CHAPTER II

LITERATURE REVIEW

Nasal drug delivery is a very attractive approach for drugs which are active in low doses and show no or minimal oral bioavailability. The nasal route can circumvents hepatic first-pass elimination associated with oral delivery, is easily accessible and also suitable for self-medication. Currently, two classes of nasally delivered therapeutics are on the market. The first one comprises low molecular weight and hydrophobic drugs for the treatment of local inflammation of the nasal mucosae and sinuses. This includes decongestants, topical steroids, antibiotics, and most over-the-counter nasal products. The second class includes a few peptide drugs like oxytocin, vassopressin, desmopressin (DDAVP), LHRH and analogues, and calcitonin. These drugs have sufficient nasal absorption to produce systemic effects. Peptides and proteins are high molecular weight and hydrophilic compounds that generally have to be administered by injection. Most of them are hardly absorbed after oral administration due to their instability in the gastrointestinal tract, poor absorption properties, and their rapid and extensive biotransformation in the body (Verhoef et al., 1994) . Therefore, nasal delivery is a particularly promising alternative route for the administration of peptide drugs.

The high potency of most peptides makes these compounds very suitable for intranasal administration as the amount per dose is mostly small. On the other hand, their physicochemical characteristics at

physiological pH may hamper the absorption through the hydrophobic epithelial membranes. In Table 1 the bioavailabilities of various peptides and proteins after nasal administration, in order of increasing molecular weight, are listed (Lee W.A., 1991). Although the data came from many investigators and a variety of animal models, the trend is clear. Small peptides like TRH and enkephalins can have quite high nasal bioavailabilities, whereas for larger peptides and proteins like calcitonin, insulin and growth hormone the transport through the nasal mucosal barrier is very limited. Several approaches can be used to improve the nasal absorption efficiency of these compounds ; for instance, addition of absorption enhancers to the nasal drug formulations.

Table 1 Intranasal bioavailabilities of peptide and protein drugs.

Drug	**Amino acids** (No.)	**Bioavailability** (%)
TRH	3	45
Metkephamid	5	100
SRIF analogue	6	75
Desmopressin	9	10
LHRH	10	1.5
ACTH analogue	17	12
Glucagon	29	<1
Calcitonin	32	<1
GHRH	40	<1
Insulin	51	<1
Growth hormone	191	<1

A major problem encountered in nasal drug delivery may be the possible ciliostatic and tissue damaging effects of drugs and additives. The nasal mucosa is covered by mucus layer which is transported backward to the throat by the nasal cilia. This process,known as the nasal mucociliary clearance, is the self-cleaning mechanism of the nose to protect the body against noxious particles inhaled. 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 olfactory epithelium, which is a pseudostratified neuroepithelium. Another connective tissue 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 goblet cells in the mucosa (Hsieh, 1994). Figure 1 shows schematic view of various cell types in the nasal respiratory 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 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

