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The relevance of pharmacokinetics in the development of biotechnology products

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SUMMARY

Biotechnology derived medicines will have an increasing impact not only upon medical practice but also upon the working lives of many pharmaceutical scientists. Whilst such medicines may be viewed as highly sophisticated to the clinician and scientist, the consumer will still rightly demand that they are both efficacious and safe. Impacting as it does upon all phases of drug development and facilitating quantitative relationship between administered dose and systemic drug concentration, pharmacokinetics has an important role to play in the development of **all** medicines. Bioanalysis is an essential prelude to any pharmacokinetic investigation. For many biotechnology products the immunoassay and bioassay methodologies employed are often relatively non-specific and imprecise and yield assay dependent pharmacokinetic parameters. Other factors may also confound the pharmacokinetic evaluation of biotechnological products. In vivo binding proteins (including antibodies) may not only interfere with bioanalytical methodology but also have a significant effect on the pharmacokinetics and biological activity of certain macromolecules. Antibody formation is a particular problem in the preclinical evaluation of human proteins. Unlike most conventional pharmaceuticals, the rate and time of delivery into the systemic circulation is a fundamental component of the biological activity of many biological molecules.

INTRODUCTION

Whilst some business analysts have predicted that within 10 years the biotechnology industry will be of an equivalent scale to today's pharmaceutical industry, other analysts have been less bullish (1). Volatility appears to be a byword within the biotech sector, but without doubt over the next 10 years we will see several biotechnology companies become major league players and for those pharmaceutical scientists currently working within the traditional pharmaceutical sector it is clear that biotechnology products will have an increasing impact upon their working lives. For society as a whole, the move to the treatment of ailments based upon a detailed understanding of imbalances or deficiencies of endogenous macromolecules or genetic material may be viewed as a significant advance in medical practice. For the individual patient, however, irrespective of the underlying sophistication, they will still expect their medicine to be efficacious and, above all, safe. Thus while biotechnology derived products and conventional drugs may differ widely in terms of molecular characteristics and modes of action, the key issues in their development, the ensurance of efficacy and safety, are identical. An outline of the drug discovery/development process is given diagramatically in Figure 1.

Pharmacokinetics impacts upon all stages of the drug development process, from the molecular structure-pharmacokinetic relationship considerations in drug discovery/design through to the increasingly prominent population pharmacokinetic considerations of phase III. The importance of pharmacokinetics in

