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Research paper

Simple method of in vitro diffusion of nicotine across porcine palatine mucosa

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Abstract

The pig's palatal mucosa is used as a simple model of in vitro vertical diffusion to study the permeation of different dosage forms of nicotine. Nicotine-loaded microspheres as well as solutions are tested and some parameters of diffusion determined (fluxes, lag-time, etc.). The nicotine fluxes of solutions and microspheres do not differ significantly, except in the case of nicotine base formulations. However, the lag-time of microspheres formulations seems to be half the lag-time of solutions. The swelling of microspheres provoking a dehydration of the mucosa, as well as the high concentration of nicotine in microspheres directly in contact with the mucosa, seem to explain this difference of lag-time. © 1997 Elsevier Science B.V.

Keywords: In vitro permeation; Pig's palatal mucosa; Nicotine; Microspheres; Franz cell

1. Introduction

In recent years, alternative routes for the administration of drugs (peptides and proteins in most cases), have received much attention. Drug administration via mucosal membranes, like nasal, rectal, vaginal and buccal membrane, has the advantage, among others, of bypassing the hepatogastrointestinal 'first pass' metabolism associated with oral administration.

In order to investigate these routes, some in vitro models have been developed, to try to elucidate some parameters of the mucosal barriers, particularly their permeability.

Two categories of diffusion cells are usually used for measuring in vitro permeation: side-by-side diffusion cells (Ussing-type cells) and in vivo mimic diffusion cells (Franz-type cells, vertical cells).

The Ussing chamber can be useful to measure the transmucosal flux of a substance across a mucosa and the effects of absorption enhancers on this mucosa. Wheatley et al. [1] have described an in vitro technique for studying the transport of compounds across the nasal epithelium and the effects of enhancing agents both on the transport of the compounds across the epithelium and on the physiological and histological integrity of the epithelium. The effect on the mucosal barrier of some enhancers has been investigated by several authors [2–7].

The Ussing chamber is useful for studying the enzymatic degradation of peptides [8] for example, as well as for comparing the absorption of a substance in different mucosae of rabbit in vitro [9]. The side-by-side Valia-Chien type cell was used by Corbo et al. [10] to investigate the permeation of model hydrophilic and lipophilic compounds through the nasal, rectal and vaginal mucosa of the rabbit. Bechgaard et al. [11] have studied the viability of the isolated rabbit nasal mucosa in the Ussing diffusion chambers and found a viability of more than 10 h. In consequence, this system may be useful for physiological and toxicological studies.

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However, side-by-side diffusion cells expose the membrane to solvent on both sides throughout the experiment leading to potential solvation effects [12]. Measurement of permeation rates under conditions similar to those encountered in vivo requires a different cell design for solid or semi-solid dosage forms (powders, films, gels, etc.). The advantage of the vertical cell design is the ability to vary the nature of the donor vehicle. The Franz-type diffusion cell is one of the most widely used systems for in vitro skin permeation studies, and was used in this work. Vyas et al. [13] have studied the diffusion of thyrotrophin across porcine buccal mucosa with Franz cells. In fact, this model allows to study the permeation of a compound through a membrane from powder dosage forms, avoiding the dispersal of the solid or semi-solid dosage form in a solvent.

Nicotine replacement therapy offers an interesting approach to help giving up smoking [14,15]. As part of a study on smoking cessation using nicotine as therapeutic agent, Cornaz et al. [16] have developed powder formulations which rapidly release nicotine from Sephadex® microspheres. These formulations were tested in this study in terms of permeation of nicotine through a model mucosa. In fact, the permeation of nicotine through the pig's palatine mucosa has been studied in different physicochemical conditions: at different ionization state of the drug and in different states (powder or solution).

A very simple mucosal model of in vitro permeation to study the diffusion of nicotine from a solid dosage form has been developed in this study, using the stripped mucosa of pig's palate as a membrane. This permeation barrier consists of a basal layer (stratum basale, a prickle cell layer (stratum spinosum), and a granular cell layer (stratum granulosum). The keratinized layer (stratum corneum) was removed in order to obtain a uniformly and easily permeable mucosa. The advantage of the striped palate of pig is that it is very easily available and isolated from the other tissues. The diffusion cell does not require any elaborate material and gives interesting information about the permeation of different drugs at different pH or in different dosage forms.