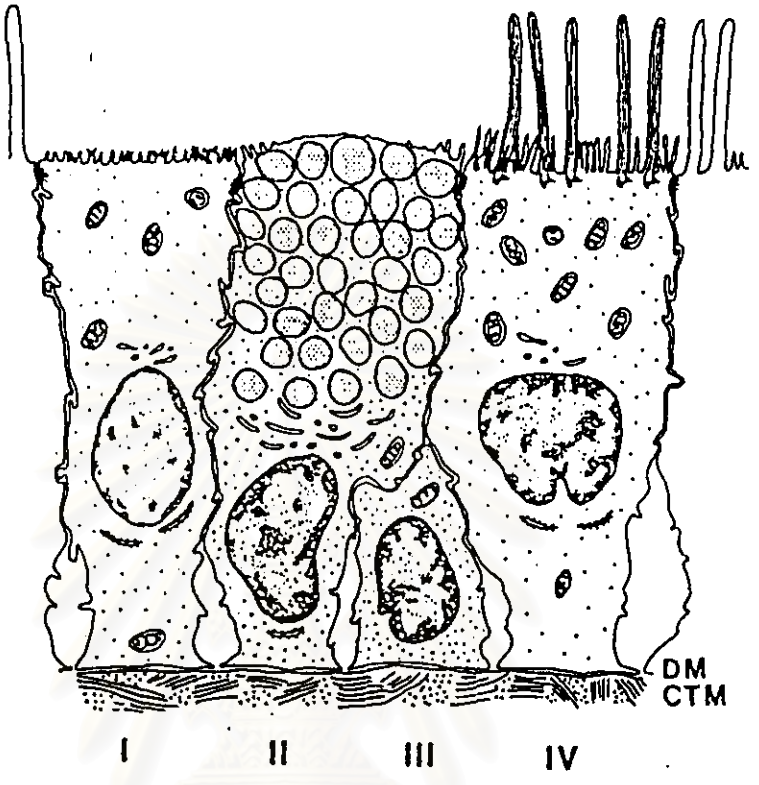

Figure 1 Schematic view of various cell types in the nasal respiratory epithelium (Su, 1991).

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 mitrocondria in the apical part.

DM = double membrane

CTM = connective tissue membrane

the nasal mucosa enter the right side of the heart for distribution to the systemic arterial circulation prior to traversing the liver (Su et al., 1991).

Barriers to Mucosal, Non -Parenteral Delivery of Protein and 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

Proteolytic activities are perhaps the most important barriers to the protein and peptide drugs. The reasons are that, in general, protein and peptide drugs have a high susceptibility to the proteolytic enzymes, and that the 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 (endopeptidases and angiotensin-converting enzyme), dipeptidases, aminotripeptidases, prolidases, prolinases and carnosinases (Zhou, 1994).

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 et al., 1990a).

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 of peptide/protein drugs. Therefore, the focus in this part of the review is placed on the epithelial mucous membranes.

The epithelial cell membranes of the nasal absorptive mucosa form physical and enzymatic barrier to drug absorption, in particular to high molecular weight and hydrophilic compounds such as peptides and proteins. The membranes are composed of phospholipid bilayers in which enzymes and other proteins are embedded via their hydrophobic segments. For the mucosal absorption, the drug is transported via transcellular route (i.e. through the cell membranes) or via the paracellular pathway (i.e. through the tight junctions) by passive diffusion. The rate and extent of

the nasal absorption of a drug is dependent upon several factors, e.g. lipophilicity, molecular weight, environmental pH, and stability to enzymatic degeneration (Chien et al., 1989).

Mechanisms of Epithelial Transport of Drugs and Macromolecules

Cell membranes are barriers to macromolecules and to most polar molecules, but they are relatively permeable to water and small hydrophobic molecules (Hsieh, 1994). Many pharmacologically important molecules and drugs cannot readily move across cell membranes. Under physiological conductions, there are several mechanisms governing the transport of molecules across cell membranes.

Pathways for the transport of molecules, either small or large, across the epithelial cells can be categorized into two groups: paracellular and transcellular transport.

I. Transcellular transport

Cell membranes are barriers to macromolecules and to most polar molecules, but they are relatively permeable to water and small hydrophobic molecules (Hsieh, 1994). Many pharmacologically important molecules and drugs cannot readily move across cell membranes. Under physiological conditions, there are several mechanisms governing the transport of molecules across cell membranes. For example, passive transport of small molecules, active transport of ionic and polar

compounds, and endocytosis and transcytosis of macromolecules (Shen et al., 1994).

1.1 Passive transport

Passive transport is the movement of a solute along its concentration and electrical gradients. Small molecules usually cross cell monolayers by passive transport. The rate at which a molecule diffuses across the lipid bilayer of cell membranes depends largely on the size of the molecule and its relative lipid solubility. The smaller and more hydrophobic the molecule, the more rapidly it will diffuse across the bilayer (Shen et al., 1994).

