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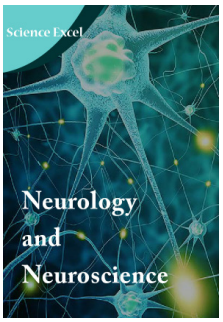

Targeted Radioprobe Delivery in Neurology

Denis Larrivee

Mind and Brain Group, University of Navarra Medical School, Spain

*Correspondence
Denis Larrivee
Mind and Brain Group, University of Navarra Medical School, Spain

Abstract

Nuclear medicine has established an irreplaceable role in the biomedical research and management of brain diseases. Its unique capacity to visualize molecular events in vivo as they unfold provides diagnostic clarity into functional performance and the option for therapeutic intervention. Limitations on access to brain tissue, however, have traditionally restricted nuclear medicine procedures to use of low molecular weight radioligands confined to the extracellular space of the brain, e.g., neurotransmitter receptors, which has precluded the interrogation of many cellular processes likely to be causal in disease etiology. Developments in radioprobe features and targeted delivery over the past two decades are overcoming these limitations, expanding the domain within which dysfunctional processes can be examined. These developments afford the prospect not only of a systemic view of brain diseases but also one likely to be more proximal to the unfolding dysfunctional events.

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Introduction

In recent decades, nuclear medicine has evolved from a modality for imaging molecular function using a handful of radionuclides into a modern specialty with the potential for assessing molecular systems. Innovation in equipment and radiopharmaceuticals has yielded a wide array of advances significantly increasing the number and range of diagnostic and therapeutic applications and propelling clinical management [1-4]. As of 2021, for example, the World Nuclear Association documented more than 40 million procedures conducted each year worldwide, with a frequency in developing countries roughly 1/10 that of developed nations [5].

Driving its growing clinical use is the ability of nuclear medicine to offer an unparalleled, non-invasive view of unlimited depth into intracellular processes. Molecular imaging techniques, such as positron emission tomography (PET) and single photon emission computed tomography (SPECT), provide for the spatiotemporal monitoring of biomarkers associated with key cellular processes in disease and tissue dysfunctions. Direct combination of medium and long range positron or gamma-emitting radionuclides with molecular markers enable the assessment not only of the distribution and concentration of biomarker molecules, but also the integrity of the function associated with a given biomarker. By combining such radionuclides with short range radioisotopes (alpha- or beta-particle emission), moreover, imaging radioligands can be transformed into a therapeutic tool, a now widely used approach termed theranostics [3,6]

While employed most frequently in oncology, nuclear medicine is also playing a growing role in neurological diagnosis and therapy. Neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Lewy body dementia, for example, are associated with the accumulation of brain proteins, leading to neuroinflammation, disruption of cellular clearance mechanisms, and neuronal death. Research advances are clarifying the underlying molecular events of these pathophysiologies and recent advances in nuclear medicine have enhanced this understanding through the direct monitoring of such proteins. Conversely, the advancement in understanding of the underlying pathophysiology has contributed to developments leading to improved diagnostic and therapeutic precision in the use of nuclear medicine [3].

A major challenge with the use of nuclear medicine in neurology is the ability to target radioligands to specific brain regions. Traditionally, small molecules have been the dominant group of drugs, providing access to what has been colloquially referred to as the 'druggable space' [7]. This term has generally been understood to include those ligands that can cross the blood brain barrier where they enter the extracellular space of the brain, typically in the vicinity of neurotransmitter receptor sites. Research into neurological dysfunction, however, is revealing the involvement of numerous processes within neurons and non-neuronal cells that are not immediately accessible to these domains. Hence, monitoring these processes by expanding the 'druggable space' is increasingly vital for providing diagnostic

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clarity.

Responding to this need new innovative modalities have emerged in the first two decades of the 21st century. These modalities are both expanding the accessible domain to radio-probes and enabling the targeting of select regions with greater precision [5]. Various formats of delivery vehicles, immunoconjugates, modified RNA molecules such as antisense oligonucleotides, reporter genes, and cellular tracking schemes are gaining traction due to their potential to enter previously 'undruggable' domains [7].

Targeted radio probe and pharmaceutical delivery with imaging has thus become a pillar to obtaining critical in vivo data on the functional well-being of specific molecular processes altered by neurological diseases and a key for therapeutic intervention [5]. In principle, such precision contains the latent possibility for monitoring and treating multiple processes affected by disease and hence of attaining to a systemic and personalized level of care.