The aim of this study is to use a model of diffusion which is very simple to perform, and to obtain some information about the passage of nicotine through a physiological membrane, from solid and liquid dosage forms.

2. Materials and methods

2.1. Materials

Nicotine-free base (NB) and nicotine hydrogen tartrate salt (NHT) were obtained from Sigma (Buchs, Switzerland).

Sephadex® microspheres G25 and G50 Fine (Pharmacia, Uppsala, Sweden) were loaded to about 10% with nicotine and characterized according to Cornaz et al. [16]. G25 is more crosslinked than G50 and needs consequently less liquid to swell than G50.

The NHT and NB solutions are concentrated to 11.1 mg/ml. In order to obtain a pH of 7.4, the NHT solution was neutralized with NaOH 1 N. Their osmolarity is measured with an automatic osmometer Knauer (no. 25647), Berlin, Germany. NHT solution has an osmolarity of 160 mOsm/kg, the NB solution of 62 mOsm/kg and the NHT solution buffered to pH 7.4 of 315 mOsm/kg.

The phosphate buffer pH 7.4 (USP) was used as receptor medium. Its osmolarity is 114 mOsm/kg.

Pig palatal mucosa.

2.2. Methods

The permeability study was carried out with stripped pig palate (without stratum corneum). The freshly excised palate mucosa with any underlying conjonctive tissue removed, was stored at −20°C before use. The mucosa was easily separated from the palatal bone. The stratum corneum was removed from the mucosa with a surgical clamp. The submucosal tissue was dissected from the palatine mucosa with a dermatome, until the ridges of the mucosa become visible. The palate mucosa was mounted in a Franz diffusion cell, the two vertical chambers being firmly clamped together to prevent leakage. The receiver compartment was filled with 8 ml of phosphate buffer pH 7.4 and maintained at 37°C, constantly stirred with a magnetic stirrer. The viability of the tissue could not be maintained for a long time, because the mucosa was not exposed to a solution on both its faces in the case of microspheres assays. In fact, with the vertical permeation system used in this study, the mucosa could not be kept living for a long time, due to the lack of liquid in the donor compartment (mucosal side). This is acceptable in the case of passive diffusion studies (which is the case with nicotine), but not in the case of facilitated diffusion, which demands living mucosa.

The exposed area of the mucosa (0.785 cm^2) was hydrated in the donor compartment during 30 min with 200 μl buffer. The excess of liquid was removed just before the beginning of the trial, in order to keep the donor chamber free of any liquid. A known amount of solution (100 μl) or of microspheres (about 10 mg) was uniformly dispersed on the surface of the mucosa. The concentration of nicotine in microspheres varied from 9

Table 1
Fluxes (F_{ss}) relative standard deviations (R.S.D.), quantification in the receiver compartment after 6 h, and lag-time of nicotine in different conditions

Samples tested	Fluxes (F_{ss}, mg/cm^2·h^{-1})	R.S.D. of the fluxes ($n = 3$, %)	NB % in the receiver compartment after 6 h	Lag-time (h)
100 μl NHT sol. pH 7.4 (11.1 mg/ml NB eq.)	0.204	7.4	58.8	0.71
NHT sol. (11.1 mg/ml)	0.217	22.7	64.0	0.41
NB sol. (11.1 mg/ml)	0.259	12.1	77.5	0.30
G25 NHT microspheres (9.4% NB eq.)	0.201	14.1	60.0	0.24
G25 NB microspheres (12.4% NB)	0.240	11.2	75.0	0.14
G25 NB microspheres (12.4% NB eq.) without liquid adjunction	0.227	13.5	58.2	0.13
G50 NHT microsphers pH 7.4 (9.7% NB)	0.228	10.6	73.5	0.24
G50 NHT microspheres (9.1% NB)	0.160	23.9	64.0	0.20
G50 NB microspheres (9.2% NB)	0.196	10.4	56.4	0.40

NB, nicotine base; NHT, nicotine hydrogen tartrate salt; G25, Sephadex® G25 microspheres; G50, Sephadex® G50 nicrospheres.

to 12%. The amount of microspheres was calculated so that the amount of nicotine present in the donor compartment was always the same (2.2 mg). When the microspheres were tested, 100 μl of buffer were added in the donor compartment to hydrate the microspheres; 200 μl of receiver compartment were periodically sampled during 6 h and replaced by an equivalent volume of fresh buffer.

