Basics of drug transport & delivery and radiopharmacy for nuclear medical applications

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Abstract **Radiopharmaceuticals are pharmaceutical substances which contain a radionuclide within their structure, not eliciting any physiological response. Radiopharmaceuticals can be used as imaging agents, function agents, in vitro studies and therapeutical agents. There are several criteria for radionuclide selection concerning in vivo applications: half-life, radiotoxicity, purity, specific activity, accessibility *vs* production cost. The design of radiopharmaceuticals is based upon physiological function of the target organ. The mechanism of localization of a radiopharmaceutical in a particular target organ depends on many processes: antigen-antibody reactions, physical trapping of particles, receptor site binding, removal of intentionally damaged cells from circulation, and transport of a chemical species across a cell membrane and into the cell by a normally operating metabolic process. There are many radiopharmaceuticls for a large number of Nuclear medicine studies, each one enabling the visualization of a specific organ function/morphology in order to achieve a diagnosis and/or therapy.**

I. INTRODUCTION

A *radiopharmaceutical* may be defined as a pharmaceutical substance containing radioactive atoms within its structure which is administered to animals/humans for research, diagnosis and/or therapy purposes, typically eliciting no physiological response [1]. Radiopharmaceuticals differ from radiochemicals in that the former have met specific requirements enabling them to be used in humans safely. They are formulated in multiple chemical and physical forms to target different organs of the body.

The gamma radiation emitted by the radiopharmaceuticals is detected or measured externally by a gamma camera. Static or dynamic images are obtained, enabling to obtain "imaging documents of organ function and structure, in contrast to diagnostic radiology, which is based upon anatomy. It is a way to gather medical information that may otherwise be unavailable, require surgery, or necessitate more expensive diagnostic tests." [2]. All nuclear medicine exams must obey to the ICRP 60 implications [3].

An ideal radiopharmaceutical should rapidly and avidly localise within the organ under investigation, remaining in it for the duration of study, being quickly eliminated from the body. No single ideal agent exists, so a radionuclide and a chemical compound are selected to achieve the best compromise. Basically, the wide range of radiopharmaceuticals available in nuclear medicine can be divided into four major groups according to their application: imaging, in vivo function, in vitro studies, and therapeutic procedures. The mechanism of localization of a radiopharmaceutical in a particular target organ can be as simple as the physical trapping of particles or as sophisticated as an antigen-antibody reaction or chemisorption of an inorganic phosphate on the hydroxyapatite crystals deposited in an acute myocardial infarction [4].

A. Imaging agents

The majority of radiopharmaceuticals are used in extremely small tracer amounts for diagnostic imaging which is the main activity of Nuclear Medicine. The selection of an imaging agent is based on its ability to localise within the organ of interest and a gamma camera is used to acquire its dynamic or static image. Dynamic scans are useful for evaluating the functional status of the organ based on the rate of accumulation and clearance of the tracer. Static images provide information on the morphology of the organ (size, shape, presence or absence of space occupying lesions) based on the pattern of radionuclide distribution [5,6,7, 8].

B. In vivo function agents

Radioactive tracer agents are also used to measure function of a particular organ by counting radioactivity emitted from the body, in blood samples or in urine. Such studies are based upon the localization, dilution, concentration or excretion of radioactivity following administration of the radiotracer. For example, ^{131}I-sodium iodide is used to assess thyroid function by determination of the percentage of administered radioiodine that is taken up by the gland within a given time, ^{57}Co and ^{58}Co - cyanocobalamin capsules are given to the patient to measure vitamin B_{12} absorption from the gastro-intestinal

tract by measuring the fraction of orally administered radioactive B_{12} that is excreted into the urine over 24 hours (Schilling's test), and ^{51}Cr-sodium chromate for determining red cell volume and red cell survival studies by measuring the dilution of a known amount of the intravenously injected ^{51}Cr-labelled red cells (and ^{125}I-HSA to measure plasma volume). It is essential that the chemical integrity of these radiotracers is not altered as a result of the radiolabelling procedure. ^{51}Cr-EDTA (ethylene diamine tetraacetic acid) is frequently used for determining G.F.R. (glomerular filtration rate) [7,8,9].