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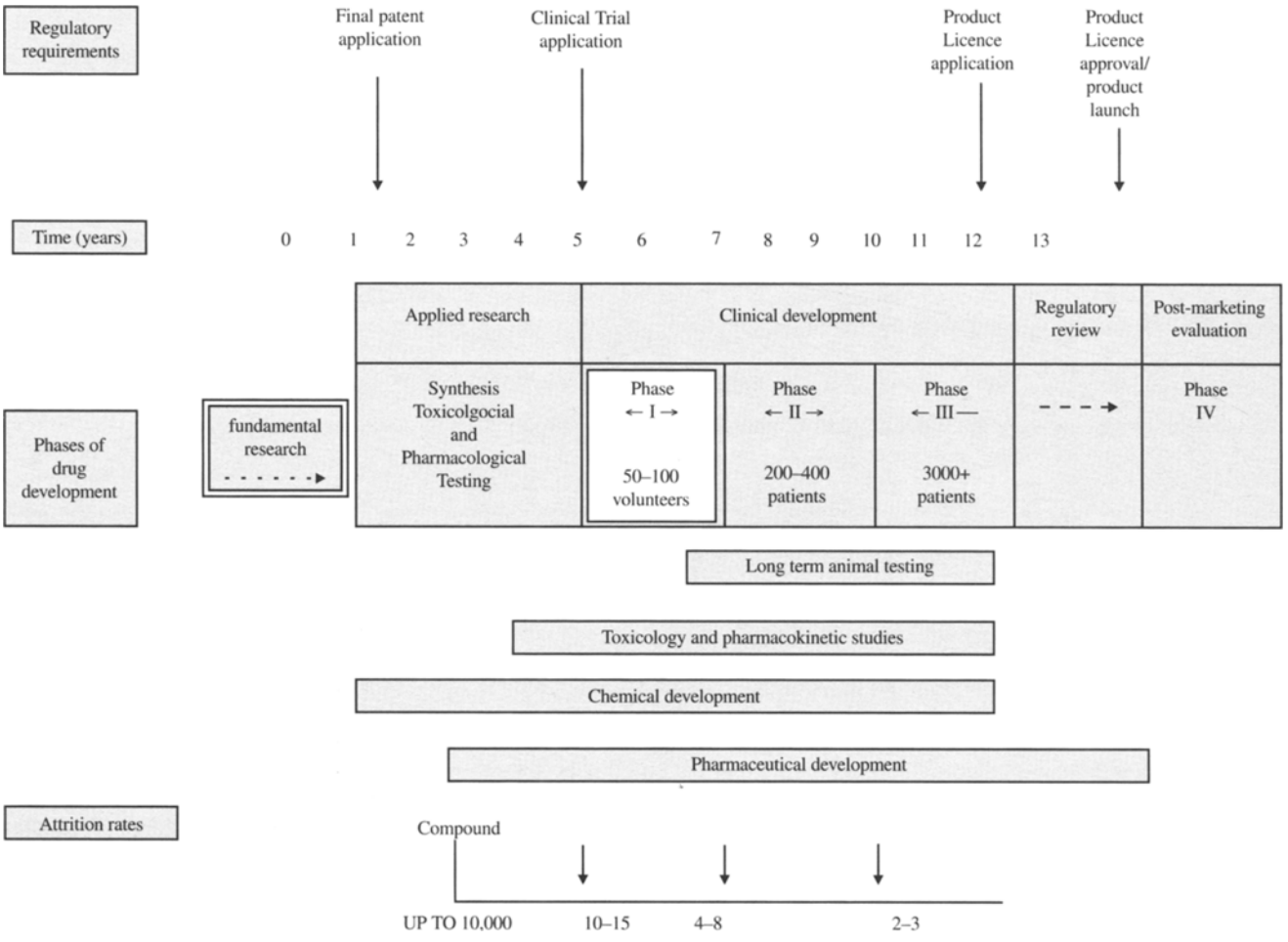

Fig. 1 : Schematic diagram illustrating the drug development process.

the drug development process may be illustrated by consideration of a New Drug Application (NDA) submission to the United States Food and Drugs Administration. Virtually every section of the submission makes reference to pharmacokinetics and there are three complete sections within the submission devoted to the topic. But why is pharmacokinetics seen as being so important? The simplest explanation is that pharmacokinetics relates the administered dose of a drug to its systemic concentration (at any point in time post-dosing) and this, in turn, is considered to be related to the biological action of the drug be it desirable (efficacy) or undesirable (toxicity). We can see, therefore, that in terms of the key elements of drug development (i.e. the ensurance of efficacy and safety) pharmacokinetics is pivotal. Efficacy and toxicity are, however, pharmacological/pharmacodynamic endpoints which potentially may be quantified directly and in turn related to the size of the dose and the time of ingestion. So is pharmacokinetics an unnecessary surrogate for biological activity? There have certainly been assertions that the importance of pharmacokinetics has been overstated at the expense of quality quantitative clinical pharmacology (2). Central to this debate is the issue of whether systemic drug concentration is always related to pharmacological response, for if it is not then perhaps one should question the routine use of pharmacokinetics in drug development. Intuitively one has to believe that pharmacological response is related to drug concentration. This does not however mean that the relationship between concentration and effect is simple or that the in vivo concentrations of interest are those within plasma or blood. Indeed, when one comes to consider certain biotechnology products, there may well be the need to modify our pharmacokinetic preconceptions. If one accepts that

pharmacokinetics has a role to play in the development of biotechnologically derived medicines, then one is immediately confronted with the problems of being able to quantify the molecular species of interest in vivo; the problems of bioanalysis.

