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Research paper

Diffusion of naltrexone across reconstituted human oral epithelium and histomorphological features

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Abstract

In transbuccal absorption a major limitation could be the low permeability of the mucosa which implies low drug bioavailability. The ability of naltrexone hydrochloride (NLX) to penetrate a resembling histologically human buccal mucosa was assessed and the occurrence of any histomorphological changes observed. We used reconstituted human oral (RHO) non-keratinised epithelium as mucosal section and a Transwell diffusion cells system as bicompartmental model. Buccal permeation was expressed in terms of drug flux (J_s) and permeability coefficients (K_p). Data were collected using both artificial and natural human saliva. The main finding was that RHO does not restrain NLX permeation. Drug transport across the epithelium was observed also in presence of various concentrations of penetration enhancers, without any significant differences. On the contrary, the flux throughout the mucosa was extensively affected by iontophoresis. Histologically, no sign of flogosis was observed in any specimen under experiment without iontophoresis, whereas cyto-architectural changes, up to nuclear pycnosis or cellular swelling, were determined as a consequence of the application of electric fields.

Keywords: Buccal permeation; Transbuccal absorption; Naltrexone hydrochloride; Reconstituted human oral epithelium (RHO); Iontophoresis; Penetration enhancers

1. Introduction

Alcohol drinking and heroin dependence are modulated by opioid receptor activity and clinical data indicated that they could be reduced by pharmacological treatment with opioid antagonists. Naltrexone hydrochloride (NLX) is a highly specific opioid antagonist with a high affinity for opiate receptor sites used as an adjunct to the maintenance of the opioid-free state in detoxified opioid-dependent individuals [1]. In humans, following oral administration, NLX is rapidly and quite completely absorbed (about 96%) from the gastrointestinal tract, but it undergoes a significant first-pass effect [2]. Thus, hepatic metabolism (>98% metabolized) will lead to a very low drug concentration in targeted tissues: i.e., the brain [1]. However, a major problem with oral NLX has been associated with a low rate of compliance in medication intake as it is generally badly accepted by patients [3], mostly because of adverse reactions to the medication. In fact, oral NLX administration is associated with a range of gastrointestinal and neuropsychiatric adverse reactions, whose patient's reports were inversely correlated with medication adherence and study retention in a 2001 analysis by Oncken et al. [4].

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It has been recently shown that NLX, in ultra-low-doses, blocks the aversive effects of withdrawal from chronic treatment with this drug [5]. Hence, there is an interest in developing alternative non-invasive delivery routes for this drug.

Transepithelial administration of NLX could be an ideal route, due to its advantages in first-pass metabolism escaping, reduction of drug peak and decreasing of the side effects observed with oral administration [2]. Moreover, a transepithelial delivery of NLX could be an alternative way to administer low drug doses in a slow and constant pattern. The therapeutic efficacy of a drug for transepithelial delivery, following its application to mucosae, mainly depends on its ability to penetrate the tissue fast enough to provide the required plasma concentrations, thus resulting in the desired pharmacological activity.

The oral cavity is an attractive site for drug delivery due to ease of administration, avoidance of hepatic first-pass metabolism and possible degradation in gastrointestinal tract since drugs are directly made available in the systemic circulation. Within the oral mucosal cavity, the buccal region offers superior performance for local/systemic pharmacological actions due to its relative permeability. Buccal drug delivery specifically refers to the delivery of drugs within/through buccal mucosa [6,7]; furthermore, the buccal mucosa has good accessibility compared to other mucosae such as the nasal, rectal and vaginal mucosa [8].

The human oral mucosa is composed of an outermost layer of stratified squamous epithelium similar to stratified squamous epithelia found in the rest of the body. It has a mitotically active basal cell layer, a number of differentiating intermediate layers and a superficial layer, where cells are shed from the surface of the epithelium. A basal membrane is interposed between the epithelium and the underneath connective tissue in which a network of blood capillaries is contained. Drugs permeated through the epithelium can diffuse into this vessels network and enter the systemic circulation [6,9].

Stratified cultured TR146 cell layers (so-called reconstituted human oral epithelium or simply RHO) are analogous to normal human buccal epithelium. Studies on bidirectional permeability through RHO have shown close correlation to the data obtained using porcine buccal mucosa [10]. In general, the cell culture approach provides some advantages compared to other *in vitro* models. The cells, derived from a human neck metastasis originating from a buccal carcinoma, have been shown to be able to grow on polycarbonate permeable inserts and form cell layers resembling the stratified human buccal tissue. The model has a similar morphology, ultrastructure and permeability barrier properties to intact buccal mucosa [11].