Targeting and Delivery

Targeting methods can vary widely to accommodate a range of objectives and can include procedures for overcoming physical and chemical barriers, identifying and tracking select cells, accessing and monitoring intracellular processes, and modulating genetic expression, among others.

Crossing the blood brain barrier

A fundamental limit on the use of radioligands in the brain is the blood brain barrier (BBB) [3,7]. Accordingly, radioligands must be designed to incorporate features enabling the ligand to overcome this barrier. For many such compounds this can be achieved by using radioligands of small molecular weight, generally not exceeding 500 daltons.

Because many of the new radiopharmaceuticals are larger than this, however, novel procedures are required for the radioligand to bypass the BBB. Among these methods, nanoparticles (NPs) have proven particularly effective as platforms that can carry larger molecular weight radioligands across the BBB [8]. For example, organically modified amino- functionalized silica NPs have been used to carry the gene encoding GDNF to striatal cells [8].

Because nanoparticles afford the unique advantage of synthetic modification, their structural and chemical properties are amenable to wide variation to optimize both transport and radioligand load. Currently, there exists a large number of different NP platforms marked by different chemistries and structures. Many have been employed for therapeutic purposes in diseases outside the nervous system - such as pancreatic cancer, diabetic nephropathy, and myocardial infarction - with a growing number in development for use in the brain [8,9].

Besides nanoparticles, viral vectors are also used to deliver radioligands to the brain. Viral vectors include a spectrum of vehicles that have been derived from multiple viral classes, including retrovirus, lentivirus, adenovirus, herpes simplex virus type 1(HSV-1) and AAV vectors, with recombinant adeno-associated virus (rAAV) vectors generally finding more frequent use [10,11]. Recombinant AAV vectors feature numerous advantages. They are nonreplicable, non-pathogenic, and do not integrate into the host genome [14]. Pre-clinical in vivo AAV gene therapy studies, for example, have been carried out for Huntington's Disease, which have utilized both shRNA and miRNA approaches [11].

Cell and molecule specific identification and tracking

Radioimmuno-diagnostic or radioimmuno-therapeutic tracers afford the opportunity to pinpoint molecular and cellular species and are typically constructed from monoclonal antibodies and their derivatives. When combined with radionuclides, it is possible to obtain critical information on antigen quantity, heterogeneity, and kinetics in real time. Among the radionuclides currently available are included actinium-225 (225Ac), astatine-211 (211At), bismuth-213 (213Bi), indium-111 (111In), iodine-123 (123I), iodine-124 (124I), iodine-131 (131I), lead- 212 (212Pb), lutetium-177 (177Lu), technetium-99m (99mTc), copper-64 (64Cu), gallium-68 (68Ga), yttrium-86 (86Y), yttrium-90 (90Y), and zirconium-89 (89Zr). The choice of radionuclide is based on several properties dictated by need and objective, which can include emitter type, energetics, and half-life, as well as tissue characteristics. Efficient radiolabeling is often carried out by binding the radionuclide to a bifunctional chelator, which possesses a binding site for the radionuclide and a linker that can attach a nucleophilic group to a carrier antibody [12-14].

Targeting extracellular proteins

Extracellular proteins can, in cases, precipitate disease symptoms. For example, much evidence reveals the involvement of mutant huntingtin protein (mHTT) in extracellular events that influence the severity, symptomatology, and propagation of Huntington's Disease across cell systems. Among the various mechanisms proposed for influencing these features are effects on brain related immunogenicity, a seeding pathology that amplifies mHTT mobilization, and prion like activity [7]. The extracellular targeting of these aspects with radioimmuno conjugates has the advantage of requiring simpler protocols than those needed for cell entry (for example, directly exposing the immune system to the mutant form of HTT), which makes it a desirable protocol objective.

Extracellular proteins can be targeted by passive or active immunization. In active immunization the immune system is exposed to an exogenous antigen to elicit an adaptive immune response. This process has the advantage of generating an acquired immune response that is relatively long lasting. In passive immunization exogenous antibodies are introduced to suppress antigenicity. While passive immunization generates a relatively rapid response, the introduction of exogenous antibodies lasts for a much shorter interval than active immunization.

