Molecular imaging with endogenous and exogenous ligands: The instance of antibodies, peptides, iodide and cupric ions

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1. Introduction

The purpose of this short review is to comment on the evolution of the meaning of molecular imaging (MI) since almost twenty years this concept has reached the role of a new paradigm in medicine and, as discussed here, in diagnostic nuclear imaging. MI is a fascinating approach that became widely accepted after the genomic revolution [1], [2], [3], [4], [5]. The discovery that cells are essentially chemical fabrics where molecules are the primary tools for storing, transferring and processing biological information necessary to sustain the cellular life cycle fueled the belief that by tracing the pathways of these molecules it could be possible to reconstruct the in vivo complex biochemical machinery of the cell. In this perspective, MI was perceived as a unique approach to fill the gap between in vitro and in vivo biochemistry as it is largely recognized that data obtained on isolated tissues always lack to disclose clues on interactions with the surrounding cellular network typically arising within a whole organism.

Albert Einstein wrote that fundamental theories could be recognized also because they usually possess some intrinsic beauty and elegance [6]. The idea at the core of MI that everything about life could be unveiled by simply monitoring in vivo biomolecular processes is astonishingly elegant and beautiful. Unfortunately, as emphasized by Eugene Wigner [7], nature does not always prefer elegance and frequently looks for complicate and cumbersome solutions (or at least, these solutions do appear to our mental categories to lack logical simplicity). Indeed, to a careful scrutiny, this intricacy seems to inherently plague also the MI paradigm, thus challenging its foundations and

much sought expectations including the highly attractive prospect of personalized medicine.

In the following, an attempt to discuss some of the basic concepts of MI from a different angle is reported. This discussion will be almost completely restricted to diagnostic nuclear MI (NMI) as this discipline still represents one of the most consistent and exhaustive examples of the use of molecular probes for investigating in vivo biological processes.

2. The molecular imaging paradigm: Which resolution?

Ideally, the most ambitious and radical application of MI would be to collect spatial and dynamic images (four-dimensional imaging) showing the behavior of endogenous biomolecules with resolutions closer to that of the atomic scale and of a single molecular event. This is exactly the strategy inspiring the imaging approach based on the use of fluorescent proteins. By genetic engineering, a fluorescent protein is linked to the protein of interest, which is then visualized by following the light emitted by the ancillary fluorescent tag [8], [9], [10], [11]. Passing over the obvious question on whether the combined protein is still identical to the original untagged protein, unfortunately this kind of optical imaging still lacks the spatial resolution typical of crystallographic diffraction methods, though its high sensitivity allows detecting extremely low concentrations (pM) of the fluorescent probe. Concomitantly, the time required to collect images is far longer that associated with a single molecular event. More precisely, the spatial resolution of the atomic scale, as detected by the most sensitive methods (e.g., X-ray crystallography and neutron diffraction [12], [13]), is of the order of 10^{2} pm ($1\ \text{pm}=10^{-12}$ m). Conversely, the spatial resolution of the most powerful optical method is approximately 10^{4}–10^{5} pm, thus 2–3 orders of magnitude lower. Similarly, a single molecular event, like the rotation of an oxygen molecule at room temperature, usually occurs within a timeframe of 10^{-9}–10^{-11} s, which is a time interval dramatically shorter than that required for collecting an image using super-resolution microscopy, a procedure usually lasting for seconds. Given that, it seems quite apparent that spatial and temporal resolutions of the most sophisticated imaging methods, having at least a potential chance to be applied to a single living cell, do not compare with the resolution typical of the atomic and molecular scale [14], [15], [16], [17], [18], [19], [20], [21]. Therefore, it should be accepted that MI is currently unable to visualize individual molecules and their dynamical changes in a living system, thus forcing to restrict the ideal MI paradigm mentioned above to a less ambitious objective.

3. The molecular imaging paradigm: Is it truly endogenous?

Essentially, MI can only collect signals emitted from a population of identical molecules all bearing the same tag without any hint of the molecular details. As mentioned above, the most obvious choice of the type of molecules to be tagged is the category of endogenous substances involved in some specific biological process. In the NMI version of this strategy, a number of relevant endogenous biomolecules are radiolabeled with some γ-emitting radionuclide and then introduced into a living organism to follow their journey by detecting radioactivity. Therefore, this full biomolecular application of the tracer principle aims at monitoring the in vivo biodistribution of the radiolabeled endogenous biomolecules, thus shedding light onto the inner biological process where they play the role of key ingredients.