Similar to other mucosal membranes, the buccal mucosa has some disadvantages as well. A major limitation could be the low permeability resulting in low drug bioavailability. Low drug transport across the epithelium could be overcome using penetration enhancers [12]: chemical enhancers act mainly by transiently altering the permeability characteristics of the outer layer which forms the rate-limiting lipophilic barrier to absorption [13], whereas iontophoresis provides a physical mechanism to enhance the penetration of hydrophilic and charged molecules across the epithelial stratum by applying an electric field [14].

However, in order to develop an alternative approach in administration of NLX, precise information on drug absorption characteristics is very important. Thus, assuming RHO epithelium as a model for buccal mucosa architecture and permeability, the aim of this study was to verify the aptitude of NLX to penetrate in it, to reach therapeutic steady-state plasma concentrations following buccal administration, and to assess the occurrence of any consequent pathological change. Tests were performed also in presence of chemical enhancers or electric fields. In addition, since the drug, and absorption promoters, could damage the structure of the biological tissue, we studied also the effects of their application on histology of RHO.

2. Materials and methods

2.1. Materials

Naltrexone hydrochloride (NLX), USP grade, was purchased from Sun Pharmaceutical Industries LTD (Gujarat, India). Sodium dehydrocholate (NaDHC), EDTA disodium salt (NaEDTA) and trisodium citrate dihydrate (TNaC) were from Polichimica s.r.l. (Bologna, Italy). 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT) and all components of buffer solutions were from Sigma–Aldrich (Milano, Italy).

Simulated saliva was prepared using a buffer solution (pH 6.8) containing NaCl (0.126 g), KCl (0.964 g), KSCN (0.189 g), KH_2PO_4 (0.655 g), and urea (0.200 g) in 1 L of distilled water [15]. Natural human saliva (pH 6.8) was obtained from one healthy donor without any conditioning habits i.e., smoking, alcohol, coffee drinking or any other further habit able to alter its composition.

Unstimulated mixed saliva was collected from one of the authors, after overnight fasting, first brushed his teeth and thoroughly rinsed the mouth using only deionized water, then sat in a relaxed position with the head in a slightly inclined forward position, allowing saliva to accumulate on the floor of the mouth. The first few millilitres of saliva was discarded. The accumulated saliva was then withdrawn using disposable sterile plastic pipettes until about 1.5 ml had been collected. The samples of saliva were not further handled to evaluate the drug behaviour in environmental conditions similar to those of the administration site.

Phosphate-buffered saline (PBS) Ca^{2+}- and Mg^{2+}-free solution, pH 7.4, was prepared by dissolving KH_2PO_4 (0.144 g), anhydrous Na_2HPO_4 (0.795 g) and NaCl (9.0 g) in 1 L of distilled water. All chemicals and solvents were of analytical grade and were used without further purification. All other reagents for cell culture were obtained from

Sigma and solutions for cell culture were prepared in endotoxin-free water.

2.2. Methods

2.2.1. In vitro drug permeation throughout RHO

The permeation of NLX was investigated *in vitro* by measuring drug fluxes throughout TR146 cell layers cultured on permeable polycarbonate inserts. Mucosal specimens, non-keratinised type (RHO/S/12), were purchased from Skinethic® Lab. (Nice, France). All samples used for drug permeation experiments and/or histomorphological analysis were incubated for 12 days. Upon arrival, the bags containing the inserts with cultured cell layers were opened under sterile airflow. Each insert (0.5 cm^2) containing the epithelial tissue was taken out and any remaining agarose adhering to its walls was rapidly removed by gentle blotting on sterile filter paper and placed in culture dishes filled with maintenance medium (Skinethic® Lab. Nice, France). Before testing, the culture dishes were placed overnight in the incubator at 37 °C, 5% CO_2 and saturated humidity. The maintenance medium was then changed by adding fresh medium. The permeability experiments were performed at a constant temperature of 37 °C [Polimix EH 2 bath equipped with a constant-rate adjustable stirrer RECO® S5 (Kinematica, Switzerland)], using the Transwell diffusion cells as a two-compartment open model. Solutions of NLX with different concentrations (15, 30 and 60 mg of NLX per 1 ml of simulated saliva) were used as samples. Solutions containing the same amounts of NLX in natural human saliva were also tested. Accurately weighed amounts of each NLX solution were applied as stagnant drug solution to the donor chamber (apical side of the cell layers representing the buccal environment).The acceptor chamber, representing the serosal side of mucosa, was filled with PBS (25 ml) to avoid cell stresses. The acceptor solution was stirred by means of a magnetic follower to avoid formation of stagnant boundary layers next to the membrane surface. An insert in which saliva was applied to the apical side of the cell layer was used as control. At regular time intervals (5 min during the first hour followed by 10 min until the end of experiment), samples were withdrawn from the basolateral side of the acceptor compartment. To avoid saturation phenomena and maintain the "sink" conditions, the sample volume taken out was replaced by fresh receiver medium. The drug transferred from the donor to the acceptor compartment was monitored spectrophotometrically (see Section 2.2.4).

