CHAPTER I

INTRODUCTION

Peptide and protein drugs are increasingly becoming a very important class of therapeutical agents as a result of the increased understanding of their role in physiology and pathology as well as the rapid advances in the field of biotechnology/genetic engineering. Improved methods of obtaining these peptides and proteins and identification of more potent or stable analogues have enhanced the feasibility of using peptides and protein therapeutically. Although they are highly potent and specific in their physiological functions, most of them are difficult to administer clinically.

Salmon calcitonin (sCT) is a polypeptide hormone consisting of 32 amino acids which plays a key role in calcium metabolism. It lowers blood calcium levels by increasing urinary calcium excretion and inhibiting bone resorption. It has been used therapeutically for the treatment of Paget's disease, osteoporosis and hypercalcemia (Stevenson et al, 1981). Because of its polypeptide nature, sCT is susceptible to degrading enzymes of the gastrointestinal tract and to inactivation by the first pass hepatic metabolism, leading to poor oral bioavailability. Therefore, it must be administered by injection, a route which is not well accepted by patients, particularly for chronic therapy (Banga et al, 1988). The alternative administration routes which included nasal , buccal, rectal, vaginal, transdermal and ocular routes have been investigated. The nasal route for drug administration has been of great interest and absorption

from this route has been studied widely. The nasal administration offers several promising advantages. Ease of administration and avoidance of needle make a preference to patients. Long term therapy is thus the more favorable aspect of nasal delivery than parenteral application. The drug is absorbed rapidly because the nasal epithelium is highly vascularized and the presence of microvilli makes a large surface area of the nasal cavity. The other important advantage of nasal delivery is an avoidance of first-pass hepatic metabolism (Shao et al, 1992).

In recent years, sCT has been commercially available for intranasal administration in the form of nasal sprays containing only synthetic sCT with no promoter. These preparations have received much attention, since absorption of the drug through the nasal mucosa is rapid and patient compliance is improved (Buclin et al, 1987). However, the relative bioavailability of sCT in humans was recently reported to be very poor, at only 1.6% that of intramuscular preparation (Lee et al, 1994). Several approaches can be used to improved the nasal absorption efficiency of sCT. Addition of absorption enhancers to the nasal drug formulations is one popular approach. The various enhancers that have been investigated included bile salts, sodium dihydrofusidate (Pontiroli et al, 1989) and sodium tauro-24,25-dihydrofusidate (Lee et al, 1994). However, most of these enhancers are associated with side effects such as irreversible changes in the nasal membrane. Consequently, these materials are unacceptable for chronic use in humans. Nevertheless, since the potential therapeutic benefits are enormous, there is considerable interest in finding novel nasal absorption enhancers that may be effective without evidence of topical or systemic toxicity following nasal administration.

Recently, chitosan has been studied as a potential enhancer of mucosal drug absorption (Illum et al, 1994) Chitosan is a high molecular weight cationic polysaccharide derived from naturally occurring chitin in crab and shrimp shells by deacetylation. Due to its good biocompatibility, biodegradability and favourite toxicological properties, it has been used for various purposes in pharmaceutical drug formulations. These include the direct tabletting and dissolution enhancement of poorly water soluble drugs (Imai et al, 1991), the sustained release of the drug and the use as constituent in liposomes (Henriksen et al, 1993).

Recent studies have investigated the effects of chitosan on the transport of small water-soluble molecules across the cultured monolayer of intestinal epithelial cancer cells (Caco-2-cell) grown in vitro (Artursson et al, 1994). They found that chitosan significantly increased the permeability of these cell. 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 molecules through the paracellular pathway. In the same year, Illum et al (1994) studied the nasal administration of insulin in sheep and rats with and without chitosan. The optimum concentration of chitosan for maximal absorption enhancement of insulin in rats and sheep was found to be 0.2 % and 0.5 % respectively. The AUC for insulin in the presence of chitosan was found to be 7 times greater than that of insulin alone. 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 preliminary results indicated the potential application of chitosan as a nasal absorption enhancer of poorly absorbed drugs like peptides. However, very few

information is available with respect to its efficacy relative to other enhancers. It is also interesting to know if chitosan could enhance nasal absorption of other peptides apart from insulin.

The purpose of this study was to investigate if chitosan could significantly improve the absorption of other peptides like sCT. The enhancing activity was also compared to that of cyclodextrin derivatives such as hydroxypropyl-β-cyclodextrin and dimethyl-β-cyclodextrin which are adjuvants reported to have a relatively mild effect on the nasal mucosa with marked enhancing activities. Furthermore, this research also investigated possible inhibitory effect of chitosan on two nasal enzymes, namely leucine aminopeptidase and trypsin, that are responsible for degradation of sCT in the nasal mucosa (Morimoto et al, 1995). The inhibition effects of chitosan on the proteolytic enzymes were also compared to that of their respective specific inhibitors such as bestatin and aprotinin.

Therefore, the aims of this study were as follows :

1. To evaluate the efficacy of chitosan as nasal absorption enhancer of sCT using in vivo nasal absorption technique.

2. To investigate the optimal pH and concentration of chitosan for nasal absorption enhancement of sCT.

3. To compare the efficacy of two different types of chitosan with that of cyclodextrin derivatives in improving the nasal absorption of sCT.

4. To study the inhibitory effect of chitosan on the proteolytic enzyme activity of leucine aminopeptidase and trypsin using in vitro technique.