The clinical pharmacology of biotechnology products
M M Reidenberg, editor

PROTEINS IN SEARCH OF A DISEASE

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Introduction

It's now not clear to me what on earth I was thinking when I accepted to talk about this subject. However, given that this was requested by the Esteve Foundation in the context of their symposium entitled :"*The clinical pharmacology of biotechnology products*" and that many of you are pharmacologists, I've somewhat nåively assumed that at least part of the subject of the title concerns pharmacology.

Are protein drugs really so pharmacologically different from organic chemical drugs? Perhaps not as much as some would think and even then not in the way implied by "looking sperendipitiously for an indication "!.

Let us explore what we understand by disease specificity and how conventional medicines are used. By comparing the labelling of many proteins with those of conventional drugs it will be possible to show that similar strenghts and weakness exist regarding their pharmacological predictiveness or not.

In order to discern whether protein drugs are really so different pharmacologically, we shall firstly review together the history and present role of proteins in medical practice, then examine certain elements which affect our attitude not only to drug development but also to their therapeutic application.

Unless otherwise stated, the use of the word protein refers to recombinant produced.

History of Protein Drugs

Non-recombinant protein drugs are as old as contemporary medicine itself if one takes Jenner's attempts at vaccination as *prima voce.* In the meantime many more injectable protein based medicinal preparations have been used and these include:- blood transfusion, plasma and plasma-derived products, Factor VIII, organ extracts , tissues isolated hormones, vaccines, preparations for immunisation and many more.

Even today the level of protein drug development is still very significant both recombinant and conventionally derived. As a rough measure of the level of importance, in the USA, in 1989 of the 37 New Drug Approvals, 23 were conventional (62%) and 14 were protein (38%) [1]. The list of protein drugs is shown in table 1.

This is probably similar to the situation in most developed countries and indicates that the number and proportion of protein drugs being approved for registration is a major component.

Table 1 FDA Approved Protein Drugs in 1989 [1]

Drug	Indication
Epogen*	anemia associated with chronic renal failure,
Alferon N	condyloma acuminatum,
Eminase	acute mycardial infarction,
Fluosol	prevent of diminish myocardial ischaemia during PTCA,
ATnativ	hereditary antithrombin-III deficiency,
Cryoprec. AHF	coagulation factor replacement,
Engerix-B*	prevention of hepatitis-B,
PedVax HIB	prevention of Haemophilus influenza type B,
Vivotif Berna	immunization against typhoid,
Oculinum	blepharospasm and strabismus,
HIVAB HIV-1	screening test for anti-HIV-1 antigen,
HIVAG-1	diagnostic/prognostic test for HIV-1 antigen,
UBI/OlympusHIV-1 EIA	test to detect anti-HIV-1 in serum or plasma,
Histatrol	positive skin test control.

* Genetically engineered.

Although not all embracing the spectrum of indications is broad. Recombinant protein drugs are still in the minority compared to more conventional methods for extracting and purifying protein drugs. Presumably this is being radically changed and it will not be long before most protein drug manufacturing needs will be switched to recombinant technology, except of course for complex drugs such as blood.

The use of protein drugs is not restricted to any field of medicine. Some of the more common protein drugs shown in table 2 reflect the broad medical applications (not indicated). There is no major area of medical practice where proteins are not at one time or another used in therapy.

Table 2 Some Commonly Prescribed Protein Drugs

- Insulin
- Calcitonin
- Vasopressin
- Growth Hormone
- ACTH
- Interferon Alfa
- Interferon Beta
- Interferon Gamma
- Interleukin-2
- Erythropoeitin
- OKT3 MAb
- Anti-lymphocyte serum
- Collagen
- Hyperimmune gamma globulins
- Vaccines
- Plasma Products
- Factor VIII
- Blood Transfusion

This in no way is an exhaustive list but makes the point about the commonness of therapeutic use of especially non-recombinant proteins.

Current research activities are enormous to the point where it is difficult to quantify. More than a hundred and fifty different human and non-human recombinant proteins are currently being evaluated in clinical trials in Europe and the USA. To give a brief overview just the pharmacological categories are given below. In some categories there may be dozens of substances (see table 3).

Table 3 Various Protein Drug Categories Under Clinical Investigation.

- Interferons *
- Growth Factors *
- Promotors of Cellular Differentiation
- Monoclonal Anibodies
- Vaccines
- Hormones*
- Anti-inflammatory
- Modulators of Tissue Matrix
- Immunosuppressives
- Immunopotentiators
- Coagulation Factors*
- Anti-Oncogenes

*Antagonists also being evaluated

Some of the most exciting modern protein drugs are growth factors which include the haematologic growth factors:- erythropoetin, G-CSF, GM-CSF, IL-3, thrombopoetin,IL-2 and IL-1. Among the non-haematopoeitic growth factors worthy of special attention are the epidermal growth factors and their inhibitors, nerve cell and bone growth factors which are likely to lead to important therapeutic agents.