BIOANALYSIS

Whilst accuracy, precision and sensitivity are important parameters to consider in the evaluation of any analytical method, perhaps the most fundamentally important parameter is specificity. An assay which provides high specificity is one which gives us assurance that measured concentrations relate exclusively to the species of interest and are not influenced by the presence of metabolites, endogenous substances, concomitantly administered drugs and so on. If we look back over the relatively short history of the development of pharmacokinetics, lack of analytical sophistication has at best been a limitation to, and often confounded, the development of the discipline. In the 1960s and early 1970s, before the development of modern chromatographic techniques, much reliance was placed upon the use of radioisotopically labelled drug material to facilitate the definition of drug kinetics. Pharmacokinetic evaluation based upon total radioactivity levels within blood were often misleading with regards to the true pharmacokinetics of the parent drug, due to the influence of circulating levels of radiolabelled metabolites (3). More recently, the recognition that the individual enantiomers of a racemic drug may differ dramatically, not only in their pharmacokinetic characteristics, but also in their pharmacological potency, or indeed action, has led to the claim that pharmacokinetic evaluation based upon 'racemic' drug concentrations and not those of the individual enantiomers leads to the generation of scientifically nonsensical results (4). Whilst recognising the need for analytical specificity, when dealing with biotechnologically derived products we are often confronted with the use of assay methods that are non-specific and, moreover, highly variable.

Modern chromatography based analytical methods are generally unsuitable for the in vivo quantification of macromolecules. The analytical methods most commonly utilised in the pharmacokinetic evaluation of such molecules are immunoassays, bioassays and the use of radiolabels.

For proteins, if the molecule contains a suitable amino acid such as tyrosine or lysine, it may be labelled externally with ^{125}I (5–7). If this is not possible, then the molecule may be labelled internally by growing the protein production cell line in the presence of amino acids labelled with ^{3}H, ^{14}C or ^{35}S (8). As with 'conventional' molecules there are many drawbacks in using radiolabelled drug in pharmacokinetic evaluation. Loss of the label from the molecule in vivo is a major problem (9). For [^{125}I]-labelled proteins, dehalogenation may occur. In studies with [^{125}I]-hGH (human growth hormone), free iodine was observed in plasma within minutes of dosing and was the major radiolabelled species within plasma within 2 h of administration of the dose (10,11). For internally labelled proteins, proteolysis may liberate labelled amino acids which, in turn, may enter the circulation or be incorporated into endogenous proteins (12,13). An inherent assumption in the use of a radiolabelled molecule is that it is only the radioisotope that differentiates it from the unlabelled molecule and that in all other respects it is indistinguishable from the 'cold' material. This need not, however, necessarily be the case as the tertiary structure of the macromolecule may be altered by the presence of the isotope (14) and, in turn, the in vivo metabolic behaviour altered (15). As with small drug molecules, pharmacokinetic evaluations based upon total radioactivity should be treated with caution.

Immunoassays fall into three major categories; radioimmunoassays (RIA), immunoradiometric assays (IRMA) and enzyme-linked immunosorbant assays (ELISA) (16–18). The latter of these methods (ELISA) is the most widely employed technique, primarily because it is non-radioactive. The major limitation of immunoassays is lack of specificity, with the method often being unable to distinguish between active and inactive forms of the macromolecule, metabolites and interfering endogenous and exogenous substances. As we will see later, endogenous binding proteins play an important role in the modulation of the biological activity and pharmacokinetics of certain macromolecular substances. By the very nature of their affinity and specificity for these macromolecules, the potential impact of these binding proteins upon immunoassay methods designed to quantify these macromolecules may be significant (19,20) due to them competing with the immunoassay antibody for the antigen (the molecule being quantified). In vivo antibody formation in response to administration of a foreign therapeutic protein may impact immunoassay methodology in the same way as endogenous binding proteins. Antibody formation is a particular problem in the preclinical evaluation of human proteins which are obviously foreign to the animal species being utilised in the preclinical programme. As with binding