2.2.2. Drug permeation in presence of chemical enhancers

Permeation of NLX in the presence of some chemical enhancers was investigated using the same system, conditions and withdrawal manner described in Section 2.2.1. To NLX solutions, prepared according to Section 2.2.1, were added, respectively, 0.1%, 0.5% and 1.0% of the appropriate chemical enhancer (NaDHC, NaEDTA and TNaC).

2.2.3. Iontophoretic permeation

The donor side of the apparatus described in Section 2.2.1 was equipped with a silver electrode (active electrode) for anode as in the acceptor chamber was applied a silver chloride-coated silver electrode (indifferent electrode) for cathode (Fig. 1). Prior to chloridation of the silver electrodes, they were dipped in distilled water, ethanol, fuming nitric acid and washed with distilled water. The electrodes were then treated with 0.1 N HCl and a current of 1 mA was applied for 24 h using silver as cathode [16]. The distance between the electrodes and TR146 cell layers was 5 mm. Current densities of 0.5, 1 and 2 mA/cm^2 (expressed as current per unit of crossing area of the epithelial layer) were applied to observe the effect of electric fields on permeation rate. Experiments were carried out in continuous current. The effect of initial donor concentration was also evaluated using solutions of NLX: 15, 30 and 60 mg of drug per 1 ml of simulated saliva or 30 mg of drug per 1 ml of natural human saliva. Withdrawals of samples were made as described in Section 2.2.1. The drug transferred from the donor to the acceptor compartment was spectrophotometrically monitored (see Section 2.2.4).

2.2.4. Drug assay

The cumulative amount permeated through the membrane was calculated from the NLX concentration in the acceptor medium and plotted as a function of time. Each experiment was performed six times for 3 h using six different culture cell inserts of one single time production batch. Each data point on the plot represents the mean of the recorded values ($P < 0.05$).

In all experiments the NLX transferred from the donor to the acceptor compartment was monitored spectrophotometrically (UV/vis Shimadzu mod. 1700 Pharmaspec instrument) by measuring the drug that reached the acceptor fluid using the appropriate calibration curve and blank (280.0 nm, $E_{1\%} = 0.0301$ in PBS). The UV absorption peak was highly reproducible and linearly related to concentration over a range of 0.001–0.4 mg/ml. At testing concentra-

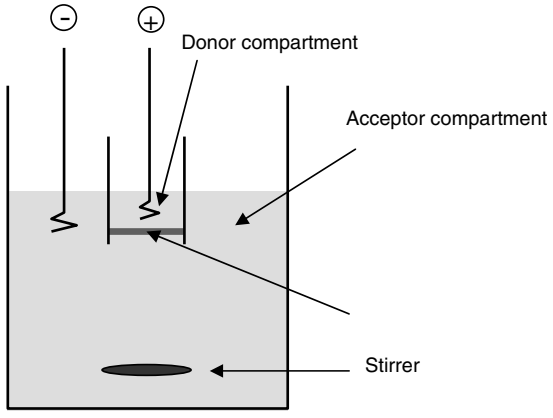

Fig. 1. Schematic picture of diffusion cells system used in experiments of iontophoretic permeation.

tions, PBS components and chemical enhancers used do not interfere significantly with the UV absorption of NLX. The sensibility was less than 0.0001 mg/ml. Intraday and interday variations, observed during collection of experimental data, were lower than sensibility.

At the end of all experiments the cell layers were treated with a freshly prepared $FeCl_3$ solution (20%) to underscore the drug eventually entrapped into the tissue.