1.2 Active transport

Active transport differs from passive transport in that the transport process is mediated by membrane transport proteins coupled to an energy source. In active transport, the cell spends energy to move molecules against the concentration gradient. In primary active transport systems such as the Na+-pump ,the system derives the energy by hydrolysis of ATP. In secondary active transport systems such as those for sugar and amino acids (Tengamnuay and Mitra, 1988), peptides, nucleosides and antibiotics with peptide-like structures, the energy source is the transmembranous Na+ gradient and/or electrical potential. An active transport mechanism must satisfy the following criteria : (a) inhibition by metabolic inhibitors such as dinitrophenol, (b) molecular oxygen requirement, (c) temperature dependence, and (d) competition for the active site of the pump among substrate analogs.

1.3 Endocytosis and transcytosis

The transport of macromolecules is different from the transport of small molecules across epithelial cells. Most of the transcellular transport of macromolecules is mediated by endocytotic processes. In endocytosis drugs or macromolecules are progressively enclosed by the cell membranes. After invagination, macromolecules migrate within membrane-bound vesicles to the perinuclear region of the cell, where the vesicles coalesce with lysosomes in which intracellular digestion occurs.

Macromolecules and drugs can also be transported across epithelial cells by transcytosis. The process of transcytosis combines elements of the pathways for endocytosis and exocytosis. Macromolecules bind to receptors on the surface of epithelial cells, and then the receptor-macromolecule complex is incorporated into vesicles and carried into the cell. The complex remains intact in endosomes and is retrieved in transport vesicles which fuse with the membranes of the opposite side of the monolayer. Therefore, intact macromolecules can be transported across the epithelial barrier.

II. Paracellular transport

There is growing interest in the paracellular pathway as a possibility to promote the transport of metabolically labile peptide drugs, on the expectation that it is deficient in proteolytic enzymes. Paracellular transport is the transport of molecules around or between the cells. Tight junctions or similar situations exist between cells. At the tight junctions, the cell membranes are brought into extremely close apposition, but are not fused, so as to occlude the extracellular space. Consequently, ions or molecules may not be able to pass through the membranes. Because of the

perception that proteolytic activity is deficient in the paracellular space, it was once commonly assumed that peptides and proteins could not be absorbed via the paracellular pathway. Therefore, in recent years the investigation of paracellular transport of peptides and proteins has gained great interest. However, without the employment of physical or chemical permeation enhancement techniques, the absorption of the majority of peptides or proteins is negligible or minimal.

Passive absorption of drugs and peptides across the intestinal epithelium involves two pathways: transcellular and paracellular. Absorption via these pathways is selective: for example, only the compounds with suitable lipophilicity can partition into the membrane lipids of the epithelial cells to be absorbed by the transcellular route. Absorption of hydrophilic drugs, on the other hand, is therefore restricted to the paracellular pathway. However, the paracellular pathway occupies only 0.1% of the total surface area of the intestinal epithelium. This, together with the rather difficult permeation through the tight junctions, often limits the paracellular absorption of large hydrophilic drugs such as peptides (Anderberg et al., 1993).

The pore radius of the mucosa can be considered an important limiting factor for the transport of peptide molecules. This was demonstrated by transport experiments across the mucosa 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 mucosa via these paracellular pores while large 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 high permeability and large vascular mucosal bed so that good absorption can be expected for molecules of up to 1kDa. In addition, for molecules less than 10 kDa in size, sufficient quantities often can be nasally absorbed into the systemic circulation by using special dosage forms such as degradable starch microspheres (DSM) without the need of absorption enhancers (Bjork et al., 1988). 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 epithelia. The mucus 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, a polymer or enhancer designed with cationic charge may help to increase the bioavailability of peptide drugs by interacting with 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 charge in membrane permeability to macromolecules and stimulation of pinocytic uptake (Chandler et al., 1991b).

Strategies to Improve Nasal Absorption of Peptides and Proteins

The possible utility of nasal administration of a large number of compounds has been reported in numerous publications (Chien et al., 1989). Nevertheless, only a few small peptide drugs for intranasal systemic absorption are presently used, and most peptides and proteins show insufficient nasal bioavailabilities. In order to enhance the nasal absorption of peptide drugs, serval approaches have been investigated during the last decade, as summarized in Table 2 (Verhoef et al., 1992) .

Table 2 Strategies for improved nasal delivery of peptide and protein drugs.