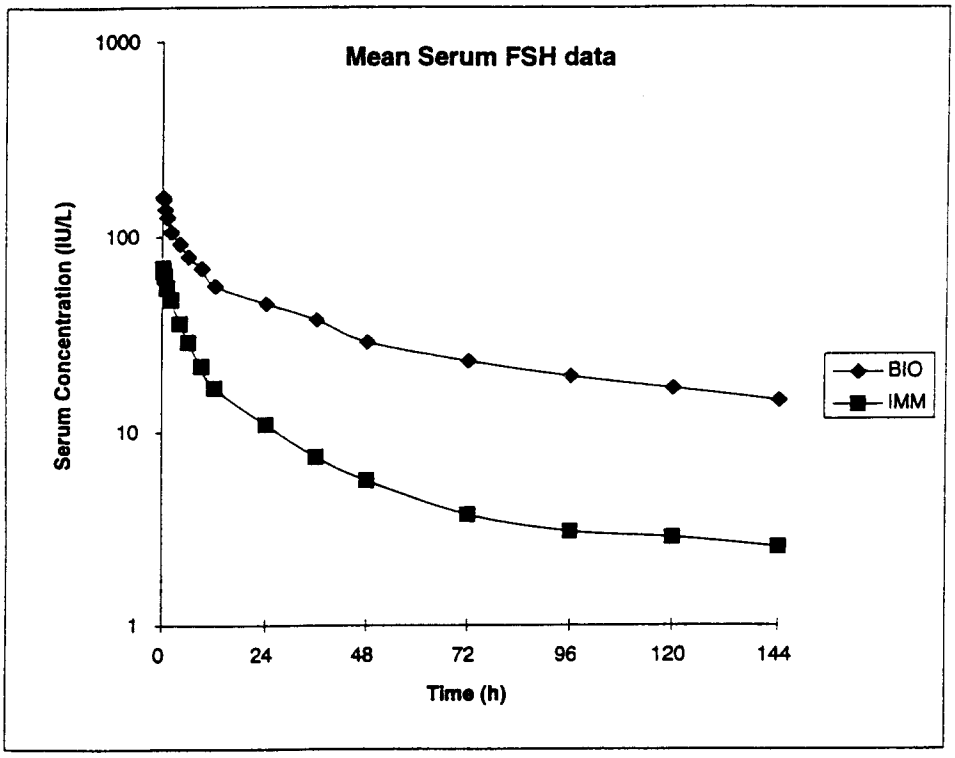

Fig. 2 : Serum FSH concentration-time curves following intravenous administration of 150 IU of r-hFSH to young healthy female volunteers with serum concentrations quantified using immunoassay and bioassay methods.

proteins, these antibodies may result in anomalously low concentrations of the therapeutic protein being detected by the immunoassay (21). Immunoassays are generally 'specific' to a particular analyte in a particular matrix (19,22). An adequate assay for the analysis of a particular analyte in serum may, therefore, be useless for the quantification of that analyte in urine or the matrix in which the molecule has been formulated. As we will see later, the inability to use a standard analytical method over all aspects of drug development can have significant implications on pharmacokinetic evaluation and interpretation.

Bioassays are cell or tissue based methods that may be performed in vitro or in vivo. The methods are often lengthy and offer an indirect method of quantification. As with immunoassays, bioassays

Table I : Mean pharmacokinetic parameters of recombinant human follicle stimulating hormone (r-hFSH) following intravenous administration at 3 dose levels to young healthy female volunteers.

	$t_{1/2}$ (h)			V (l)			Cl (l/h)		
	75 IU	*150 IU*	*300 IU*	*75 IU*	*150 IU*	*300 IU*	*75 IU*	*150 IU*	*300 IU*
Immunoassay									
	14.0	16.9	19.0	4.25	4.40	4.38	0.50	0.42	0.40
	± 8.82	± 5.00	± 5.88	± 0.58	± 0.43	± 0.61	± 0.14	± 0.07	± 0.07
Bioassay									
	28.0	29.4	32.0	2.22	1.73	2.19	0.25	0.12	0.12
	± 19.9	± 7.40	± 14.7	± 0.81	± 0.59	± 0.48	± 0.20	± 0.05	± 0.05

Table II : Absolute bioavailability of r-hFSH following intramuscular and subcutaneous administration of 150 IU to healthy female volunteers with serum concentrations quantified using either immunoassay or bioassay methods.

