Targeting of Drugs 2

Optimization Strategies

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PREFACE

The NATO Advanced Studies Institute series "Targeting of Drugs" was originated in 1981. It is now a major international forum, held every two years in Cape Sounion, Greece, in which the present and the future of this important area of research in drug carriers is discussed in great depth. Four previous ASIs of the series dealt with drug carriers of natural and synthetic origin, their interaction with the biological milieu and with ways by which the latter influences such interaction. The present book contains the proceedings of the 5th NATO ASI "Targeting of Drugs: Optimization Strategies" held in Cape Sounion during 24 June-5 July, 1989. A logical sequel to the last one, the ASI deals with strategies by which milieu interference curtailing the function of drug carriers is circumvented or removed.

We express our appreciation to Drs. R. Langer and E. Tomlinson for their valuable advice throughout the planning of the ASI and to Dr. G. Deliconstantinos who, as Chairman of the Local Committee contributed so effectively to its success. The ASI was held under the sponsorship of NATO Scientific Affairs Division and co-sponsored and generously financed by Smith Kline French Laboratories (now SmithKline Beecham), Philadelphia, USA. Financial assistance was also provided by CIBA Geigy (Horsham), Schering (West Berlin), Farmitalia Carlo Erba (Milan), Liposome Technology Inc. (Menlo Park), Pfizer (Sandwich), Dior (Paris), Syntex Research (Palo Alto), ICI Pharmaceuticals (Mereside), Boehringer (Mannheim), Wyeth (Taplow), Merck Sharp Dohme (Rahway), Sandoz A.G. (Basle) and Lilly Research Centre Ltd. (Windlesham).

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4. Targeting of Drugs: Anatomical and Physiological Considerations (eds. G. Gregoriadis and G. Poste), Plenum, 1988.

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BIOLOGICAL DISPERSION AND THE DESIGN OF SITE-SPECIFIC PROTEIN THERAPEUTIC SYSTEMS

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INTRODUCTION

A drug acts when it reaches its pharmacological site of action. However, ideal clinical effectiveness relies additionally on the right amount of drug reaching its site(s) of action at the right rate and frequency, on (often) a drug-free interval (at the receptor site), and on not critically interacting with non-target sites. Few drugs in use today attain such ideality. As more attention is paid to drug/receptor interactions, (often through the use of molecular modelling procedures and/or the use of cloned endogenous proteins which can act as templates for the designed fit of agonist or antagonist drugs), increased effort is being focussed on controlling the biological dispersion of drugs. This activity, which was once reserved almost exclusively for cytotoxic drugs, is an increasingly important aspect of the discovery and drug development process, particularly in the design of therapeutic proteins. Approaches to site-specific delivery include simple low molecular weight prodrugs activated at sites of disease, suicide enzyme substrate inhibitors; polymeric soluble and particulate macromolecular carriers; and unique site-specific therapeutic proteins.

Site-specific drugs and carriers are often trivially referred to as targeting systems or magic bullets. The magic bullet concept is a poor one, since it conjures up the image of an aimed missile have a defined and preordained trajectory. However, in reality, site-selectivity relies on the drug or carrier having a higher affinity for a particular feature of the normal or diseased body. Conceptually, it is more a case of "I don't know where I'm going to, but I'll know when I get there".

THERAPEUTIC PROTEIN SYSTEMS

Many polypeptides and proteins having unique pharmacological propertie are expected to be used clinically in the coming decades. These usually have regulatory or homeostatic functions, and include both endogenous polypeptides and proteins, and their (heterologous) derivatives. This latter class of molecules may be produced by inter alia, site-directed mutagenesis, proteolysis, ligated gene fusion, protein aggregation and/or conjugation with (other) biologically active effector functions (Tomlinson, 1989). Proteins can be considered as drugs (for example, drug/antibody complexes).

