CHAPTER V

CONCLUSIONS

With the continuing development of a number of peptide-and protein-based pharmaceuticals, noninvasive routes of absorption for these macromolecular drugs are receiving extensive scrutiny. The nasal route especially is receiving considerable attention. However, the peptide and protein drugs generally are poorly absorbed across the nasal mucosa and often require some types of absorption enhancers to effect significant absorption. Besides, the nasal permeation enhancement mechanisms of the enhancers may be different. So they should be investigated in terms of the efficacy and toxicity as well as the extent of membrane reversibility. The final step, an *in vivo* human model was used to study the nasal absorption of peptide in the presence of selected enhancers before further clinical testing can be conducted. For this study, salmon calcitonin (sCT) was selected as the model peptide drug.

The results from this study can be summarized as follows:

1. Five enhancers, namely 0.5% CS J, 0.5% CS G, 1.25% DM-β-CD, 5.0% HP-β-CD and 0.1% LCC, were investigated for the efficacy of their absorption enhancing activities based on comparison of percentage [D-Arg^2]-Kyotorphin remaining in the perfusate after 60 min nasal perfusion and the apparent first order absorption rate constant.

2. ANOVA and Duncan's test on the % [D-Arg^2]-Kyotorphin revealed that there were significant differences among the five enhancers in their absorption promoting activities. 5% HP-β-CD was not effective from control whereas 0.1% LCC, 0.5% CS J, 1.25% DM-β-CD and 0.5% CS G were all effective over the control. Furthermore, 0.1%LCC was significantly more effective than 0.5% CS G

whereas 0.5% CS J and 1.25% DM-β-CD gave absorption enhancing activities somewhat intermediate between 0.1% LCC and 0.5% CS G.

3. The membrane-damaging or membrane-perturbing effects of these enhancers were evaluated by measuring the extent of intracellular enzyme (LDH) release into the nasal perfusate during the first period of perfusion. ANOVA results showed that there was a significant difference in the LDH release at T_{60} among these enhancers ($p < 0.05$), which can be classified into 3 groups with different membrane-damaging effects. 0.1% LCC constituted the first group having the greatest effect on the nasal membrane. The second group, which demonstrated moderate membrane interactions, consisted of 1.25% DM-β-CD, 0.5% CS J and 0.5% CS G. The third group, which exerted the least effect on the nasal membrane, consisted of 5% HP-β-CD and the control saline.

4. Significant correlation ($p < 0.05$) was observed between the percent dipeptide and the concentration of LDH found in the nasal perfusates at T_{60} of the first perfusion. This indicates that the enhancers under study were able to enhance the nasal absorption of the model dipeptide at least by direct interaction with the nasal membrane, i.e. through the transcellular pathway.

5. Comparison of the LDH activities and percent dipeptide remaining in the perfusate before and after removal of the enhancer revealed that 0.1% LCC, 1.25% DM-β-CD, 0.5% CS J and 0.5% CS G exhibited good mucosal recovery in terms of both membrane integrity and permeability to peptide absorption. On the other hand, 5% HP-β-CD and the control saline showed no signs of such reversibility. Clearly, this was due to their weak effects on the nasal mucosa.

6. One-tenth of a percent LCC and 1.25% DM-β-CD appear to give faster recovery than 0.5% CS J and 0.5% CS G with respect to the LDH release. This could be due to different mechanisms of membrane interactions exerted by different enhancers. It is possible that CS J and CS G may have a lower but more sustained

effect on the nasal membrane than LCC and DM-β-CD. Also, the mucoadhesive properties of the two chitosans may have resulted in incomplete removal of the polymer. Some residual amount of chitosans may remain in the nasal cavity during the second perfusion and could be responsible for the slight increase in the LDH level.

7. Although 1.25% DM-β-CD gave LDH release at T_{60} similar to 0.5% CS J and 0.5% CS G, it appeared to stimulate faster release of LDH than the two chitosans, especially during the first 15 min of perfusion. This observation suggests that DM-β-CD may be more membrane-irritating than chitosans despite its more rapid recovery. Its toxicity became much more pronounced when the concentration was increased from 1.25 to 2.5 %, as evidenced by bleeding of the nasal mucosa.

8. On the other hand, the extent of recovery with respect to dipeptide absorption was similar among 0.5 % CS J, 0.5% CS G, 1.25% DM-β-CD and 0.1% LCC. The ranking was not correlated with the extent of recovery based on the LDH release. No clear explanations could be offered for this observation. However, the differential extent to which these enhancers can increase the nasal permeability via the paracellular pathway is not presently known. Since the absorption of [D-Arg^2]-Kyotorphin can occur through both the transcellular and paracellular pathways, it is possible that the differences among these enhancers in their extent of absorption enhancement via the latter route could be responsible for the lack of correlation.

9. Comparison of the peptide absorption enhancing activity and membrane-damaging effect between 0.1% and 0.5% of both chitosans revealed no significant differences ($p > 0.05$, unpaired Student's t-test). This indicates that the effects of CS J and CS G on the nasal membrane integrity and mucosal permeability were not dependent on concentration, at least in the range 0.1-0.5%.

10. One-half percent CS G was selected as a representative of chitosans to evaluate its nasal absorption enhancing effect *in vivo* using healthy male volunteers as

subjects in a three-way crossover study with a completely randomized block design. Salmon calcitonin (sCT) was used as a model peptide and 1.33% DM-β-CD as a reference enhancer. The extent of sCT nasal absorption was assessed from the decrease in plasma calcium following its nasal administration. Area under the plasma calcium versus time curve ($AUC_{0\text{-}9hr}$) was calculated for each subject and the results were compared among the control (no enhancer), 0.5% CS G- and 1.33% DM-β-CD-treated groups using ANOVA.

11. ANOVA and subsequent Duncan's test revealed that 0.5% CS G was superior to the control and 1.33% DM-β-CD-treated groups in enhancing the nasal absorption of sCT ($p < 0.05$). After nasal administration with CS G, the plasma calcium decreased gradually, reaching minimum of 89.5 % of the initial value at 6 hr post dose. The calcium level was still significantly lower than the control and 1.33% DM-β-CD even at 9 hr after administration. On the other hand, plasma calcium for the control and 1.33% DM-β-CD decreased only slightly during the first 3 hr before returning to the initial value, indicating a smaller extent of sCT nasal absorption.

12. In conclusion, it has been demonstrated in this study that the cationic polysaccharide chitosans possess significant nasal absorption enhancing efficacy, safety as well as fairly good reversibility on the rat nasal mucosa. *In vivo* absorption study in human volunteers also substantiated the superior property of chitosans over DM-β-CD, another novel class of enhancer, in promoting the nasal absorption of peptide like sCT. Therefore, chitosans especially CS G appears to have a promising potential for use as a safe and effective nasal absorption enhancer in nasal peptide formulations provided that more clinical testing has been conducted.