetic patterns of MIBI, both at the whole organism and the cellular level, e.g., furofosmin [Q12] (*2*) and other ^{99m}Tc-complexes of the Q-series (*3,4*), tetrofosmin (*5–8*). Conversely, several lipophilic compounds with neutral charge (e.g., CDO-BOH, CDO-MEB, Tc-NCPI, Tc-*bis*(dithiocarbamato) nitrido) (*1,9,10*) exhibit patterns of intracellular accumulation and whole-body pharmacokinetics that are significantly different from the lipophilic cations. It is thus clear that the combined properties of modest lipophilicity and monocationic charge are essential requisites for the specific pharmacokinetic features, contrary to prior conventional ^{99m}Tc-labeled radiopharmaceuticals in which ^{99m}Tc merely serves as the radioactive tracer to image organs or tissues as a function of biodistribution properties peculiar to the nonradioactive portion of the compound. These new agents distribute as a function of the coordination chemistry of technetium per se. In these compounds, technetium is not simply chelated to some more or less complex molecules with their own patterns of in vivo distribution, but it constitutes instead the essential core around which a totally new molecule is spatially arranged.

The above consideration is particularly relevant when trying to elucidate the mechanism(s) of specific tissue accumulation of these new compounds. Classical procedures aimed at identifying specific mechanisms for active transport across biologic membranes are generally based on the use of inhibitors and/or promoters for the transport of naturally occurring substances (*11,12*) (e.g., rotenone, iodoacetate, ouabain, metabolic blockers of enzyme activities). The transport mechanisms are tentatively identified by analogy with known biochemistry of preexisting compounds. This reasoning is limiting in that we are trying to characterize totally new and/or artificial compounds as if they are naturally occurring substances with known biologic mechanisms. Because of the extensive literature on the transport properties of lipophilic cations across membrane bilayers, it is simplistic to conclude that, since no promoters and/or inhibitors for the intracellular accumulation of MIBI have been identified, this radiopharmaceutical freely diffuses across cell membranes by a passive phenomenon based on its electrical charge (cationic) and partition coefficient (lipophilicity) (*13,14*).

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Experimental in vitro models for the study of intracellular accumulation of MIBI and other similar compounds by tumor cells (as also by cardiac myocytes) consistently show that, although this process is relatively slow if compared to the in vivo situation (with a plateau usually attained within 40–60 min in in vitro models versus a few minutes in vivo), each cell type exhibits a specific uptake pattern. In fact, some cell lines behave as better MIBI accumulators than other cell lines (*15*), despite identical conditions of extracellular MIBI concentration. These findings are not consistent with the simple hypothesis that the diffusion of MIBI across cell membranes is a purely passive event based on an extracellular:intracellular concentration gradient. These varying uptake patterns may reflect differences in the intracellular density of storage organelles.

Paradoxically, while relatively little is known about the exact mechanism(s) of MIBI diffusion/transport from the extracellular to intracellular space, we now have many insights on the mechanism(s) of intracellular distribution of this compound and on its exit from the cells. These advances have been made possible by pharmacologic manipulation of cells and subcellular organelles (such as the mitochondria) with calcium ion channel modulators and other ionophores (e.g., valinomycin, tetraphenylborate, nigericin, etc.) (*16,17*).

It is thus now clear that, upon entering the intracellular space (whether by simply passive and/or by some form of facilitated diffusion), MIBI is stored (actively) in a subcellular compartment (the mitochondria) (*11,12,18,19*). Its relatively long retention at this site constitutes its very basis for imaging specific tissues, such as the myocardium, in a blood flow-dependent manner. Some important links are missing in the chain of pharmacokinetic events that define specific scintigraphic phenotypes of different tissues (for instance, thyroid and parathyroid), particularly as they concern the differential washout phase of MIBI from such tissues. In fact, the exit of this radiopharmaceutical from its accumulation sites is consistently described as being monoexponential (see, for instance, thyroid, parathyroid, tumor). This definition leaves some critical issues unanswered:

1. How can the washout of MIBI be monoexponential if at least two compartments (the mitochondria and the cytoplasmic space) can be identified at the level of each cell?
2. How does MIBI exit from the mitochondrial to the cytoplasmic space?
3. How does MIBI normally exit through the cell membrane of various tissues?
4. Why do different tissues (e.g., the thyroid and parathyroid) exhibit different monoexponential washout rates of MIBI after reaching similar peak levels at about the same time? Does this difference merely reflect different intracellular densities in storing organelles?

