The clinical pharmacology of biotechnology products.
M.M. Reidenberg, editor

TOXICOLOGICAL DEVELOPMENT OF HEMATOPOIETIC GROWTH FACTORS

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INTRODUCTION

Currently, a range of growth factors involved in the proliferation of hemopoietic cells were identified and characterized. Molecular cloned hematopoietic growth factors have now been used in many clinical trials for the therapy of life-threatening diseases.

This has provided toxicologists with an appropriate challenge to develop a reasonable strategy for safety testing and the design of toxicity studies with these products.

Factors influencing growth and differentiation of hematopoietic cells include interleukins, colony stimulating factors and erythropoietin. In most cases they are named according to the cells they stimulate which include granulocytes, macrophages, erythrocytes, megacaryocytes etc. In part they stimulate also the secretion of other cytokines demonstrating that the knowledge of the regulation of hematopoiesis is increasing in complexity. Recent studies have implicated additional factors in the control of hematopoiesis. Different factors act sequentially at different stages of the growth process. For example, the combination of GM-CSF and IL-3 leads to a synergistic increase in platelet and leukocyte counts.

GUIDELINES AND RECOMMENDATIONS

In the recent years, many proposals for meaningful testing procedures for biotechnology products were published (1, 2). Most scientists agree that routine toxicological experiments developed for safety testing of xenobiotics cannot be applied fully to recombinant drugs. However, it is known that naturally occurring human polypeptides, including hematopoietic growth factors and their recombinant counterparts may have adverse effects sometimes not detected in preclinical studies. Examples

are the fluid retention with pulmonary edema after IL-2 administration to human patients or the toxic effect of interferons. These findings may be produced by intrinsing toxicity - no induced by their pharmacological potential - or by exaggerated pharmacodynamic mechanisms.

This experience led to different guidelines and recommendations in major countries.

In the U.S. a more pragmatic approach is used allowing the exclusion of inappropriate examinations.

In the "Cytokines and Growth Factor Pre-Pivotal Trial Information Package" (3) studies in relevant models are proposed necessary to assess the risk in clinical trials and to support dose, route, frequency and duration of dosing. The amount of animal studies can be discussed with the FDA at an early stage of the preclinical development. This program may include acute, subacute, chronic tests, and if conducted in relevant species, testing on reproductive toxicity, neurotoxicity and immunotoxicity with the active substance, as well as with excipients or contaminating substances taking into consideration species-specificity and immunogenicity in non-host species limiting the relevance of many usual routine toxicity experiments.

On the other hand, Japanese regulations for biotechnology products are similar to the requirements of normal drugs (4). Some routine experiments may be omitted if there are specific reasons not to conduct them. Normally this set of safety data has to include examinations on acute, subacute, chronic, and reproduction toxicity, antigenicity, mutagenicity, carcinogenicity, local tolerance, and on general pharmacology parameters.

In the European Community the guideline for the pre-clinical safety testing for products derived from biotechnology (5) adopts a middle course.

The amount of studies depends on individual product characteristics and their biochemical group. Testing is classified into three categories:

Category I are recombinant products identical to naturally occurring human polypeptides and proteins. Pharmacodynamic, pharmacokinetic and some toxicological studies are required.

For category II, products closely related to the human factor with known or not verified differences of their structure, more data must be submitted including reproduction and immunotoxicity experiments.

For polypeptides and proteins distantly related or unrelated to humans more detailed testing will be necessary.

No fixed battery of studies is recommended, the usefulness of tests should be discussed with the competent regulatory authorities case-by-case.

TEST SUBSTANCES

Test substances used in preclinical toxicity studies should be manufactured and formulated identical to the product used in clinical trials. This includes cloning and expression systems, purification steps, impurity profile, and the final formulation. Significant changes in the manufacturing procedure, the production facility, product specification, or formulation may lead to additional preclinical and clinical studies because recombinant products are characterized by these factors to a great extend. This means that pivotal toxicity studies should only be performed with material from a fixed validated manufacturing process with established release specifications for the final product with known stability including compatibility with the container system (vials and stoppers).

The quality of the test substance may be influenced by the production process that should prevent and eliminate possible content of host cell or inducer contaminants or those introduced by the process, such as proteins derived from the substrate, endotoxins, DNA, culture medium and viruses. Therefore, established and relevant tests for bulk material and the final container are very important.