Targeting of Drugs, Edited by G. Gregoriadis *et al.*
Plenum Press, New York, 1990

Many of the proteins proposed for therapeutic use are glycoproteins whose biological disposition is primarily due to three properties: namely, their chemical and metabolic stability, size and shape, and surface features. The biological half-life of most polypeptides and proteins is short, due generally to a poor chemical stability, and/or rapid liver metabolism and kidney excretion. Also, the instability of paracrine- and autocrine-like mediators is largely due to their degradation by peptidases and proteinases in the vascular endothelium, liver, and kidneys, etc. Further, the terminal amino acids of proteins may serve to control their intracellular metabolic stability (and hence their intracellular residence time). Protein-engineering methods may be used to replace labile amino acids, with, for example, the oxidation-resistant amino acids alanine, serine or threonine, or to produce proteins having differing foldings, potentially leading to proteins which are protected from inactivation.

Pharmacology

For most therapeutic proteins the relation between applied dose and effect is highly critical, particularly as non-linear dose-effect relationships are often found (e.g. with parathyroid hormone, substance P and δ-sleep inducing peptide). As we and others, notably George Poste, have pointed out (Tomlinson, 1989), in selecting proteins for therapeutic use, little rational thought has been applied to their site of action, namely, whether the putative therapeutic protein is to act systematically (i.e. endocrine-like), or whether it is a mimic of an endogenous molecule that is normally produced to act locally (i.e. as an autocrine- or paracrine-like mediator). Endogenous endocrine proteins (e.g. hormones such as insulin), act over long distances from their site of manufacture; they are also stable in blood and, if relevant, their size and surface character enable their (specific) extravasation. However, paracrine-/autocrine-like mediators are produced and released to act locally, and/or have very short chemical half-lives. Such properties ensure that they do not give rise to untoward effects on non-target neighbouring cells. Autocrine- and paracrine-like mediators are often produced at sites of inflammation, tumors and injuries (e.g. transforming growth factors alpha and beta, angiogenenin, fibroblast growth factors, and epidermal growth factor, etc.). As described elsewhere (Tomlinson, 1989), in the site-specific delivery of these mediators, one needs to consider the issues of chronicity in the activation of cells (including their temporal localisation and responsiveness) and, since such agents may be acting as part of a polymediator cascade of events, also the staging sequence through which they act.

CELL RECOGNITION AND PROCESSING

The pathway to a pharmacological site may involve passage into and through various cells. To survive and to maintain their function, both normal and diseased cells take up and process numerous types of materials by a variety of mechanisms. The uptake of hydrophilic macromolecules at the plasma membrane involves invagination and vesiculation of the lipid bilayer to form vesicles. These processes have a putative applicability for cell-selection and cell-access by site-specific therapeutic systems (Tomlinson, 1986). Movement along biological pathways can be either passive or active and include cell fusion, fluid-phase pinocytosis, phagocytosis, and both constitutive and non-constitutive receptor-mediated endocytosis. The binding to specific and/or non-specific regions of cell surfaces can aid other processes which cause cell access of macromolecules - including membrane fusion and simple diffusion. There are two classes of vesicular routings, i.e. those which involve constitutive recycling, and those which occur upon a specific ligand/receptor interaction. For the former class, these processes occur independently of external stimuli, and are for the

purpose of imbibing, cell growth, and intra- and intercellular communication. Recognition and processing can be very sensitive processes, affected dramatically by slight changes in structure. For example, by analogy, an alteration in the gp 120 tryhptophan at position 432 of the HIV envelope can abrogate CD4 binding and thus affect its tropism (Cordonnier et al, 1989).

The capacity and kinetics of cell trafficking events are important considerations in the design of site-specific systems (as are the abundance, specificity, avidity and (cellular) fate of any receptor system). For the use of systems which rely on transport receptors to effect their site location, there is a need to know the dose/uptake relations. For example, studies on hEGF show an edxtraordinary dose-dependency of the pharmacokinetic behavior after iv administration in rats, due not to the saturation of excretion processes such as biliary and urinary excretions, but to the binding saturation of transport receptors (Murakami et al, 1989).

Site-specific delivery with soluble proteins relies on a combination of anatomical and (patho)physiological events, each bringing its own constraints and opportunities. These can involve either anatomically accessible and discrete compartments, as well as normal and dysfunctioning cellular processes of both a passive and an active type.

