THE ROLE OF ANIMAL MODELS IN PHARMACOKINETIC-PHARMACODYNAMIC MODELLING FOR THE EVALUATION OF CONJUGATED ANTIBODIES

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Abstract: The purpose of this analysis is to explain the usage of animal model in pharmacokinetic-pharmacodynamic (PK/PD) behavior of conjugated antibodies intended for radioactive isotope labelling in the form of radiopharmaceuticals for therapy and diagnosis. This is especially important for the prediction of human pharmacokinetics and dosing of radiopharmaceuticals, especially those that would be used systemically for therapy and are beta or alpha emitters.
For this purpose, it is very helpful to use computer modelling. However, pre-clinical trials of animal models remain a key starting point for further clinical trials. Pre-clinical animal models in translational research are fundamental to the understanding of disease and drug pharmacology but are often limited in their utility to robustly define an efficacious dose in the clinic.
Animal models in the pharmacokinetic/pharmacodynamic (PK/PD) evaluation of conjugated antibodies will serve an important role in preclinical assessments of new radiopharmaceuticals, dosing optimization for those that are clinically approved, and setting or confirming susceptibility breakpoints. This will be very helpful during early development of potential radiopharmaceuticals.
PK/PD modeling can optimize the design of clinical trials, guide the dose and regimen that should be tested further, help evaluate proof of mechanism in humans, anticipate the effect in certain subpopulations, and better predict interactions.
All of these effects could lead to a more efficient development process. Because of certain peculiarities of conjugated antibodies, of used monoclonal antibodies, ligands and especially radioisotopes such as PK and PD characteristics, PK/PD modelling could be particularly relevant and thus have an important impact on decision making during the development of these potential radiopharmaceuticals.
Keywords: animal models, pharmacokinetic-pharmacodynamic modeling, conjugated antibodies, radiopharmaceuticals

1. INTRODUCTION

The pharmacokinetics and pharmacodynamics of a pharmacologically active components are important parameters that can answer how to model the behaviour of a molecule in the body as a potential drug, to explain the mechanism of action, side effects or secondary effects. In this way, the best is to predict the dose at which the potential drug can be administered, the frequency and mode of administration and be the basis for individual targeted therapy especially in special groups of patients.
Through these studies, it is important to analyze the behaviour of the active molecule as well as its concentration as a function of time.
We generally have three approaches to assessment:
1. exponentials, explained as empirical models
2. compartmental models
3. physiological flow patterns models
The first two models have advantages because they are simple and can easily describe and predict the individual behaviour of the active molecule and its concentration in different tissues in the body, distribution and elimination, the effect of altered perfusion, the specifics of the membranes it passes through, and plasma proteins binding.

These two approaches have certain limitations and shortcomings of specific physiologically, anatomically and biochemically significant parameters.
These shortcomings are eliminated when using the third approach. Through this approach we include a combination of different levels of information at the same time. Data from experimental studies, chemical data, biological knowledge, certain assumptions based on published papers and mathematical models are used in order to understand the way a complex biological system (organism) works.
This modeling of the behavior of an active molecule as a potential drug is called physiologically based pharmacokinetic modeling (PBPK).
Physiologically based pharmacokinetic modeling in the human body or animal model is important to answer certain clinical questions:
- which are the effects of external factors - simultaneous usage of others drug, multiple drugs, food, liquids usage
- which are the effects of internal factors - age of the patient, presence of chronic diseases, stage of the disease, dysfunction of certain organs, genetic predisposition
When using PBPK it is important to consider all the parameters on which this approach is based, such as organ / tissue volume, blood flow, specific pharmacokinetic parameters for the given drug - absorption, distribution, metabolism and elimination. The physicochemical characteristics of the drug such as pKa for absorption, partition coefficient, distribution coefficient, molar mass, etc. should also be considered.
The pharmacokinetics and pharmacodynamics are especially important for the prediction of human pharmacokinetics and dosing of potential drug including radiopharmaceuticals as a specific type of drug for diagnosis and therapy.
Radiopharmaceuticals especially those that would be used systemically for therapy and are beta or alpha emitters become a new field of interest, especially through the possibility of monoclonal antibodies becoming specific vectors for appropriate radioactive isotopes and thus being attractive for use for individual target therapy in oncology.
Usage of these complex structures as potential drugs, especially biologic drugs with the additional presence of radioactive isotopes (especially alpha or beta emitters) requires very careful pre-clinical and clinical trials. The use of PK/PD modeling using computer programs to predict the behavior of these potential drugs, as well as predicting the dose and frequency of dosing can be critical. PK/PD modeling can optimize the design of clinical trials, guide the dose and regimen that should be tested further, help evaluate proof of mechanism in humans, anticipate the effect in certain subpopulations, and better predict interactions.
All of these effects could lead to a more efficient development process. Because of certain peculiarities of conjugated antibodies, of used monoclonal antibodies, ligands and especially radioisotopes such as PK and PD characteristics, PK/PD modelling could be particularly relevant and thus have an important impact on decision making during the development of these potential radiopharmaceuticals.
The use of animal models in the examination of these potential drugs can only further confirm the reliability of the results obtained from PK/PD modeling and be complementary in reaching appropriate conclusions.