I. Synthesis of stabilized and more lipophilic (or more potent) analogues

II. Use of peptidase and protease inhibitors

III. Use of absorption enhancers

IV. Formulation approach :

- sprays vs drops
- viscous agents e.g. Methylcellulose (MC), HPMC
- powders vs solutions
- bioadhesives e.g. starch microspheres
  polyacrylic acid gel
  hyaluronate
  chitosan

Peptide Analogues

The first approach includes the synthesis of potent peptide analogues with increases stability to enzymatic degradation and increased

lipophilicity, which may be accomplished at the NH_2-terminus by cyclization,and by the use of C-terminal blocking agents. For example, nasal delivery of salmon calcitonin (sCT) may be advantageous over that of human CT. Due to increased lipophilicity and greater resistance to metabolic degradation, salmon CT is about 40 times more potent than CT from human origin. Intranasally administered sCT has been shown to be clinically effective in patients with postmenopausal osteoporosis, Paget's disease, multiple myeloma and also useful for the prophylaxis of corticosteroid-induced osteoporosis.

Peptidase and Proteases Inhibitors

The second strategy is dealing with coadministration of protease and peptidase inhibitors (Lee et al., 1988). The nasal epithelial tissue contains substantial amounts of peptidases and proteases, both at membrane bound and intracellular levels, quite similar to the intestinal mucosae and other alternative absorptive mucosae (e.g., buccal, vaginal). The predominant enzymes appear to be aminopeptidases among other exo- and endopeptidases, dipeptidyl peptidase, dipeptidyl-carboxy-peptidase, trypsin and cathepsin B. The amino peptidase inhibitors such as bacitracin, bestatin and amastatin have been found to promote the nasal absorption of LHRH , salmon calcitonin, leucine enkephalin and human growth hormone (O'Hagan et al., 1990) in rats. Moreover, the compound camostat mesilate has been found to improve the nasal absorption of vasopressin, desmopressin and salmon calcitonin in rats by inhibiting both aminopeptidase and trypsin-like activities (Morimoto et al., 1991b).

Absorption Enhancers

The most frequently used approach to improve the absorption efficiency of intranasally administered peptides and proteins is the use of absorption enhancers(Verhoef et al., 1992). Presently, there are seven major types of nasal absorption enhancing compounds (Table 3). Most of the nasal absorption enhancers are capable of increasing membrane fluidity, either by creating disorders in the phospholipid domain in the membrane or by facilitating the leaching of proteins and lipids from the membrane. They also improve peptide and protein absorption probably by one or combination of several mechanisms, such as decreasing the viscosity of the mucus layer, solubilization of drugs and membrane components, and formation of the reversed micelles in the nasal epithelium, and by inhibiting enzymatic degradation.

Table 3 Absorption enhances used for nasal of peptides and proteins.

Surfactants :	Polyxyethylene-9-lauryl ether (Laureth-9) Saponin
Bile salts :	Trihydroxy salts (cholate,glyco-and taurocholate) Dihydroxyl salts (deoxycholate, taurodeoxycholate) Fusidic acid derivatives (e.g. STDHF)
Chelators :	Salicylates EDTA
Fatty acid salts :	Oleic acid Caprylate (C_8)
Phospholipids :	Lysophosphatidylcholine (lyso-PC)
Glycyrrhetinic acid derivatives :	Carbenoxolone Glycyrrhizinate
Cyclodextrins :	α-, β- and γ-Cyclodextrin Cyclodextrin derivatives (e.g., DM-β-CD)