C. Agents for In Vitro Studies

These agents are radiotracers that are *not* injected into patients, but used to measure chemical substances, hormones, or drugs in patient's blood sample. The majority of tests are based upon the radioimmunoassay (RIA) principle, using an antigen-antibody immune reaction. These tests are frequently used to measure plasma levels of thyroid hormones, cortisol, digoxin, and a number of other compounds. In many institutions, RIA falls under the domain of Clinical Chemistry/ Chemical Pathology [7,8,9].

D. Therapeutic Agents

The commonest therapeutic procedure is the use of 131I-sodium iodide to treat hyperthyroidism and ablation of residual functioning thyroid tissue in differentiated thyroid carcinoma. Intending to selectively destroy diseased tissue, several other beta emitters are occasionally used as an adjunct to conventional procedures cancer therapy. For instance, 111In-pentetreotide (111In-octreoscan) has been used as an adjunct for diagnosis and management of somatostatin receptor-bearing gastro-enteropancreatic neuroendocrine and carcinoid tumours. In clinical trials, 166Ho-DOTMP (1, 4, 7, 10-tetraazcyclo-dodecane-1, 4, 7, 10-tetramethylenephosphonate) has been used for bone marrow ablation as an attempt to treat terminal stage multiple myeloma. 89Sr-Strontium chloride ('metastron') and 153Sm-EDTMP [ethylenediamine-tetra (methylene phosphonic acid)] are used as alternatives and adjunct to external beam therapy for the palliation of bone pain from bone metastasis secondary to prostate carcinoma following failure of hormone or chemotherapy. Occasionally, 32P-sodium phosphate has also been used to treat polycythemia [7,8,9,10].

E. Radionuclides

There are several criteria for radionuclide selection concerning in vivo applications: half-life, radiotoxicity, purity, specific activity, accessibility *vs* production cost [8,11, 12].

The half-life must be adequate for the clinical investigation purposes (long enough to enable optimal uptake by the target organ, and short enough to achieve minimal irradiation of the patient). Radiotoxicity concerns the cellular damage or radionecrosis due to emitted rays. It depends on the nature and energy of the emitted radiation, tissue uptake and pharmacokinetics of elimination. As for purity we must envisage three different aspects: radionuclidic (ratio between the isotope radioactivity and total activity); chemical (due to chemical impurities arised during the production process); and radiochemical purity (ratio between the activity of the radionuclide, present in the chosen chemical form, and the total activity of the radionuclide in the solution). Specific activity (the amount of radioactivity per gram of present element) should be high. The production cost is significative, mainly depending on the price of the target material, irradiatin time, and irradiation efficiency. The location of the research/clinical facility regarding the production site and transport fees also contribute for the radionuclide accessibility.

Table 1: Commonly used radionuclides in Nuclear Medicine.

Radionuclide	Half-Life	Uses
Technetium 99m	6.1 hrs	Brain, bone, liver, spleen, kidney and blood flow imaging
Iodine 123	13.22 hrs	To monitor thyroid and adrenal function
Fluorine 18	109.9 mins	Diagnosis of certain types of cancer
Iodine 131	8.04 days	Diagnosis and treatment of thyroid cancer
Phosphorous 32	14.3 days	Treatment of excess of red blood cells
Strontium 89	50.5 days	Treatment for bone pain
In 111 Octreotide	67.2 hrs	To diagnose gastro-entero-pancreatic neuroendocrine (GEP) tumours and carcinoid tumours
Yttrium 90	64.1 hrs	Radiation synovectomy
Samarium 153	46.7 hrs	Pain reduction associated with bone metastases

