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Radiopharmaceuticals: Preparation, Evaluation and Applications

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The radioactive agents used in the nuclear medical field are called radiopharmaceuticals and are required to exhibit high and specific localization of radioactivity into target tissue. Among radionuclides used in radiopharmaceuticals ^{99m}Tc plays an important role in widespread applications. The preeminence of ^{99m}Tc is attributable to its optimal nuclear properties of a short half-life and a gamma photon emission of 140 keV, which is suitable for high-efficiency detection and which results in low radiation exposure to the patient. Radionuclides in nuclear medicine are all synthetic and fall into four categories namely, generator produced, thermal neutron reactor produced, cyclotron produced and pile produced. Radiopharmaceuticals undergo a very lengthy and expensive regulatory process as well as extensive chemical and physical testing (pH, isotonicity and chemical parameters) to insure that the final product is sterile, pyrogen free, safe for human use and is efficacious. These include both animal and human studies prior to release of the product for sale. Clinical application of radioactive diagnostic and therapeutic agents constitutes one of the great advances in nuclear medicine. Various radiopharmaceuticals have been applied clinically in the diagnosis of various malignant and benign conditions. This article highlights the preparation, evaluation and applications of radiopharmaceutical preparations, which contain such useful radio atoms.

Constructive research on the nucleus of atom has not only resulted in the means to harness this tremendous power for production of electricity and other forms of energy but has also provided scientists with more than 1400 species of atoms. These find innumerable applications in industry, medicine, pharmacy, agriculture and other disciplines. Radiopharmaceuticals are those preparations, which contain one or more radionuclides, i.e. radioactive heavy metals for diagnosis and therapy. For diagnostic applications, they are administered in very small quantities so that they should not be pharmacologically active to produce any physiological effect. But for the therapy, the radiopharmaceutical preparation contains enough radioactivity to produce the intended specific changes in tissue. The radiopharmaceutical preparations may be simple solutions of inorganic salts or suspensions with complex organic substances. Radioisotopes are used in medicines in two different ways, either as radiation sources or as radioactive tracers.

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PRODUCTION OF RADIONUCLIDES[1-5]

Radionuclides used in radiopharmaceuticals are produced artificially by the radioactive decay of other radioactive atoms. This production can be carried out by any of the following three methods

Using radionuclide generator:

The radioisotope generator is an ion exchange column

containing resin or alumina upon which a long-lived parent nuclide has been adsorbed. Typical radionuclide generator is a glass or plastic column and the bottom of this is filled with adsorbent material on which parent nuclide is adsorbed. After the secular equilibrium i.e. after 4-5 half lives, the daughter nuclide growth is eluted in carrier free state with appropriate solvent. It includes production of ^{68}Ga, ^{81m}Kr, ^{82}Rb, ^{99m}Tc, and ^{113m}In radionuclides out of which ^{99m}Tc is of particular importance. Due to its ideal imaging energy and physical half-life as well as the ability to bind to so many compounds, approximately 85% of all imaging procedures are performed following administration of ^{99m}Tc. The versatile chemistry of technetium emerging from the 8 possible oxidation states, along with a proper understanding of the structure-biologic activity relationship, has been exploited to yield a plethora of products meant for morphologic and functional imaging of different organs[6]. Recent efforts have been directed toward the design of ^{99m}Tc-labeled compounds for estimating receptor or transporter functions. A number of bifunctional chelating agents that provide ^{99m}Tc labeled proteins and peptides of high *in vivo* stability with high radiochemical yields have also been developed. More recently, organometallic technetium and rhenium compounds have been introduced as another class of ^{99m}Tc radiopharmaceutical design[7]. Ponto[8] examined preparation problems occurred in the production of ^{99m}Tc radiopharmaceuticals and identified causes of substandard ^{99m}Tc radiopharmaceuticals products.

Principle of operation of Mo/Tc generator:

Prior to shipping the generator to the production unit, ^{99}Mo sodium molybdate is immobilized on a column of alumina due to its very high affinity for alumina. The eluant (0.9% saline solution) is passed through the column and sodium pertechnetate, the daughter of ^{99}Mo decay, is eluted from the column due to its almost total lack of affinity for alumina. The pertechnetate is collected in a shielded, evacuated sterile vial and calibrated prior to use. It is referred to as the eluate. Quantitative removal of pertechnetate is attributed to the lack of affinity of pertechnetate for alumina, whereas molybdate is essentially completely and irreversibly bound to the alumina. When eluting the generator, the elution volume should be carefully controlled so a relatively constant radioconcentration is obtained every day.