EXTRAVASATION OF MACROMOLECULES

To be effective, on occasions, macromolecular site-specific systems will need to leave the cardiovasculature in order to reach either extravascular-extracellular, and/or extravascular-intracellular target sites. Extravasation is under strict anatomical and (patho)physiological control. Hence, systems can either be incorporated into phagocytic cells which can extravasate, or pass directly through either interrupted endothelia, or through the cell barrier itself by exploiting fluid-phase and/or receptor-mediated, constitutive and non-constitutive cell transport processes.

Passage through Normal Endothelia via Passive Processes

The structure of the endothelium is complex and varies greatly in different organs and tissues. It is generally comprised of four layers, namely, the plasma membrane/plasma interface (which is formed by the glycocalyx of the cell and the proteins adsorbed onto it); the endothelium (a monolayer of cells which are metabolically very active and effect and monitor the bidirectional exchange of fluid between the plasma and the interstitial fluid); the basal lamina (which supports the endothelium); and the adventitia (a connective tissue which surrounds the lamina and fuses with the surrounding fibro-areolar tissue). Capillaries having a continuous endothelium and an uninterrupted basement membrane are the most widely distributed. Fenestrated capillaries are morphologically distinct from these, and are typified by having a very thin cytoplasm on each side of the nucleus (30-60 nm), and gaps of between 20-100 nm diameter at irregular intervals. Some tissues have sinusoidal endothelial membranes where the membrane is very thin and there is hardly any connective tissue separating the endothelial cells from the parenchymal cells of the underlying organ. These areas are often lined by phagocytic cells. Endothelial cells contain a large number of spherical vesicles of uniform diameter (plasmalemmelar vesicles). These are generally between 60 to 80 nm in diameter. Plasma molecules are selectively transported across the endothelium according primarily to their size, but their charge and their physicochemistry (i.e. hydrophilic/lipophilic balance) are also contributing factors. The capillary wall permeability for soluble macromolecules is well-documented. Soluble materials of less than 30 nm diameter are able to permeate through continuous endothelia (e.g. see Fig. 1). Rippe and Stelin (1989) have

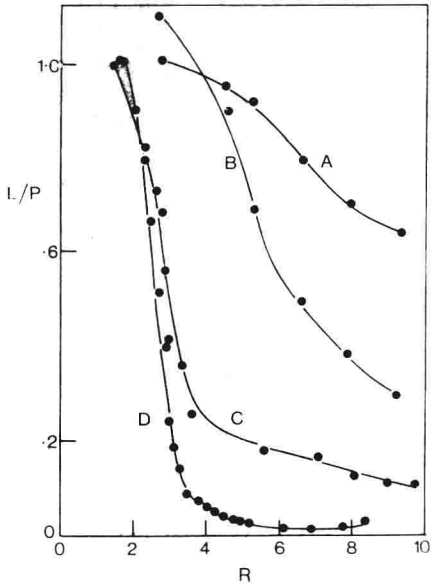

Fig. 1. Relationship between lymph-to-plasma ratios (L/P) for uncharged dextrans and their molecular radius (R, nm) for different organs in the rat; A-D are liver, intestine, leg and lung, respectively (from Tomlinson, 1987). (Reproduced with the permission of the copyright holder.)

recently examined the blood/peritoneal clearances of various endogenous solutes in patients undergoing continuous ambulatory peritoneal dialysis. They demonstrated that solute transport is compatible with a functional blood/peritoneal barrier consisting of a two-pore membrane containing both a large number of paracellular small pores of radius 4.0 to 5.5 nm, and a smaller number of larger pores of radius 20 to 30 nm. In addition, they found that whereas solutes smaller than 2.5 nm in radius permeated across the peritoneal membrane mainly by diffusion across the small pores, solutes larger than 4 nm were calculated to cross exclusively by unidirectional convection across the large pores. Further, molecules larger than 2.5 to 3.0 nm radius (approximately 25 kDa) were simulated to be lost from the peritoneal cavity by non-size-selective lymphatic drainage.