These analytical tests have to be supplemented by pharmacological quality control assays, such as for abnormal toxicity and pyrogenicity. These additional tests cannot however take the place of a toxicological test as they involve normally only small numbers of animals which receive only one standard dose.

Excipients, diluents, preservatives etc., chosen for

formulation, must be compatible with the active substance. Diluents used in toxicity studies should be identical as those intended for marketing of the factor.

TEST STRATEGY

Toxicity studies in animals should define the toxic potential of the drug, as far as possible. However, with recombinant products there are no guarantees that these experiments will generate always relevant information. But some of these gene products are capable of producing toxic effects as severe as xenobiotic drugs. Therefore, a careful evaluation of their toxic potential in animals prior to clinical use is necessary taking into account special problems which may arise with these substances. Target organs of human toxicity may sometimes - but not always - be predicted on the basis of animal studies.

Considering known guidelines and recommendations and balancing a more scientific approach against regulatory positions, a strategy of preclinical tests for hematopoietic growth factors must be developed to satisfy both the regulators and the clinical investigators.

The design of studies should answer scientific questions and reflect the intended application with correlation to the pharmacological response which may be linear or follow a bell-shaped curve.

Immunogenicity of these human polypeptides in animals may represent a major problem to meaningful toxicological evaluation in other than studies with a limited number of applications. Antibody formation may be induced even in closely related species, such as non-human primates. This effect could be demonstrated in Cynomolgus monkeys in a one-month subacute study with GM-CSF. Therefore, the evaluation of antibody formation should be evaluated in studies of longer duration. However, it is noteworthy to state that an antibody response may also be seen in humans receiving human proteins which may be related to genetic variability. The relevance of these antibodies for possible toxic effects during long-term therapy is not clear at the moment. Further research is needed and this issue will remain a major topic in the future.

As one of the first steps of preclinical development it would be desirable to perform pharmacokinetic and screening pharmacodynamic experiments to serve as the basis for the choice of species for toxicity studies.

Sometimes, however, comprehensive ADME (adsorption, distribution, metabolism, excretion) studies are possible only to a limited extend due to the very small amounts injected. Therefore, sensitive methods for the measurement of blood levels must be developed. Such findings will also give useful information for dosage selection, duration of longer-term toxicity studies, and comparison to data gained during clinical trials.

Because some hematopoietic growth factors tend to be species-specific, well-founded animal models must be selected based on the findings of these preceding pharmacokinetic and pharmacological studies. Therefore, initial investigations in several rodent and non-rodent species should be conducted to demonstrate presence or absence of the desired pharmacodynamic response. This approach is difficult to perform with factors where the physiological effect in man is unknown.

Some hematopoietic growth factors exhibit a pattern of species cross-reactivity but are most efficient to stimulate the target cells of the same species. Human GM-CSF is not effective in rodents and responds best to human and non-human primate progenitor cells. It stimulates canine hematopoiesis (6) but the activity on the peripheral blood cells is different to that in primates (decrease of platelets, eosinophil counts remain unchanged). In this case only monkeys are an appropriate species to perform preclinical testing. In contrast to this cytokine, the pharmacologic action of erythropoietin is also exhibited in lower species usually used in toxicity experiments. Therefore, also mice and rats can be used for these studies. As a general rule, it cannot be expected to observe toxicity relevant to man when the factor is not efficacious in the animal model selected. One of the most important aims of the toxicological experiments is to demonstrate effects when the physiological response is pushed to an extreme. Some companies conduct pharmaco-toxicological studies in monkeys first, the animal species closest to man,

lower species are used if the pharmacological response is similar to those of the non-human primate or of humans. But primates should only be selected if clearly needed and this should be scientifically justified. But because the monkey is closest to man, the subhuman primate is - in some cases - the only relevant model available for testing.

In the experiments the same route of administration as intended to be used in man should be used, usually the intravenous and subcutaneous routes.

The selection of preclinical doses is sometimes difficult because during clinical trials these may change from the anticipated clinical dose. If possible also dosages should be used that induce toxic findings in the animals, therefore, a multifold (at least 100-fold) of the first anticipated human dose may be determined. If a pharmacological effect can be measured in the animals this can also form a basis for dose selection in the toxicity studies.

