Chapter 4

*In-vitro* permeation mechanism of sumatriptan succinate across sheep nasal mucosal membrane
4.1 INTRODUCTION

Absorption of drug from nasal cavity is very complex and is influenced by many factors, which has been discussed earlier. Mathematical analysis of kinetics and dynamics of these processes indicated solubility and permeability as the fundamental properties controlling drug absorption. Permeability is an important, but still unpredictable, determinant of absorption, and it is informative to explore mechanisms contributing to permeability. Transport across nasal mucosa or nasal cell cultures can be mediated through one or a combination of several routes. Until now, permeation studies showed that both the paracellular and transcellular transport pathways are possible. In the case of the paracellular route, passive diffusion between epithelial cells is limited by the barrier function of the tight junctions. The alternate transcellular transport can be either passive or active. Transcytosis, an active transcellular transport process, is either receptor mediated or non-specific (pinocytosis). Whether the administered compounds will be transported through the transcellular or paracellular route will be determined by the physical and chemical properties of the drug. Usually, highly lipophilic compounds diffuse passively across the barriers by the transcellular pathway, whereas hydrophilic, membrane impermeable drugs diffuse to a higher extent through the paracellular pathway, which is controlled by the tight junctions. To discriminate between passive and active transport processes, permeation studies performed in diffusion chambers are most useful. Passive diffusion is indicated by low energy dependency. So the determination of Peff at different initial donor concentrations should exhibit non-saturable kinetics. In contrast to passively transported permeants, active transepithelial transport should show saturation kinetics and direction-specificity. Equally typical are substrate specificity and dependence on metabolic energy (Schmidt et al., 1998).

There are no previous reports available on exact permeation mechanism for sumatriptan succinate across nasal membrane. Therefore the objective of this chapter is to identify potential permeation pathway for sumatriptan succinate across sheep nasal mucosal membrane by preliminary in vitro permeation study. The preliminary knowledge about the probable permeation mechanism across nasal mucosal membrane would be helpful if developing formulations for nasal delivery of sumatriptan succinate.
4.2 EXPERIMENTAL

4.2.1 In-vitro Permeation Studies

In vitro permeation studies were done as described previously by many research groups (Ceschel et al, 2000 and Pisal et al, 2004). Briefly, nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse and used immediately. Franz diffusion cell setup was used to determine drug transport across the sheep nasal mucosal membrane. To start the permeability experiments, 1 ml of Kreb’s bicarbonate buffer solution containing the sumatriptan succinate was placed in donor chamber, whereas the receiver chamber was filled with 20 ml of pure buffer solution. Usually, the pH of buffer was kept at 7.0 at a constant temperature of 37 °C. The solutions were perfused with carbogen gas consisting of 95% oxygen and % carbon dioxide, and stirred continuously during transport experiments. At appropriate time points, samples (1 ml) were taken from the receiver phase, and replaced by an equal volume of fresh buffer. The samples withdrawn were filtered and used for analysis. Blank samples (without sumatriptan) were also run simultaneously throughout the experiment to check for any interference. The samples were diluted with water and assayed by spectrophotometry at 283 nm. The viability of the tissue in the permeation study was investigated before and after the experiment by staining with trypan blue (0.1% solution in PBS). After an incubation time, medium from both compartment was removed, tissues were washed and were examined by light microscopy for exclusion of the marker. Exclusion of the marker from the tissue cells was considered to be viable as described by Verena et al (Verena et al, 2004).

To characterise the transport mechanism of sumatriptan succinate across the sheep nasal mucosal membrane the influence of several parameters was investigated. The initial drug concentration was varied between 0.25 and 5 mg ml⁻¹, sodium ions were replaced by N-methyl-D-glucamine (NMDG). Furthermore, effects of addition of 2.5 mM EGTA (ethylene glycol-bis(β-aminoethylether)-N,N',N''-tetraacetic acid) on nasal mucosal membrane were investigated.
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4.2.2 Permeability coefficient
The apparent permeability coefficient ($P_{app}$, cm sec$^{-1}$) was calculated according to the following equation:

$$P_{eff} = \left( \frac{dC}{dt} \right)_{ss} \frac{V}{AC_D}$$

where $(dC/dt)_{ss}$ (µg ml$^{-1}$ s$^{-1}$) is the time dependent change of concentration in the steady-state, $A$(cm$^2$) is the permeation area, $V$(ml) the volume of the receiver compartment and $C_D$(ug ml$^{-1}$) the initial donor concentration (Lang et al, 1996).

