CHAPTER II

LITERATURE REVIEW

The number of peptide hormones clinically available has progressively expanded during the century. A limiting factor in the therapeutic use of these drugs is the lack of appropriate delivery system. Peptides and proteins are scarcely absorbed following oral delivery as a result of enzymatic degradation in the gastrointestinal tract. Therefore, the only effective route of administration of these drugs is by parenteral routes (Pontiroli.,1990). However, the parenteral route suffers several serious drawbacks such as patient compliance, high risk of overdose, infections and local thrombophlebitis as a result of its invasive nature of administration. These are some of the reasons that led to the search for alternative routes of administration.

Non-injection, non-oral routes of administration such as nasal, sublingual, rectal, pulmonary, and transdermal routes have received greater attention as alternative routes for systemic delivery of peptide drugs. Among these routes, nasal administration has been of great interest and absorption from this route has been studied widely. The nasal epithelium is highly vascularized and the presence of microvilli makes a large surface area of the nasal cavity. As a result, the drugs are absorbed rapidly (Donovan, Flynn and Amidon, 1990). In addition, the volume of the nasal secretion is also much less than the GI-secretion, leading to smaller dilution of the administered drug and better contacts with the absorptive epithelium (Stanford and Lee, 1986). The important advantage of nasal delivery is an avoidance of first-pass hepatic metabolism (Shao and Mitra, 1992). Besides, the drugs can be given in a pattern which simulates the release cycle of the endogenous peptides, thereby

preserving their biological rhythms and reducing unwanted side effects (Banga and Chien, 1988). Nasal administration is also considered to be non-invasive as opposed to the parenteral route, thereby encouraging self-administration and increasing patient compliance.

The mucosal membranes of the nasal cavity are the moist lining epithelium, including several types of epithelia. A small portion extending into the nasal cavity from the nares is a stratified squamous epithelium. The remainder of the nasal membrane is made up of respiratory epithelium, which is composed of ciliated cuboidal and columnar cells, goblet cells and the olfactory epithelium, which is a pseudostratified neuroepithelium. Another connective tissue layer called the submucosa usually connects the mucosa to the underlying structures. On the surface of the mucosal epithelium, there exists a layer of mucus composed of mucopolysaccharides secreted from the goblet cells in the mucosa (Hsieh, 1994). Figure 1 shows a transitional electron microscopic diagram of the nasal epithelium.

The nasal vasculature consists of a rich capillary network in the subepithelium and around the nasal glands and a cavernous plexus deep to the glandular zone. It is characterized by fenestrated endothelium. The cavernous plexuses receive blood from the capillary bed and from arteries by means of arteriovenous anatomoses. Venous drainage occurs through a number of venous plexuses, some of which communicate with intracranial venous sinuses. Thus, the nasal mucosa is obviously well suited for heat exchange and for potential drug absorption. In general, drugs absorbed via the nasal mucosa enter the right side of the heart for distribution to the systemic arterial circulation prior to traversing the liver (Su, 1991).

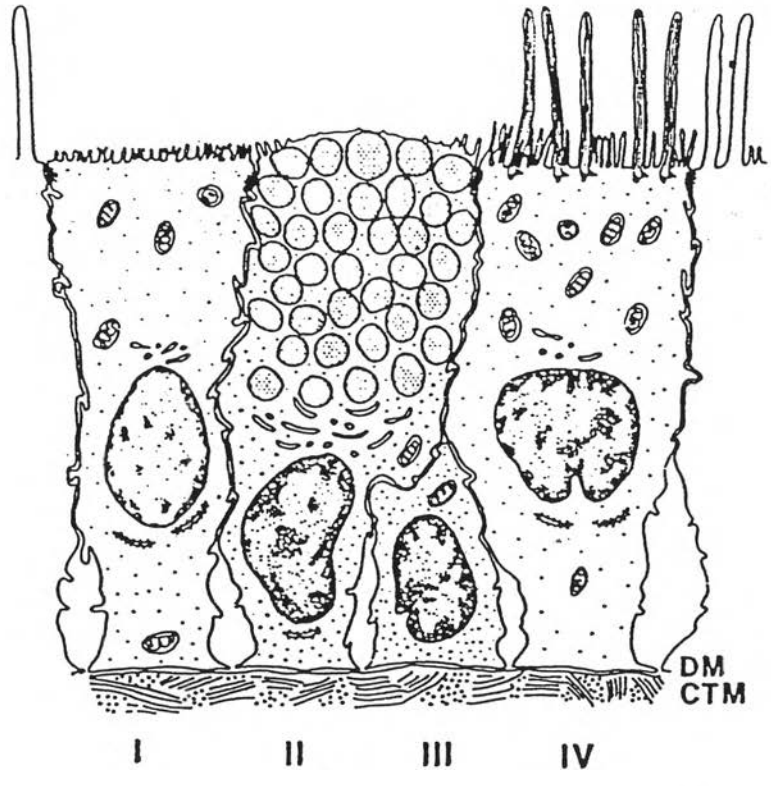


Figure 1 Transmission electron microscopic diagram of the four cell types in the nasal epithelium. (Su, 1991)