It could be a matter of an infinite debate to precisely define which (and when) a compound can be considered endogenous or exogenous. For the purpose of this commentary, endogenous is any substance that is assembled by the cellular fabric from basic chemical building blocks. Usually, these elementary building blocks are supplied to the organism from the outside as nutrients and further processed through some metabolic pathway. Therefore, they should be considered as exogenous substances. This is a sufficiently simple definition that may help to narrow down the analysis. A few examples can be mentioned here along with some borderline situations. No discussion is required to recognize that proteins are endogenous. Similarly, there is no doubt that glucose is exogenous. Pyruvic acid can be made from glucose through glycolysis. Therefore, it is endogenous. However, pyruvate can be also metabolically converted to ethanol and to the amino acid alanine. Are ethanol and alanine endogenous or exogenous? For those drinking a glass of whiskey or eating a beef both are surely exogenous. As emphasized in the introductory note, nature is not always elegant and simple, and the distinction between endogenous and exogenous substances is just another example. However, a key point worthy to be considered here is that, presumably, there exist no true endogenous substances when these are isolated, or synthetically compounded, and then administered to a living organism. Living tissues produce endogenous substances in response to an internal stimulus and assemble them close to the target to avoid having to cross through hard biological barriers. Thus, it is unlikely for an exogenously administered compound to preserve its whole native biological properties and ubiquitously follow exactly the same natural biological pathway as it occurs for the true endogenous counterpart.

A popular example is offered by radiolabeled antibodies and peptides [22], [23], [24], [25], [26], [27], [28], [29], [30], which are commonly considered as endogenous molecules. The receptor-like mechanism responsible for the accumulation of these molecules onto the target is particularly simple and, when there exists a sufficient number of cellular receptors, this would make these endogenous molecules a perfect choice as imaging probes. However, the biological fate of endogenous proteins and peptides is far different from that of an identical compound prepared outside the organism and administered intravenously. Synthetic equivalents should be heavily manipulated to allow them reaching the target, as nicely demonstrated by the development of minibodies, affibodies, nanobodies [24], [25], [26] and somatostatin-like peptides [28], [30]. Again a comparison with optical imaging with fluorescent proteins may help to further clarify this point. Current gene transfer technology allows a fluorescent protein being incorporated into a host protein by genetic fusion. The resulting chimeric fluorescent protein can be expressed within cells in its native environment and, as a result, it can be surely viewed as an endogenous probe simply because it is assembled by the same cellular machinery.

This observation also holds for simpler molecules as exemplified by L-DOPA. The amino acid L-tyrosine is converted to L-DOPA by the action of the enzyme tyrosine hydroxylase, particularly inside neurons in the central nervous and peripheral sympathetic systems. Along this pathway, L-tyrosine (or L-alanine) is the exogenous precursor. Consequently, the whole process depends on the existence of efficient mechanisms able to transport this amino acid to the target neurons. However, L-DOPA can be also isolated from some plants or chemically synthesized and, thus, administered as a drug. Obviously, this dramatically changes the biological scenario since it will be the exogenous L-DOPA that has to be transported and pass through a longer journey to reach target cells. It follows that its metabolic pathway will be significantly altered from that of the truly endogenous L-DOPA [31], [32].

In summary, although these exogenously produced mimics bear close or integral chemical resemblance with their native biological counterparts, in reality, they are different molecules. They should be necessarily considered as exogenous substances modeled on the bioactive site of their endogenous analogues. Thereby, it appears that, when confronted with the puzzling complexity of biological systems, the NMI paradigm requires some further adjustment. This leads to the obvious formulation that NMI is not intended to image biological processes, but more realistically, it investigates the interaction of a biological substrate using exogenous single-molecule radiolabeled probes. This implies that, despite NMI is unable to visualize single biomolecular events,

it is intrinsically molecular simply because it makes use of molecular-sized objects to accomplish its task.