Many reports have been published on the efficacy of various absorption enhancers in nasal peptide and protein delivery (Chien et al., 1989). For example, bile salts and STDHF can significantly enhance the nasal absorption of insulin and growth hormone in rats, rabbits and sheep (Longenecker et al, 1987). Medium chain fatty acid salts like sodium caprylate, sodium caprate and sodium laurate are also strong enhancers of nasal insulin absorption. Among these fatty acids sodium caprylate, at a concentration of 1%, exhibited the strongest insulin absorption promoting effect. This effect appeared to be partly associated with the chelating ability for calcium ions and with the inhibitory action on leucine aminopeptidase activity. However, the fatty acid salts showed higher haemolytic activity than glycocholate (Mishima et al, 1987). The absorption enhancing effect of carbenoxolone, glycyrrhetinic acid salt and glycyrrhizinia acid salt on the nasal insulin absorption was also investigated in rats. These compounds have structures comparable with triterpenes, and show a promoting action similar to that of bile acids or saponins (Mishima et al, 1989). Carbenoxolone turned out to be the most effective agent. Nasal absorption of 10 IU/kg insulin in the presence of 1% carbenoxolone was 26.5% of that in the case of a 5 IU/kg intravenous dose. Nasal leucine aminopeptidase activity was more strongly inhibited by carbenoxolone than by glycocholate. Intranasal insulin solutions in combination with 0.5% of L-α- lysophosphatidylcholine in rat produced a reduction in blood glucose levels similar to laureth-9. The two main constituents of L-α- lysophosphatidylcholine , being the palmitoyl component (72%) and the stearoyl component (24%), produced similar effects to that of L-α- lysophosphatidylcholine , thus indicating that both of these lysophos-pholipids are equally potent absorption enhancers (Illum

et al , 1989). Using the enhancer didecanoyl- L-α- lysophosphatidylcholine in human volunteers insulin bioavailability was 8.3% and 11.5% when compared to intravenously and subcutaneously administered insulin, respectively (Drejer et al, 1990).

In the past years scientists have concentrated their research on the effect of a relatively new class of compounds, i.e. cyclodextrins (CDs), on nasal drug absorption. CDs are cyclic oligosaccharides of 6, 7 or 8 glucose units: α-CD, β-CD and γ-CD, respectively. They can form inclusion complexes with lipophilic drugs, thereby increasing their water-solubility. Moreover, CDs may also have direct effects on epithelial membrane barriers, thereby facilitating transmucosal transport of hydrophilic drugs such as peptides and proteins. Studies in rats have shown that DM-β-CD in concentrations ranging from 2 to 5% is a very potent enhancer for the nasal absorption of insulin and an ACTH hexapeptide analogue, resulting in absolute bioavailabilities of approx.100 and 70%, respectively (Merkus et al 1991a). In a recent report Irie et al. (1992) confirmed the absorption promoting effect of some CDs, especially the methylated derivatives, on nasal insulin delivery in rats. Moreover, the authors provided evidence that this CD effect is mainly caused by a combination of increased permeability of the nasal mucosa probably through an interaction of CD molecules with lipids and/or calcium ions and reduced enzymatic degradation of the polypeptide. The CDs as absorption enhancing compounds will be discussed in more detail in relation with the observed interspecies differences.

Pharmaceutical Formulation

Sprays vs Drops:

The site of drug deposition in the nose is highly dependent on the dosage form. Nasal sprays deposit more anteriorly, resulting in lower clearance of sprays than of drops. The nasal bioavailability of desmopressin has been found to be significantly increased following spray administration as compared to nasal drops (Hardy et al., 1985).

Powders vs Solutions :

Powder dosage forms of peptides and proteins have advantages over liquid formulations. For example, in powders the chemical stability of the drug is usually increased, a preservative in the formulation is not required, and it is possible to administer larger amounts of drug and excipients (Lee W.A., 1991). Intranasal delivery of a nafarelin powder dosage form with high molecular weight dextrans resulted in increased peptide absorption over a liquid formulation (Vickery et al, 1989). Pontiroli et al (1989) compared the administration of powders and solutions of glucagon and human calcitonin with dihydrofusidate as enhancer in a clinical study. They found that the powder formulations were as effective as the spray solutions. In contrast, Provasi et al (1992 a,1992b) reported that in rabbits nasal powder dosage forms of salmon calcitonin were twice as effective as solutions.

Bioadhesives :

In order to reduce the nasal clearance and to improve nasal drug absorption, Illum et al. (1987) introduces the concept of bioadhesion in nasal drug delivery. Bioadhesive microspheres based on materials such as starch, albumin and Sephadex. Evidence is available that the absorption enhancement by starch micropheres is not only relates to their mucoadhesive properties, but also to their own inherent absorption promoting effect by widening the spaces between the tight junctions.