Protein drugs are not new to medicine. They have always been used in the treatment of disease even though the quality of their production left, at times, much to be desired. The advent of recombinant technology has not only considerably increased the scope of discovery and potential application, but has also rendered the whole approach more pharmacologically acceptable. Many modern protein drugs could not emerge from the obscurities of experimental science without the advent genetic engineering.

Mono- and Polymodal Origins of Disease

Many clinicians feel more comfortable treating a disease with a single known aetiology with a specific therapy. Often this is not always possible. Polymodal disease both interms of aetiologies, pathogeneses and multiple non-specific therapies are not rare.

There is a widely held belief that if a disease has a specific cause, more effective therapy is possible compared to developing therapies for

polymodal or multi-faceted pathologies. In fact this is not always as simple as it sounds. When the cause of a disease is not known much confusion and uncertainty may dominate the clinical picture including its treatment. When the cause is finally identified and the appropriate specific therapy discovered, all becomes so simple !

To show how such simple considerations affects ones feelings as a clinician an unusual lisiting according to monomodal and polymodal origins of disease is given in table 4.

Table 4 Some examples of Mono- and Polymodal Diseases

Monomodal	Polymodal
Pneumococcal pneumonia	AIDS
Phenylketonuria	Osteoporosis
Pernicious anaemia	Crohn's Disease
Acromegaly	Dementia
Trisomy 21	Diabetes
Scurvy	Rheumatoid arthritis
Xeroderma pigmentosa	Leprosy

Before the spirochaete responsible for syphillis was identified there were some very varied and bizarre explanations for the multitude of clinical syndromes associated with chronic infection. Even after it had been identified but penicillin was not yet discovered, therapies were both complex and troublesome [2].

The lack of an effective treatment for AIDS, even knowing that the disease is caused by HIV virus, is probably clinically more frustrating than treating diabetes with insulin which is effective in controlling some acute aspects of the disease without curing it.

Protein and non-protein drugs are both used to treat monomodal and polymodal diseases.Monomodal diseases treated with proteins, include human growth hormone to treat pituitary dwarfism, erythropoetin to treat EPO deficiency in renal dialysis patients and t-PA to reverse the process of early thrombsis in acute myocardial infarction. Whereas organic chemical drugs are used to treat polymodal diseases such indomethacin in rheumatoid arthritis, tetracycline in acne, steroids in atopic eczema just to mention a few. Similarly alpha interferon is used to treat chronic hepatitis B, chronic myeloid leukaemia and chronic granulomatous disease (polymodal disease and therapy). Anexate reverses benzodiazepine induced sleep [3], penecillin cures pneumococcal pneumonia, antihistamines cures anaphylaxis, all examples of monomodal diseases and organic chemical drugs.

Hence no apparent inherent difference with regard to the simplicity or complexity of the diseases treated by non-protein and protein drugs.

Mechanistic and Specific Target Approach to Drug Development

Some types of drugs emanate from a discovery process that might be discribed as mechanistic whereas others clearly do not (see table 5). Certain pathologic mechanisms are less frequently or not at all used as the primary driving force for drug discovery, often because they are too complex or their applicability unproven. Hence it will be assumed that the discovery of a new anti-inflammatory drug will be active in many different disease states where inflammation is a dominant factor, whereas a drug found to be active against breast tumours may not necessarily be active against lung cancer or leukaemia.

Table 5 Pathologic Mechanisms Used and Unused in Drug Discovery

Commonly Used	Uncommonly Used
Pain	Malignancy
Inflammation	Congenital abnormality
Edema	Ageing
Anaphylaxis	Psychosis
Immunological Adjuvant	Pappillomatosis
Microorganism growth	

Specific target directed drug discovery programs are highly successful if the target is appropriate. Targets can be of all levels of molecular complexity from a heavy metal such as magnesium or calcium, or an enzyme, to a nuclear receptor or a plasma membrane receptor and even an entire organism such as a virus or bacterium. Some examples of these are given in table 6.

Table 6 Examples of Specific Targets for Drug Development.