The nicotine was simply quantified by a UV spectrometer Lambda 12 (Perkin Elmer, Uberlingen, Germany) at 260 nm, after an appropriate dilution of the sample. No absorption peak was found interfering with the nicotine peak at 260 nm and the concentration of the samples was sufficient to quantify the percentage of nicotine in the receptor compartment.

The residual concentration of nicotine in the donor compartment was quantified at the end of the trial, as well as the concentration of nicotine in the donor compartment. The nicotine concentration during the permeability study was corrected for dilution. All trials were performed 3–4 times.

Every assay showed a region of linearity between 0.5 and 2 h ($r^2 \geq 0.995$), except for NHT buffered solution (linearity between 1 and 3 h) and for G25 microspheres loaded with nicotine base (linearity between 0.5 and 1.5 h).

The steady-state fluxes (F_{ss}) were calculated from the slopes of the linear portion of the plots of cumulative amount of permeated nicotine (in mg nicotine base) per unit surface area (cm^2) vs. time of sampling. The lag-time of diffusion is the x-intercept of the linear permeation profile. The error (relative standard deviation) associated with them is situated between 8 and 24% and is directly proportional to the error associated with the fluxes (slope of the linear portion of the permeation profile).

The nicotine concentration in the case of nicotine microspheres in the donor compartment was not determinable. This is the reason why the coefficients of permeability were not calculated.

3. Results and discussion

Solutions and microspheres of nicotine were tested in solution and solid state, at different pH, and the results of fluxes, lag-time, % in the receiver compartment after 6 h, and relative standard deviations are listed in Table 1. After a lag-time of 0.2–0.7 h, a linear relation was found in all experiments. There are some statistical differences noted in the fluxes collected in Table 1. According to ANOVA test ($\alpha = 0.05$), followed by a Duncan's test, NB solution, NB-loaded G25 and NB-loaded G50 (Fig. 2) have statistically different fluxes.

The highest fluxes were observed with NB in solution as well as with NB loaded G25 microspheres. That could be explained by the fact that the buccal mucosa is more permeable for lipophilic (non-ionized) compounds [17,18].

It can be observed in Fig. 1 that NB solution presents a statistically higher nicotine flux than NHT or pH 7.4 NHT solutions (Fig. 1), at the same concentration, according to the fact that the rate of transfer of a

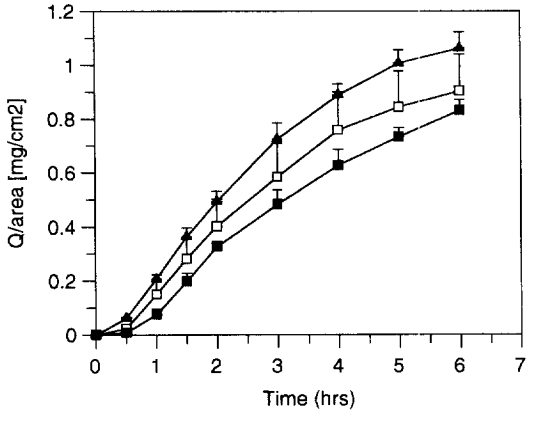

Fig. 1. Permeation of different nicotine solutions through the stripped mucosa of pig's palate ($n = 3$, mean ± standard deviation). (▲) Nicotine base (NB) solution, (□) nicotine hydrogen tartrate (NHT) solution, (■) buffered pH 7.4 nicotine hydrogen tartrate solution.

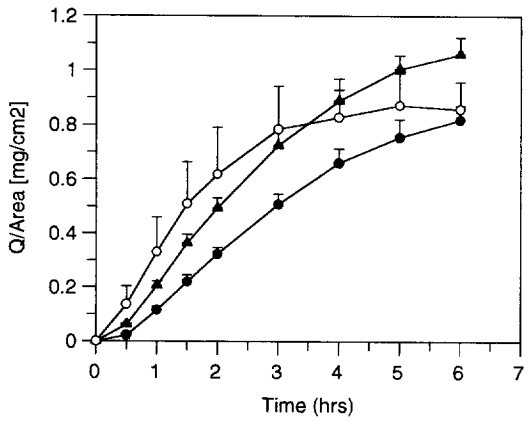

Fig. 2. Permeation of nicotine base solution and microspheres through the stripped mucosa of pig's palate ($n = 3$, mean ± standard deviation). (▲) Nicotine base (NB) solution, (○) nicotine base (NB)-loaded G25 microspheres, (●) nicotine base (NB)-loaded G50 microspheres.

molecule is dependent on the concentration gradient of the non-ionized species, rather than the total concentration (pK_a nicotine = 7.9) [17].