Cyclotron produced radionuclides:

The cyclotron and similar particle accelerators can be used only with charged particles such as electrons, protons and deuterons. This is because the operation of such machines depends upon the interaction of magnetic and/or electrostatic fields with the charge of particles undergoing acceleration. A beam of charged particles is produced by accelerating ions around a widening circle using magnetic field for control and electric current for acceleration. Various separation techniques are available to separate product from target. It is required that chemical forms of target and product must be different to effect separation. It includes production of positron emitting isotopes such as ^{11}C, ^{13}N, ^{15}O and ^{18}F. Cyclotron yield is dependent upon number of target atoms, energy of particles, decay of product after it is formed, length of irradiation and isotope enrichment of target.

Pile produced isotopes:

Most of the radioactive materials produced today for use in industry, academic research and medicines are produced in a nuclear pile (nuclear reactor). Uranium fission reaction produces a large supply of neutrons. One neutron for each uranium atom undergoing fission is used to sustain the reaction. The remaining neutrons are used either to produce plutonium or used to produce radioactive products by causing the neutrons to interact with specific substances, which have been inserted into the pile, the latter process being known as neutron activation. Production of ^{133}Xe, ^{99}Mo, and ^{131}I is carried out by this method. These isotopes are produced in great quantity in nuclear reactors and are regarded by the nuclear power industry as waste products. Once they have been purified adequately, they are perfectly suitable for human use. The fission reaction may be represented by the following equation. The reaction becomes self-propagating and requires special care to prevent it from quickly running out of control.

$U^{235} + n \rightarrow {}^{**}U^{236} \rightarrow 2.3\ n + -I^{131}$, Mo^{99}, Xe^{133}, and Others

Thermal neutron reactor-produced radioisotopes:

Radioisotopes used in nuclear medicine are almost all synthetic. For thermal neutron reactor-produced radioisotopes, reactor is source of thermal neutrons. An (n, gamma) reaction occurs. It causes increase of atomic weight by one and no change in atomic number. Same element is therefore present, e.g., ^{98}Mo after reaction produces ^{99}Mo. Reactor yield is dependent upon neutron flux in reactor (n/sec/cm^2), nuclear capture cross section, number of target atoms, isotope enrichment of target, decay of product after it is formed and length of irradiation. The product of an (n, gamma) reaction or other reaction may be described in terms of specific activity, i.e., radioactivity per unit mass of the element present. Efficient separation requires that chemical

forms of element in target and product must be different.

The radionuclides produced by these ways are used in radiopharmaceuticals in the form of proper dosage forms. The physical form of radiopharmaceutical depends upon the type of study or characteristics of organ. They can be in the form of gases, gases in solution, liquids, true solutions, colloidal solutions or suspensions, macroaggregates, microspheres, emulsions, freeze dried solids and capsules.

EVALUATION OF RADIOPHARMACEUTICALS

The basic principle of evaluation of radiopharmaceuticals is same as that of other pharmaceutical products. Strict quality control tests should be carried out for purity, potency, product identity, biological safety and efficacy. Quality control tests for radiopharmaceuticals are of two types i.e. physicochemical and biological.

Physicochemical tests, Particle sizing[9]:

Two major types of diagnostic radiopharmaceuticals consist of particulate suspensions. One type is used for the investigation of perfusion defects and includes preparations such as macroaggregated human serum albumin and microspheres. The other type is used for investigation of the reticuloendothelial system and includes preparations such as colloids and phytate formulations. Safe and reproducible use of particulate preparations requires control over the number and size of the particles in the product. The preferred range of particle size is 20 to 80 μm with no particles larger than 100 μm and the minimum of particles below 20 μm. Light microscopy is the best feasible method for the determination of particle size in these preparations. A sample is placed in a haemocytometer chamber and the number of particles are estimated by counting particles in the centre and four corner squares; multiplication by the appropriate factor will give the number of particles per cm^3.

Not all pharmaceutical colloids are true colloids, and the mean particle size of colloidal preparations varies widely between different formulations of the same agent, and between different agents. Some sulfur colloids have mean particle sizes of around 0.4 to 0.6 μm while antimony trisulphide colloids have mean size below 0.1 μm. No single technique is suitable for sizing over such a large range. Filtration through nucleopore membranes (polycarbonate membranes having closely controlled etched pores) gives useful data for routine quality control. The method is not suitable for particles smaller than 0.005 μm, Electron microscopy of the samples evaporated on carbon grids can be used for accurate sizing of some colloids but can not be recommended for routine examination. Light scattering and photon correlation techniques are now used for the estimation of colloidal particle size distributions.

pH and ionic strength:

pH meters are universally employed to measure the pH of pharmaceuticals. Narrow range pH indicator papers are suitable if standard pH buffers are used in conjunction with the sample to give reference colours corresponding to upper and lower pH limits. A drop of each pH standard should be placed on the paper along with a drop of the sample, so providing a permanent visual record of the pH test[10].