Other bioadhesive polymer systems have also been used in nasal peptide drug delivery. A polyacrylic acid gel base improved the absorption of insulin and calcitonin in rats (Morimoto et al. 1985), while cellulose derivatives and neutralized polyacrylic acid increased nasal insulin absorption in dogs (Nagai et al.1984). Hyaluronate solutions showed an enhancing effect on the absorption of vasopressin and desmopressin in rats (Morimoto et al. 1991a). Moreover, the water-soluble bioadhesive polymer chitosan has recently been found to improve the nasal transcellular pathway of peptide absorption (.

Salmon Calcitonin as Model Drug for Nasal Administration

Salmon calcitonin (sCT) has been chosen as a model polypeptide in this study for two reasons. First of all, this peptide is among 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 2). The structure of sCT differs significantly from human and bovine calcitonin throughout the chain; this probably accounts for salmon calcitonin's greater potency and stability. All 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

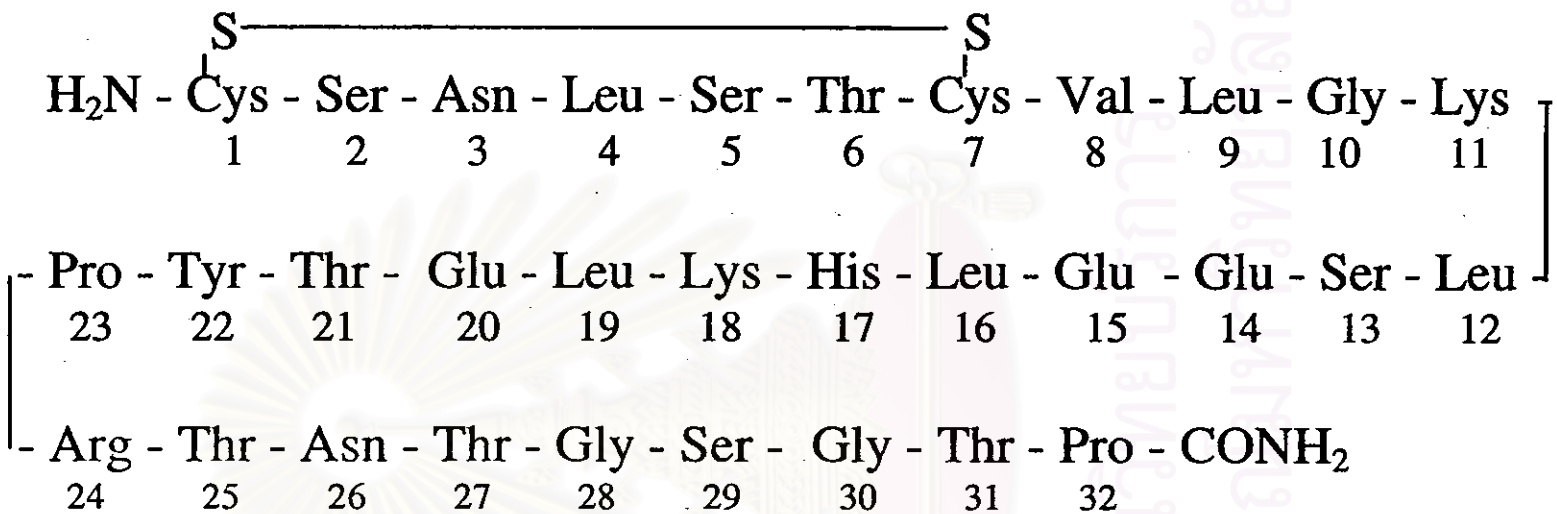

Figure 2 Amino acid sequence of salmon calcitonin

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 et al, 1974). A secondary action occurs through an inhibition of the tubular reabsorption of both calcium and phosphorous 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 alkaline phosphate (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 associated with the clinical use of sCT usually have been infrequent and mild, although adverse effects may occasionally be severe enough to require discontinuance of the drug.

