Auger Electrons: Lethal, Low Energy, and Coming Soon to a Tumor Cell Nucleus Near You

Radiopharmaceuticals labeled with low-energy electron emitters have several key advantages over traditional agents that emit higher-energy particles. Sadly, the majority of such agents described in the literature to date have harnessed only a small percentage of the actual cytotoxic potential of Auger-emitting radionuclides because of poor design. Rather than specifically trafficking to the tumor cell nucleus, a requirement for optimal cytotoxicity, the vast majority of Auger emitter–labeled peptides reported thus far have achieved nuclear localization only after receptor-mediated endocytosis, lysosomal degradation, and "residualization" of metal radionuclides into the cell nucleus. Given the current status

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of molecular biology, this strategy seems rather crude, akin to tossing a message in a bottle into the sea and hoping it finds its way to the intended recipient. The article by Ginj et al. in this issue of *The Journal of Nuclear Medicine* (*1*) introduces a refreshing alternative and advance over traditional Auger-emitting peptide conjugates in the form of a new class of trifunctional somatostatin analogs that are not only transported into the desired tumor cell but are also further transported into the nucleus where Auger electrons are most lethal.

Auger electrons are extremely low-energy atomic orbital electrons that are emitted as an alternative to x-ray emission after electron capture, a form of β^--decay (*2,3*). Auger electron therapy is a useful strategy for specific tumor cell killing with a low level of damage to surrounding cells, originating from subcellular (nanometer) ranges and highly localized energy deposition (10^6–10^9 cGy) in an extremely small volume (several cubic nanometers) around the decay site (*4*). Auger electron emitters produce an array of reactive radicals (e.g., OH·, H·, e^-_{aq}, etc.) similar to α-emitters, which are regarded as the classical form of high linear-energy-transfer (high-LET) radiation. The estimated absorbed dose rate at the center of a cell delivered by ^{99m}Tc, ^{123}I, ^{111}In, ^{67}Ga, and ^{201}Tl is, respectively, 94, 21, 18, 74, and 76 times higher if the radioactivity is localized within the nucleus versus being on the cell membrane (*5*).

Several therapeutic agents containing Auger-emitting isotopes have been studied, including the somatostatin analog, octreotide, labeled with ^{111}In via the chelator diethylenetriaminepentaacetic acid (DTPA) (Octreoscan [^{111}In-pentetreotide; Mallinkrodt]). Binding of this peptide to specific cell-surface receptors prompts internalization of the ligand–receptor complex via invagination of the plasma membrane, a process known as receptor-mediated endocytosis. The resulting endosomes rapidly acidify, causing dissociation of the ligand from the receptor, and [^{111}In-DTPA]octreotide is metabolized therein to ^{111}In-DTPA-D-Phe (*6*). Assuming no metal complex dissociation, this radiolabeled metabolite should be retained inside the lysosomes. However, partial translocation of ^{111}In to the perinuclear area and into the nucleus after internalization of [^{111}In-DTPA-D-Phe]octreotide has been reported (*7*), suggesting migration of ^{111}In to the cell nucleus via an unknown biochemical mechanism. In vitro studies using [^{111}In-DTPA]octreotide have revealed that the therapeutic effect of ^{111}In is indeed dependent on internalization (*8*) but did not account for possible trafficking of the radiometal to the nucleus.

There have been similar reports of other radiometals "leaking" from peptide conjugates and subsequently migrating to the nucleus. In an attempt to rationalize higher observed therapeutic effects for somatostatin analogs labeled with the Auger-emitting isotope ^{64}Cu relative to the corresponding ^{111}In-labeled peptides, Wang et al. found a 3-fold higher amount of ^{64}Cu relative to ^{111}In localized to the nucleus of tumor cells after incubation of [^{64}Cu-TETA]octreotide (TETA is 1,4,8,11-tetraazacyclotetradecane-*N,N′,N″,N‴*-tetraacetic acid) and [^{111}In-DTPA]octreotide, respectively (*9*). This difference in nuclear uptake may be attributed to higher in vivo stability of the ^{111}In-DTPA complex relative to ^{64}Cu-TETA.