F. Radiopharmacy facilities

The **Radiopharmacy** is an integral part of a Nuclear Medicine Department that deals largely with the preparation, labelling, quality control (QC), and dispensing of radiopharmaceuticals and radionuclides for human use. Usually, the radiopharmacy comprises a radiochemistry laboratory especially equipped with a dose calibrator, a well counter, a biological safety class III cabinet for cell labelling or cold kit manufacturing, or a Biological Safety cabinet class II for cell labelling. Radiochemists/radiopharmacists perform these functions at large hospitals or medical centres., being involved in manufacturing cold kits and in developing new agents and procedures. In research institutes the resources are adapted to the particular needs and developed work, such as hot cells for radiosynthesis, in vitro and in vivo studies, radiation level monitoring, etc. [8,13].

II. MECHANISMS OF LOCALIZATION OF RADIOPHARMACEUTICALS

A. Biological Membranes

Lipids of cell membranes include phospholipids composed of glycerol, fatty acids, phosphate, and a hydrophobic organic derivative such as choline or phosphoinositol. Cholesterol is a lipid of cell membranes that regulates membrane fluidity and is a part of membrane signaling systems. The lipids of membranes create a hydrophobic barrier between aqueous com-partments of a cell. The major structure of the lipid portion of the membrane is a lipid bilayer with hydrophobic cores made up predominately of fatty acid chains, and hydrophilic surfaces. Membrane proteins determine functions of cell membranes, includingserving as pumps, gates, receptors, cell adhesion molecules, energytransducers, and enzymes. Peripheral membrane proteins are associated with the surfaces of membranes while integral membrane proteins are embedded in the membrane and may pass through the lipid bilayer one or more times. Carbohydrates covalently linked to proteins (glycol-proteins) or lipids (glycolipids) are also a part of cell membranes, and function as adhesion and address loci for cells. Cell junctions are a special set of proteins that anchor cells together, occlude water passing between cells (tight junctions), and allow cell-to-cell direct communica-tion (gap junctions) [14].

The accepted model for biological membranes, the fuid mosaic model, describes membranes as a fluid lipid bilayer with floating proteins and carbohydrates [15].

There are some special types of biological membranes, **type 1**: Blood-brain barrier (BBB) - the membranes between the blood and brain have effectively no pores. This will prevent many polar materials (often toxic materials) from entering the brain. However, smaller lipid materials or lipid soluble materials, such as diethyl ether, halothane, can easily enter the brain. These compounds are used as general anesthetics; **type 2**: Renal tubules - in the kidney there are a number of important regions for drug elimination. In the tubules drugs may be reabsorbed. However, since the membranes are relatively non-porous, only lipidic compounds or non-ionized species (dependent of pH and pKa) are reabsorbed; **type 3**: Blood capillaries and renal glomerular membranes - these membranes are quite porous allowing non-polar and polar molecules (up to a fairly large size, just below albumin - M.Wt 69,000) to pass through. This is especially useful in the kidney since it allows excretion of polar (drug and waste compounds) substances [14].

B. Design of radiopharmaceuticals

The design of radiopharmaceuticals is based solely upon physiological function of the target organ. The mechanism of localization of a radiopharmaceutical in a particular target organ depends on many processes: antigen-antibody reactions, physical trapping of particles, receptor site binding, removal of intentionally damaged cells from circulation, and transport of a chemical species across a cell membrane and into the cell by a normally operating metabolic process. Radiochemistry plays a significant part in the development of these compounds and methods for their quality control to insure radiochemical purity. The Guide to Good Pharmaceutical Manufacturing Practice (GMP) defines Quality Assurance as the “sum total of organized routine made with the object of ensuring that products will be of the quality required”. Great emphasis must be placed on process control to assure final product quality. All radiopharmaceuticals must abide to the Regulations, Directives and Guidelines, respectively for Europe and/or US [16,17,18].