I. nonciliated columnar cell with microvilli

II. goblet cell with mucous granules and a well-developed Golgi apparatus

III. basal cell

IV. ciliated columnar cell with many mitochondria in the apical part.

DM = double membrane

CTM = connective tissue membrane

Barriers to nasal delivery of protein/peptide drugs

Since protein and peptide drugs are recognized as foreign peptides, the mammalian system possesses several extremely efficient barriers to restrict the entry of the macromolecules. These barriers may completely or partially obstruct the penetration of the drugs and thereby physiologically protect the safety of the mammalian body. The major barriers affecting the administration of protein/peptide drugs are the significant proteolytic enzyme activities and the presence of various epithelia at different locations (Zhou, 1994).

Proteolysis barriers

The transport barrier of nasal epithelium is considered to be weaker for the absorption of peptides and proteins than that of the intestinal epithelium. However, the absolute bioavailability of nasally administered polypeptides is still less than that by parenteral administration (McMartin et al., 1987). For example, nasal administration of insulin (Tengamnuay and Mitra, 1990b) and calcitonin (O'Hagan and Illum, 1990) resulted in only 1 % absorption. It thus appears that several absorption barriers may be present in the nasal mucosa which are responsible for their lower-than-expected nasal bioavailabilities. There barriers include the presence of many mucosal proteolytic enzymes which are responsible for biochemical degradation of peptides before and during absorption as well as the epithelium itself which acts as an efficient barrier against membrane penetration.

The proteolytic enzyme barrier is perhaps the most important barrier to the protein/peptide drugs. The reasons are that, in general, protein/peptide drugs have a high susceptibility to the proteolytic enzymes, and that many enzymes with high

activities are widely distributed in the human body, especially between the entry point into the systemic circulation and the target site. These enzymes include exopeptidases (aminopeptidase and carboxypeptidase), endopeptidases (endopeptidase and angiotensin converting enzyme), dipeptidases, aminotripeptidases, prolidases, prolinases and carnosinases. Some examples of these enzymes and their locations are shown in Table 1.

Table 1 Proteolytic enzymes and their location in mammalians (Zhou, 1994)

Enzyme	Tissue	Animal
Aminoligopeptidase	intestinal,nasal,rectal, vaginal, buccal and dermal	rat, rabbit, dog and quinea-pig
Angiotensin-converting enzyme	intestinal, nasal, rectal, vaginal and buccal	human,rat
Leucine aminopeptidase	Intestinal, nasal, rectal, buccal and dermal	rat, rabbit, dog and quinea-pig

Enzymatic activities also differ among differ among different species. It was found that the hydrolysis rate of Leu-enkephalin to des-tyrosine metabolite in rat nasal cavities was much higher than the rate in human nasal cavities (Hussain et al, 1990). Indeed, various tissue homogenates from various animals show different proteolytic activity profiles, suggesting that species differences are to be expected when comparing the same tissue from the different species using the same peptide

drug. Little is known about the proteolytic barriers in different tissues of the human body and this requires further study. However, reliable data can be predicted from rat, dog, rabbit and guinea pig models which are, at the enzyme levels, histologically similar to the human body, once the proteolytic barriers in the human tissues are characterized (Zhou and Li Wan Po, 1990).

Absorption barriers

Absorption barriers include clearance systems at administration sites, obstacles of the epithelial membrane to the drugs, as well as transcellular and pinocytosis processing. Various organs may have different clearance systems, but little information is available about particular transcellular and pinocytosis methods of processing peptide/protein drugs. Therefore, the focus in this part of the review is placed on the epithelial mucous membranes.

The epithelial membranes constitute a highly efficient barrier to drug absorption. These membranes universally cover the organ tissues which are used in common non-parenteral administration of peptide/protein drugs. In order to pass the membrane, the drugs have to pass through the membrane cells (transcellular transport) by means of passive or concentration-gradient diffusion, by active transport or by transport via vesicles. Alternatively, the drug may have to pass through the tight junctions between cells (paracellular pathway). The transport of molecules across the membrane is dependent on several factors such as the molecular weight, structural conformation, pH and hydrophilic characteristics of the drugs themselves (Zhou, 1994).