4.2.3 Statistical analysis
All the experiments were repeated three times. Results are expressed as mean ± S.D. Statistical differences between two mean values were evaluated using unpaired student's t-test. Differences between mean values of more than two groups were evaluated using one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test. Difference between means was considered significant if the $P<0.05$. 
4.3 RESULTS AND DISCUSSION

In general, drugs can be transported by different routes across the nasal epithelium (e.g. passive diffusion, transcellular or paracellular transport or carrier mediated either primary or secondary active transport or transcytosis). The series of experiments addressed the questions of the transport mechanisms for sumatriptan succinate.

4.3.1 Effect of Drug concentration on Drug transport

First the effect of different initial concentration of sumatriptan succinate on the amount of drug transport was determined. Experiments were carried out over a wide concentration range 0.25 to 5 mg ml⁻¹ for a time period of 240 min. Results show that the percentage of drug being transported per unit time was independent of the initial drug concentration.

Figure 4.1 Effect of different concentrations of sumatriptan succinate on the drug transport represented as percentage of drug being transported after 240 min, data points are mean ± S.D (n=3).
4.3.2 Effect of sodium concentration on drug transport
The following experiments addressed a potential sodium dependency of sumatriptan succinate transport. Therefore, the sodium gradient was reduced by replacement of sodium by N-methyl-D-glucamine (NMDG). Neither partial replacement of sodium by NMDG in the donor or in the acceptor phase nor total sodium depletion affected the transport of sumatriptan across the nasal mucosal membrane suggesting that no sodium dependent carrier system is involved in the process of drug absorption.

Table 4.1 Effect of sodium on the permeability (P_eff determined at 240 min) of sumatriptan succinate across sheep nasal mucosal membrane

<table>
<thead>
<tr>
<th>Effect of sodium</th>
<th>Permeability (x 10^{-5}, cm/sec) Mean ± S.D (n=3)</th>
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<tbody>
<tr>
<td>with sodium</td>
<td>5.04 ± 0.167</td>
</tr>
<tr>
<td>sodium replaced by NMDG in acceptor</td>
<td>5.01 ± 0.214(n.s)*</td>
</tr>
<tr>
<td>sodium replaced by NMDG in donor phase</td>
<td>4.97 ± 0.291(n.s)*</td>
</tr>
<tr>
<td>sodium replaced by NMDG in both phase</td>
<td>5.14 ± 0.282(n.s)*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D (n=3).

* Levels of significance between standard condition and sodium replacement

4.3.3 Effect of coadministration of EGTA on permeability
The functions of gap junctions in stabilizing tissue integrity are highly dependent on the presence of calcium. By cheating calcium the importance of paracellular pathway for drug absorption can be studied. We used the calcium chelator EGTA because of its higher calcium affinity than other chelators like citrate or ethylenediaminetetraacetic acid (EDTA). Control experiments demonstrated that viability was not affected after a 4 h incubation period with EGTA containing buffer. Exposure of sheep nasal mucosal membrane to EGTA resulted in an increased absorption of sumatriptan succinate in pure drug solution as indicated by the upward shift of the time-transport profile, which was significant over the whole time period of 240 min. Concerning local tissue damage, staining of sheep nasal mucosa with trypan blue after permeation studies with various formulations did not show any dead cells.
Figure 4.2 Influence of EGTA on the transport of sumatriptan succinate across sheep nasal mucosal membrane using drug solution (5 mg/ml), drug transport in the presence of 2.5 mM EGTA is given by triangles and square is the controls without addition of 2.5mM EGTA, date are expressed as mean ± S.D(n=3), * indicates significance with P<0.05.