Particulate contamination:

All products administered by injection should be free from gross particulate contamination. Control is exercised by using glassware, vials and reagents, which are themselves free from particulate matter. Visual examination of the product either directly through a glass screen or between illuminated cross-polaroid screens provides adequate control of most small volume radiopharmaceuticals. Those which can not be examined visually (e.g., colloids, macroaggregates, microspheres) must be controlled through strict vigil with reference to cleaning of glassware and containers and purity of reagents[11].

Radionuclide purity:

The specified radionuclide impurity limits reflect the radiological hazard associated with the impurity, the clinical use of the radiopharmaceutical, and the practicality of achieving better standards. For example, the BP 1993 specifies a limit of 1.0 % of ^{126}. In ^{125}I iodinated human albumin and a similar limit for ^{60}Co in ^{58}Co cynacobalamin. In both the examples, the radionuclide impurities have longer half-lives than the principal radionuclide and hence it degrades slowly in comparison to principal radionuclide and results in increased proportion of radionuclidic impurity. Another important consequence is that the BP radionuclidic impurity limit also effectively defines a shelf life of these materials[12]. The method for determining radionuclidic impurity is usually the technique of gamma spectroscopy. In many cases, the Pharmacopoeias control radionuclidic impurities by specifying that the sample spectrum (beta or gamma) does not differ significantly from that of a standardized solution, so that the limit for impurities is effectively that of the standard solution for which no details are provided. In other cases, the characteristics of the

impurities are listed so that they may be identified in the spectrum.

Radiochemical purity:

Radiochemical purity is defined in the BP as the ratio, expressed as a percentage, of the radioactivity of the radionuclide concerned that is present in the source in the chemical form compared to the total radioactivity of that radionuclide present in the source. For most diagnostic radiopharmaceuticals, a purity of above 95% is desirable since the radiochemical impurities will certainly have a different biodistribution, which may distort the scintigraphic image and so invalidate a clinical diagnosis based on the scintigram. Although extraction and phase separation methods are coming into use, separation methods are universally employed in radiochemical purity determinations, planner chromatographic and electrophoresis being the most popular. Their main advantage over column and other elution methods is that the entire applied radioactivity remains on the developed chromatoplate of electrochromatogram, which can then be examined and quantitated by a number of techniques. Planner chromatography includes paper, thin layer and high performance thin layer chromatography[13-16]. The techniques are quite similar to each other as a sample is applied to the stationary medium and developed with a suitable mobile phase. Table1 shows the planner chromatography of selected ^{99m}Tc-radiopharmaceuticals[17-21].

Radio assay:

It is the heart of whole quality control operation. Total radioactivity is to be measured before dispensing and administration using isotope dose calibration. It is a well-type chamber filled with air or gas coupled with electrometer, and activity of samples is measured in curies.

Biological tests[22]:

These include test for sterility, pyrogens and toxicity. The BP method is followed to test the sterility. A quantity of radiopharmaceutical more than the human dose is kept with a proper nutrient medium, e.g. soyabean casein medium at 20-25° for 2-14 d and observed for bacterial growth. The conventional pyrogen test may be performed wherein the rise in rabbits temperature is noted after i.v. injection of the preparation. Alternatively, LAL test can be performed. Toxicity may be manifested as alteration of histology and physiology of the tissues. Acute and chronic toxicity test are carried out in mice, rat, rabbit and dog for 2-6 w, animal is sacrificed and the organs are observed for pathological changes. The value of LD_{50}, i.e., the dose that shows 50% mortality in 30 d is then estimated.

APPLICATION OF RADIOPHARMACEUTICALS

Diagnostic applications of radiopharmaceuticals:

For diagnosis, isotopes are used as radioactive tracer. As mentioned earlier, when radioisotopes are used for diag-

TABLE 1: PLANNER CHROMATOGRAPHY OF SELECTED ^{99m}TC- RADIOPHARMACEUTICALS.

Radiopharmaceutical	Mobile phase	Support	Complex	Rf values TcO_4^-	Colloid
Tc-HAM(microspheres)	Acetone	Wh31ET	0	1	-
Tc-Ethylenedicysteine	Acetone	ITLC-SG	0	1	0
	0.5 m AcOH	ITLC-SG	0	1	0
Tc-MAG3	Saline	ITLC-SG	0	1	0
	Acetone	ITLC-SG	0	1	0
Tc-HAS	Acetone	Wh31ET	0	1	0
	$EtOH+NH_3+H_2O$	ITLC-SG	1	1	0
Tc-Lidofen	20%NaCl	ITLC-SA	0	1	0
	Water	ITLC-SG	1	1	0
Tc-exametazine	Butan-2-one	ITLC-SG	0	1	0
	Et_2O	Wh1	1	0	-

nosis, the radiation dose delivered to the patient is maintained at a low level.