In addition to the difficulties in assessing potential levels of intracellular radiometal transchelation and trafficking to the nucleus, there exists a second source of complexity surrounding the biologic effects of Auger electrons. The "bystander effect" is a phenomenon wherein radiobiologically damaged cells induce cell death in nonirradiated cells through the release of cytokines and free radicals (*10*). For example, Reilly and coworkers demonstrated strong antitumor effects against breast cancer xenografts using ^{111}In-DTPA-hEGF (hEGF is human epidermal growth factor), an agent previously shown to rapidly internalize into EGF receptor–positive cells (*11,12*). Radiotherapeutic effects exceeded microdosimetry estimates and this inconsistency was partially attributed to the bystander effect. However, in vitro experiments revealed that ^{111}In radioactiv-

Received Sep. 2, 2005; revision accepted Sep. 16, 2005.

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ity localized into the cell nucleus, suggesting a trafficking mechanism. As a result, it is challenging to deconvolute the possible influences of both the trafficking and bystander phenomena versus the observed radiotherapeutic effects.

Targeted radiotherapy of somatostatin-positive tumors has also been widely investigated using peptides labeled with β^--emitting radionuclides such as ^{90}Y and ^{177}Lu. When coupled to octreotide, ^{177}Lu shows great promise for therapy of small, metastatic tumors with less renal toxicity as compared with ^{90}Y-labeled somatostatin analogs (*13–15*). The longer range of β^--particles from ^{90}Y, typically 1–10 mm, results in irradiation of neighboring receptor-negative tumor cells as well as potentially normal cells by "crossfire"—a term used to describe the deposition of energy from a radionuclide in a particular tissue into a distant target. For this reason, lower-energy β^--emitters such as ^{177}Lu and very low-energy Auger electron–emitting radiopharmaceuticals offer distinct advantages for treating much smaller lesions and micrometastases because of their much shorter ranges in tissue. A successful ^{177}Lu/^{90}Y combination radionuclide therapy has already been reported (*16*); therefore, adding a nuclear-targeted peptide labeled with an Auger emitter could even further enhance these results. One may envision that the addition of ^{111}In to the combination of ^{177}Lu- and ^{90}Y-labeled octreotide could conceivably reach an even broader spectrum of tumor burden, from relatively large tumors to the smallest micrometastases.

In a revealing study, Behr et al. directly compared the therapeutic effects of an internalizing monoclonal antibody labeled with ^{125}I, ^{131}I, ^{111}In, or ^{90}Y (*17*). Both Auger emitters (^{125}I and ^{111}In) showed better therapeutic results than the β^--emitters. In addition, a trend toward better therapeutic results with the radiometals compared with radioiodine was demonstrated. The latter finding can be rationalized by the fact that radiometals attached to antibodies are residualized intracellularly. In contrast, radioiodinated antibodies undergo lysosomal degradation to mono- or diiodotyrosine that is rapidly released from cells.

In this issue, Ginj et al. introduce a more robust approach: specifically targeting the Auger-emitting isotope, ^{111}In, into the nuclei of tumor cells in a controlled manner to ensure rapid nuclear localization of high levels of intact ^{111}In-labeled peptide (*1*). To our knowledge, this is the first reported targeted peptide radiopharmaceutical rationally designed to deliver therapeutic Auger electrons to tumor cell nuclei. The demonstrated ability of this agent to achieve selective cytotoxicity for micrometastatic tumor burden in an animal model is anxiously awaited.

However, one might predict even further dramatic improvements through combining this agent with a low-energy β^--emitter such as ^{177}Lu. Assuming no saturation of entry through nuclear pores, both ^{177}Lu- and ^{111}In-labeled octreotide conjugates could be targeted to the nucleus to reap the therapeutic benefits of increased tumor cell retention of both isotopes. Both local and bystander effects of the Auger emitter ^{111}In would act in synergy with the extended range of ^{177}Lu. For larger tumor burdens, one might even choose to include ^{90}Y in this cocktail because of the longer range of this higher-energy β^--emitter.

In an Invited Commentary in a 2003 issue of *The Journal of Nuclear Medicine*, Kassis posed the question "Cancer Therapy with Auger Electrons: Are We Almost There?" (*18*). It seems that we have yet to reach "there" as the therapeutic success of this new class of trifunctional molecules in tumor-bearing animal models remains to be established. Likewise, it is unclear how general the nuclear localization signal and aminohexanoic linker strategies will prove to be when applied to other targeting vectors. Nevertheless, the development of a somatostatin derivative that is localized to and retained within the interior of tumor cell nuclei is certainly a welcome and long overdue step in the right direction.

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