The pore radius of the mucosa can be considered an important limiting factor of the paracellular transport of peptide molecules. This was demonstrated by transport experiments across membrane with macromolecules of different sizes (McMartin et al, 1987). The equivalent pore radius diameter of various mucous tissues appears to have a range from 4 to 8 A°. Amino acid, dipeptides and tripeptides are therefore able to penetrate the mucous membrane via these paracellular pores while larger peptides are hindered. The pore radius of the mucosa was found to be reversibly enlarged by absorption enhancers such as sodium cholate (Hirai et al, 1981).

Thus far, the nasal mucosa seems to have the lowest absorption barrier for administered peptides. The reasons for it are the relatively high permeability and large vascular mucosal bed so that good absorption can be expected for molecules of up to 1 kDa. In addition, for molecules less than 10 kDa in size, sufficient quantities often can be absorbed into the systemic circulation by using special dosage forms such as degradable starch microspheres (DSM) without the need of absorption enhancers (Bjork and Edman, 1988 and 1990). These forms of formulation presumably enhance peptide absorption by affecting tight junctions. This aqueous channel route (tight junctions) may also be present in other mucosa such as buccal, rectal, vaginal and ocular membranes. The mucous barrier may also be of particular importance for peptide drugs due to the peptide-mucus interactions. It appears that the peptide drugs or prodrugs designed with anionic or neutral charge have less interaction with negatively charged cell membrane surfaces. Conversely, enhancers designed with cationic charge may help to increase the bioavailability of peptide drugs via interactions with the mucosal membrane surface. A good example is DEAE-dextran, a polycationic polysaccharide. This compound was demonstrated to enhance the bioavailability of intranasal insulin. The interaction between the cationic

charged enhancer and mucus may lead to a change in membrane permeability to macromolecules and stimulation of picnocytic uptake (Chandler et al., 1991b).

General approaches to bypassing enzymatic and epithelial barriers

In order to bypass the enzymatic and epithelial barriers for the purpose of increasing the nasal bioavailability of high molecular weight protein and peptide drugs, several approaches are available: (I) inhibition of their enzymatic degradation, (ii) improving their resistance to breakdown or their permeability across the membrane by structural modification; (iii) by pharmaceutical formulations which prolong their retention time with mucus at the administration site; and (iv) increasing their permeability across the relevant membrane by using chemical absorption enhancers (Zhou, 1994).

Inhibition of proteolytic enzymes

For several years it has been known that protease inhibitors increase the absorption of protein drugs. Compounds such as boroleucine and phosphinic acid dipeptide have been suggested as having good potential in enhancing the bioavailability of peptide and protein drugs due to their appropriate molecular sizes and potent inhibitory effects on various proteolytic enzymes (Hussain et al., 1989). However, the safety of these inhibitors after nasal administration is doubtful and must be further tested (Hussain et al., 1992). Bestatin and puromycin are another class of inhibitors that have been studied. Nevertheless, their effects on peptidases were much less effective than boroleucine (Hussain et al., 1989).

Chemical modification

Hydrogen bonding potential or lipophilicity of peptides also can be altered by chemical modification. This often leads to conformational changes of the peptides and thereby may increase their permeability and/or stability during transport across the cellmembrane. For example, when four methyl groups were added to the peptide, acetamido-D-Phe-D-Phe-D-Phe-carboxamide by methylation, it was found that the penetration rate of this peptide through Caco-2 cell membrane was significantly enhanced (Conradi, Higers and Burton, 1992). Tengamnuay and Mitra (1990a) also reported that by substituting L-Arg with D-Arg in the L-Tyr-L-Arg structure resulted in a dipeptide analogue which was highly resistant to hydrolysis by the nasal mucosal enzymes.

Formulation approach

The formulation approach has been employed to develop an effective nasal delivery system for peptide and protein drugs for many years. The most popular dosage form is a powder or microspheres system since the nasal solutions and sprays tend to provide lower peptide drug availability. In a recent study by Bjork and Edman (1988), insulin (0.75 and 1.7 IU/kg) which was dispersed in degradable starch microspheres (DSM) and administered nasally as a drug powder resulted in a dose-dependent decrease in blood glucose in rats. The bioavailability of the nasal insulin was found to be 30 %, whereas the administration of DSM alone or soluble insulin alone produced no effect. The effectiveness of the nasally delivered peptide in this dosage form was due to the uptake of water by DSM and subsequent swelling which produced dehydration of the epithelial cells, leading to a widening of the tight junctions and thereby facilitating paracellular transport of large hydrophilic molecules

(Bjork and Edman, 1990). When DSM was combined with an enhancer, lysophosphatidylcholine (LPC), the extent of insulin nasal absorption was improved even further (Farraj et al., 1992). This improved formulation also has been used for enhanced nasal absorption of human growth hormone (Illum et al., 1990).