5. Is the rate-limiting step the exit of MIBI from the mitochondria to the cytoplasmic space or the exit from the cytoplasmic space to the extracellular space?

Available experimental evidence based on in vitro cell culture studies do not help us understand the issue of different washout rates of MIBI from different cells/tissues better. In fact, studies to date have focused mostly on the accumulation phase, showing that tumor cells reach intracellular plateau levels that are maintained far longer (e.g., over experimental periods of 40–60 min) (*11,13,15–18*) than the scintigraphic observations in vivo would suggest. This is perhaps because in vitro experiments are usually conducted with excess MIBI concentrations in the incubation culture medium, while the washout phase would be better characterized in conditions mimicking the parameters actually taking place in vivo; that is, with continuing removal of MIBI from the incubation medium similarly as done, for instance, by Chiu et al. (*16*).

Lipophilicity, one of the molecular bases for easy and fast transport of MIBI across the cell membranes (and a base for its favorable tumor imaging properties), has so far resulted in some important limitations in the clinical applications of MIBI for tumor imaging. With the same excretion mechanisms as other lipophilic compounds, MIBI undergoes hepatobiliary clearance in addition to some degree of renal excretion. This combined process, taking place within a few minutes after intravenous injection, results in a very high radioactivity accumulation in the abdominal area, a confounding factor that has prevented useful application for tumor imaging in the abdomen. Recent reports, however, indicate that at least in some selected conditions, there may be useful time-windows for tumor imaging with MIBI in the abdominal area. As shown by Cesani et al. (*20*), the first useful time-window may be very early (within 1–2 min postinjection), before hepatobiliary clearance becomes so significant as to obscure the abdominal organs. With this approach, these authors were successful in imaging a VIPoma located in the pancreas. This observation opens the way for possible useful application of fast SPECT (for instance with a triple-head gamma camera) for tumor imaging with MIBI in the abdominal area. Conversely, for tumor lesions located in the liver, one could simply wait until completion of the hepatobiliary clearance has resulted in removal of most of the radioactivity in the blood pool and in the liver itself, thus achieving favorable target-to-nontarget ratios based on the much longer retention time of MIBI in the tumor than in the hepatocytes. Al-Sebaie and Rush (*21*) have described scintigraphic visualization of metastatic liver lesions incidentally discovered in the course of a myocardial perfusion scan performed 1 hr after tracer injection.

As with other scintigraphic agents whose tumoral accumulation is linked to increased metabolic activity (chiefly [^{18}F]FDG but also, to some extent, ^{67}Ga and ^{201}Tl), the potential of MIBI as a prognostic indicator of tumor response to treatment (chemotherapy or radiotherapy) has been explored. Results, however, have been quite disappointing, in that reduced MIBI accumulation in tumor lesions was not found to be systematically associated with a favorable response to treatment (*22*). This apparent limitation in the possible clinical uses of MIBI scintigraphy in oncology is perhaps linked to another peculiarity of MIBI that might explain some heterogeneity in its pattern of intracellular accumulation (or, better, in its intracellular residence time) in tumors: the interaction of MIBI with the multidrug resistance phenomenon.