2. EFFECT OF ANIMAL SPECIES ON PHARMACOKINETICS

The **type** of animals used as models in pre-clinical trials for testing a potential drug is of great importance in its pharmacokinetics.
Smaller mammals, such as mice, rats, and rabbits, often show faster metabolism and elimination, and therefore the half-lives in these models may be significantly shorter than in larger mammals such as humans.
The **route of administration** also affects the pharmacokinetics of the drug and may vary depending on the animal species and the different drugs. This is especially important to check if they are a pharmacokinetic test of the same drug in different dosage forms, ie there is a different way of application (parenteral, oral, local).
The **strain of the same animal** may affect pharmacokinetics. For example, BALB / c and NUDE immunodeficient mice may show significantly different concentrations of certain drugs
Age is a parameter that can have major effects on pharmacokinetic studies in many mammalian species. However, the clinical applicability of the use of age-related pharmacokinetics in an animal model and its correlation with age-related pharmacokinetics is limited.
It should be noted that only adult animals should be used for experimental models, unless otherwise requested and approved.
Changes that may occur in animal models when examining the pharmacokinetics of the same drug in different age groups of animals may be taken as a reason to study pharmacokinetics in different age groups in humans. However, the absence of age-related differences in the animal model does not necessarily mean that there are no clinically significant differences in drug pharmacokinetics in different adults.

Generally, drug concentrations are higher in adult animals compared to young animals for a given dose.
However, pre-clinical trials of animal models remain a key starting point for further clinical trials. Pre-clinical animal models in translational research are fundamental to the understanding of disease and drug pharmacology but are often limited in their utility to robustly define an efficacious dose in the clinic.
Animal models in the pharmacokinetic/pharmacodynamic (PK/PD) evaluation of conjugated antibodies will serve an important role in preclinical assessments of new radiopharmaceuticals, dosing optimization for those that are clinically approved, and setting or confirming susceptibility breakpoints. This will be very helpful during early development of potential radiopharmaceuticals.
PK/PD modeling can optimize the design of clinical trials, guide the dose and regimen that should be tested further, help evaluate proof of mechanism in humans, anticipate the effect in certain subpopulations, and better predict interactions.

3. MATERIAL AND METHODS

The compilation and comparative method were used which enabled us to create and draw our own conclusions using an individual and specific approach by comparing literary data. This paper represents a starting point for comparison with the results obtained in our experimental studies.