Use of nasal absorption enhancers

Many researchers in nasal drug delivery have included certain adjuvants in nasal peptide formulations in an attempt to increase their systemic bioavailability. The compounds that have been studied as nasal absorption enhancers are, for example, anionic and cationic surfactants (Hirai et al., 1981), bile salts (Gordon et al., 1985, Pontiroli,1990), bile salt-fatty acid mixed micelles (Tengamnuay and Mitra, 1990a and 1990b), fusidic acid derivatives (Longenecker et al., 1987, Baldwin et al., 1990), medium chain fatty acids (Mishima, Wakita and Nakono, 1987) and cyclodextrins (Merkus et al., 1991).

The mechanisms of action of these absorption enhancers are not clearly known, but several possibilities have been postulated. The first is the increased solubility of the drugs brought about by the surfactant-type enhancers. Because proteins and peptides usually form aggregates in aqueous solutions, their dissociation into more readily soluble monomers by these enhancers may facilitate their paracellular transport (Brange, Havelund and Hougaard, 1992). Secondly, surfactant-type enhancers like hydrophobic bile salts may also facilitate transcellular transport of insulin probably by reverse micelle formation (Gordon et al., 1985). A third mechanism of the enhancer is to inhibit the activity of proteolytic enzymes. Some enhancers such as bile salts, fusidic acid derivatives and cyclodextrins have been shown to inhibit mucosal proteolytic activity. However, these compounds are not

specific inhibitors or substrate analogues of the proteinases or peptidases (Zhou and Li Wan Po, 1991). It is postulated that binding of the peptide drug with the enhancer may prevent the formation of the enzyme-substrate (enzyme-protein drug) complexes which are intermediates of the degradation process. Fourthly, the positively charged enhancers may react with the negatively charged membrane surface, and thereby reduce the peptide drug-mucus interactions (Chandler, Illum and Thomas, 1991a) resulting in an increase of the drug bioavailability. The fifth possibility is that the enhancers can lower the barrier function of the mucosal membrane and associated mucus layer. For example, bile salts, including sodium taurodihydrofusidate (STDHF), have been shown to reduce the viscosity of the mucus layer adherent to all mucosal surfaces and increase the pore size within the cell membrane, thereby allowing diffusion of insulin through the cells (Longenecker et al., 1987). The barrier function of the mucosal membrane could also be lowered by a more severe mechanism, e.g., the removal of certain membrane components like membrane proteins and phospholipids. Several enhancers, especially the surfactant-type compounds, have strong solubilizing capacity which may not only dissolve or dissociate the peptide aggregates but also may effectively cause leaching of these membrane components, leading to a substantial increase in membrane permeability (Tengamnuay, 1989).

Although these enhancers are able to significantly increase nasal absorption of peptides, many studies have later revealed that their use can cause damages to the membrane. This has caused great concerns and prevented them from potential application in clinical setting, particularly during chronic administration. For example, many enhancers can cause cellular changes in the mucosa which include loss of nasal membrane components (Shao et al., 1992: Shao and Mitra, 1992), ciliotoxicity (Hermens et al., 1990), and severe alterations in the morphology of the

nasal mucosa such as necrosis and even complete loss of epithelium (Tengamnuay and Mitra, 1990b; Donovan et al., 1990; Ennis, Borden and Lee, 1990). The membrane damaging properties of these enhancers, especially in long term therapy of many hormone-deficient diseases, have precluded then from clinical application. As a result, more efforts have been pushed toward finding novel absorption enhancers which can give better safety and efficacy.