Despite continuing progress in modern chemotherapy for cancer, with few exceptions, remission induced by such drugs is only temporary. The same chemotherapy effective in attaining remission is almost invariably ineffective at the time of tumor relapse. Malignant cells resistant to a single chemotherapeutic agent (either de novo, that is at the time of initial observation, or after exposure to the cytotoxic agent) are also resistant to other, structurally unrelated cytotoxic compounds. This variant of multidrug resistance is mediated by the expression on the cell membrane of a glycoprotein with a molecular weight of about 170 KDaltons (the so-called permeability glycoprotein, or P-gp), the phenotypic product of the MDR1 gene, which functions as an active, ATP-dependent mechanism pumping cytotoxic agent out of the cells (*23–26*). Of considerable interest to nuclear medicine physicians, is the fact that MIBI is actively extruded out of the tumor cells by the P-gp pump, in a manner similar to the cytotoxic agents involved in multidrug resistance.

Knowledge is rapidly evolving in this field. After the seminal observations by Piwnica-Worms et al. (*27*), there is potential for new understanding of the mechanisms involved in multiple drug resistance.

In vitro studies have demonstrated that the active extrusion of MIBI from the inner space of tumor cells is directly correlated with the degree of expression of the MDR-1 gene. This process can be inhibited by known inhibitors of the P-gp activity, such as verapamil and cyclosporin (*27*), but efficient inhibition is achieved at suprapharmacologic levels. These observations confirm the need to develop more potent, less toxic compounds capable of inhibiting the P-gp pump mechanism (*28–31*). Preliminary in vivo observations also show that the degree of scintigraphic visualization with MIBI of human tumors implanted in the experimental animal model is inversely correlated with the degree of P-gp expression (*27*). These two sets of in vitro and in vivo experimental observations suggest the possibility of developing a quantitative test, based on MIBI scintigraphy in baseline conditions and after challenge with a P-gp inhibitor, as a prognostic indicator of the level of P-gp expression in the tumor lesions of the individual patient, as well as possible pharmacologic manipulation to make that particular patient responsive to cytotoxic chemotherapy.

These concepts linking the degree of MIBI accumulation to the level of P-gp expression in tumors are gradually being translated into clinical practice (*32*). In particular, Moretti et al. (*33*) reported on one patient in whom there was nonvisualization with MIBI of a small-cell lung cancer which was well visualized with ^{111}In-pentetreotide and by CT, which was linked to high expression of P-gp, as suggested by the total lack of response to chemotherapy (*33*). Similar conclusions, based on a comparative evaluation with [^{18}F]FDG PET and MIBI and by clinical follow-up in a group of 14 patients with Hodgkin's lymphoma, were reached by Dimitrakopoulou-Strauss et al. (*34*), i.e., that tumor scintigraphy with MIBI might represent a useful tool for the prognostic value of chemotherapy in cancer patients. In both studies, the degree of P-gp expression was estimated only indirectly on the basis of poor clinical response to chemotherapy.

Recent preliminary data, however, reported by Ciarmiello et al. (*35*) link the washout rate of MIBI from breast cancer lesions to the degree of P-gp expression, which is directly evaluated (using an anti-P-gp monoclonal antibody labeled with ^{125}I) in surgical tissue specimens. This observation opens the way to further studies.

CONCLUSION

Scintigraphic in vivo evaluation of complex cell functions is being made possible by new radiopharmaceuticals based on the peculiar coordination chemistry of ^{99m}Tc. Although the exact

mechanisms of tissue accumulation and washout of these compounds are not yet fully understood, evidence is emerging that their interaction with subcellular organelles and complex macromolecules might provide a new basis for advancing knowledge in important aspects of tumor biology that bear some relevance to the therapy of cancer patients.

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MIRDOSE: Personal Computer Software for Internal Dose Assessment in Nuclear Medicine

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The calculation of internal dose estimates is performed by summing the radiation absorbed in various target tissues from a number of source organs in the body that contain significant quantities of radioactive material. In nuclear medicine, the most commonly used method for the calculation of internal dose estimates is that developed by the Medical Internal Radiation Dose (MIRD) committee, as described in various documents, but most recently summarized in the MIRD Primer (*1*). In this article, the expression given for the absorbed dose is:

$$D_k = A_0 \sum_j \tau_j \sum_i \frac{\Delta_i \phi(r_k \leftarrow r_j)_i}{m_k}, \qquad \text{Eq. 1}$$

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