International Journal of Pharmaceutics, 75 (1991) 97–115

ADONIS 037851739100307L

IJP 02512

Invited Reviews

Peptide and protein drugs: I. Therapeutic applications, absorption and parenteral administration

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(Received 20 February 1991)
(Modified version received 4 May 1991)
(Accepted 10 May 1991)

Key words: Peptide delivery; Protein delivery; Stability; Bioavailability; Absorption barrier; Proteolytic activity; Absorption enhancer; Proteinase inhibitor; Liposome

Summary

In this first part of a two-part review of peptide and protein drugs, the pertinent terminology is introduced and the therapeutic applications of those drugs summarised. Their absorption and the methodology commonly used for study on it are discussed. Approaches to optimising delivery of the peptide and protein drugs are highlighted.

Introduction

With the recent advances in recombinant DNA technology, the commercial production of proteins and peptides for pharmaceutical purpose is now routine. The list of available therapeutic agents produced by this technology is expanding rapidly to include interferon, macrophage activation factors, tissue plasminogen activator, neuropeptides and experimental agents that may have potential in cardiovascular disease, inflammation, contraception and so on. Unfortunately, protein and peptide drugs possess some chemical and physical properties, including molecular size, susceptibility to proteolytic breakdown, rapid plasma clearance, immunogenicity and denaturation, which make them unsuitable for delivery using the normal absorption routes and in particular, the oral route. In part one of this review protein and peptide drugs are considered with particular emphasis on their pharmacological profiles, potential routes of delivery and their associated problems.

Recent major reviews on the subject include the general article by Gardner (1984) on the intestinal absorption of intact peptides and proteins and that by Humphrey and Ringrose (1986) on the absorption, metabolism and excretion of peptide and related drugs. In a further review, Lee (1988) discussed enzymic barriers to peptide and protein absorption. Banga and Chien (1988)

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broadened the scope and considered systemic delivery of those agents in general.

Terminology

Peptide or protein drugs are derived from amino acids by peptide bond linkages. Proteins are large peptides. Peptides containing less than eight amino acid residues are called small peptides. Peptide drugs in this group include enalapril, lisinopril and thyroid releasing hormone analogues. The term polypeptide drugs refers to peptide drugs with eight or more amino acid residues and includes cyclosporin, leuproline and luliberin. Polypeptide drugs containing from about 50 to as many as 2500 amino acid residues are named protein drugs. These include insulin, growth hormone and interferons. Some protein drugs, such as insulin or IgG containing two or more polypeptide chains, are called oligomeric proteins and their component chains are termed subunits or protomers.

Therapeutic Uses of Peptide and Protein Drugs

Peptide and protein drugs can be conveniently classified according to their activity profiles as follows:

Enzymes

Some exogenous enzymes have been used as enzyme replacement therapy in the treatment of enzyme deficiency diseases such as lysosomal storage and mannosidosis (Table 1). Because enzyme deficiency in humans is usually genetic in origin, enzyme replacement is often the only available therapy. Some exogenous enzymes have also been utilized in the treatment of diseases other than inborn enzyme deficiency. Good examples include t-PA (tissue plasminogen activators), urokinase and streptokinase. These enzymes activate circulating plasminogen and fibrin clot-associated plasminogen equally well and, because of this, they have been marketed in the U.K. and U.S.A. (Robinson and Sobel, 1986; *British National Formulary*, 1989). Thrombin-like enzymes of snake venoms have also been developed for dissolving blood clots through enhanced release of fibrinopeptides from fibrinogen (Kornalik, 1985).

Hormones

Hormones represent the largest class of protein or peptide drugs used in medical therapy. All hormones have 'target cells' on which they act and these may be located in a specific organ or be more widely distributed in the body. Some hor-