The results suggest that sumatriptan succinate is preferentially taken up passively via a diffusion-limited process. First of all, the transport of sumatriptan succinate across sheep nasal mucosal membrane was not saturable since increasing drug concentration resulted in an increased drug flux. Carrier-mediated transport should be saturable as the amount of carriers is limited. However, carrier-mediated transport cannot be excluded totally since at the sumatriptan succinate concentration used all carriers may not be saturated. Diffusion-limited passive transport processes may occur transcellularly or paracellularly. Usually lipophilic substances are absorbed via the transcellular route, whereas hydrophilic agents are transported paracellularly. Since sumatriptan succinate is hydrophilic in nature, paracellular transport would have to be expected. Transport via the paracellular route can be enhanced using EGTA which interrupts the integrity of the junctional complex by chelating calcium, whereas transport of lipophilic agents remains unchanged. EGTA indeed enhanced the permeability of the epithelial barrier for...
sumatriptan succinate, which is consistent with paracellular transport. Furthermore, the sumatriptan succinate transport was not significantly affected by the Na\(^+\) gradient. In contrast to this, most carriers for amino acids, glucose etc. are coupled to this inward-directed sodium gradient, which is established by the action of Na\(^+\)/K\(^+\) - ATPase. From these findings it can be concluded that sumatriptan succinate seems to be transported via a passive non-saturable route, most probably via the paracellular pathway. In one of the earlier study, data from permeability profiling using the parallel artificial membrane permeability assay (PAMPA) and cell monolayer (Caco-2 and MDR1-MDCKII) methods were compared for sumatriptan by Kerns et al (Kerns et al, 2004). The sumatriptan succinate had higher cell monolayer permeability than PAMPA permeability and therefore it belonged to the group of compounds that are subject to absorptive mechanisms like paracellular, active transport, or increased passive diffusion under a pH gradient. These results are based on the fact that there was poor correlation between transport rates across PAMPA and cell monolayer. Moreover permeability in PAMPA was less than that of cell monolayer, further ruling out possibility of secretory transport mechanism (efflux mechanism). As sumatriptan succinate is a weak base transport by pH gradient is ruled out Varma et al (Varma et al, 2004) reported that sumatriptan succinate is a non-substrate to p-glycoprotein, which further rules out the possibility of efflux mediated transport for sumatriptan succinate. So there lies possibility of only passive paracellular transport or active transport for sumatriptan succinate based on earlier reports. However, there is no report available on commenting on the exact mechanism of permeation across membranes for sumatriptan succinate. Based on the results obtained in this study active transport for sumatriptan succinate across sheep nasal mucosal membrane is almost ruled out, therefore it can be concluded that although there may be other pathways affecting transport of sumatriptan succinate it is preferentially and significantly transported via passive paracellular mechanism across sheep nasal mucosal membrane.

Taking above results into consideration mucoadhesive polymers like chitosan glutamate and carbopol 934P can act as supporting systems for enhancing transport across nasal membrane. By taking the known mucoadhesivity of chitosan glutamate and carbopol 934P into consideration, the proposed increased permeability may be explained as a consequence of the adhesion of the formulations to the surface. Moreover, the contact time of the drug with the surface is increased, which leads to a facilitated delivery of sumatriptan succinate. Recently it was shown that chitosan interacts with cytoskeletal F-
actin, thereby changing the F-actin distribution. Since F-actin is associated with the proteins in the tight junctions, the simultaneous widening of the intercellular space may occur. The resulting opening of tight junctions would lead to increased paracellular permeability. In addition, chitosan as well as carbopol 934P reacts as a chelator for metal ions. The depletion of calcium from the electronegative site of the membrane, which requires coordination with cations for dimensional stability, also leads to opening of tight junctions. Thus formulations containing above mentioned polymers can be useful in enhancing permeability of sumatriptan succinate across nasal mucosal membrane.

4.4 CONCLUSION
In summary, these experiments demonstrate that the transport was independent of initial drug concentration as well as complete or partial depletion of Na+ ions and was enhanced by pre-treatment with EGTA. Taken together, our results indicate that the paracellular pathway seemed to be the main route of sumatriptan succinate transport across nasal mucosal membrane.
4.5 REFERENCES