Cyclodextrins, one class of cyclic polysaccharides, have been suggested as potential enhancers for non-parenteral peptide absorption. Merkus, Schipper et al. (1991) reported that 109 % of nasal bioavailability (2.0 IU/ kg) could be obtained when insulin was co-administered with 5 % dimethyl-β-cyclodextrin (DM-β-CD), a highly soluble cyclodextrin derivative. A recent report by Irie et al. (1992) also provided similarly promising results for this compound. However, it was found later that DM-β-CD caused the release of many membrane and cellular components such as phospholipids, enzymes and proteins (Shao et al., 1992). These researchers also found that hydroxypropyl-β- cyclodextrin (HP-β-CD), another derivative, was the least membrane damaging. However, its absorption enhancing effect was also minimal (Verhoef et al., 1994). DM-β-CD also shows, to some extent depending on concentration (1-5 %), damages to the ciliary system of chicken embryo trachea, although its ciliostatic potency is much less than that found for STDHF (0.5 %), LPC (0.5 %) and bile salts such as deoxycholate (0.2 %), glycocholate (1.5 %) and taurocholate (1.3 %) (Merkus et al., 1993). Therefore, the long term clinical use of DM-β-CD as a potent absorption enhancer in nasal peptide formulations appears to be questionable and more studies are needed to verify its safety profiles.

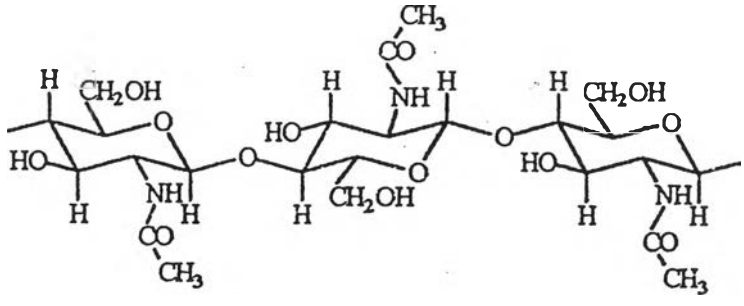
Recently, acylcarnitines, endogenous amino acid-like compounds, which play a key role in the mitochondria transport system in cells by carrying fatty acids across

the mitochondria membrane have been suggested as nasal absorption of peptides is micelle formation. Acylcarnitines with a fatty acid chain length less than 12 carbons were essentially ineffective in enhancing drug absorption from the rectum of rats, whereas acylcarnitines with a chain length of 12-18 carbon units significantly increased the bioavailability of sodium cefoxitin (from 2% to an average of 60%) (LeCluyse, Appel and Sutton, 1991). The 12 carbons lauroylcarnitine chloride was found to be the strongest enhancer among acylcarnitines and it had the strongest enhancing effect for sCT nasal absorption in rat at the concentration of 0.1% (Kagatani et al., 1996). However, more studies about the toxicity of LCC on the nasal membrane are needed before it can be used in long term clinical setting.

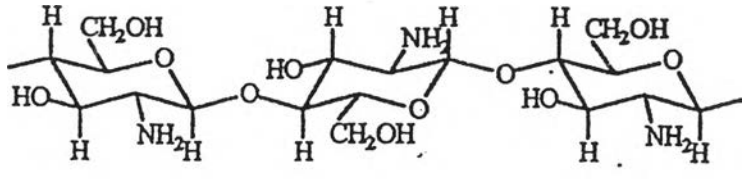
Chitosan as potential nasal absorption enhancer of peptides

Chitosan is a polymer obtained from deacetylation of chitin, a naturally-occurring structural polymer abundant in crab and shrimp shells. Chitosan, or partially N-deacetylated chitin, is a cationic polysaccharide with linear chain consisting of two monosaccharides, i.e. N-acetyl-D-glucosamine and D-glucosamine, joining together by β-(1,4)-glycosidic linkage. The greater the extent of deacetylation, the smaller is the proportion of N-acetyl-D-glucosamine in the polymer chain. Figure 2 shows the chemical structures of chitin and chitosan. Since chitin and chitosan are obtained from crab and shrimp shells which are the waste products of Thailand's marine food industry and can be manufactured and purified in large scale, any research attempt to increase the applicability of chitosan is always highly attractive.

There are several pharmaceutical applications of chitosan. For example, it has been used as a pharmaceutical excipient to increase water solubility of several oral