In vivo cell tracking using direct cell labeling

Radioligand imaging using single photon emission computed tomography (SPECT) or positron emission tomography (PET) has several advantages over other imaging modalities for cell tracking, including its high sensitivity and whole-body quantitative imaging capability (with clinically available scanners). For cell tracking, ex vivo direct cell radiolabeling – i.e., radiolabeling cells before their administration - is the simplest and most robust method, allowing labeling of any cell type without the need for genetic modification [15].

Cells are usually radiolabeled ex vivo by incubation with a radiotracer [14], followed by injection of the radiolabeled cells into the imaging subject. In vivo PET or SPECT imaging can then be performed over time to assess the distribution of the cells. The radiolabeling mechanism can vary depending on the type of probe. Cells can be radiolabeled either using radiotracers designed to bind to the cell membrane or specifically designed to penetrate the membrane where they become trapped

intracellularly. A limitation of direct cell labeling is that the temporal imaging window is restricted by the half-life of the radionuclide used, with progressive loss of signal strength.

Direct cell labeling can also be affected by the efflux of the radiotracer from the radiolabeled cells in vivo. Hence, ideal, direct cell labeling agents should facilitate fast, high yield, cellular uptake, with high cellular retention of the radionuclide, while not affecting cell viability. Furthermore, they should allow imaging over relatively long periods of time if needed for a given imaging application. Accordingly, long-lived radionuclides (such as 111In, 89Zr) are usually preferred.

Most compounds used for direct cell radiolabeling are "radiometal−ionophore" complexes, which consist of a radiometal and an ionophore, a ligand which binds to a metal ion reversibly for transport across lipid membranes [16]. The generated radiometal complex is sufficiently hydrophobic to allow passage across cell membranes but not stable enough to remain intact within the cell. Once inside the cell, the radiometal can be captured by intracellular proteins or other macromolecules [17] resulting in the trapping of the radionuclide−and generation of a radiolabeled cell. Despite the successful use of ionophore ligands for transport of label into cells, the potential radiotoxicity associated with delivery of ionizing radiation intracellularly can pose a danger to cell health and viability. One approach to mitigating this danger is to radiolabel cells on the cell membrane, further away from the nucleus, with the prospect of reducing Auger-electron toxicity that can originate from some nuclides. For example, direct cell tracking has been successfully employed to track the migration of cancer cells in the brain.

Accessing and monitoring intracellular processes

Reporter genes

A key question in neurological disease has been that of determining which protein interactions mediate dysfunctional processes. An increasingly important method for addressing this question is that of reporter gene imaging. In this method reporter genes are introduced into brain cells using a suitable vector. Currently, recombinant adeno-associated viral (AAV) vectors are the most frequently used delivery method for effective cell transduction and stable expression of a modified gene in the brain. When activated the reporter gene produces a protein receptor or enzyme that binds, transports, or traps a subsequently injected imaging radioprobe, which becomes the contrast agent [18,19].

Several gamma-emitting radionuclides are available for radiolabeling injected agents, ranging from small molecules and peptides to antibodies, nanoparticles, and cells. In the clinic, the most widely used radionuclide is 99mTc, which has a moderately short half-life (6 hours). This is long enough for convenient synthesis of radiotracers (while not imposing prolonged radiation exposure to the subject), offers favorable emission properties, and has convenient production methods. Because of its metallic character, 99mTc radiotracers employ coordination complexes to bind the radionuclide with a chelating agent [12,13]. Despite significant limitations initially with the biodistribution and specificity of reporter gene products, there is a growing repertoire of available reporter genes that could be used for tracking cellular processes and protein-protein interactions within them. Following the advent of standardized genetic editing techniques, researchers have isolated a large collection of reporter and modifier proteins (RPs and MPs, respectively) from a variety of species that have since been instrumental in characterizing many biological processes [19]. For example, RPs are frequently used to tag endogenous proteins or to track the behavior of individual cells in vivo.

Antibody fragments

Especially promising is the use of radiolabeled antibody fragments as reporter probes [19,20]. Antibody fragments retain the ability to target specific protein domains that may be critical in mediating protein-protein interactions and are more accessible to restricted sites than whole antibodies due to their smaller size. Their mode of action is to suppress protein activity by directly binding to functional domains, thereby interfering with the ability of aberrant proteins to interact with binding partners. Research with intrabodies has been conducted for several decades. The first intrabodies directed to functional sites on huntingtin protein, for example, were generated from a human spleen, single- chain variable fragment (scFv) phage library [21].

