

PROPOSING NORMS FOR CLINICAL APPLICATION OF BIOLOGICAL RADIOLABELLED COMPOUNDS. PHARMACODYNAMIC AND TOXICOLOGICAL RECOMMENDATIONS FOR PRECLINICAL STUDIES

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Abstract

In October of 2005 a small group of researchers from different Countries had a meeting at the International Atomic Energy Agency headquarter in Vienna, Austria; the aim was to prepare a tentative user-friendly document for personnel involved in preparation of radiopharmaceuticals based on peptides, proteins and antibodies for human use. This document should cover all practical, methodological and ethical concerns relating to radiolabelled products mentioned above and should clarify the complicated road-map that one has to follow in this area. This document does not cover the use of radiolabelled oligonucleotides, cells and other autologous products and does not provide technical protocols on actual methodologies. Herein, we will like to present you some pharmacodynamic and toxicological recommendations for in vivo preclinical studies. This guidance only represents my own current thinking in this topic.

Key words: radiopharmaceuticals, clinical trials, drugs, pharmacology, recommendations, IAEA, evaluation, labelled compounds, radiation protection

PROPUESTA DE NORMAS PARA LA APLICACIÓN CLÍNICA DE COMPUESTOS BIOLÓGICOS RADIOMARCADOS. RECOMENDACIONES FARMACODINÁMICAS Y TOXICOLÓGICAS PARA LOS ESTUDIOS PRECLÍNICOS

Resumen

En el mes de octubre de 2005 un pequeño grupo de investigadores de diferentes nacionalidades se reunieron en Viena, Austria, bajo los auspicios del Organismo Internacional de Energía Atómica con el objetivo de preparar un documento sencillo y útil para el personal involucrado en la preparación de productos biológicos radiomarcados como péptidos, proteínas y anticuerpos. Este documento debe incluir los aspectos prácticos, metodológicos y éticos relacionados con los productos radiomarcados anteriormente mencionados y debe esclarecer la ruta crítica que se debe seguir en esa área. Ese documento no cubre el uso de oligonucleótidos, células y otros productos antológicos radiomarcados y no ofrece protocolos técnicos sobre metodologías. En el trabajo presento recomendaciones farmacodinámicas y toxicológicas para los estudios preclínicos in vivo que deben seguir estos productos. Esta guía sólo representa mi forma actual de pensar sobre este tema.

1.0 Preclinical pharmacodynamic studies:

1.1 Aim

The objective of the present preclinical pharmacodynamic guidance is to provide recommendations to practitioners and predict the pharmacological effects of a new biological radiopharmaceutical prior to initiation of human studies. Previous in vitro assays (e.g. cell lines and/or primary cell cultures) could be useful to examine the effect in animals.

Although there is no international accepted definition, pharmacological studies could be classified as:

a- Primary pharmacodynamic studies. Studies related to the desired diagnostic or therapeutic effect.

b- Secondary pharmacodynamic studies. Studies not related to the desired diagnostic or therapeutic effect.

c- Safety pharmacodynamic studies (USA) or general pharmacology studies (Japan, EC).

Studies related to the potential undesirable pharmacodynamic effect of the test substance on physiological vital functions.

In practice, secondary and safety pharmacodynamic studies can be evaluated independently or as part of toxicological and/or primary pharmacodynamic studies. In this section we will focus the discussion on the preclinical diagnostic and therapeutic primary pharmacodynamic effect of radiolabeled peptides, proteins, monoclonal antibodies and their fragments. The safety pharmacodynamic, and toxicity studies will be discussed in section 2. As secondary pharmacological effects (when they exist) may of course be desirable or undesirable,

further primary or safety studies should be performed following the recommendations established in sections 1 or 2.

Biological radiopharmaceuticals are typically administered into the circulation (i.e. intravenously or intra-arterially) and are used for diagnosis, monitoring, and therapy. In some special cases the biological radiopharmaceutical can be administered into a body compartment (e.g., locoregionally into a tumour cavity of a cerebral tumour or intraperitoneally in case of a peritoneal carcinomatosis) with the same purposes. While the diagnostic and monitoring uses include different diseases, the therapeutic use is practically limited to treat cancer diseases.

Radiolabeled peptides are included in these sections due to their exponential growth in the diagnostic and therapeutic applications in the last decade. The automated means of synthesizing these compounds in large quantities and the simplified methods of purifying, characterizing, and optimizing them have kindled attention to peptides as carrier molecules. These new techniques have accelerated the commercial development of radiolabelled peptides, which has provided additional radiopharmaceuticals for the nuclear medicine community. Peptides have many key properties including fast clearance, rapid tissue penetration, and low antigenicity, and can be produced easily and inexpensively. However, there may be problems with in vivo catabolism, unwanted physiologic effects, chelate attachment, and toxicity due to binding to receptors expressed by non-tumour tissues [1,2].