G50 microspheres loaded with NB or NHT seem to have lower fluxes due to their more important swelling volume. In fact G50 absorbed all the liquid available in the donor compartment and the dissolution of nicotine is slow compared to G25 microspheres which do not need so much liquid to swell. This fact is confirmed in both Figs. 2 and 3 which show a lower permeation profile for G50 than for G25 and solutions, independently of the nature of nicotine (NB in Fig. 2 or NHT in Fig. 3). It is interesting to note that in the case of the characterization of G25 and G50, the microspheres were allowed to freely swell and release nicotine, absorbing water by capillarity [16]. The release of nicotine from G25 and G50 did not differ significantly. But in the present study, the liquid available in the donor compartment is limited and probably does not allow a complete swelling of G50 and an efficient release of nicotine. This could explain the reason why the permeation profile of G50 seemed to be rate-limiting.

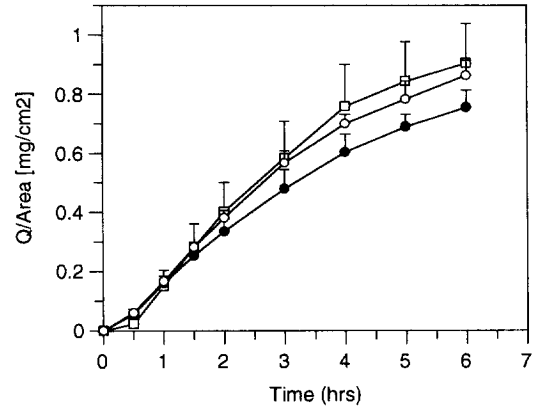

Fig. 3. Permeation of nicotine hydrogen tartrate solution and microspheres through the stripped mucosa of pig's palate ($n = 3$, mean ± standard deviation). (□) Nicotine hydrogen tartrate (NHT) solution, (○) nicotine hydrogen tartrate (NHT)-loaded G25 microspheres, (●) nicotine hydrogen tartrate (NHT)-loaded G50 microspheres.

The lag-time of diffusion has been defined as a measure of the time it takes for the permeant's concentration gradient to become stabilized across the membrane [19]. Table 1 showed that lag-times of nicotine microspheres are generally shorter than lag-times of nicotine solutions. In Fig. 2, nicotine from NB G25 formulation appeared more rapidly in the receiver compartment than from a solution. This could be explained by the fact that when microspheres are in contact with water, they swell and release nicotine which must be in high concentration in the layers surrounding the microspheres. This situation seems to provoke a rapid passage of nicotine through the mucosa, affecting the lag-time, even though it should be independant of diffusant concentration [19]. Another possible explanation of the short lag-time observed with G25 and G50 was proposed by Edman et al. [20]. The swelling of microspheres provokes a loss of water in the epithelial cells, a shrinking of the cells and an opening of the tight junctions. But tight junctions are rare in the buccal mucosa, and the dehydration of the surface of the mucosa due to the swelling of microspheres could simply enhance the contact of the dosage form (high concentrated in nicotine) with the mucosa, generating a good diffusion of nicotine through the epithelial cells (Fig. 4).

The relative standard deviations (R.S.D.) of the fluxes varied from 8 to 24% ($n = 3$), due principally to the variations of the thickness of the mucosae.

The percentage released after 6 h did not differ significantly from one formulation to another, except in certain cases, when the liquid available in the donor compartment was not sufficient to allow the maximal release of nicotine from the microspheres. One can observe that NB G25 microspheres without adjunction of liquid had a lower percentage of release (58.2%) than the same microspheres with 100 μl of buffer (75.0%).

The concentration of nicotine in the donor and in the receiver compartment after 6 h was determined and it was found that about 20% of the initial amount of nicotine remained in the mucosa. The amount remaining in the mucosa depends of the thickness of the sample used in the trial (1–2 mm).