***In vitro* studies[23]:**

Radioactive materials are used in performing assays on biological materials. In this, the radioactive materials are not administered to the individual but are used as reagents in carrying out measurements of substances in biological fluids removed from the body. These *in vitro* assays are of the following types:

Radiometric analysis requires the use of a standard reagent having a known relationship between chemical concentration and radiological concentration. For example, determination of serum calcium using standard ^{14}C-oxalic acid solution and determination of serum citric acid by use of ^{52}Br. Competitive radio assay also known as saturation analysis, follows the basic principle of a competitive reaction in which a radioactive substrate (ligand) and non radioactive substrate (analyte P) compete with each other for a binding agent Q. The substrate P may be a vitamin, hormone, drug or other substance, the concentration of which is to be determined. To perform the assay, the substrate must be available with a radioactive tag. ^{125}I is the most frequently used tag.

Immuno radiometric assays are those in which, a large excess of binder (antibody) is used to bind all the analyte. In one type of immunometric assay, a radioactively tagged antibody is added and by measuring the radioactivity remaining in solution, the analyte concentration in the sample can be determined. Various receptor systems identified in body tissues and organs to be of physiological importance, also act as binders to specific hormones and other compounds having physiological activity. One receptor system of particular importance is the estrogen receptor of breast cancers. Estrogen receptor site assay in breast tumors has become a routine clinical assay.

***In vivo* quantitative studies[24-25]:**

In these studies, the radioactive materials are administered to the individual and then the distribution of the radioactive materials is measured by detection of the radioactivity in the body fluids or tissues within the body (external counting) or after removal from the body (urine, blood and exhaled air). The isotope dilution technique can be employed for measuring red blood cell and plasma volume. The more popular procedure uses radio iodinated human serum albumin injected intravenously. After 10 min, a blood sample is withdrawn and blood volume is calculated from the measured decrease in radioactivity of the injected sample upon its dilution by blood. RBC volume can be determined by the use of cells labeled with ^{51}Cr in the form of sodium chromate. Radioactive hydrogen (^{3}H) in the form of tritiated water can be used to determine total body water.

The RBC destruction mechanism and RBC half-life are measured by means of a disappearance rate technique. If erythrocytes are labeled *in vitro* with ^{51}Cr and then reinjected, the fate of the tagged cells can be followed by assay of serial blood samples taken every 2 or 3 d for at least 2 w. This study is a valuable aid in the diagnosis of haemolytic anemia. In certain types of haemolytic anemias, red blood cells disappear rapidly from the blood stream, being trapped and eventually destroyed by the spleen. The extent of RBC uptake by spleen can be determined by tagging the cells *in vitro* with ^{51}Cr reinjecting them intravenously, counting externally over the spleen and liver and calculating the spleen/liver radioactivity. A high ratio, activity associated with decreased RBC survival, may indicate a need for a spleenectomy.

Campylobacter pylori, a microorganism suspected for causing chronic gastritis and peptic ulceration could be detected through the large amounts of urease, which it produces. ^{14}C labeled carbon dioxide in the exhaled breath is analyzed by absorption in hyamine hydroxide, followed by measurement of ^{14}C in a liquid scintillation counter.

Schiiling test is useful for detection of pernicious anemia and for differentiation from other macrocytic anemias. In a normal individual, over 50% of an oral dose of vitamin B_{12} is absorbed through the walls of the GIT. This absorption occurs only in presence of an intrinsic factor. In pernicious anemia patient there is a deficiency of intrinsic factor, which causes poor absorption of the vitamin and most of the ingested vitamin B_{12} will therefore, be found in the feces. The degree of absorption or of fecal excretion can be measured by the use of ^{60}Co labeled vitamin B_{12}. Other anemias such as those associated with sprue and idiopathic steatorrhea, are also accompanied by a decrease in vitamin B_{12} absorption. They may be differentiated from the pernicious anemia through the oral administration of intrinsic factor. A marked increase in vitamin B_{12} absorption results in pernicious anemia patient but not in patients of sprue and other malabsorption syndromes.

If a soluble radioisotope is introduced into a moving stream of liquids, the time taken for the activity to reach a given point along the direction of flow can be used to calcu-

late the rate of flow. The method is valid only if the rate of flow is high compared with the rate of spreading of isotope by diffusion or turbulent flow. The principle is used in the measurements of blood circulation rates in patients suffering from thrombosis. Aqueous injection of sodium chloride containing 10-20 μg of ^{24}Na can be used for this purpose.