A flexible strategy is therefore required which must take into consideration indication in the human patient, life-threatening or not, intended use, administration route, dosages, frequency of application, and duration of the therapy, and the pharmacological profile of the factor case-by-case.

On the other hand, over a period of many years much experience has been obtained in testing the safety of biological drugs, e.g. human plasma proteins. This has led to the development of safe drugs and forms a good foundation on which to base appropriate tests for these new recombinant preparations. This experience has been supplemented by the results of toxicity studies with many recombinant drugs during the last years.

TEST PROGRAM

In the acute studies with single administration, by the route intended to be used in man, parameters studied are clinical observations, body weight and autopsy findings, supplemented by selected laboratory tests.

The repeated-dose toxicity experiments are usually carried out in a relevant rodent and/or a non-rodent species. The dosages employed are normally the single human dose and

multiples of this dose, administered by the route intended for clinical use. The duration is usually to last for as long as requested by the clinical application, or up to such a time as an immunological response occurs which makes continuation of the study inappropriate. With hematopoietic factors the normal duration is 2-4 weeks eventually followed by a recovery period with some of the animals. Endpoints to be studied and autopsy and histological examinations are chosen on the basis of current guidelines for standard toxicity studies. In addition pharmacokinetic and immunological tests should be carried out.

The test on local tolerance at the injection site is performed by the usual methods used for other drugs.

Some of the most important experiments are the pharmacological safety tests - i.e. the effect of the factors on physiological body functions and organ systems. These should include the cardiovascular and the respiratory systems to exclude the possible effect of vasoactive substances by partial degradation of the polypeptides in relevant animal models.

In the majority of cases tests for mutagenicity, teratogenicity and carcinogenicity are not necessary. Such studies should be included in justified cases only.

There is no clear rationale for the conduct of mutagenicity tests on naturally occuring peptides or those produced by recombinant DNA technology. No reports are available that hematopoietic growth factors may interact with the DNA. For regulatory purposes perhaps some in vitro tests in mammalian cells could be selected.

It is unlikely that meaningful or realistic data could be obtained from experiments on the carcinogenic potential of these factors. It cannot be excluded that growth factors may have a tumor promoting potential leading to a possible risk especially after therapy with known mutagens or carcinogens (e.g. cytostatic drugs). But at the moment no validated models exist to exclude this possibility.

Selected studies on possible reproduction toxicity should be performed when relevant animal models exist or when factors may have additional functions on the generative organs. For example CSF-1 uterus organ levels are increased during pregnancy and effects on implantation of the ovum has been detected in

mouse experiments (7).

TOXICITY STUDIES WITH r GM-CSF AND r EPO

The mainly species-specific recombinant human GM-CSF stimulates granulocytes and macrophages after cancer chemotherapy, bone marrow transplantation, radiation etc. Acute and 30-day subacute studies were performed in Cynomolgus monkeys, single-dose experiments also in the non-relevant rabbit. In vitro mutagenicity tests included two studies in mammalian cells. Examinations of pharmacological safety endpoints were conducted in Cynomolgus monkeys. The influence of low and high doses on circulation, respiration, hematology, coagulation and serum chemistry parameters was studied. Additional experiments included tests on antibody formation in different species, on pyrogenicity and on the absence of E. coli proteins from the cell culture process.

Recombinant human erythropoietin is a non species-specific glycoprotein from murine fibroblasts. Main indication is the therapy of anemia due to chronic renal failure.
Acute studies were performed in mice, rats and rabbits, subacute 30-day experiments in rats and Cynomolgus monkeys. Reproduction studies were conducted in rats (segments I, II, III). In vitro mutagenicity tests, studies on local tolerance, absence of cell culture proteins and antigenicity supplemented the safety data set of this product. Safety pharmacological experiments in monkeys and rats excluded the adverse effect on vital organ functions.

CONCLUSION

Animal studies for the assessment of the toxicological profile of hematopoietic growth factors are only meaningful if they provide relevant information to their use in humans. It requires detailed information on their structure, their pharmacodynamic and pharmacokinetic potential to define the risk for adverse effects in preclinical experiments.