150 IU i.m.		*150 IU s.c.*	
Immunoassay	*Bioassay*	*Immunoassay*	*Bioassay*
60.8 ± 17.8	32.7 ± 13.6	74.3 ± 29.0	66.8 ± 38.9

suffer from lack of specificity due to the potential confounding influence of substances which may modulate the biological activity of the substance of interest. Intuitively, one might feel that concentrations of an analyte obtained by bioassay may be more appropriate (as opposed to immunoassay data) to relate to pharmacological response in any PK-PD modelling exercise, but this was not borne out with the PK-PD modelling of follicle stimulating hormone (FSH) (23).

As can be seen, all analytical methodologies commonly applied to the evaluation of the pharmacokinetics of biotechnology products have severe limitations which may significantly influence pharmacokinetic interpretation. As a consequence, common practice is to employ (sometimes out of necessity) more than one analytical method. This, however, can itself lead to difficulties. Figure 2 shows serum concentration-time profiles for human follicle stimulating hormone (r-hFSH) with concentrations derived from both immunoassay and bioassay procedures. Whilst the pharmacokinetics of FSH are independent of the assay method from a structural model viewpoint, the individual pharmacokinetic parameters do exhibit significant assay method dependent differences (Table I). Choice of analytical method may significantly influence the answers to specific pharmacokinetic questions. For instance, Table II shows the absolute bioavailabilities of r-hFSH following intramuscular (i.m.) and subcutaneous (s.c.) administration.

Earlier, the point was made that an important aspect of pharmacokinetics is the ability to quantitatively link the administered dose of drug to ensuing systemic concentrations. With low molecular weight 'conventional' drugs, we perhaps take for granted that the 'labelled' dose provided with the formulation to be administered is the pharmacokinetically relevant dose. For many biotechnology products this may be not so straight forward, as the analytical method suitable for analysis of the material of interest in vivo may not be suitable for the analysis of drug content within the formulation and *vice versa*. This may lead to significantly different assay method dependent 'doses' and the problem of having to choose which dose is pharmacokinetically relevant.

POTENTIAL IMPACT OF BASAL CONCENTRATIONS

Conventional drugs are often sufficiently discrete from a structural point of view from the molecular morass that is the human body, that bioanalysis of in vivo concentrations of the drug is at least a theoretical possibility. For many of the biotechnology derived drugs there is often already present relatively high and non-constant in vivo concentrations of the molecule in question. This not only presents significant bioanalytical problems but also complicates pharmacokinetic analysis and interpretation. Experimentally, one may be forced to utilise model systems wherein endogenous production of the molecule under investigation is absent or has been suppressed (24,25). Alternatively, pharmacokinetic analysis may necessitate the use of a pharmacokinetic model which incorporates within it terms which account for the baseline concentration-time characteristics of the molecule under investigation (26).

PRECLINICAL DEVELOPMENT

In the development of any medicine, a key stage is the transition from animal experimentation to the very first human exposure. There has been much debate as to how the size of the first dose to humans is selected. Preclinical pharmacokinetic/toxicokinetic data gathered in different mammalian species have, on occasion, been utilised to quantitatively scale

pharmacokinetic observations between species to predict key pharmacokinetic parameters of the compound in humans and thus help identify an appropriate dose-range for the first administration (27).

Whilst the supply of adequate amounts of a NCE (new chemical entity) for the early human studies is important for conventional drugs, it is a major issue for many biotechnology products with manufacturing costs being typically 100 × those of small molecules (28). If material is of limited supply for preclinical and early clinical development, then it is essential that investigations carried out at this stage in the development process are optimal for the generation of the maximum amount of relevant information. Interspecies (allometric) scaling has been successfully applied to several macromolecular drugs to expedite preclinical development (27–30). The preclinical development of human therapeutic proteins does, however, present with specific problems. Certain proteins such as human interferon-γ are species specific with regards to their biological activity. A further problem is that human proteins may be seen as foreign in other mammalian species and thus elicit an immune response which may dramatically influence the pharmacokinetics and pharmacological response (31).