Imaging studies:

In recent years, scanning techniques have developed rapidly and are now among the most useful tools in diagnostic medicines. By means of scanning, tissues and organs can be visualized and such visualization facilitates the detection of abnormalities in their function. Radioactive materials are administered to the individual and distribution of the radioactive material in the body is measured by using imaging techniques.

Cardiovascular imaging:

Radiopharmaceuticals are useful in cardiac imaging as agents that provide information of regional myocardial blood perfusion. They are administered to provide information at peak cardiac output. The study involves stressing the patient with exercise on a treadmill or giving an i.v. injection of dipyridamole. An injection of thallium chloride or technitium-99m (^{99m}Tc) labeled methoxy isobutyl isonitrile is then given and imaging is carried out[26]. ^{99m}Tc sodium pyrophosphate injection is primarily a skeletal imaging agent has been used as a cardiac imaging agent as an adjunct to the diagnosis of acute myocardial infarction[27].

Bone imaging:

This method is widely used in the diagnosis of benign and malignant, primary and metastatic bone tumors. It is also useful in the study of patient with suspected fracture, arthritis, metabolic bone disease, vascular necrosis, bone infarcts and bone healing. Bone imaging radiopharmaceuticals consist of diagnostic (primarily single photon emitters) and therapeutic agents. The therapeutic radiopharmaceuticals are utilized on the basis of their particulate emissions (primarily beta-) and thus are treated differently than the single photon bone imaging agents[28]. Tuncay *et al.* prepared microsphere formulations of diclofenac sodium (DS) using a natural biodegradable polymer as a carrier for intraarticular administration to extend the duration period of the dosage form in the knee joint. For the *in vivo* studies, ^{99m}Tc labeled polyclonal human immunogammaglobulin (^{99m}Tc-HIG) was used as the radiopharmaceutical to demonstrate arthritic lesions by gamma scintigraphy. After the induction of arthritis in knee joints of rabbits, the radiolabeled microspheres loaded with DS were injected directly into the articular cavity and gamma scintigrams were obtained at periodic intervals to find the residence time of the microspheres in the knee joints in order to determine the most suitable formulation[29]. Tuncacy *et al.* have also prepared controlled-release parenteral formulations of DS using Poly (lactide-co-glycolide) polymers for intra-articular administration. For *in vivo* studies, ^{99m}Tc-HIG was used as the radiopharmaceutical to demonstrate arthritic lesions by gamma scintigraphy[30].

Caglar *et al.* presented a prospective analysis of ^{99m}Tc-MDP and ^{99m}Tc citrate scintigraphy in 29 patients clinically suspected of having chronic osteomyelitis. The authors concluded that Tc-99m citrate is a promising agent for localizing and showing the extent of bone infection that help the surgeon to determine areas of debridement before surgery[31].

Lung imaging:

The main purpose of the lung imaging is the diagnosis of pulmonary emboli and to evaluate pulmonary perfusion and pulmonary ventilation, and to assess pulmonary function prior to pneumonectomy. The agent used is ^{99m}Tc macroaggregated albumin. Iseri *et al.* prepared albumin and gelatin microspheres incorporating a tuberculostatic agent, rifampicin and its *in vivo* distribution was studied by causing its accumulation in the target organ, i.e., lung. Biodistribution was determined by intravenous administration of particles of 25 to 27 microns ^{99m}Tc-labeled microspheres to Swiss mice. The radioactivity of the lungs was compared with that of the liver, spleen, kidney, stomach and heart. The percentage accumulation was higher in the lungs than in the other organs for both albumin and gelatin microspheres[32].

Radioembolization is used in diagnostic imaging of the lungs and for therapy of hepatic tumors. Ergiin *et al.* prepared ^{99m}Tc microspheres of poly lactic acid (PLA) and evaluated its uptake and biodegradation in lungs. The high lung uptake obtained in mice and rabbits indicated the suitability of PLA microspheres for lung imaging[33].

Renal imaging:

This is used to determine renal function, renal vascular flow and renal morphology. These are also used for evaluation of renal transplant patients for complication such as obstruction, infarction, leakage, tubular necrosis and

rejection.^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) and ^{131}I-ibdohippurate are commonly used radiopharmaceuticals[34].

Spleen imaging:

Radiopharmaceuticals are used for spleen imaging for various purposes. Spleen imaging is performed using ^{99m}Tc-denatured erythrocytes. After a blood sample is withdrawn from the patient the erythrocytes are labeled with ^{99m}Tc *in vitro*; the labeled cells are then denatured by heating at 49.5° for 15 min following reinjection into the patient, the cells are then taken up by the spleen. Imaging with ^{99m}Tc-denatured erythrocytes is used to detect spleen nucleus[35]. Ercan and Bernay[36] presented a new method of labeling human RBCs with ^{99m}Tc by the use of Sn-alpha-D-glucose 1-phosphate for spleen imaging.