Modulating genetic expression

Nucleic acid therapies for chronic diseases typically adopt one of two approaches, interference with protein specific, RNA translation mechanisms or direct modification of the genes of protein products. RNA translation (RNAis) approaches employ short-interfering RNAs (siRNA) or microRNAs (miRNA) [22]. These molecules target mature mRNA in the cytosol, triggering degradation through the RNA-induced silencing complex and eventually reducing protein expression [23]. Gene therapy, on the other hand, entails the use of altered genes termed transgenes to treat and prevent disease [24]. Gene therapy approaches use virus vectors to transfect brain cells with a therapeutic gene, which under the regulation of a suitable gene promoter. Nucleic acid-based approaches have traditionally employed gapmer anti-sense oligonucleotides (ASO) and anti-sense oligonucleotides [25,26]. The former are a string of nucleotides with a central unmodified region flanked by modified nucleotides. Such radiolabeled drug oligonucleotide probes can provide a reliable quantitative tool for distribution, mass balance, and metabolite profiling studies [27]. Several cancers and/or tumor-specific promoter's systems have been developed. Tumor- specific promoters that are over expressed in the tumor can induce specific therapeutic genes, enhancing their localized activity [28]. Therapeutic genes developed for neurodegenerative diseases include, for example, Huntington's, Alzheimer's, and Parkinson's diseases.

Conclusion

Nuclear medicine has confirmed its place as a versatile and powerful technique for in vivo diagnosis and therapy of the brain, traditionally providing insight into metabolic and neurotransmitter dysfunction. Findings from molecular research on the complex factors affecting symptomatic expression, however, have shown that many steps outside of transmission functions are potentially dysfunctional. Access to such targets is critically dependent on targeted delivery both as to cell type and molecular process, requiring a significant expansion in the range of processes traditionally amenable to analysis.

Addressing this need, recent decades have seen the development of an array of procedures used for radioprobe targeting. The current procedural armamentarium includes natural and synthetic vectors, gene activation, immunogenic selectivity, and nucleotide manipulation. This spectrum of methods is auspicious for precision interrogation and systems directed, molecular medicine.