Organic Chemistry	Biotechnology
Chemotherapy	Gamma interferon receptor *
Specific Proteinase inhibitors	Thrombin
Angiotension converting enzyme	Hematopoietic growth factors
Monoamine oxidase	HIV/CD_4 receptor
Prostaglandin synthetase	MAb against cell or soluble antigens
Analogues of active drugs	Hormone & coagulation deficiences
Histaminic receptors *	Epidermal growth factor inhibitors
	Vaccine

* antagonists

Rapid "chemical" progress in both conventional drug and protein based discovery can be derived from this highly specific targetted orientation. Pharmacologic and therapeutic progress depends on the appropriateness

of the target to the disease process and whether its inhibition or stimulation is clinically acceptable.

These approaches are used in the discovery of both protein and conventional drugs. The real difference arises with proteins that have species restricted activites, whereby certain animal models or receptor binding experiments cannot be used outside a restricted range of animal species, as is seen with say gamma interferon [4] but not with erythropoetin [5].

There are of course a number of specific fundamental differences on how one discovers different types of proteins, or steroids, or sugars, or protein kinase inhibitors from a chemical viewpoint, but this exceeds the domain of pharmacology.

Modes of Treatment

Surgical practice and thinking had a major impact on early therapeutics. At one time surgical practice was dominated by removal. Removal of diseased or traumatised tissue. Of course this has changed, surgery is now much more sophisticated. Surgeons still spend a lot of their time removing tissues and organs, but in addition they repair, they restore function and they even replace diseased organs or parts of them.

Physicians however did not like to think of themselves as "removers " but rather as modifiers and replacers. Continuing in this very simplistic approach, modern medical treatment may be classified as therapy by:

- Replacement
- Removal
- Blocking, and
- Stimulation,

some examples are given in tabels 7 and 8.

Table 7 **Functional Classification of Medical Therapy: Replacement and Removal.**

Replacement	Removal
Hypothyroidism	Calories in obesity
Pernious anemia	Sodium in essential hypertension
Scurvy	Glutamine in hereditary intolerance
Kwashikor	Tryamine in Migraine
BT in haemolysis	UV light in Xeroderma pigmentosa
Pituitary dwarfism	*Immune Complexes by plasmapharesis*
G-CSF in Neutropenia	*Antigens in allergy*

Footnote: therapies in italics indicate protein therapy.

It would be difficult to say from the examples chosen above that there were major differences between the way protein and non-protein drugs are used, both the pharmacologic and the therapeutic modalities seem to be very similar.

Table 8 **Functional Classification of Medical Therapy: Stimulation and Blocking.**

Stimulation	Blocking
Caffeine in Narcolepsy	ACE inhibitors in Hypertension
Ephedrine in Asthma	H_2-receptor in gastro-duodenal ulcers
Isoprenaline in Asthma	H_1-receptor in allergy
Bisacodylum in constipation	Proton pump blocker in GI ulcers
Androgens in Wasting diseases	5HT blockers in Migrain
GTN in angina pectoris	Pilocarpine in Glaucoma
LHRH agonist in Ca Prostate	ß-blockers in hypertension and anxiety
Interferon alfa 2 antiviral	Heparin in blood coagulation
	IL-1 or G-IFN receptor antagonists

Footnote: therapies in italics indicate protein therapy.

Similarly if one compares the package insert labelling of a selection of conventional and a selection of protein drugs (table 9) it really isn't very difficult to find examples of drugs that have a narrow indication label and those that have a broad one. From this viewpoint also it is not possible to describe protein drugs as fundamentally so different in their pharmacologic development.

Conclusions

Protein drugs are no more or no less in search of a disease than their organic counterparts. The availability of meaningful and predictable preclinical and clinical pharmacologic models is equally sparse for both. Modalistic and mechanistic analyses show that protein drugs are in fact evaluated and developed in a similar fashion to traditional pharmaceuticals.

Protein drugs are not new to medicine and in fact have been around for more than a century whereas biotechnologically produced drugs have only been around for just over a decade. They have a broad application in medicine and rather than be dismayed by the advent of genetic engineering, the improved quality and quantity of these protein drugs both as substances as well as preparations, is a real pharmaceutical advantage. Certain types of protein drugs could only exist because of this technological advance. Of course there are new problems, but these are not proving to be insuperable.

Protein pharmacology however, is different in a number of important respects and are worthy of consideration. Protein drugs may modulate specific cellular functions, for example: 1) growth factors not only of the reticulo-endothelial system but probably for every tissue in the body including nervous tissue, 2) anti-oncogenes, 3) suppression of Philadelphia positive clones in CML; whereas others modulate biological

Table 9 **Types of Package Insert Labelling Real & Imaginary for Conventional and Protein drugs**