Imaging of inflammatory lesions:

Scintigraphic imaging of infection and inflammation is a powerful diagnostic tool in the management of patients with infectious or inflammatory diseases[37]. Many radiopharmaceuticals have been introduced for the scintigraphic demonstration of infectious and inflammatory lesions and some of them are currently in clinical use. They can be classified into two major categories according to their specificity. Specific radiopharmaceuticals include *in vitro* labeled leukocytes, radiolabeled monoclonal antibodies, and receptor specific small proteins and peptides. Nonspecific radiopharmaceuticals include, radiolabeled nanocolloids, liposomes, macromolecules such as human immunoglobulin, dextran and human serum albumin, various small molecules and ions[38]. Nowadays, a few radiopharmaceuticals are available that could replace radiolabeled leukocytes, such as ^{67}Ga-citrate, ^{99m}Tc-IgG and ^{99m}Tc-labeled antigranulocyte antibody preparations. Furthermore, various agents labeled with ^{99m}Tc are currently developed for this application[39]. Tutus *et al.* investigated that ^{99m}Tc-GSH is a potential alternative to the currently used radiopharmaceuticals for imaging ocular lesions[40]. Ercan *et al.* prepared and evaluated ^{99m}Tc(V) DMSA as an agent for the visualization of inflammatory lesions in comparison to ^{99m}Tc(III) DMSA and ^{99m}TC-HIG[41].

Gastrointestinal imaging:

Latex particles coated with either amino or carboxyl groups can be efficiently labeled with ^{99m}Tc and used in the studies of gastrointestinal function. Ercan *et al.* prepared ^{99m}Tc-labeled monodisperse latex particles coated with amino or carboxyl groups for studies of GI function[42].

Brain imaging:

Brain imaging is performed using radiopharmaceuticals by single photon emission computed tomography (SPECT) and positron emission tomography (PET). SPECT and PET radiopharmaceuticals are classified according to blood-brain-barrier (BBB) permeability, cerebral perfusion and metabolism receptor-binding, and antigen-antibody binding. The blood-brain-barrier SPECT agents, such as ^{99m}Tc-DTPA, ^{201}Ti and ^{67}Ga-citrate are excluded by normal brain cells, but enter into tumour cells because of altered BBB[43]. Samnick *et al.*[44] prepared p-amino-3-[^{123}I]iodo-l-phenylalanine, p-[^{123}I]iodo-l-phenylalanine, L-8-[^{123}I]iodo-1,2,3,4-tetrahydro-7-hydroxyisoquinoline-3-carboxylic acid and L-3-[^{123}I]iodo-alpha-methyl-tyrosine and investigated their uptake in human pancreatic carcinoma and glioblastoma cells as well as the mechanisms promoting the tumour uptake. The radiopharmaceutical uptake into tumour cells was rapid and temperature and pH-dependent. The radioactivity concentration in tumour cells varied from 10 to 33% of the total activity following 30 min incubation at 37° (pH 7.4). In comparison, accumulation of the radiopharmaceuticals into normal brain and pancreatic tissue remained relatively low.

Imaging of brain tumours requires a disrupted BBB, however, it is intact in the early stages of brain tumour growth, when diagnosis is most critical. Relative to normal brain, brain tumour cells frequently overexpress peptide receptors, such as the receptor for epidermal growth factor (EGF). Peptide radiopharmaceuticals such as radiolabeled EGF could be used to image early brain tumours. Kurihara and Pardridge described a bifunctional molecule that contains both biologically active human EGF radiolabeled with ^{111}In and an anti-transferrin receptor monoclonal antibody that undergoes transcytosis through the BBB via the endogenous transferrin transport system[45].

THERAPEUTIC APPLICATIONS OF RADIOPHARMACEUTICALS

Treatment of hyperthyroidism:

^{131}I-iodine is used extensively for the treatment of hyperthyroidism. Upon oral administration of the radionuclide, approximately 60% of the radioactivity is taken up by the overactive gland[46]. The principal disadvantage of the radioiodine therapy is the high incidence of early and late hypothyroidism, making it necessary to monitor patients

adequately after treatment. Although there is a theoretical possibility of radiation-induced cancer or genetic defects, there is no substantial evidence to support this on the basis of several large follow up studies conducted over the past 20 years or so[47]. As a consequence, treatment is no longer restricted to older patients and now adolescencents[48] and even children, being treated with radioiodine[49].