1.2. Legislations and facilities for animal work

Depending mainly on the radionuclide used, there are special considerations to be taken into account with the design and performance of preclinical studies with radiopharmaceuticals.

Animals, animal wastes and materials used during the experimentation are radioactive. Facilities and investigators should have the adequate conditions and experience to protect personnel, general public and animals (e.g. controls from treated ones) from any contamination. Facilities and personnel should also be in compliance with good laboratory practice (GLP) for laboratory animals. When the laboratory animal regulations are in disagreement with the radiological protection regulations, additional considerations should be taken (e. g., ventilation systems).

Personnel and institutions should be licensed by authorities for using the specific radionuclide in experimentation. Despite this inconvenience, these studies are necessary to predict the pharmacological/toxicological profile of a biological radiopharmaceutical prior to initiating human studies.

1.3. Good laboratory practice (GLP)

It is desired to perform preclinical studies with pharmaceuticals in compliance with GLP. Nevertheless, it is recognized that due to the specific and unique design frequently used for biopharmaceuticals and in particular for biological radiopharmaceuticals, it may not be possible to fully comply with GLP.

Primary and secondary pharmacodynamic studies do not necessarily need to be conducted in compliance with GLP [3]. Safety and toxicity studies should be conducted in compliance with GLP to the greatest extent possible.

It is important to emphasize that areas of non-compliance with GLP should be identified. Data quality, documentation of the study, and archived data should be ensured throughout and after the study. In these special cases, lack of full GLP compliance does not necessarily mean that the data can not be used to support clinical trials [3,4].

1.4. Animal models

The species specificity of many peptides, proteins and monoclonal antibodies has demanded the determination of species relevance before pharmacological/toxicological studies initiation. A relevant species is one in which the test material is pharmacologically active due to the expression of a receptor or an epitope (in case of monoclonal antibodies). The selection of the species is usually accomplished by in vitro comparison of binding affinity or functional activity of the product in animal and human cells followed by in vivo demonstration of the pharmacological activity [4,5].

Absolute equivalence of antigen density or affinity for the biopharmaceutical, however, is not always possible or necessary for an animal model to be useful. Differences in binding for example may be compensated for by alterations in dose or dosing frequency [6]. It is important to show that the biological radiopharmaceutical maintains activity and biological properties equivalent to that of the unlabeled material. In some cases, for studying the primary pharmacodynamic properties of biological radiopharmaceuticals, xenograph or transgenic animal models expressing the adequate receptor or epitope can be performed.

In case of therapeutic biological radiopharmaceuticals for distinguishing specific radiation effect from potential pharmacological/toxicological effects of the «cold» non-radioactive labeled material or from the unlabelled peptide, protein or monoclonal antibody (if their therapeutic profiles are not previously known), appropriate control groups should be included. Diagnostic biological radiopharmaceuticals typically achieve their intended pharmacological effect due to the radioactivity administered and

therefore these control groups are not necessary. When conducting safety/toxicity studies appropriated control groups should be included.

Gender of animals

Both genders should generally be used or justification given for specific omissions (e.g. ovarian or prostate cancers).

Anaesthesia

When conducting in vivo studies, especially when safety pharmacological studies on physiological vital functions (i.e. central nervous, cardiovascular and respiratory systems) are performed, it is preferable to use unanaesthetized animals. Data from unrestrained animals that are chronically instrumented for telemetry, data gathered using other suitable instrumentation methods for conscious animals, or data from animals conditioned to the laboratory environment are preferable to data from restrained or unconditioned animals. In the use of unanaesthetized animals, the avoidance of discomfort, pain as well as the possible radioactive contamination during the injection period, and the radioscintigraphy uptake quality is a foremost consideration.

As the use of unanaesthetized animals is not always possible, when necessary, the adequate anaesthesia and dose level according the animal species should be selected.

Administration. Dose selection

In general, the expected clinical route of administration should be used when feasible. The use of other routes may be acceptable if the route must be modified due to limited bioavailability, limitations due to the route of administration, or to size/physiology of the animal species. Most biological radiopharmaceuticals in clinical use are administered systemically (e. g., intravenously or intra-arterially for radioimmunotherapy of unresectable hepatocellular carcinoma [7]). In some cases the radiobiopharmaceutical can be administered locoregionally (into glioma resection cavities [8] or intraperitoneally in advanced ovarian cancer patients [9]) with the objective to increase the radio biopharmaceutical concentration at administration site and to decrease the systemic radio-toxicity. In cases of therapeutic radiobiopharmaceuticals administered systemically or intraperitoneally and until we have a better understanding of the data extrapolation to humans, the radiation dose should be expressed in terms of body surface (MBq/m²).