Extravasation of macromolecules occurs by diffusion and convection and transcytosis through the vesicle/plasmalamellar pathways. For macromolecules, the large proportion of extravasation is due to convection. This is related to the relative vascular and (interstitial) extravascular pressure, and is porportional to the rate of fluid movement from the vessel lumen to the interstitium. As pointed out by Jain (1989), this event is proportional to the surface area and the difference between the vascular and the interstitial hydrostatic pressures minus the difference between the vascular and interstitial osmotic pressures. Additionally, transmural arterial pressure has an effect on endothelial (percolation-driven) transport of colloidal particles (Fig. 2) (Chien et al, 1984). Since the osmotic reflection coefficient describes the effectiveness of the transluminal osmotic pressure differences in causing movement of fluid across an endothelium, we can write that the transport of a macromolecule across endothelia is characterised by the three transport parameters of (i) vascular permeability, hydraulic conductivity and reflection coefficient, (ii) surface area, and (iii) both the transvascular concentration and pressure gradients (Jain, 1987).

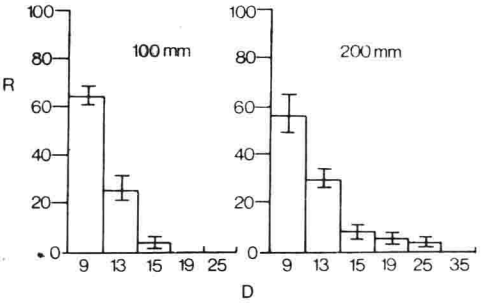

Fig. 2. Effect of transmural pressure (100 mm Hg and 200 mm Hg) on the distribution (R, percent) of Ag and Au colloid particles of different diameter (D, nm) found in the carotid endothelium and subendothelial space (in dogs) (Chien et al, 1984). (Reproduced with the permission of the copyright holder.)

Pathophysiological Opportunities for Extravasation

Inflammation. The hyperpermeability of endothelial barriers at various sites of inflammation is well established. This has been regarded as being of potential use for the selective delivery of anti-inflammatory drugs to inflamed extravascular regions. For example, in areas of inflammation induced by carrageenin, the accumulation of lipid microspheres of approximately 200 nm diameter around endothelial cells of blood vessels, and the penetration of these to the outer layer of blood vessels, has been reported. Inflammation can cause regional changes in the structure, chemical composition and permeability of the endothelium. Permeability changes appear to be due to the effect of histamine and bradykinin which act directly on the capillary venule endothelia vessel wall, with various other mediators (including leukotriene B4 and the complement enzyme C5), effecting a rapid interaction between venular endothelial cells and circulating neutrophils. It is unclear what such hyperpermeability means in terms of the pathogenesis of the underlying disease and the adequate retention of the carrier, particularly when one appreciates that inflamed sites often contain phagocytic cells. Interestingly, intravenously administered radiolabelled 'small' liposome particles can be used to image joints of patients affected by rheumatoid disease (Williams et al, 1986); though it has been found that when the disease is in remission, with no active synovitis, then accumulation of the radiolabel does not occur, suggesting that some accumulation is due to phagocytic activity.

Ischemia/hypertensive vascular lesions. An increased permeability in the endothelia is seen as an important factor in the pathogenesis of hypertensive lesions leading to infiltration and accumulation of plasma material. For example, in experimental malignant hypertension, colloidal iron and carbon particles of between 5 to 50 μm diameter are able to extravasate. Capillaries have also been shown to be permeable at sites of tissue ischaemia, both in the mesenteric artery and myocardium. However, this is not the case with other hypertensive states.

Tumor endothelia. Ultrastructural studies of both animal and human extravascular tumors indicate that a significant fraction of tumor blood vessels have wide interendothelial junctions, a large number of fenestrae and transendothelial channels formed by vesicles, and discontinuous and/or absent basal lamina. In addition, various tissue uptake studies have also demonstrated that the vascular permeability of tumors is significantly higher than where there is a continuous membrane such as in skin and muscle.