Treatment of thyroid carcinoma:

Radioiodine has been used for several decades in the treatment of differentiated thyroid carcinoma, a tumour which metastases to bone, lungs and other soft tissues[50]. However, it is slow growing and the prognosis is relatively good, allowing long term follow up of treated patients. Repeating radionuclide imaging with radioiodine can assess response to therapy and, if necessary, further therapeutic dosages of ^{131}I may be required in advanced or resistant cases[51].

Treatment of neuroendocrine tumours:

With the development of new radiopharmaceuticals there is a tendency to apply nuclear medicine therapy for malignancies of higher incidence (lymphoma, prostate) than the ones that have been treated for many years (thyroid cancer, neuroendocrine tumours). One of the most important areas of current development in radionuclide cancer therapy is the monotherapeutic use of new or already available radiopharmaceuticals in preclinical or phase I studies and to a lesser degree in phase II trials. In this context, the radioimmunotherapy is showing important advances in the treatment of medullary thyroid carcinoma, malignant lymphomas and brain tumours with potential extension to neuroblastoma therapy[52].

Metaiodobenzylguanidine is similar in structure to the adrenergic neuron blocker guanethidine and the neurotransmitter noradrenaline. Due to the structural similarity, it is taken up by the adrenal medulla and other tissues with rich sympathetic innervation but unlike noradrenaline it is not metabolised and is largely excreted unchanged in the urine. ^{131}I-metaiodobenzylguanidine (^{131}I-MIBG) was initially investigated as a means of locating phaeochromocytomas, but was found to be taken up also by other neuroendocrine tumours such as neuroblastoma, carcinoid tumours, and medullary carcinoma of the thyroid. Subsequent to its development as a diagnostic agent, ^{131}I-MIBG has been used with success in the treatment of these tumours. More recent development include the use of ^{131}I-MIBG as a first line treatment in high risk neuroblastoma patients and the use of agent in combination with chemotherapy and whole body irradiation for the treatment of neuroblastoma[53]. Several other agents are being investigated as alternatives to ^{131}I-MIBG and include 161-Terbium-diethylenetriaminepentaacetic acid-octreotide and 111-Indium-diethylenetriaminepentaacetic acid (^{111}In-DTPA) for tumours containing somatostatin receptors[54].

Treatment of bone tumours:

Bone metastases are a common finding in patients suffering from cancers of the prostate, breast and lungs, and in these patients control of bone pain is a significant clinical problem. External beam radiotherapy, in combination with analgesic drugs, remains the mainstay of treatment but the proportion of the body that can be treated is limited. In patients with breast and prostate carcinoma, bony metastases are usually multiple, and often widespread, and in theory the systemic administration of a suitable therapeutic radiopharmaceutical offers the opportunity of a more specific and less toxic method of treating disseminated disease[55].

Several beta emitting radionuclides, in a variety of chemical forms, can be used for the treatment of bone metastases. Apart from ^{32}P and ^{89}Sr, and a number of clinical studies using the newer agents 186-Rhenium and 153-Samarium have been carried out. Other agents such as ^{117m}Strontium-diethylenetriaminepentaacetic acid (^{117m}Sn-DTPA), which are at an earlier stage of development, will have an improved therapeutic effect with minimal myelosuppression[56].

Treatment of myeloproliferative disease:

^{32}P has been used for more than 50 years in the treatment of a variety of haematological disorders. Following intravenous injection, ^{32}P as the orthophosphate, is concentrated selectively by rapidly proliferating tissue and there is also bone uptake. In this way, a significant radiation dose is delivered to the bone marrow and results in growth retardation of the haemopoeitic cell lines[57]. The primary application of ^{32}P-phosphate is its role in the treatment of polycythaemia rubra vera. In this condition there is an abnormal increase in the number of red cells in the circulation. Symptoms are often widespread and non-specific, resulting from increased blood viscosity and if the disease is left untreated the prognosis is poor. However, treatment with phlebotomy, radioactive phosphorus or chemotherapy all results in significant increase in life expectancy.

Intracavitary therapy[58-59]:

Direct intracavitary administration is a means of delivering radiopharmaceuticals in high concentration to tumours that are spread out over the serosal linings of cavities and tumour cells present in the malignant effusions. In order to minimise leakage of the radionuclide from the cavity, it is usually given in the form of a radiocolloid. Intracavitary therapy is applied to the peritoneal, pleural and pericardial cavities as well as to cystic brain tumours and to the spinal canal.

Colloidal ^{198}Au was formerly the most widely used agent, but the radionuclide emits unwanted gamma radiation, leading to unnecessary exposure of non-target tissue within the patient, and of other personnel. The agents of choice are now ^{32}P and ^{90}Y radiocolloids, with perhaps radiolabeled antibodies having a wider role to apply in the future.