References

1. Crișan G, Moldovean Cioroianu NS, Timaru D G, Andrieș G, Căinap C, Chiș V. Radiopharmaceuticals for PET and SPECT imaging: A literature review over the last decade. Int J Mol Sci. 2022;23(9):5023. doi:10.3390/ijms23095023
2. Hutton BF, Erlandsson K, Thielemans K. Advances in clinical molecular imaging instrumentation. Clin Transl Imaging. 2018;6(1):31–45. doi:10.1007/s40336-018-0264-0
3. Vaz SC, Oliveira F, Herrmann K, Veit-Haibach P. Nuclear medicine and molecular imaging advances in the 21st century. Br J Radiol. 2020;93(1110):20200095. doi:10.1259/bjr.20200095.
4. Enlow E, Abbaszadeh S. State of the art challenges and emerging technologies in radiation detection for nuclear medicine imaging: A review. Front Phys. 2023;11:1106546.
5. Witney TH, Blower PJ. The chemical tool kit for molecular imaging with radionuclides in the age of targeted and immune therapy. Cancer Imaging. 2021;21:18.
6. Lawn T, Howard MA, Turkheimer F, et al. From neurotransmitters to networks: Transcending organisational hierarchies with molecular-informed functional imaging. Neurosci Biobehav Rev. 2023;150:105193. doi:10.1016/j.neubiorev.2023.105193.
7. Edelmann MR. Radiolabelling small and biomolecules for tracking and monitoring. RSC Adv. 2022;12(50):32383–32400.
8. Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. Nat Rev Drug Discov. 2021;20(2):101-124. doi:10.1038/s41573-020-0090-8.
9. Ramanathan S, Archunan G, Sivakumar M, et al. Theranostic applications of nanoparticles in neurodegenerative disorders. Int J Nanomedicine. 2018;13:5561-5576. Published 2018 Sep 20. doi:10.2147/IJN.S149022.
10. Choudhury SR, Hudry E, Maguire CA, Sena-Esteves M, Breakefield XO, Grandi P. Viral vectors for therapy of neurologic diseases. Neuropharmacology. 2017;120:63-80. doi:10.1016/j.neuropharm.2016.02.013.
11. Słyk Ż, Stachowiak N, Małecki M. Recombinant Adeno-Associated Virus Vectors for Gene Therapy of the Central Nervous System: Delivery Routes and Clinical Aspects. Biomedicines. 2024;12(7):1523. doi:10.3390/biomedicines12071523.
12. Tabrizi SJ, Estevez-Fraga C, van Roon-Mom WMC, et al. Potential disease-modifying therapies for Huntington's disease: lessons learned and future opportunities. Lancet Neurol. 2022;21(7):645-658. doi:10.1016/S1474-4422(22)00121-1.
13. Gawne PJ, Man F, Blower PJ, T M de Rosales R. Direct Cell Radiolabeling for in Vivo Cell Tracking with PET and SPECT Imaging. Chem Rev. 2022;122(11):10266-10318. doi:10.1021/acs.chemrev.1c00767.
14. Steinbrueck A, Sedgwick AC, Brewster JT 2nd, et al. Transition metal chelators, pro-chelators, and ionophores as small molecule cancer chemotherapeutic agents. Chem Soc Rev. 2020;49(12):3726-3747. doi:10.1039/c9cs00373h.
15. Thakur ML, Segal AW, Louis L, Welch MJ, Hopkins J, Peters TJ. Indium-111-labeled cellular blood components: mechanism of labeling and intracellular location in human neutrophils. J Nucl Med. 1977;18(10):1022-1026.
16. Gao T, Wang P, Gong T, et al. Reporter Genes for Brain Imaging Using MRI, SPECT and PET. Int J Mol Sci. 2022;23(15):8443. Published 2022 Jul 30. doi:10.3390/ijms23158443.
17. Nerella SG, Michaelides M, Minamimoto T, Innis RB, Pike VW, Eldridge MAG. PET reporter systems for the brain. Trends Neurosci. 2023;46(11):941-952. doi:10.1016/j.tins.2023.08.007.
18. Freise AC, Wu AM. In vivo imaging with antibodies and engineered fragments. Mol Immunol. 2015;67(2 Pt A):142-152. doi:10.1016/j.molimm.2015.04.001.
19. Marks JD, Hoogenboom HR, Bonnert TP, McCafferty J, Griffiths AD, Winter G. By-passing immunization. Human antibodies from V-gene libraries displayed on phage. J Mol Biol. 1991;222(3):581-597. doi:10.1016/0022-2836(91)90498-u.
20. Wild EJ, Tabrizi SJ. Therapies targeting DNA and RNA in Huntington's disease. Lancet Neurol. 2017;16(10):837-847. doi:10.1016/S1474-4422(17)30280-6.
21. DiFiglia M, Sena-Esteves M, Chase K, et al. Therapeutic silencing of mutant huntingtin with siRNA attenuates striatal and cortical neuropathology and behavioral deficits. Proc Natl Acad Sci U S A. 2007;104(43):17204-17209. doi:10.1073/pnas.0708285104.
22. Kordasiewicz HB, Stanek LM, Wancewicz EV, et al. Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. Neuron. 2012;74(6):1031-1044. doi:10.1016/j.neuron.2012.05.009.
23. Tabrizi SJ, Ghosh R, Leavitt BR. Huntingtin Lowering Strategies for Disease Modification in Huntington's Disease. Neuron. 2019;102(4):899. doi:10.1016/j.neuron.2019.05.001.
24. Denis HL, David LS, Cicchetti F. Antibody-based therapies for Huntington's disease: current status and future directions. Neurobiol Dis. 2019;132:104569. doi:10.1016/j.nbd.2019.104569.
25. Volpe A, Pillarsetty NVK, Lewis JS, Ponomarev V. Applications of nuclear-based imaging in gene and cell therapy: probe considerations. Mol Ther Oncolytics. 2021;20:447-458. doi:10.1016/j.omto.2021.01.017.
26. Montaño-Samaniego M, Bravo-Estupiñan DM, Méndez-Guerrero O, Alarcón-Hernández E, Ibáñez-Hernández M. Strategies for Targeting Gene Therapy in Cancer Cells With Tumor-Specific Promoters. Front Oncol. 2020;10:605380. doi:10.3389/fonc.2020.605380.
27. Zhuang HH, Qu Q, Teng XQ, Dai YH, Qu J. Superenhancers as master gene regulators and novel therapeutic targets in brain tumors. Exp Mol Med. 2023;55(2):290-303. doi:10.1038/s12276-023-00934-0.