Chitin



Chitosan

Figure 2 Chemical structures of chitin and chitosan

drug formulations (Imai et al., 1991). It has also been used as polymer matrix in thesustained release drug preparations (Miyazaki et al., 1988). Being a substance of natural origin with biocompatibility, chitosan has found many applications in other areas such as food and cosmetic industries. Recently, Artursson et al. (1994) have studied the effects of chitosan on the transport of water-soluble molecules across the cultured monolayer of intestinal epithelial cancer cells (Caco-2 cells) grown *in vitro*. They found that chitosan significantly increased the permeability of these cells. They also postulated that chitosan may react with the protein of the cellular tight junctions, leading to the opening of the tight junction and subsequent passage of hydrophilic macromolecules through the paracellular pathway. In the same year, Illum,Farraj and Davis(1994) studied the nasal administration of insulin in sheep and rats with and without chitosan. They found that inclusion of chitosan at concentrations from 0.1 to 1.0 % w/v in the nasal insulin solutions could significantly enhance the nasal absorption of this peptide over the control group (nasal insulin without chitosan). Recently, Sahamethapat(1996) found that CS J (0.1-0.5% at pH 4.0) and CS G (0.1-0.5% at pH 6.0)were highly effective in enhancing nasal absorption of [D-Arg^2]-Kyotorphin and at concentration of 0.1% both chitosans showed very small irritating effects on the rat nasal mucosa as judged from the extent of mucosal protein and phosphorus release. Afterwards, Sinswat (1997) found that 1%w/v CS J and CS G could increase by two-fold the rat nasal systemic bioavailability of sCT relative to that of control group. These preliminary results indicated the potential application of chitosan as absorption enhancer of poorly absorbed drugs like peptides. However, very few information is available with respect to its safety and efficacy. It is interesting to know if chitosan could enhance nasal absorption of other peptides apart from the commonly studied insulin. Moreover, its safety profiles with respect to the membrane damaging effects need to be established as well as its possible mechanisms

of absorption enhancement. The primary purpose of this research project is to further characterize chitosan in terms of its safety and efficacy.

However, the efficacy and safety of nasal absorption enhancers have been studied mostly in animal models. There are great anatomic differences in the nasal tissues between small laboratory animals and humans. Therefore, it is difficult to extrapolate the results obtained from a particular animal study to man. And it is advised to perform human experiments at an early stage in the development of nasal drug formulations. Animal studies will then remain useful for a first screening of the efficacy and safety of nasal drug formulations as well as for the subsequent optimization of the formulations once the effectiveness in man has been established (Merkus et al.,1993).

There are many approaches to identifying the toxic potential of nasal formulations. Short-term tests have distinct disadvantages when compared to repeated dose studies. However they can provide a basis for early decisions about intranasal formulations. The toxicity of nasal absorption enhancers has been estimated in recent years by measuring their effect on the mucocilliary transport rate, nasal morphology, ciliary beat frequency, saccharin clearance test and biochemical evaluation (Aspden et al., 1995; Ennis, Borden and Lee, 1990; Schipper,Verhoef and Merkus, 1991; Aspden et al. 1997; Shao and Mitra, 1992).

Mucociliary transport rate

The potential toxicity of some absorption enhancers has been tested with the frog palate model, measuring the mucociliary transport rate before and after application of an absorption enhancer formulation (Gizurarson et al., 1990).

Nasal morphology

Several research groups have investigated in animal tissues the histological effects of absorption enhancers, such as deoxycholate (Hersey and Jackson, 1987), laureth-9 (Chandler, Illum and Thomas, 1991b), cyclodextrin (Gill et al., 1994) on the nasal morphology. Using different contact times with the nasal epithelium all these enhancers were reported to cause more or less severe epithelial disruption. The scanning electron microscopy was used to characterize gross structural and cellular changes. Following different times exposure to the enhancers, micrographs of the nasal tissues were evaluated mainly for surface integrity, ciliary morphology, and mucus extracellular debris (Ennis et al., 1990).

For example, in a study by Illum et al. 1994, it was found that exposure of the rat nasal mucosa to a chitosan solution at a concentration of 0.5 % over a period of 60 minutes failed to cause any significant change in the morphology of the mucosa as compared to the control, indicating a potential safety of this novel class of absorption enhancers. Furthermore, Sahamethapat (1996) also found that daily nasal administration of intact rats' nostrils for 2 weeks caused only mild to moderate irritation to the nasal mucosa, with the most prevalent effects being just mucus hypersecretion and increase in goblet cell activity.

Ciliary Beat Frequency (CBF)

Ciliary beating is the most important parameter in nasal mucociliary clearance. It should not be hampered by nasally administered drugs and additives such as preservatives and absorption enhancers (Merkus et al., 1993). Ciliary beat frequency measurements were performed on ciliated tissues of chicken embryo trachea or human adenoid tissue with a photoelectric registration device. (Donk, Zuidema and Merkus, 1982). However, the inhibitory effect of absorption enhancers on the CBF

in vitro is much more pronounced than that *in vivo*. *In vitro*, the ciliated tissue is directly exposed to the compounds investigated, whereas *in vivo* the cilia are protected by the mucus layer. Moreover, under the *in vivo* conditions the nasally administered drugs will be diluted by the mucus and subsequently eliminated by the mucociliary clearance (Merkus et al., 1993).