GI Effects

Adverse effects of sCT most frequently involve the GI tract. Transient nausea, with or without vomiting, is the most common adverse effect and usually is mild and diminishes or disappears with continued therapy. Nausea and vomiting may occur within 30 minutes after an injection of sCT and may be minimized by administering the drug at bedtime. Other adverse GI effects of sCT include anorexia/poor appetite, diarrhea , pediatric discomfort, and abdominal pain . An unusual (e.g., salty, metallic) taste sensation has also been reported.

Dermatological, Sensitivity, and Immunologic Reactions

Flushing of the face, ears, hands and feet also occurs commonly within minutes after injection of sCT, but usually is well tolerated. Flushing may be minimized by administering the drug at bedtime. Tenderness and/or tingling of the palms and soles have also been reported. A local inflammatory reaction may occur at the site of subcutaneous or intramuscular injection of sCT. Rashes, including a maculopapular

eruption, erythema, and urticaria, have been reported occasionally in patients receiving calcitonin.

However, side effects are less common with the intranasal than with the injectable form.

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 milliliter. 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.

Chitosan as Novel Nasal Absorption Enhancer

Chitosan is partially deacetylated chitin. Commercially, chitin is extracted from shrimp and crab shells, and transformed to chitosan to obtain a water-soluble product. Figure 3 presents the chemical structures of chitin and chitosan. Chitosan is a linear biopolymer, specificlly a polysaccharide, which consists of two monosaccarides: N-acetyl-D-glucosamine and D-glucosamine linked together by β- (1→4) glycosidic bonds. The relative amount of the two monosaccharides in chitosan may vary, giving samples of diffent degrees of deacetylation.

Chitosan, a natural, non-toxic, biodegradable, high molecular weight polymer, is now produced commercially in north America on a