4. Which type of exogenous probes?

At least in principle, any type of exogenous molecule could be effectively used as imaging probes according to the revised formulation of the NMI paradigm. As brightly demonstrated for receptor interactions, a variety of exogenous compounds can efficaciously bind to receptors with the same or greater affinity of endogenous substances. Actually, many new receptors have been found by investigating the pharmacological effect of exogenous ligands. It might be speculated that the machinery of receptors has been evolved as an adaptive response of organisms to the invasion of exogenous molecules as specific receptors are commonly discovered for almost all alien substances. The key question is always whether the selected interaction with the target site is capable of accumulating a sufficient number of probes to generate a detectable signal. This prompts to distinguish between high-capacity sites and low-capacity easily saturated sites [4], [33].

Considering that proteins are the ubiquitous regulators of cellular cycles, targeting proteins that are at the origin of a specific pathological condition with a suitable probe is a widely accepted strategy toward the goal of obtaining a more fundamental understanding of the disease [34], [35]. However, the biological scenario is again extremely complex as many categories of proteins and enzymes have a low capacity and it may happen that some proteins could not be efficiently targetable [36], [37], [38]. Hence, the usual challenge is to target a key protein with enough binding capacity. Though a low-capacity site may not constitute a formidable problem for diagnostic imaging, it represents a tremendous limitation for radionuclide therapy of cancer [38], [39]. Evidently, high-capacity sites do appear to provide a more adequate mechanism for delivering a very high-dose payload to the tumor. Fortunately, the class of transport proteins relies on an alternative type of substrate-target interaction that frequently allows transferring a high number of imaging probes inside the cell. This class includes transporters that are responsible of ferrying nutrients and ions trough the cellular membrane [40]. An illustrious example is represented by the passage of iodide ions through the sodium-iodide transporter pump. Radioactive iodine is employed in the treatment of thyroid cancer and it was measured that approximately 35% of the initial activity is retained into a thyroid cancer upon injection of ^{131}I in the form of aqueous iodide [40], [41]. This still remains an unparalleled application of radionuclide therapy.

Recently, another relevant example has emerged. In fact, some preliminary description of the selective uptake of [^{64}Cu]Cu^{2+} ions in an animal model of prostate tumor was published a few years ago [42]. After this observation, an impetuous flow of evidence on the accumulation of copper ions by a variety of tumors has been reported both in animal and human studies [43], [44], [45], [46], [47], [48], [49]. In Fig. 1 a collection of PET/CT images from patients with prostate and brain tumors administered with [^{64}Cu]$CuCl_2$ is reported [50], [51], [52]. Interestingly, a number of studies have been recently published showing the key role played by copper(II) ions in the genesis and growth of tumors [45], [46]. These results are surely outstanding and very promising, particularly because they provide evidence of the potential utility of a simple injection of radioactive Cu^{2+} ions for the treatment of different tumors. In the context of the present discussion, the selective uptake of radioactive copper by a variety of cancer cells strongly supports the view that transporters of exogenous substances may constitute the most suitable avenue for delivering high doses of radioactivity to the target.