Saccharin clearance test

In 1989, Liote et al. showed that nasal mucociliary transport time, as determined by a saccharin clearance test, correlated to transport rates of nasal mucus but not to ciliary beat frequency (CBF) in healthy human volunteers. Clearance rates were assessed by a standard saccharin taste test. The saccharin clearance test is a noninvasive test that involves administering a small piece of a saccharin tablet to one nostril and recording the time taken for the volunteer to taste the sweetness of the saccharin. The advantages of the saccharin clearance test as an indicator of mucociliary transport rates (MTR) include its simplicity and relative inexpensiveness, which make it a routeinly used and popular procedure in rhinology clinics. The test has previously been shown to give results similar to those obtained using radiolabeled particles and gamma scintigraphy. For example, in 1997, Aspden et al. showed that a once daily nasal application of a 0.25 % solution of the chitosan for 7 days had no effect on saccharin clearance.

Biochemical evaluations

A biochemical approach has been developed by Shao and Mitra (1992) and Shao et al.(1992) to assess toxicity to the nasal membrane by various groups of absorption enhancers. Total protein, phosphorus, lipid phosphorus, nasal membrane-bound and intracellular enzyme releases have been examined to provide a wide range of information concerning the extent of nasal irritation or epithelial cell damage. The

extent of release of total protein and the enzymes, lactate dehydrogenase (LDH) and 5'-nucleotidase (5'-ND), indicates directly the extent of damage sustained by the nasal mucosa. Membrane-bound 5'-ND release in the nasal perfusate gives an indication of the level of membrane perturbation while LDH, being a cytosolic enzyme, indicates the amount of cell leaching and / or lysis thereby providing additional information about the intracellular damage sustained by the nasal mucosa. The total protein release data although not very specific in the type of damage, provide a general indication about the extent of irritation. In addition, the reversibility of permeability enhancement can be assessed by measuring the release of biochemical marker(s) from epithelium before and after removal of the absorption enhancer (Swenson, Milisen and Curatolo, 1994).

Since large interspecies differences appear to exist in the nasal absorption of drugs, nasal absorption enhancers, may differ substantially in their efficacy and safety (Merkus et al., 1993). The absorption enhancers which have been tested for efficacy and safety in animals should also be tested in humans. In this study, the efficacy chitosan, together with other nasal absorption enhancers were therefore evaluated in human volunteers using sCT as a model drug.

Salmon calcitonin as model drug for *in vivo* nasal administration

Salmon calcitonin (sCT) has been chosen as a model polypeptide in this study for two reasons. First of all, this peptide is one of the most widely studied peptide drugs for possible absorption through alternative routes, particularly the nasal route. Approximate comparison between the results obtained in this study and other reported results would be possible. Secondly, improved methods of sCT delivery could significantly facilitate the treatment of Paget's disease, hypercalcemia, osteoporosis

and postmenopausal disorders. sCT is a straight-chained polypeptide composed of thirty-two amino acids, and is produced synthetically. The hormone is joined at positions one and seven by disulfide linkage and contains a proline amide at positions thirty-two (Figure 3). The structure of sCT which is obtained from salmon differs significantly from human and bovine calcitonin throughout the chain. This probably accounts for salmon calcitonin's greater potency and stability. All the thirty-two amino acids are important for activity, as manipulation of the arrangement and studies with isolated portions of the chain resulted in reduced activity. The potency of sCT is expressed in International Units (IU), which are equal to Medical Research Council Units (MRC Units). One unit corresponds to 0.2 μg of the pure peptide. A polypeptide containing the 32 amino acids in the same sequence as in sCT has been synthesized and is commercially available in sterile and lyophilized form.

Description

White, fluffy powder; lyophilized

Physicochemical characteristics

Molecular weight : 3431.88

Solubility : Very soluble in water ; slightly soluble in alcohol and insoluble in chloroform, ether

Mechanism of action

Hypercalcemia : sCT has been shown to effectively lower serum calcium concentrations in hypercalcemic patients with carcinoma, multiple myeloma, or, to a lesser degree, primary hyperparathyroidism. Two mechanisms have been proposed whereby calcitonin exerts its hypocalcemic effects. The primary action is through an inhibition of bone resorption, a rapidly occurring process during the active stages of

Paget's disease. It causes a downward shift in the number of osteoclasts formed (osteoclasts are elevated during bone resorption) in favor of an increase in osteoblast production (osteoblasts are elevated during bone formation) leading to a greater bone surface area with a reappearance of normal histological structure (Brodier, 1974). A secondary action occurs through an inhibition of the tubular reabsorption of both calcium and phosphorus resulting in initial hypercalciuria and hyperphosphouria.