TABLE 1

Therapeutic application of some enzymes

Enzymes	Therapeutic application	Reference
Adenosine deaminase	Enzyme deficiency	Hershfield et al. (1987)
Dextranase	Lysosomal storage	Colley and Ryman (1974)
β-Fructofuranosidase	Storage disease	Gregoriadis and Ryman (1972b)
α-Mannosidase	Mannosidosis	Patel and Ryman (1974) Fishman and Citri (1975)
L-Asparaginase	Cancer	Abuchowski et al. (1984)
β-Glucosidase	Adult Gaucher's disease	Braidman and Gregoriadis (1976)
Tissue plasminogen activators	Thrombosis	Robinson and Sobel (1986)
Urokinase	Thrombosis	Robinson and Sobel (1986)
Streptokinase	Thrombosis	Robinson and Sobel (1986)
Thrombin-like enzymes of snake venoms	Thrombosis	Kornalik (1985)

mones like luliberin (luteinizing hormone releasing hormone, LHRH) function solely to bring about the release of other hormones from different endocrine glands. It is also well known that many hormones act by means of a second messenger and quite often this is cyclic AMP (cAMP) which is formed from ATP. On reaching its receptor in the cell membrane, the hormone causes the release of cAMP, which is the actual regulator of the metabolic process. In this way, the physiological effect of one molecule of the hormone is amplified many times (Wills, 1985). Because hormones are very specific and a tiny amount can produce large pharmacological effects, they are ideal for biotechnological development which is more suitable for relatively small outputs. Perhaps the best known hormone drug is insulin which has been used as an endocrinotherapeutic agent since the 1920's (Banting and Best, 1922).

Enzyme inhibitors

Enzyme inhibitors have been used as drugs for a long time. These include proteins such as aprotinin, and peptide drugs such as enalapril and lisinopril. Captopril is an inhibitor of angiotensin converting enzyme (ACE), which catalyses in vivo generation of angiotensin II from the decapeptide, angiotensin I, to constrict arterioles and increase cardiac output, leading to hypertension in man. Captopril is now a widely used antihypertensive agent (Romankiewicz et al., 1983). Enalapril and lisinopril are subsequent developments which are also becoming widely adopted for the treatment of hypertension and congestive heart failure (Todd and Heel, 1986; Lancaster and Todd, 1988).

Antimicrobial agents

A number of antimicrobial agents are peptide drugs, for example, the penicillins, cephalosporins, polymyxin B sulphate, actinomycin and bleomycin. Structurally, these drugs are small peptides, mostly containing a non-peptide moiety. All of these antimicrobial drugs are microbial metabolites.

Immunomodulating peptides and proteins

Endogenous immunomodulating agents

These agents are now produced by molecular genetic approaches. Well-known examples are the interferons (IFNs) which are families of inducible secretory proteins produced by eukaryotic cells in response to viral and other stimuli. Interferons are not directly antiviral but they act prophylactically by inducing antiviral proteins. These protect cells from viral infection by inhibiting virus-directed translation and transcription (Moore and Dawson, 1989). Another example is interleukin-2 (IL-2) which exerts its biological effect through cell surface receptors on activated T and B cells and on NK cells (natural killer cell). Interleukin-2 has been administered clinically in attempts to restore immunocompetence in patients suffering from the acquired immunodeficiency syndrome (AIDS), and to improve the immunocompetence of cancer patients (Dawson and Moore, 1989).

Exogenous immunomodulating agents

Some exogenous immunomodulating agents are also used to promote immunocompetence in man. For example, cyclosporin (CS-4), a cyclic undecapeptide which is isolated from *Tolypocladium inflatum* Gams, is widely used as an immunosuppressive (Calne et al., 1978; Cantarovich et al., 1987; Mehta et al., 1988; Borel, 1989), whereas muramyl dipeptide has been used as an immunological adjuvant (Kreuger et al., 1984; Bomford, 1989).

Vaccines

Vaccines derived from the infective microorganisms are introduced into the mammalian body to induce antibody formation against the pathogens. Well-known examples include measles vaccine and polio vaccine. It is anticipated that an increasing number of such vaccines will be biotechnologically produced, to give more specific and pronounced antigenic responses.