Table 1. Average Time for MoAb and Fab to move in the tumour interstitium (from Jain, 1989)

Distance	Average time	
100 μm	about 0.5 h	about 0.2 h
1 mm	" 2-3 days	" 0.5-1 day
1 cm	" 7 months	" 2 months

However, macromolecular drugs such as immunotoxins and drug-antibody complexes, extravasate very poorly and percolate minimally into tumor masses. Jain (1989) has concluded that the poor extravasation of macromolecules could be due to tumors containing regions of high interstitial pressure, coupled with a decreased vascular pressure. This serves to lower fluid extravasation, which in turn leads to low levels of macromolecules extravasating since their transport normally occurs primarily by convention (Jain, 1987). (As tumors grow, their interstitial pressure increases correspondingly.) Also, since large tumors have a relatively lower vascular surface area than small vascularised tumors, then this should lead to a lower transvascular exchange in large tumors compared to small ones (Jain and Baxter, 1988). After extravasation, the flux of a macromolecule in the interstitium occurs via diffusion and convection, the former being proportional to the concentration gradient in the interstitium, and the latter to the interstitial fluid velocity (which, in turn, is proportional to the pressure gradient in the interstitium). In normal tissues, the matrix is composed of collagen, elastin, nidogen, etc. within which is the fluid and a hydrophilic gel made mainly of polysaccharides. In tumors, the large interstitial space and low concentrations of polysaccharides favor the movement of macromolecules in the tumor interstitium (Jain, 1989). But this event does not appear to occur readily, as indicated by a heterogeneous distribution in tumors of administered exogenous macromolecules. It appears that two events serve to inhibit such movement. First, and especially for systems targeted to bind to a surface marker, the binding of a macromolecule to an antigen will lower its apparent diffusion coefficient, and second, because of the large distances and the nature of the network, large molecules such as immunoglobulins can take considerable time to diffuse (Table 1). Related to this is the question of the fate of any low molecular weight drug released from a macromolecular carrier at the tumor surface. Notwithstanding that the concentration gradient for such a drug will permit its diffusion in all directions, Levin and coworkers have computed that the time taken for low molecular weight drugs to diffuse from a well-perfused tumor shell inwards to a poorly-perfused tumor core is long (Levin et al, 1980). For example, even for drugs with high diffusion coefficients (in the region of 10^{-6} to 10^{-5} cm^2 sec^{-1}), to travel 5 nm into the tumor mass would take between 24 and 48 hours. To achieve even this would require high levels of drug to be constantly present at the surface of the tumor mass. It is evident that this could lead to systemic toxicity.

There have been numerous attempts to selectively deliver drug to a tumor target and to maintain it there using various recognition ligands. These include hormones, porphyrins, lectins, sugars and anti-receptor monoclonal antibodies able to recognise some feature on the tumor surface (such as transferrin and low-density lipoprotein receptors). It appears that many of the markers suggested for targeting are not tumor-specific (except perhaps oncofetal markers), but that they are present in an abnormal abundance. In addition, it is known that tumor masses have a clonal heterogeneity - which may preclude the approach of using selective recognition

Table 2. Some Key (Patho)Physiological and Biochemical Issues Affecting the Pharmacokinetic and Pharmacodynamic Behaviour of Site-Specific Macromolecules Acting on Extravascular Tumours

Exclusivity, abundance and trafficking capacity of surface markers
Clonal heterogeneity and biochemical resistance
Intra/extravascular location
Diffusion, convection and binding of drug and/or drug/carrier complex
Multiple sites of drug resistance
Lower hydraulic conductance
A reducing relative endothelial surface
An increasing interstitial pressure
Lower blood pressure
Slow interstitial diffusion
Membrane permeability
Toxic side-effects
Non-linear dose/response relationship
Chronicity of growth and responsiveness

ligands in site-specific drug delivery due to the possibility for the selection of cells which have a biochemical drug resistance.