POTENTIAL IMPACT OF ANTIBODY FORMATION

Formation of antibodies to therapeutic proteins undergoing clinical development can have significant impact upon the evaluation of their pharmacological and pharmacokinetic characteristics. Whilst an immune response to proteins foreign to man may be anticipated, with the advent of recombinant human proteins it was thought that immunogenicity would no longer be a major problem. This, however, has proven not to be this case with antibodies having been found to a variety of recombinant human interferons (32–35), recombinant human growth hormone (36), recombinant human insulin (37) and a variety of other recombinant human proteins (38). From a therapeutic viewpoint, antibodies which alter the biological activity of the therapeutic protein are of most concern and are known as neutralising antibodies. From a pharmacokinetic viewpoint, the pharmacokinetics of a therapeutic protein may be influenced by both neutralising and non-neutralising antibodies. One may envisage a scenario where the therapeutic impact of a neutralising antibody is a direct consequence of antibody induced changes in the pharmacokinetics of the therapeutic protein. This appeared to be the basis of the observations made by Queseda and Gutterman (39) with r-IFN-α_{2a}. In this study, 3 out of 37 cancer patients developed antibodies to the protein whilst receiving treatment, an observation which appeared to coincide with a significant increase in the clearance of the molecule. Antibody formation against a therapeutic protein need not always result in an acceleration of its elimination. Indeed, there have been several instances where the formation of an immune complex has slowed the elimination of the therapeutic protein. If the antibody is non-neutralising then the duration of biological activity may be extended as a consequence of the decrease in clearance of the 'protein' (immune complex). This is the case with human leukocyte IFN-α. When administered to rats, the formation of anti-human-IFN-α antibodies decreased its clearance by 15-fold without influencing the biological activity of the cytokine (40). As may be envisaged, this type of phenomenon (i.e. an antibody 'induced' decrease in clearance) has been used by molecular biologists as a means of prolonging the action of a therapeutic protein (41). Such an approach is only of use therapeutically, however, where the antibody formed is non-neutralising as a decrease in clearance of the therapeutic protein with obliteration of biological activity is useless.

The potential of antibody formation has implications with respect to clinical study design. If antibody formation is likely, then we cannot easily use single-dose studies of a crossover design even though alternative designs may be statistically less powerful. Similarly multiple-dose studies pose particular problems.

Immunogenicity may also be dependent upon the route of administration. Due to the precipitation of antigens which is known to occur following subcutaneous and intramuscular administration, these routes tend to result in more marked antibody response as aggregated proteins have been shown to be more immunogenic than soluble proteins (42).

POTENTIAL IMPACT OF BINDING PROTEINS

Interactions with in vivo proteins other than antibodies are an important factor to be taken into account in the pharmacokinetic and pharmacodynamic assessment of several macromolecules of therapeutic interest. For small molecules, the passive interaction with circulating proteins, such as albumin and α_1-acid glycoprotein, is well recognised. In general, the