Absorption of Peptide and Protein Drugs

Analytical problems

Several methods have been employed for studying the absorption of peptide and protein

drugs. However, high molecular weight proteins and polypeptides present some unique difficulties. Techniques such as gel filtration and ion-exchange HPLC usually have to be used. Even so, it is still very difficult to assay them in the presence of body fluids such as blood and urine. In such cases, radioassays or radioimmunoassays are often the most appropriate and hence, these techniques have been widely used in the measurement of the bioavailability of peptide or protein drugs. However, radioassays may be non-specific, and many chemical assay procedures may by themselves influence the conformation of protein drugs, thereby causing the loss of their biological activities. The entity being chemically assayed may not be the biologically active moiety and in such cases, in vitro or in vivo bioassays are often used during absorption studies. For protein/peptide hormones, the measurement of pharmacological responses may be the assay method of choice. For enzymes or enzyme inhibitors, specific enzyme reactions may be the best analytical method. The bioavailability of immunomodulating and antimicrobial agents may be evaluated using some specific animal models and indicator microorganisms. For example, the prophylactic

TABLE 2

Instability of protein and peptide drugs

Effect factor	Protein or peptide drugs	Reference
Physical instability		
Aggregation	Interferon-γ	Hsu and Arakawa (1985)
		Arakawa et al. (1987)
	Bovine growth hormone	Brems et al. (1986)
		Brems et al. (1988)
Precipitation	Insulin	Brennan et al. (1985)
		Loughead et al. (1980)
Chemical instability		
β Elimination	Lysozyme	Nashef et al. (1977)
	Phosvitin	Sen et al. (1977)
Deamidation	Bovine growth hormone	Lewis and Cheever (1965)
	Human growth hormone	Lewis et al. (1970)
		Becker et al. (1988)
	Insulin	Berson and Yalow (1966)
		Fisher and Porter (1981)
	r-Immunoglobulin	Minta and Painter (1972)
	Epidermal growth factor	Diaugustine et al. (1987)
	Prolactin	Graf et al. (1970)
	Gastrin releasing peptide	McDonald et al. (1983)
	ACTH	Graf et al. (1971)
		Bhatt et al. (1990)
Disulphide exchange	Lysozyme	Volkin and Klibanov (1987)
	Ribonuclease A	Zale and Klibanov (1986)
Racemization	ACTH	Geiger and Clarke (1987)
		Meinwald et al. (1986)
Oxidation	Corticotropin	Dedman et al. (1961)
	α-, β-Melanotropins	Dixon (1956)
	Parathyroid hormone	Tashjian et al. (1964)
	Gastrin	Morley et al. (1965)
	Calcitonin	Riniker et al. (1968)
	Corticotropin releasing factor	Vale et al. (1981)

TABLE 3

Liposomes as peptide and protein carrier

Liposome composition	Peptide or protein	Route	Animal model	Reference
Phosphatidyl-choline : cholesterol 7 : 2	semipurified glucocerebroside β-glucosidase	i.v.	man	Belchetz et al. (1977)
Phosphatidyl-choline : cholesterol 7 : 7	highly purified glucocerebroside β-glucosidase	i.v.	man	Gregoriadis et al. (1982)
Phosphatidyl-choline : cholesterol : phosphatidic acid 7 : 2 : 1	bacterial amyloglucosidase	i.v.	man	Tyrell et al. (1976)
Dimyristoyl phosphatidyl-choline : choles-terol : dicetyl phosphate 1 : 0.75 : 0.11	cholera toxin human malaria sporozoite antigen	i.v.	rabbit	Alving et al. (1986)
Phosphatidyli-nositol	insulin	i.v.	mouse rat	Dapergolas and Gregoriadis (1976)
Phosphatidyl-choline : choles-terol : dicetyl phosphate 10 : 2 : 1	insulin	oral	rat	Patel and Ryman (1976)
Phosphatidyl-choline : choles-terol : dicetyl phosphate 3 : 9 : 1	insulin	oral	rat	Tanaka et al. (1975)
Phosphatidyl-choline : phos-phatidylserine 7 : 3	muramyl peptide	i.v.	mouse guinea-pig	Fidler et al. (1985)
Phosphatidyl-choline : choles-terol : dicetyl phosphate 7 : 1 : 2	lysozyme			Sessa and Weissmann (1970)
Phosphatidyl-choline : choles-terol : phospha-tidic acid 20 : 1.5 : 0.2	adenovirus type 5 hexon protein	i.v.	mouse	Six et al. (1988)
Phosphatidyl-choline : choles-terol : phospha-tidic acid 7 : 1 : 2	lysozyme			Sessa and Weissmann (1970)

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