Thus, access to solid tumors is highly limiting for the use of macromolecular drugs/carriers in cancer, because (a) in humans, the dilation of tumor blood vessels leads to a lowering in hydraulic (transmural) conductance; (b) as tumor masses grow, their relative endothelial surface area (for extravasation) is reduced; (c) interstitial pressure increases, further reducing conductance; (d) the interstitial movement of macromolecules in tumor masses can be in the order of days to move a millimetre; and (e) specific (antigen) binding (by an antibody) serves to delay this movement and to reduce tumor cell penetration. Table 2 defines many of the factors which affect the pharmacokinetics and pharmacodynamics of cytotoxic macromolecular drugs. Clearly, a complex interplay exists between all of these parameters. The overwhelming conclusion that must be drawn from this is that macromolecular drugs are inappropriate agents for directly treating extravascular tumors located in tissues having a continuous endothelium. The same arguments do not apply to accessible tumors such as those in the spleen or the blood. Attempts to improve on the selectivity of anticancer agents has been made by an indirect two-stage approach, in which a bacterial enzyme carboxypeptidase G2 (CPG2) is conjugated to a $F(ab')_2$ fragment (against a subunit of human chorionic gonadotrophin). After localisation (which, following on from the above discussion, must be minimal), a prodrug of a cytotoxic agent able to be selectively cleaved by the enzyme, is introduced (Bagshawe et al, 1988). In mice this has been shown to lead to a reduction in tumor growth.

RETENTION IN THE CENTRAL COMPARTMENT

In many cases, macromolecular drugs and carriers need to persist in the central blood compartment. This may be either because of a need to access a target (cell) within the cardiovascular system, or to remain in the central compartment long enough to be able to remain intact and to extravasate (via either passive or active means). Size, surface charge, chemical stability, and surface physical and physicochemical stability, are the most important features for achieving this persistence. For therapeutic (site-specific) proteins needing to remain in the blood compartment, two prime methods have been adopted. Namely, increasing the apparent size of the protein and/or reducing its (untoward) interactions with blood and tissue components.

Uptake by the Mononuclear Phagocyte System (MPS)

Recognition of macromolecular therapeutic systems by the immune system is mediated through physicochemical interaction. Frequently, opsonization by fibrinogen, fibronectin and other blood components, is a prelude to recognition and thence removal by cells of the formed complex; antigen-antibody interaction and Fc-mediated removal also occurs. Opsonized materials are taken into cells by engulfment after adjerence to, and vesiculation of, phagocytosing cell membranes. Both opsonization and adherence can be diminished if the attractive forces between the interacting therapeutic protein, blood macromolecule and, for example, a cell-surface macromolecule are diminished. Adsorption and adhesion are complex phenomena which are controlled by many factors including hydration, and electrostatic, dispersion and steric forces, and by other short-range interactions (Norde, 1984). Interfacial adsorption is dependent upon a balance between these forces. Colloidal particles will attract each other through van der Waals interactions (short-range), and repel each other through long range, repulsive (e.g. Coulombic) forces. As proteins approach one another there is a net attraction, with a potential energy barrier to interaction at closer proximities, and with strong interaction at very short ranges. Interaction can be avoided by creating a high potential energy barrier. Although in vitro this may be achieved by charge/charge effects, this is likely to be diminished in vivo. Napper and Netschey (1971) have argued that for (particulate) colloids, a high potential energy barrier can be formed by creating a sterically stabilized surface upon introducing a hydrated (i.e. hydrophilic) polymer at the surface of the colloid. The hydration effect is enthalpic in origin; the stabilization effect being manifested by both osmotic effects and chain entanglements, both of which are entropic in origin (Ottewill, 1977). The size of any repulsive barrier should be determined by both the thickness of the polymer layer and its density, as well as by polymer-polymer interactions caused by specific interactions along the polymer chain. It is probable that steric stabilization is akin to the mechanism whereby blood cells and various bacteria and parasites escape detection by the mononuclear phagocyte system (MPS). Surface modifications to proteins can be made to improve their tolerance within the vasculature, due largely to the formation of a surface which makes it energetically unfavorable for other macromolecules to approach.