Quality of biological radiopharmaceutical drugs

Biological radiopharmaceuticals used in the primary pharmacodynamic studies will have appropriate chemical, pharmaceutical, radiochemical, and radionuclide standards of identity, strength, quality, and purity to be of such uniform and reproducible

quality as to give significance to the research study conducted. The radiation dose should be sufficient and not greater than necessary to obtain valid measurements. It is important to use an acceptable method of radioassay of the biological radiopharmaceutical drug to assure that the dose calculations actually reflect the administered dose.

Frequently, the radionuclide and/or the peptide, protein, or monoclonal antibody come from different manufactures who are independently responsible for the final control of their products. It is recommended that the formulation used in the primary pharmacodynamic studies be identical to the formulation that will be used in the follow-up preclinical and clinical studies. However, as primary studies are evaluated for establishing the proof of concept, some reasonable changes in manufacturing and/or formulation are expected. In this case the decision to repeat some or all primary pharmacological studies should depend on an assessment of the impact or likely impact of these changes on the biological radiopharmaceutical properties.

1.5. Pharmacokinetic studies

It is difficult to establish uniform guidance for pharmacokinetic of biological radiopharmaceutical. Single and multiple dose pharmacokinetic and tissue distribution (percent of the injected dose per gram of target tissue and various normal tissues, target/normal tissue ratios) studies in relevant species and immunodeficient animals bearing human tumour xenografts are useful. The animal models do not represent an absolute reliable system to predict the behaviour of the biological radiopharmaceutical in humans due to the biological differences of the animal models and the pathology in humans, alterations in the pharmacokinetic profile due to immune-mediated clearance mechanisms and they are not helpful at identifying areas of normal tissue cross reactivity. However, the results obtained from these experiments can give important information for the characterization of the compound.

Available radiation dosimetry software programs (e.g. Medical Internal Radiation Dose (MIRDose) and Organ level Internal Dose Assessment (OLINDA)) can be used to provide estimates of radiation absorbed doses received by specific organs. Autoradiography (light and/or electron microscopy) and immunohistochemistry studies are useful in order to determine the histopathologic localization of the biological radiopharmaceuticals.

The pharmacokinetic parameters of biological radiopharmaceuticals should be defined using one or more assay methods (e.g. by ELISA and by measurement of radioactivity).

In general, the expected clinical route of administration should be used when feasible. Due to the mechanism of action of diagnostic biological radiopharmaceuticals, the optimal

imaging time is as important as the optimal dose. Organ distribution and washout information will generally establish a theoretically ideal imaging time. The time window of effective imaging (i.e. how soon after administration and for how long) should be established.

The expected consequence of metabolism of radiolabelled peptides, proteins and antibodies is the degradation to small peptides and individual amino acids. Therefore, the metabolic pathways are generally understood. Classical biotransformation studies as performed for pharmaceuticals are not needed.

2.0. Preclinical safety and toxicity studies

2.1. Aim

The objective of this section is to provide recommendations to nuclear medicine practitioners to design safety and toxicity studies for determining the potential radiation effect of diagnostic and therapeutic biological radiopharmaceuticals. Because there are other guidances available for preclinical safety/toxicity evaluation of pharmaceuticals [3,4], this guidance focuses mainly on radiation effects associated to biological radiopharmaceuticals. In case of the biological radiopharmaceutical is intended to be used in paediatric patients, studies in juvenile animals should be also performed. It is important to take into account that ionizing radiation causes injury not only to pathological but also to normal cells and tissues by damaging nuclear DNA [10], which is a known and accepted as unavoidable effect. For consideration of the legislations and facilities for animal work, good laboratory practice (GLP), and animal models, see sections 1.2, 1.3, and 1.4 respectively.

2.2. Safety studies

Safety pharmacology is defined as: those studies that investigate potential undesirable pharmacodynamic effects on physiological functions in relation to exposure in the diagnostic or therapeutic range and above, investigating the mechanism of adverse effect observed and /or suspected [3].

The safety pharmacology study should be designed to identify a dose-response relationship, and doses should elicit moderate to severe adverse effects in this or in other studies of similar route and duration.

The organization of safety pharmacology studies begins with the cardiovascular, respiratory and central (as well as peripheral) nervous system (CNS), which if acutely affected, can have a significant impact on the ability to sustain life. These three organ system make up the «safety pharmacology core battery», studies which should be completed prior to first administration in humans. Supplemental studies may include, but are not limited to renal, gastrointestinal, endocrine, or immune systems [3,11].