Toxicity testing should not follow rigid guidelines, it must be adapted to the properties of these factors case-by-case. What tests should be conducted must be decided pragmatically.

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Discussion - TOXICOLOGICAL DEVELOPMENT OF HEMATOPOIETIC GROWTH FACTORS

J.A. Galloway

I would like to make a comment about immunogenicity and antigenicity. I think that all of us would like to avoid this in our recombinant products. On the other hand, antibodies are not necessarily bad. Immunotherapy is an accepted intervention for the treatment of allergic diseases in man. In addition in the University Group Diabetes Program where patients received beef insulin for ten years and developed antibodies, they showed no long therm effect ascribable to those antibodies.

H. Ronneberger

The problem is that different regulatory agencies may react in different ways to the same problem. Some may be quite pragmatic (e.g. the F.D.A.), but some may show overconcern. For instance, in the case of GM-CSF, it was suggested that one would induce an AIDS-like state by the administration of small amounts of antigenic substances over a longer period

A.J.H. Gearing

Can I comment also that for many cytokines there are naturally existing antibodies.

R.G. Werner

I would like to make a comment on changes in production process. A change in the manufacturing process should only be considered to be significant if there is a change in product quality and the product does not meet the specifications, we have right now a real large number of quality control methods for proteins and also for impurities and I think we will be able to detect changes of product quality after a change of a production process.

You mentioned that there are natural variants of hirudin and that there are also second generation hirudins. Is there any improvement in specificity or activity compared to the natural compound?

H. Ronneberger

What is a minor change and what is an essential change you can discuss it with the authorities and if you have, for example, another purification step, of course, this is

a very essential change of your product; your product is characterized by the production procedure. Perhaps it is nonsense, but nobody knows it exactly. This is always difficult to decide.

M.M. Reidenberg

As a somewhat disinterested observer to this, it seems to me that what these studies are doing is really establishing safety of the regulators and of the company rather than of the product. It seems as if we are asking the regulators what to do, as if they and we are not part of the single scientific community really investigating a brand new classification of drugs and trying to figure out what to do. And I would wonder if there is any possibility of accumulating this experience over these next few years in a systematic way so that one can review the subject in two years time or three years time and contribute to determining what should be done based on experience and knowledge so that a meeting such as this in 1993 wouldn't end up with the same kind of discussion and no scientific advance.

H. Ronneberger

Most authorities, I think, have no real experience with these products, and they want to be on the safe side and therefore they ask for these experiments and the companies have to do such experiments otherwise they will not get the approval. And if mutagenicity tests for monoclonal antibodies are required you have to perform these studies even if everybody knows that it is nonsense.

M.M. Reidenberg

I accept that as a state of the world right now, but what I am saying is that we need to think about how to make it better. In fields such as law, the academic lawyers write law review articles (at least in common law countries) that review cases and end up with an analysis that is then used to help advance law, as it has to deal with problems that never existed before. I would hope that in medicine we can do the same thing. We should be accumulating data now to indicate that certain things that regulators are now requiring for the safety of the regulators do not make scientific sense. But unless somebody can bring together 50 or 70 such examples where this was done and it doesn't make sense, three years from now they will continue to require the same things, because they have no scientific support for no longer requiring them. The data that we are talking about exists in company files; usually it's not made part of the peer reviewed literature and so an academic doesn't have the access to the data as things

stand right now in order to write the equivalent of an academic law review article. What I am requesting or urging is that we change that so that we will be able to have science evolve and regulatory science evolve so that it will make sense in the future, because as things stand right now this identical meeting held three years from now would end up with the identical kind of complaints, the identical kind of data and no advance in the identical kind of excessive costs in development for which there is no value.

P. du Souich

By prerogative of the chairman, I would like to add the following: in Canada, the preclinical and clinical studies, and even the review process, are facilitated because industry, in agreement with the government, may create a panel of scientists to evaluate the progress of the dossier. The panel reports to the government. The members of the panel are not involved in the development of the drug so to avoid conflicts of interest, and will only act as referees to evaluate all aspects of the drug. This procedure fastens the development of the drug and its acceptance by the government.

R. Ronneberger

Yes of course I will include also the Canadian authorities to what I said about the FDA, it is very similar.