Drug	Label
ACE Inhibitor	Hypertension and CHF
Levodopa	Parkinson's Disease
Aminophylline	Asthma
G-CSF	*Congential & iatrogenic neutropenia*
EPO	*EPO deficient anaemia*
anti-LPS MAb	*Endotoxic shock*
anti-TNF MAb	*Shock & Cachexia*
5FU	Ca.breast,colo-rectal ca., Pancreatic ca.,carcinomas of H & N., Ca.bladder and ca. Prostate
Prostaglandin Synthetase Inhibitor	Rheumatoid arthritis,Osteo arthritis, Ankylosing spondylitis,acute Gout, Uveitis,Pleurisry,Pericarditis, Bartter's Syndrome, Patent ductus arteriosus in neonates.
Corticosteroids	Anti-inflammatory, Leukaemai and lymphoma, Asthma and Eczema, Urticaria, Granuloma, Hypercalcaemia...
Interferon alfa-2	*Hairy cell leukaemia, Chronic myeloid leukaemia, Myeloma and lymphoma,Renal Cell Ca. Malignant Melanoma,Kaposi's Sarcoma in AIDS, Laryngeal pappilomatosis,Chronic hepatitis-B,Chronic hepatitis-C, Condyloma acuminata.*
Interferon Gamma	*Congenital Granulaomatosis,Rheumatoid arthritis, Renal Cell ca., Cutaneous Leishmaniasis,Leprosy, AIDS,adjuvant carcinoma.*

Footnote: therapies in italics indicate protein drugs.

mechanisms that can also be altered or influenced by organic or even inorganic substances, such as: 1) promotion of cellular differentiation, 2) inhibition of inflammation, and 3) inhibiton of immunity. It is interesting to note that hypothalamic and pituitary "controlling hormones" are peptides whereas the local tissue hormones they control are not. Peripheral "independent hormones" are peptides.

Exogenously administered proteins similar to their endogenous counterparts renders pharmacokinetics and metabolism evaluation

particularly challenging in those circumstances where endogenous levels vary physiologically or in disease. At this time protein drugs are administered topically or by injection. This is changing.

An appreciation of the complex cell surface receptor ligand interactions, post-receptor activation events and gene modulating activities of protein drugs is basic to understanding how they work.

Proteins play special roles in nature and disease. Protein drugs are special in many, but not all ways.

Acknowledgement

I am especially grateful to Sergio Enrill and the Esteve Foundation for providing me with a set of circumstances which forced me to reflect on this subject. As usual my colleagues provided me with a wealth of information and lively critique.

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Discussion - PROTEINS IN SEARCH OF A DISEASE

C. Dionne

I would like to comment on the problems at the earlier stages of drug development with, for example, FGF and FGF receptors. When we first started two or three years ago we were dealing with two FGFs and one receptor. Now it is much more complicated. Establishing a research programme that is directed during the early stages now has become much more difficult because there are so many options and mostly not enough time or hands to develop all those options.

L. Gauci

Yes, it is a problem related to the area in which you are working. Most clinicians would agree this area is very important. How it would actually turn out in a few years time we don't know. For example, supposing somebody comes along with a recombinant thrombopoietin, from the beginning we know what we want to do with that drug. We know what it should do and what it shouldn't do. We know how many patients we would want to be treating. It represents an easier risk investment type of decision. But no one could deny that the type of work that you are doing is very, very important, the full implications of which remain unknown. So, someway your work has to be financed to allow it to proceed until it is known what its therapeutic applications will be. But that is no different to the sort of problems of the people in the early days had working on prostaglandins.

W. Aulitzky

LHRH learned us, that one substance can have two totally different effects. A stimulating effect and a blocking effect. But this has not really anything to do with the substance or with the hormone itself, it has something to do with receptor phenomenon. Don't you think that the misunderstanding about the effect of the different interferons could also be due to the fact that we are dealing with hormones and that we do not fully understand the receptor phenomenon involved in those actions?

L. Gauci

Yes, I entirely agree with your comment and I would take it one step further. Interferons stimulated a very interesting study aimed at understanding what happens at membrane receptors. At one time we had the very naive idea that down-regulation was responsible for loss of activity but this would appear not to be true. In order for

something to be active it has to have a receptor. The binding to the receptor however is not enough, it's got to activate it and stimulate post-receptor activation events.

W.M. Wardell

I'm not convinced that the pharmacology and the clinical pharmacology are really "new" at least the principle. Isn't the position still in part that we have new drugs, and we are finding clinical facts about those drugs?

L. Gauci

I think there is a new pharmacology and I don't think it is restricted to proteins. For instance, I am very impressed with what is happening in the area of sugar chemistry and I think we are going to have to learn about the pharmacology and applications of sugars.

J.A. Galloway

I think that an exceedingly important term in the equation of future drug development and medical treatment will be the impact of the results of mapping the human genome. It seems likely to me that this could well be the topic of an Esteve Symposium before the end of this century.