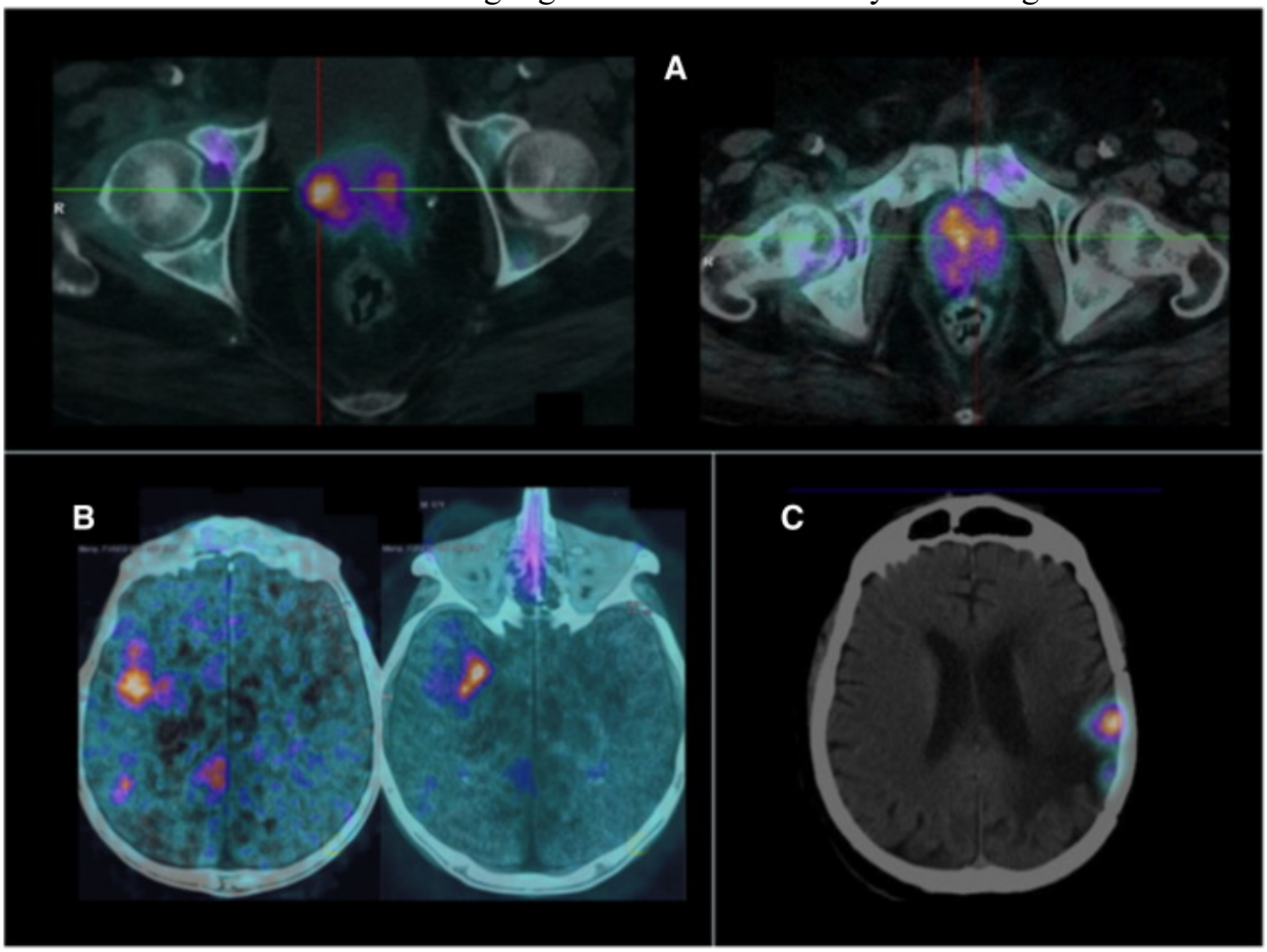

Fig. 1. Hybrid PET/CT images collected after i.v. injection of [^{64}Cu]$CuCl_2$ in a patient with (A) prostate cancer, (B) cerebral tumor and (C) glioma (courtesy by P. Panichelli, Advanced

Center Oncology Macerata, Italy). Selective uptake of [^{64}Cu]Cu^{2+} ions is observed in all cancerous lesions.

5. Conclusions

A major limitation for a genuine molecular application of the MI paradigm still originates from the complexity and incompleteness of the biological description of the cellular chemical fabric conjoined with the lower spatial resolution of imaging methods as compared to the atomic scale. In this respect, a more rigorous analysis of the fundamental concepts of MI based on available experimental evidence could be beneficial to ensure future progress of the field and avoid its misuse. Although several proposed approaches to MI may look esthetically attractive, sometime they lack a solid experimental foundation because of the tremendous intricacy of biological systems. Consequently, a constant progress of the NMI field still relies on pursuing studies at the fundamental level including the chemistry of radioisotopes, the search of more sensitive imaging technologies and the production of novel radionuclides that might reveal unexpected biological properties. In this context, it is a remarkable finding, the observation of a selective uptake of bare radionuclides by tumors when they are administered under a simple ionic form and without any particular surrounding chemical decoration as recently reported for simple copper-64 chloride salt. Since copper has well-established biological functions, these results may point to the need of investigating the production of other radioisotopes of elements having a biological role such as zinc [53], manganese and iron. In conclusion, although nature does not always come across with elegance, certainly simple solutions look always elegant.

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