4. RESULTS FROM THE RESEARCH MATERIAL AND DISCUSSION

The number of therapeutic monoclonal antibodies (mAbs) and their medical indications are increasing daily. As result that many studies are needed to improve the affinity of the molecular target, clinical activity, and a full understanding of their pharmacokinetic behaviour.
Dose selection is a major challenge in the development of targeted therapies, especially for mAbs with complex PK and PD, which as drugs of the complex biological structure are very different from those of drugs with a precise chemical structure.
Because the volume of distribution is related to body size, most mAbs are dosed based on body weight or body surface area, especially since the therapeutic window of mAbs is wide. However, each patient is an individual and significant variations in the pharmacokinetics of certain mAbs and many factors that affect patients' exposure to mAbs can occur.
A number of strategies have been devised for the improvement of therapeutic monoclonal antibodies for some patients that do not respond fully to the therapeutic monoclonal antibodies. The approaches include, among others, the deployment of antibody-drug conjugates and therapeutic radiolabelled antibodies. Some of which have already obtained regulatory approval for clinical application.
For the clinical use of therapeutic monoclonal antibodies-based radiopharmaceutical, the in vivo dynamic and kinetic stability are vital. Hence, derivatization of the therapeutic monoclonal antibodies to introduce strong chelator groups is necessary. Chelator groups that are potentially useful for a variety of radioactive isotopes have already been synthesized and widely studied.
This should be specifically examined and explained when using radioactive monoclonal antibodies for which we have several additional critical causes:
- the presence of a radioactive isotope with its own pharmacokinetics may contribute to the increase of organ irradiation if it is not well bound to the antibody which is a specific vector
- the antibody used for labelling should have high specific binding to the target tissue and accurate previously examined pharmacokinetics which should not differ from that when it is radioactive
- conjugates antibody - radioactive isotope (radioimmunoconjugates) have in their composition different ligand-molecules that enable efficient binding of the isotope. These ligands can alter the pharmacokinetics of antibodies, that is, the size and stability of the antibody molecule.
PK/PD modelling of the behaviour of each individual used antibody, its radioimmunoconjugates, and the influence of the present ligands and any additional chemical agents present should performe regularly. This is necessary to optimize doses and dosage schedules, to introduce an individually targeted approach and to prevent the risk of treatment failure.
Pharmacokinetic studies, especially that in relation to pharmacodynamic data, are important to support effective and safe dosing regimens, and the use of animal models supports the clinical efficacy and tolerance studies in treated animals.
PK/PD modelling allows us to consider a number of key issues at different stages of the drug discovery and development process.
One of the key goals of translational, mainly mechanical, PK/PD modelling is to generate a priori simulations that help to support performance predictions between different types such as in vitro in vivo predictions and animal-

clinical correlations. Animal model studies have a distinct advantage over both in vitro models and clinical trials in the ability to discern which PK/PD dosing index is most closely associated with efficacy.

The use of radioactive preparations can significantly facilitate the determination of mass equilibrium, as radiolabeled molecules can be relatively easily detected in tissue samples and body fluids. Determining the model of disposition of the radioactive molecule may be adequate to predict the doses to be used in toxicity studies where the results of the total exposure of the test animal to the substance (base compound and metabolites obtained) are of concern. the radiolabeled molecule should not be biologically unstable; when a radioactive element is present at more than one position of the test compound, the radioactivity should be evenly distributed throughout the molecule

The radiochemical purity of the test substance (radioactivity that is firmly bound to the test compound, eg antibody) is another important consideration.

If the test compound contains radiochemical impurities, i.e. chemical degradation products (e.g. antibody fragments) containing a bound radioactive isotope and if their distribution in the organs / tissues of interest is determined, together with the distribution of the test compound the interpretation of to be difficult.

For example, a compound with 95-99% radiochemical purity will have different binding to the target tissue (tumor) compared to a compound having 50% radiochemical purity and 50% different radioactive fragments with different behavioral kinetics.

All of these effects could lead to a more efficient development process. Because of certain peculiarities of conjugated antibodies, ligands and especially radioisotopes such as PK and PD characteristics, PK/PD modelling could be particularly relevant and thus have an important impact on decision making during the development of these potential radiopharmaceuticals.

Because PB/PK modelling in estimating the predicted patterns available for monoclonal antibody-based radiopharmaceuticals can be obtained in the same way as other human drugs by adjusting biochemical parameters in animal-validated models. Adjustments are usually based on experimental results from in vitro tests on animals and humans and by substituting appropriate human tissue sizes and blood flows. The development of these models always requires a computer program capable of simultaneously solving multiple differential equations that correspond to the processes that occur.

5. CONCLUSIONS

The development of safe and effective dosing regimens is a significant challenge in the development of many new drugs, especially those with a biological structure such as monoclonal antibodies. Radiopharmaceuticals as pharmaceuticals by definition use the same method of testing, especially if their specificity is taken into account due to the presence of a radioactive isotope and the risk of its presence if it is not integrated in a stable manner.

This can be achieved by integrating PC and PD information into preclinical experimental models. Therefore, an accurate and predictable animal model together with PK/PD models can be an extremely powerful tool that can guide the drug development process and optimize the therapeutic effect.

In this paper we have tried to present the basic factors that can influence the animal model and PK / PD studies of conjugated monoclonal antibodies as potential radiopharmaceuticals and the general approach to designing PK/PD studies.

We are convinced that carefully controlled animal studies will continue to make a significant contribution to the development of radiopharmaceuticals, especially those based on monoclonal antibodies and optimization of the dosing regimen at those sites for alpha or beta radiation therapy.

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