Paget's disease of bone : sCT is effective in the treatment of Paget's disease. This disease is commonly found in axial skeleton, but may involve any bone. The earliest pathology lesion appears to be an increase in osteoblastic bone resorption. This in turn is followed by a compensatory increase in osteoblastic activity. The resultant clinical symptoms include bone pain at the site of lesion, increased skin temperature over the affected area due to an increased vascularity of affected bone and an increase in the incidence of fractures. Calcitonin reduces the rate of bone turnover, possibly by an initial blocking of bone resorption, resulting in decreases in serum alkalinephosphatase (reflecting increased bone formation) and decrease in urinary hydroxyproline excretion (reflecting decreased bone resorption, i.e., breakdown of collagen).

Osteoporosis, postmenopausal : sCT may be used in conjunction with adequate calcium and vitamin D intake in the management of postmenopausal osteoporosis to prevent progressive loss of bone mass. The evidence of efficacy was based on increases in total body calcium. Specific therapeutic effects (e.g., the effect of the drug on fracture rates) remain to be fully evaluated. sCT alone appears to be ineffective in the management of osteoporosis.

Adverse Effects

Adverse effects of nasal salmon calcitonin have included facial flushing, hot flashes, nasal intolerance (stinging, rhinitis, rhinorrhea, nasal dryness, epistaxis), back pain, arthralgia, nausea, hypotension and allergic reactions(Kurose et al., 1987). Adverse effects, particularly flushing and nausea, often lessen or disappeae with continued therapy (Gagel et al.,1988). Other common side effects include influenza-like symptoms, fatigue, rash, myalgia, arthrosis, bronchospasm, hypertension, angina pectoris, dyspepsia, constipation, abdominal pain, diarrhea, cystitis, dizziness, paresthesia, abnormal lacrimation, conjunctivitis, lymphadenopathy, and depression (Overgaard et al.,1994).

Alternations in nasal mucosa or transient nasal conditions (e.g., nasal crusts, dryness, redness or erythema, nasal sores, irritation, itching, "thick feeling," soreness, pallor, infection, stenosis, runny/blocked passages, small wounds, bleeding wounds, tenderness, uncomfortable feeling, and soreness across bridge of nose) have occurred in both patients treated with placebo and patients receiving active drug therapy. The most frequent problems have been rhinitis (12%), epistaxis(3.5%), sinusitis(2.3%), and isolated nasal ulcerations. Repeated rhinoscopy in 100 postmenopausal women treated with nasal salmon calcitonin revealed that the drug is well tolerated. The only abnormal finding was a diffuse mucosal hyperemia of mild to moderate intensity in 11 patients (Foti et al.,1995).

Bioassay, Preparations and Dosage

Bioassay of calcitonin preparations is performed by assessing their ability to lower the plasma concentration of calcium in rat. Salmon calcitonin is available for clinical use as Calcimar®or Miacalcic®, a synthetic preparation supplied in vials containing 50 or 100 I.U. per mililiter. The recommended dosage (administered

intranasally) is 200-400 IU daily in several divided doses for hypercalcemia and an initial dose of 200 IU twice daily is used for Paget's disease.

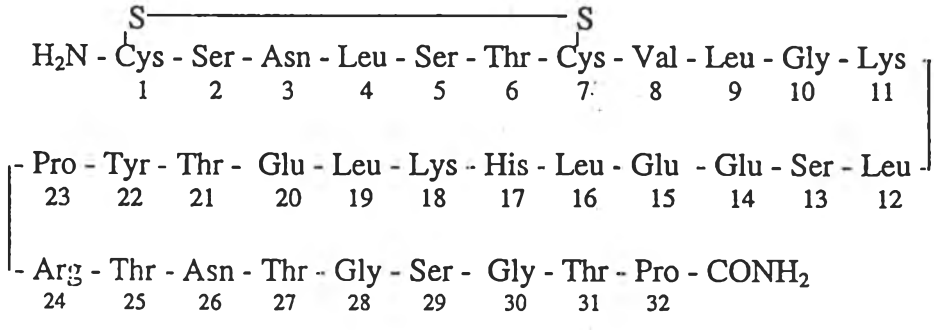


Figure 3 Amino acid sequence of salmon calcitonin