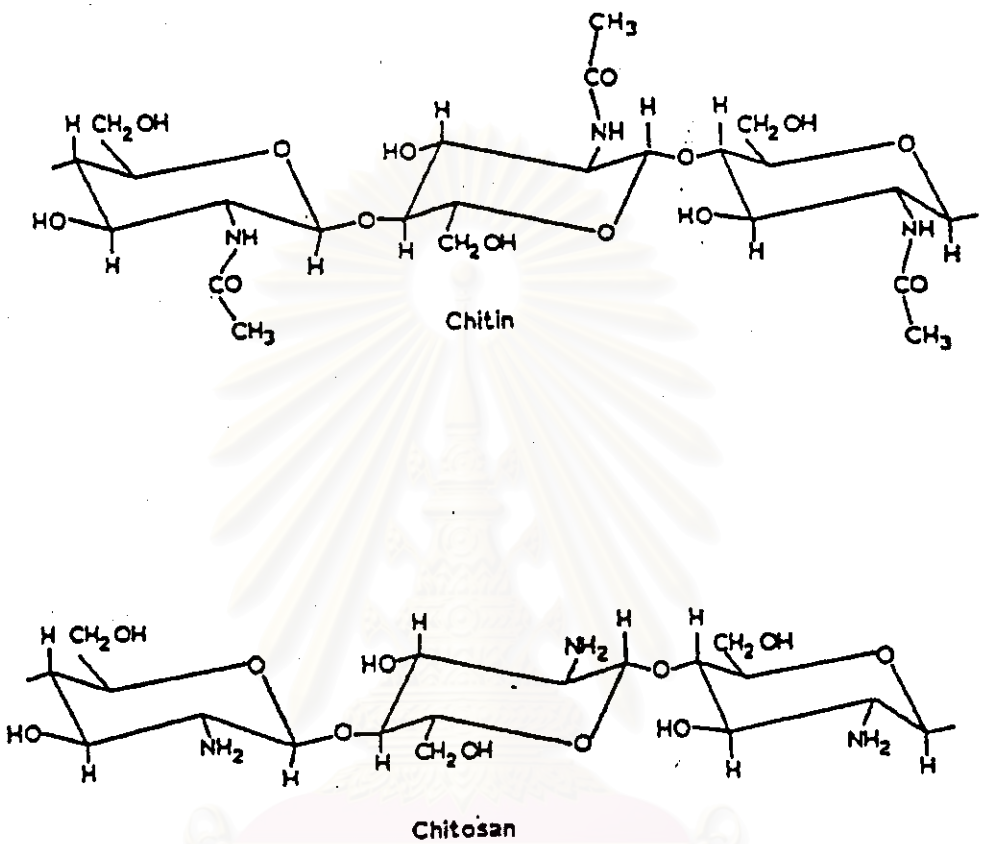

Figure 3 Chemical Structures of chitin and chitosan

large scale . For some time, it has been sold commercially as a solution, flake and fine powder, and more recently in bead and fiber forms. Chitosan's availability in a variety of useful forms and its unique chemical and biological properties make it a very attractive biomaterial. Chitosan's key properties are its activity to act as a cationic flocculant, humectant, viscosifier, and selective chelator of metal ions (Sandford, 1991). Chitosan's ability to be made into films, fibers and beads as well as powders and solutions lead to many commercial applications. It is used as hair treatment and in skin care products. Clear solutions form clear films that adhere to skin or hair. Chitosan's largest uses continue to be a non-toxic cationic flocculant in treatment of wastes and as a chelator of toxic (heavy and radiative) metals. It is also used as a water purification aid.

For medical uses, chitosan is being evaluated in a number of medical applications. These include wound dressings, hemostatic agent, and drug delivery system (Sandford et al, 1991). In these uses, the properties of chitosan are 1) biocompatability, 2) its ability to improve wound healing and/or clot blood 3) ability to absorb liquids and to form protective films and coatings. Chitosan has been shown to be suitable as eye bandage material and even have utility as a contact lens materials (Sanford et al, 1991). Chitosan can be made into strong fibers that may be useful as a suture material and as a wound dressing when chopped fibers are incorporated into a non-woven matrix. Chitosan beads can be used as a biocompatible matrix to deliver drugs. Due to chitosan's ability to be depolymerized by lysozyme, an enzyme found in various mammalian tissues, drug impregnated chitosan beads can be a bioerodible system to deliver pharmaceuticals (Sandford et al, 1991).

Chitosan has been employed as a pharmaceutical excipient in oral drug formulations in order to improve the dissolution of poorly soluble drugs (Imai et al, 1991). According to mucoadhesive properties, chitosan was investigated for nasal drug delivery of many poorly absorbed drugs in order to improve their systemic bioavailability. These properties probably are mediated by ionic interaction between the positively charged amino groups in chitosan and the negatively charges sialic acid residues in mucus (Lehr et al, 1992). Illum and coworkers (1987,1988 and 1990) have introduced the principle of employing bioadhesive microphere systems for nasal delivery of such poorly absorbed drugs in order to improve the systemic bioavailability without the use of enhancer systems. Starch microspheres have been suggested to exert two different effects on the nasal membrane. The bioadhesive effect of the microspheres decreases the rate of clearance of the drug from the nasal cavity and thereby allows a longer contact time with the absorptive epithelium. Further, it has been shown in a study employing monolayers of Caco-2 cells that the microspheres promote a transient widening of the tight junctions between cells thereby allowing larger hydrophilic molecules to pass through the paracellular pathway (Edman et al, 1992).

Recently, it has been reported that chitosan enhanced considerably the absorption of peptides such as insulin across the nasal epithelium (Illum et al, 1994). The result showed that the inclusion of chitosan at concentration 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 (without chitosan). The mechanism of action was suggested to be a

combination of mucoadhesion and an effect on the gating properties of the tight junction (Illum et al, 1994). These mechanisms were also confirmed in Caco-2 cells, a human intestinal cancer cell line. The enhancing activity of chitosan was found to be pH and concentration dependent (Artursson et al, 1994). However, not enough information is known about its specific effect on the nasal mucosal integrity, its effect on the proteolytic enzymes of the nasal mucosa, as well as its enhancing effect on other peptides like sCT, another important peptide hormone.