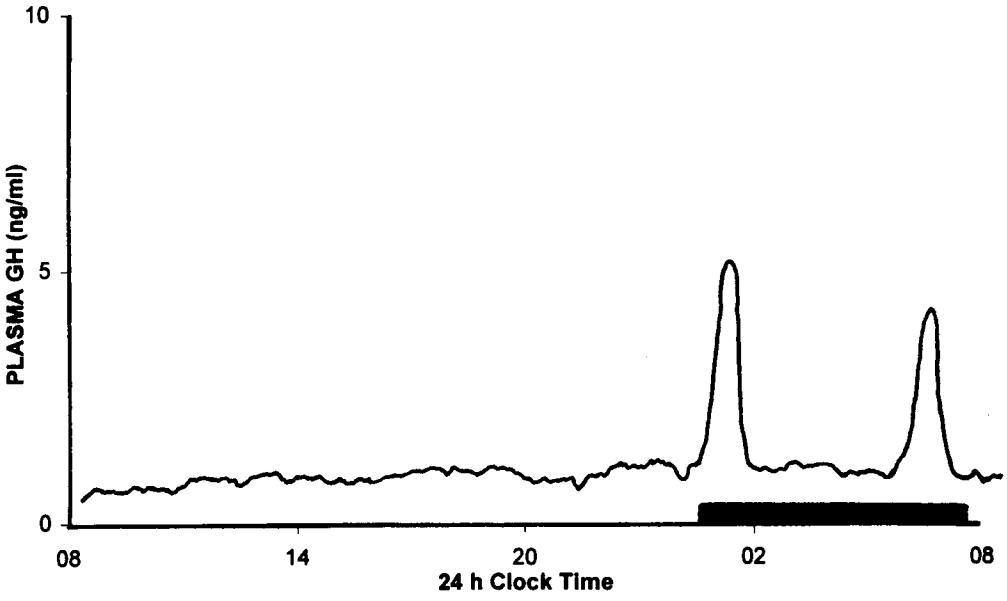

Fig. 3 : Endogenous plasma growth hormone concentrations measured over a 24 h period [based upon data provided in (62)].

binding of small molecules by these proteins is simply governed by the physicochemical characteristics (i.e. lipophilicity, pk_a, etc.) of the molecule and has no obvious intrinsic physiological purpose. This having been said, however, the potential impact of such plasma protein binding on the pharmacokinetics of small molecules is fairly well understood and may be taken into account in any pharmacokinetic analysis and interpretation. The generally held belief that only drug not bound to plasma proteins is able to distribute throughout the body and exert a pharmacological effect, or that plasma protein binding impairs the elimination of certain types of small molecule, need not hold true for all macromolecules. Specific in vivo binding proteins have been identified for a range of therapeutic proteins. These binding proteins may have an inhibitory or stimulatory effect on the biological activity of the therapeutic protein and may also significantly influence pharmacokinetics.

For many years, growth hormone (GH) was believed to circulate in an unbound form (43), however, over recent years, at least two GH binding proteins have been identified; one with a high binding affinity and the other with low affinity (44,45). GH binding protein binds approximately 50% of GH within the circulation and of this 50% approximately 90% is accounted for by the high-affinity binding protein (46,47). Bound growth hormone has a lower volume of distribution than free GH and is cleared approximately 10-times more slowly (48,49). There is still some debate on the role of GH binding proteins on the physiological action of GH. Whilst the extended half-life of bound GH may be seen as a mechanism by which in vivo activity is prolonged, it is known that GH binding protein competes with in vivo receptor binding. Indeed it is believed that the high-affinity binding protein is shed GH receptor (50). There are a variety of disease states and conditions which influence GH binding protein levels (47,51) and there are two types of short stature which appear to be associated with GH binding protein deficiencies (52,53) but it may be that the observed binding protein deficiency is merely indicative of a GH receptor deficiency which is the true basis of these particular types of dwarfism.

Insulin like growth factors (IGFs) have a variety of actions, being involved in anabolic metabolism, mitogenesis and the stimulation of cell differentiation. There have been identified numerous IGF binding proteins which appear to have modulatory function on localised actions of IGF (54). In addition, IGF binding proteins also influence the pharmacokinetics of IGF (55,56).

Specific binding proteins have also been identified for, amongst others, the interferons (57), interleukins (58,59) and tumour necrosis factor (60) all of which appear to have specific physiological functions and a potentially significant influence on the

Table III : Time taken to reach maximum serum concentrations of a range of peptide and protein drugs following subcutaneous administration*.

Drug	*Tradename*	*MW (kD)*	T_{max} *(h)*
Calcitonin	Cibacalcin	3.4	0.5
Insulin	Huminsulin Normal	5.7	1–3
GM-GSF	Leucomax	14.7	3–4
IL-2	Proleukin	15.5	3.3
IFN alpha-2a	Roferon A	19	7.3
Growth hormone	Genotropin	22	4–6
IFN gamma-1b	Imukin	20–25	7
EPO	Expert	30	12–18

*Data provided courtesy of Dr A Supersaxo.

pharmacokinetics of these molecules.