Some tests were performed with fresh mucosa, in order to compare the nicotine diffusion through this tissue to the nicotine diffusion through a stored mucosa (−20°C). The results (unpublished) indicate that no significative differences were observed between these two kinds of mucosae. In fact, with the vertical permeation system used in this study, the mucosa could not be kept living for a long time, due to the lack of liquid

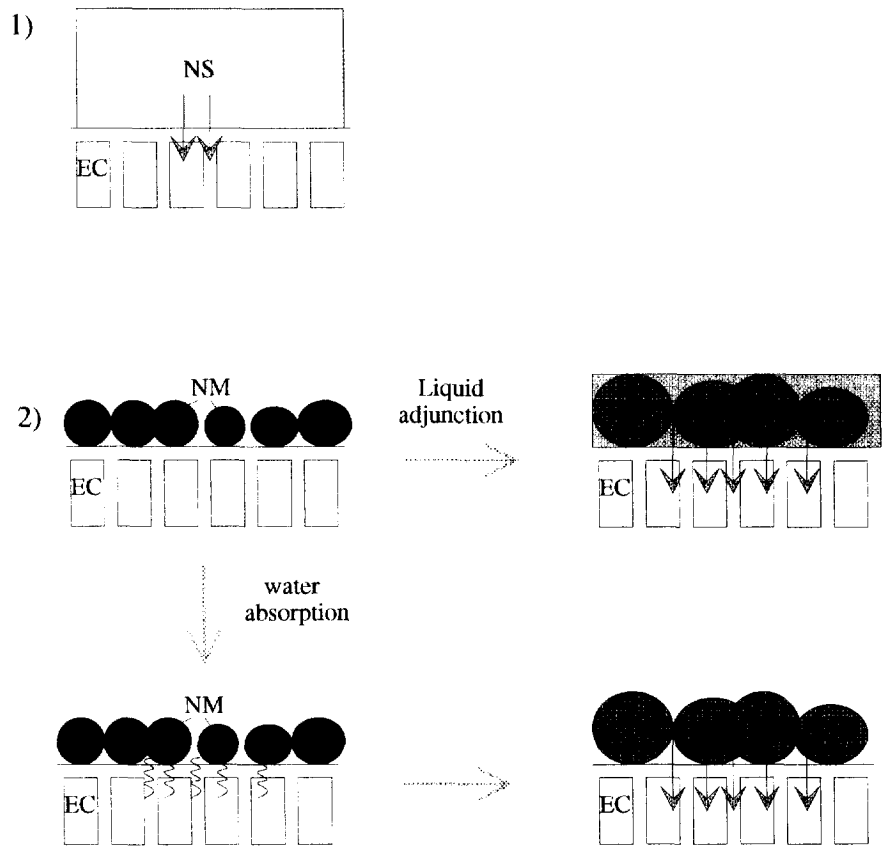

Fig. 4. Passage of nicotine through the buccal mucosa from a solution or from a microspheres formulation. (1) Nicotine is available from a solution (NS, nicotine solution) and passes via a transcellular or a paracellular pathway through the epithelial cells (EC). (2) Nicotine microspheres (NM) are highly concentrated with nicotine and swell after liquid adjunction or water absorption from the mucosa, provoking a release of nicotine in the surrounding liquid or directly from the microspheres. The gradient of concentration is higher in the case of microspheres than with a solution.

in the donor compartment (mucosal side). This is acceptable in the case of passive diffusion studies (which is the case with nicotine), but not in the case of facilitated diffusion, which demands living mucosa.

4. Conclusion

In conclusion, the microspheres used in this study allow a good diffusion of nicotine, as well as a short lag-time, probably due to the direct contact of the microspheres with the mucosa and to the high concentration of nicotine available in this dosage form. This could be interesting in the case of poorly absorbed drugs as well as hydrophilic drugs. The method used in this study allows to rapidly test the diffusion of a molecule through a biological membrane, in a well reproducible way, both from solutions and from solid formulations, which is not possible with the classical Ussing-type diffusion cells. In fact, this method makes it possible to obtain rapidly information about the diffusion of a molecule through a biological mucosa, information which is essential when preformulation of new drug delivery systems is in progress.

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