C. Involved localization mechanisms

Simple diffusion, which means that the net movement of particles (molecules) is from high concentration to low concentration areas., describes a mechanism whereby a radiotracer diffuses across cell membranes and then redistributes itself elsewhere in the body (e.g. ^{133}Xe gas diffuses across lung membranes and circulate in the blood stream). Exchange diffusion involves the diffusion of a radiotracer into a cell where a chemical exchange takes place (e.g. $^{18}F^-$ exchanges with the OH^- on the hydroxy-apatite structure of bone tissue to form ^{18}F-fluorapatite) [14].

Facilitated transport occurs if the particles can only pass through protein channels; the rate of diffusion being determined by the number of available channels, as well as the number of particles to transport [14].

Active transport involves use of a normally operative energy-dependent metabolic pathway in the body to move a radiopharmaceutical across a cell membrane and into the cell (e.g. thyroid uptake of radioiodide; myocardial perfusion imaging with ^{201}Tl; renal imaging with ^{131}I-o-

iodohippurate or ^{99m}Tc-MAG3 (mercaptoacetyltriglycine) for tubular secretion studies; etc.) [8,19].

Phagocytosis involves the physical entrapment of colloidal particles by Kupffer cells in the reticulo-endothelial system following an intravenous injection. Colloidal suspensions contain particles in the range of 0.05 to 4 µm. The most commonly used phagocytic agents, Tc-sulfur colloid and Tc-microaggregated albumin, typically have particle sizes ranging from approximately 0.1-2.0 µm. The smaller the particles, the greater the bone marrow uptake; larger particles tend to localize in the liver and spleen. Due to the small size of the colloid compared to the diameter of the average capillary (7 µm), capillary blockage does not occur [8,20].

Capillary blockage involves the intentional microembolization of a capillary network with particles, enabling external visualization of its perfusion. This is achieved by i.v. injection of a radiolabeled, precipitated, biodegradable substance, ^{99m}Tc-MAA 34 (macroaggregate of human serum albumin) [8,21,22].

Cell sequestration involves radiolabeling and then heat damaging of a small volume of the patient's red cells (usually 10 ml) to take advantage of the spleen's normal function, i.e., removal of damaged red cells [8,20,22].

Compartmental localization is defined as the placement of a radiopharmaceutical in a fluid space and maintaining it there long enough to image that fluid space [e.g. ^{133}Xe, ^{127}Xe, or ^{81m} Kr- gases; autologous ^{99m}Tc- red blood cells or ^{99m}Tc-HSA; cisternogram following injection of ^{111}In-DTPA (diethylene triamine pentaacetic acid) directly into the cerebrospinal fluid] [8,22].

Physicochemical adsorption or chemisorption is another important localization mechanism. The phosphate or phosphonate groups on currently used bone agents bind avidly and essentially irreversibly i.v. to the hydroxy-apatite structure of bone tissue. In addition, by the same mechanism, they localize in metastatic lesions to bone [e.g. ^{99m}Tc- MDP (methylenediphosphonate), HDP (hydroxyl-methylenediphosphonate), and PYP (pyrophosphate)] [8,23].

The antigen/antibody (Ag/Ab) reaction is a more recently exploited mechanism - highly purified radiola-belled monoclonal Ab with high specificity for a particular Ag are injected i.v. and imaged later in time (e.g. ^{111}In-Oncoscint) [8,22].

An increasingly important mechanism to consider is the receptor binding of radiopharmaceuticals specifically designed to trace receptor sites [e.g. ^{123}I-IQNB (3-quinuclidinyl-4-iodobenzilale) for muscarinic receptors, ^{123}I-iomazenil for benzodiazepine receptors, and ^{123}I-mIBG (m-iodobenzylguanidine) for myocardial adrenergic receptor] [24,25]. Critical features of drug development in this case include special attention to the specific activity and radiochemical purity of the starting material, otherwise the specific activity of the final product may be unsuitable for localizing receptor sites due to saturation with cold (non-radioactive) material.

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