2.3. Toxicity studies

The number and types of toxicity studies recommended would depend in part on the phase of development, what is known about the agent or its pharmacologic class, its proposed use, and the indicated patient population.

Due to the inherent toxicity effects of biological radiopharmaceuticals, the uptake of targeting agents in normal tissues has to be minimized for successful diagnosis and/or therapy and some methodological developments have been made applying extracorporeal elimination of the excess of targeting agents in the systemic circulation [12], and reduction of renal uptake by amino acid infusion [13]. Another method is to use antibodies with specificity for the targeting agent to form large molecular complexes [14], which are taken up and degraded by the reticuloendothelial system (RES). Various methods using pretargeting [15,16] have also been tried for improved selective tumour uptake.

Single dose and repeated dose toxicity studies

Medical imaging drugs, unlike most of other products, are typically administered in single dose or infrequently, they are not administered to achieve a steady state. Therefore, the development program can omit long-term (i.e., 3 month duration or longer) repeat dose toxicity studies, and if toxicity studies are performed on the combined components of the test compound and no significant toxicity is found, toxicological studies of individual components are seldom required [17]. Radiation toxicity studies of therapeutic biological radiopharmaceuticals should include some levels (the maximal dose should be at least twice the maximum planned human radiation dose) to identified the no observed adverse effect level (NOAEL) as well as dose related mild to severe radiation toxicity, establishing the maximal tolerated dose (MTD) to be used to define the starting dose in Phase I clinical trials. The study should also include the cold formulation as a control group to distinguish specific radiation effects from potential effects of the cold formulation [18,19].

The studies should identify organs at risk and establish a margin of safety for early and late radiation toxicity. The time period in which radiation injury becomes clinically apparent is determined in part by the turnover time. In organs with a rapid cell turnover, as happens with bone marrow, epidermis, and small intestine, radiation injury can cause bone marrow failure, desquamation, nausea, and vomiting and diarrhoea within days or weeks of an acute dose radiation (an accepted time is less than 60 days). Radiation injury to these organs is called early or acute radiation toxicity and is often reversible. However, in organs with slow cell turnover rate as happens in brain, liver and kidneys, symptoms of radiation injury can cause brain radionecrosis, and liver or kidney failure within several months to years with latency period of relatively normal organ functions (an accepted

time is more than 60 days). Radiation injury to these organs is referred as late radiation toxicity and is usually progressive and irreversible. Therefore, animal studies designed to elucidate late radiation toxicity effects of a biological therapeutic radiopharmaceutical should last for at least one year post dosing and study duration of less than one year should be justified. A recovery period should generally be included to determine the possible reversal effect. When possible, these studies should also include a toxicokinetic design.

Immunogenicity

Biological radiopharmaceuticals are frequently immunogenic, and the development of antibodies after intermittent, repeated administration can alter the pharmacokinetic/toxicokinetic, biodistribution, safety and/or imaging/therapeutic properties and greatly complicates the study interpretation. The development of such antibodies should be tested and characterized during the study.

Local tolerance studies

Local tolerance should be evaluated. In some cases, the potential adverse local effect of the product can be evaluated in single or repeated dose toxicity studies, thus obviating the need for separate local tolerance studies. The effect of misadministration should be evaluated in a manner that it is appropriate for the intended route of administration (e.g., in the case of biological radiopharmaceuticals intended for intravenous administration, extravasation or spill on the skin effects should be evaluated).

2.4. Interpretation of results

High grade organ toxicities have been reported with therapeutic biological radiopharmaceuticals. Therefore, dosimetry estimates should be required prior to clinical studies; they should be developed with simulation models using an appropriate diagnostic or therapeutic radioisotope. Information on pharmacokinetics/toxicokinetics should be sufficient for radiation dosimetry calculations.

It is recommended that calculations of absorbed dose to organs should be carried out in accordance with the Medical Internal Radiation Dosimetry (MIRDose) or Organ level Internal Dose Assessment (OLINDA) schedules. The model used for calculations of the cumulated activity (time integral of the activity) in source organs should be explained and the origin of data used, such as animal studies, should be stated.

The absorbed dose to the organ receiving the highest exposure and to all organs included in the calculation of the effective dose-equivalent should be stated. The unit must be milliGrays per unit of activity administered: mGy/MBq.

The estimation of the radiation dose should be summarized in terms of the effective dose

equivalent using the weighting factors given by the International Commission Radiological Protection (ICRP). The unit must be milliSieverts per unit of activity: mSv/MBq [20].

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