POTENTIAL IMPACT OF THE RATE AND TIME OF DRUG DELIVERY

For small molecules, the rate of input or the time of input into the body has, on the whole, been believed to be of little importance with regards to the pharmacological activity of the molecule. Indeed, formulation scientists during the 1970s and 1980s looked to remove (or at least reduce) pharmacokinetic variability brought about by rate of absorption by the development of zero order (constant-rate) drug delivery systems. Work with the calcium channel antagonist nifedipine indicated that the rate of delivery into the body has a significant influence on the pharmacological profile of the compound (61) showing that the rate of drug delivery may have important therapeutic implications. For biotechnology products, the rate and time of delivery is often an intrinsic feature of the therapeutic profile of the substance. Figure 3 shows the normal secretary pattern for growth hormone, with maximal secretion occurring within a few hours of the onset of sleep. Translating this observation through to therapeutic use of GH, it has been shown that a continuous infusion of GH is far less effective at promoting growth than a pulsatile delivery (63–65). Similar observations (intermittent as opposed to continual administration) have been seen for parathyroid hormone (66) and tissue plasminogen activator (t-PA) (67). Gonadotropin releasing hormone (GnRH) controls the in vivo production of follicle stimulating hormone and luteinising hormone. Whereas a pulsatile delivery of GnRH stimulates the production of FSH and LH, a continuous delivery suppresses production of these hormones (68).

ROLE OF THE LYMPHATIC SYSTEM

For most, if not all, protein and peptide drugs, the oral route of administration is not feasible due to GI enzymatic degradation of these molecules. The most commonly employed routes of administration for these substances are therefore i.v., i.m. and s.c. An important feature of several biotechnology derived products is that their biological activity is dependent upon the route of administration (69). One possible explanation for such observations relates to the pathway by which the drug enters the systemic circulation. Following s.c. administration, a molecule may enter the systemic circulation via the blood capillaries or via the lymphatics. Supersaxo and co-workers have indicated that as molecular weight increases, the lymphatics become the predominant pathway (70,71). This phenomenon when viewed from blood presents as an increase in time to reach maximum blood concentrations post-dosing with increasing molecular weight (Table III). These observations are of particular importance for those biotechnology products with biological targets within the lymphatics (e.g. interferons and interleukins). Indeed blood concentrations of such molecules may have little bearing on their biological activity.

CONCLUSIONS

The title of this article could be construed as an argument for or an argument against the role of pharmacokinetics in the development of biotechnology products. As the title is not posed as a question this perhaps gives a clue as to the concluding thoughts of the author.

If one were to simply view pharmacokinetics as a surrogate measure of pharmacological response, then perhaps one could question the relevance of pharmacokinetics in the development of biotechnology products. But pharmacokinetics offers much more than this. Whilst the article has highlighted potential problems, pitfalls and discrepancies associated with pharmacokinetic analysis and interpretation, without this knowledge our scientific and clinical understanding of the range of biotechnology products would be much weaker.

Undoubtedly, the major impediment to the development of pharmacokinetics in the biotechnology arena is bioanalytical methodology. Without specific, precise, accurate and sensitive bioanalytical methods our ability to undertake rigorous pharmacokinetic analysis is much compromised. Although it is not too long ago that pharmacokinetics relied upon administration of radiolabelled drug and measures of total systemic radioactivity, the major difference between then and now, however, is our ability to quantify biological response whether it be directly or via a therapeutic surrogate.

Wherever possible, therefore, biological response should be quantified and viewed together with pharmacokinetics. Although the relationship between pharmacokinetics and response is not always obvious for many biotechnology products, it is often through our attempts to understand this relationship that we uncover and come to understand more fundamental issues. Pharmacokinetics is certainly not a mere surrogate for therapeutic response and without pharmacokinetics we loose a potential handle on the mechanisms underlying biological activity.

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