Chemical Protectants

Many examples of hydrophilic (bio)polymeric protectants have been described for conjugation to therapeutic proteins. Synthetic and biological materials have been used or suggested, and include polyethylene glycols, poloxamers, poloxamines, albumin, immunoglobulin G, carboxymethycellulose, natural xanthans and sorbitans, etc. Conjugations of proteins with hydrophilic polymers have often been reported as being very successful in altering their potency as well as for reducing their immunogenicity and increasing their duration of action. Abuchowski and Davis (1977) first adopted this approach for stabilizing therapeutic proteins by forming protein conjugates with hydrophilic polyethylene glycol chains. Others have increasingly used this approach, and modifications of it, for lengthening the blood half-lives of a number of peptidergic mediators, including lymphokines, and enzymes, such as catalase, asparaginase and urokinase, whilst still maintaining their reactive functionalities. Steric stabilization (or polymer-excluded volume) approaches to avoiding opsonization have also received attention for modifying antibodies (Rihova et al, 1986), and the modification of antibodies with hydrophilic polymers can be additionally utilised to enable the chelation of small inorganics for dianosis purposes (Torchilin et al, 1986).

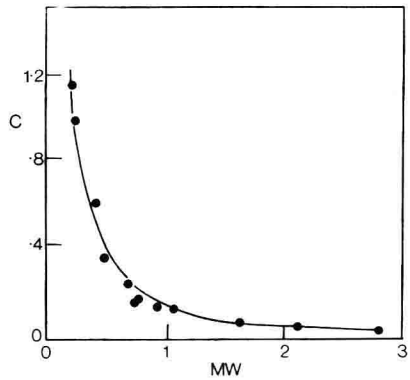

Fig. 3. Effect of size on the systemic clearance (C) of recombinant interleukin 2 (rIL-2) in rats, showing the relationship between C ($ml.min^{-1}$) and effective molecular weight (MW x 10^{-5}) of rIL-2 modified with polyethylene glycol (Katre et al, 1989). (Reproduced with the permission of the copyright holder.)

Covalent attachment of a hydrophilic polymer such as PEG to a small protein will significantly increase its size. This can be modulated by the extent of protein modification and the size of polymer used. Figure 3 shows the experimentally found relationship between the systemic clearance rates of PEG-modified recombinant IL-2 in rats and their effective size and shows that for such systems an abrupt change in clearance occurs at around 70kDa, which of course is predictable from known information on serum proteins and filtration (Katre et al, 1989). IL-2/PEG is reported to have a half-life 10 times that of non-pegylated IL-2, a lower toxicity, whilst still retaining its antitumor activity in patients with advanced cancers.

White et al (1989) have shown that by prolonging the circulating time of SOD and catalase with PEG, conjoint treatment with these increased survival and consistently decreased lung injury and neutrophil recruitment and activation in rats exposed to hyperoxia. In addition, they found that polyethylene-attached antioxidant enzymes decrease pulmonary oxygen toxicity (in rats); indeed, in vitro studies even suggest that PEG itself may be contributing to protection by scavenging hydroxyl radical but not superoxide or hydrogen peroxide.

A decrease in immunogenicity of therapeutic proteins may result from either a reduction in their aggregation, or simply by a masking of any antigenic determinants. It has been found that the primary and secondary IgE antibody responses to protein may be suppressed by chemically conjugating protein with a derivative of polyaspartic acid, i.e. α,β-poly[(2-hydroxyethyl)-DL-aspartamide) - chosen since it had been used as a plasma expander without apparent toxicity (Okada et al, 1985). Although the mechanism of this effect is not fully defined, it has been shown that the suppressive effect of protein modified with polyethylene glycol (Lee et al, 1981) or fatty acid (Segawa, 1981) is due to the induction of suppressor T cells and that conjugation with a copolymer of D-glutamate and D-lysine leads to suppression via tolerance of B cells (Katz et al, 1972).

Conjugation of proteins with hydrophilic polymers can also increase their chemical and physicochemical stability and is known to enhance resistance to proteolysis and heat denaturation (Wileman et al, 1986). Thus, such chemical protection can affect clearance, and, perhaps, immune response.