Regional use of radiopharmaceuticals:

The use of the arterial route to deliver radioactive formulations which preferentially lodge in arterioles or capillaries of tumours has long been seen as an attractive method of therapy. Over the past 30 years, number of radionuclides including ^{32}P and ^{90}Y have been labeled to a variety of carriers especially microspheres. The preparations have been administered regionally to treat tumours resident in liver, lung, kidney, tongue, spleen and soft tissue tumours of the extremities[60]. Most clinical experience with intraarterial microspheres has been gained in the treatment of metastases to the liver, which are known to derive their blood supply predominantly from the hepatic artery. Ariel and Padula[61] used ^{90}Y labeled microspheres together with two chemotherapeutic agents in 105 patients with liver tumours arising from colo-rectal cancer, and reported average survival time of 26 and 31 mo. These figures compare favourably with average survival figures of 6-12 mo in patients treated using alternative methods.

Other clinical studies using activated ^{90}Y-glass microspheres and ^{90}Y-resin microspheres have been carried out in the treatment of liver tumours[62]. In a tracer study using radiolabeled microspheres, angiotensin II was used to modify the pattern of arterial blood flow in patients with liver metastases, to produce an increase in tumour to normal ratio by a factor of 3[63].

Radiolabelled antibodies (Radioimmunotherapy):

With the introduction of hybridoma technology it was expected that antibodies could provide the vehicle for specific targeting of therapeutic agents and for destroying malignant diseases. A variety of techniques for preparing suitable antibodies and labeling these with radionuclides have been developed. Clinical experience to date has largely been confined to the use of antibodies derived from mouse or rat as *in vivo* diagnostic agents in radioimmunoscintigraphy (RIS). A vast number of studies have been undertaken with a host of different monoclonal antibodies (MAbs) or their fragments. These have been radiolabeled with a range of different radionuclides and used in a variety of studies on patients. No generalisation of RIS as a technique can be made, since experience varies considerably with a range of reported sensitivities and specificities. The accumulation of radiolabeled MAbs in tumour tissue is in most cases very low and is associated with relatively short retention times, which limit their usefulness for therapeutic purposes. There are many factors leading to low antibody uptake at target sites which, include technical problems during preparation, such as low antibody purification efficiency and loss of immunoreactivity resulting from damage incurred in the radiolabeling process. Other problems encountered are alterations in antigen expression by the tumour, effects of dilution after *in vivo* administration, shedding of antigen into the circulation and the induction of human anti-mouse antibody response[64]. With regard to solid tumours, regional or intracavitary administration of radiolabeled antibodies is considered to be more appropriate, as this could lead to increased tumour uptake at sites of localised disease. Promising results have been obtained after intraperitoneal administration of radiolabeled antibodies in patients with ovarian carcinoma. Although percentage uptakes per gram of tumour tissue still tend to be quite low, some encouraging clinical responses using radiolabeled MAbs have been obtained especially in patients with limited disease[65]. Radiolabeled peptides are an emerging class of radiopharmaceuticals that share chemical and biological properties. From the chemical point of view they have a poly-amino acid structure varying from 3 to more than 200 amino acids, and they are labeled with different isotopes directly or by a linker. Biologically, they bind to specific cell membrane receptors, thus providing *in vivo* histopathological information for diagnostic purposes, therapy follow-up or targeted radiotherapy[66].

CONCLUSIONS

Production and use of radiopharmaceuticals has revolutionised the pharmaceutical and biomedical field. Radiopharmaceuticals are radioactive drugs that when used for the purpose of diagnosis or therapy, typically elicit no

physiological response from the patients. The design of these compounds is solely based upon physiological function of the target organ. Radionuclides that are used in nuclear medicine are mostly artificial. At present, more than 1500 radionuclides have been produced artificially primarily in a cyclotron or a reactor. Since, radiopharmaceuticals are intended for human administration, it is imperative that they undergo strict quality control measures. Quality control of radiopharmaceuticals is usually a complex and time-consuming activity in a hospital radiopharmacy involving several specific tests and measurements to ensure their purity, potency, product identity, biological safety and efficacy. A variety of radionuclides have been exploited for their diagnostic and therapeutic potential. Although conventional radiotherapy plays a vital role in the treatment of several diseases, it doesn't guarantee the safety of normal tissues. In contrast, systemic administration of a radiopharmaceutical offers the opportunity of treating widely spread diseases, the object being to selectively irradiate the diseased site sparing normal tissues. It is obvious that radiopharmaceuticals play a key role in the diagnostic and therapeutic field, however, safety of the patients due to hazardous effects of the radiation is of utmost importance that can be regulated by strict quality control measurements.

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