2.2.5. Data analysis

The flux values (J_s) across the membranes were calculated at the steady state per unit area by linear regression analysis of permeation data following the relationship:

$$J_s = \frac{Q_r}{A\,t}\,(\mathrm{mg\,cm^{-2}\,h^{-1}}) \quad (1)$$

where Q_r is the quantity of NLX which passes through the cell layers into the receptor compartment (mg), A is the active cross-sectional area available for diffusion (cm^2) and t is the time of exposure (h).

The permeability coefficient (K_p) was then calculated by the relationship

$$K_p = \frac{J_s}{C_d}\,(\mathrm{cm\,h^{-1}}) \quad (2)$$

where J_s is the flux calculated at the steady state ($mg\,cm^{-2}\,h^{-1}$), C_d is the drug concentration in the donor compartment ($mg\,cm^{-3}$).

All data analyses were elaborated according to Vergnaud [17], Korsmeyer et al. [18] and Peppas et al. [19] equations using Curve Expert program version 1.3. Linear or non-linear least squares fitting methods were used to determine the optimum values for the parameters present in each equation. Statistical analysis was performed using a chi-square test. A P value less than 0.05 was considered to be statistically significant.

2.2.6. Cell viability

At the end of the experiments cell viability of tissue cultures was measured by the quantitation of mitochondrial dehydrogenase activities (MTT assay) according to Mosmann [20]. Briefly, 0.5 ml MTT solution (0.5 mg/ml) was added to all test wells containing RHO cultures and incubated for 30 min at 37 °C. After incubation, the MTT solution was discarded and the RHO cultures washed twice with 1 ml PBS, pH 7.4, after which 1 ml isopropanol was added to each well. The extraction process lasting 1 h was performed at room temperature on a shaking platform. The optical density at 540 nm was determined spectrophotometrically. The mean optical density of the untreated control tissues was set to represent 100% of viability (MTT test, $n = 1$, $OD = 0.999$) and results were quantified as percentage of the untreated controls.

2.2.7. Histological methods

For histological analysis the epithelial tissue was cut out from the insert together with the polycarbonate filter using a sharp scalpel. The filter samples were fixed in 10% neutral-buffered formalin for 2 h, washed in water for 1 h, dehydrated in graded ethanol (60%, 80%, 90%, 95%, and 100%) and, after permeation in xylene, embedded in paraffin using the standard procedures. Formalin-fixed, paraffin-embedded samples were cut in 4-μm-thick sections on a microtome with a disposable blade and conventionally stained with hematoxylin–eosin. Three different types of negative controls were included in the histomorphological analysis. Blank RHO controls were considered those samples not subjected to the experimental phase; permeation controls were those samples subjected to the experimental phase in the absence of drug; iontophoresis controls were those subjected to iontophoresis in the absence of drug.

The aim of histomorphological analysis was evaluating the pathological changes occurring in cell morphology and tissue organization; samples treated with 5-fluorouracil (5-FU) were included in the analysis as markers of the cytopathic alterations. Slides were evaluated by optical microscopy by two of the authors in a blind and independent fashion. Photomicrographs of relevant fields were taken at 40× and 100× magnification. Digital images were captured using a Leica DC 300F camera (Leica, Nidau, Switzerland) mounted on a Leitz DMRB microscope with the Leica IM50 Image Manager program version 1.20.

3. Results and discussion

The Transwell system was used as a two-compartment linear open model to assess the *in vitro* permeation kinetic of NLX throughout TR146 cell layers cultured on polycarbonate. In order to avoid possible uncertainties attributable to the saliva composition, comparative experiments were carried out using artificial or natural human saliva. All experiments were carried out for 3 h to avoid changes in permeability characteristics; in fact we experienced that cultured tissues, out of the maintenance medium, live and remain intact for only 3–4 h [21].

In compartmental analysis the drug transfer rate from the donor to the acceptor phase is often affected by the initial drug concentration, so in our experiments solutions with increasing amounts of NLX were tested. A steady-state membrane flux was attained within the first hour of application as reflected by the short diffusion lag time for solutions with different drug concentration. The drug permeation increased with concentration as shown in the profiles of cumulative NLX permeated amount against time (Fig. 2A and B, respectively). This effect was observed using either artificial or natural human saliva.

In the first period of drug permeation (about 20 min), a time lag was observed; it was ascribed to the NLX concentration in the donor compartment. In particular, the time lag was more evident when the permeated amount of NLX was reported as percentage of the administered dose (instead of mg) versus time (Fig. 3). The time lag was significant when the lowest drug concentration was applied using natural human saliva.

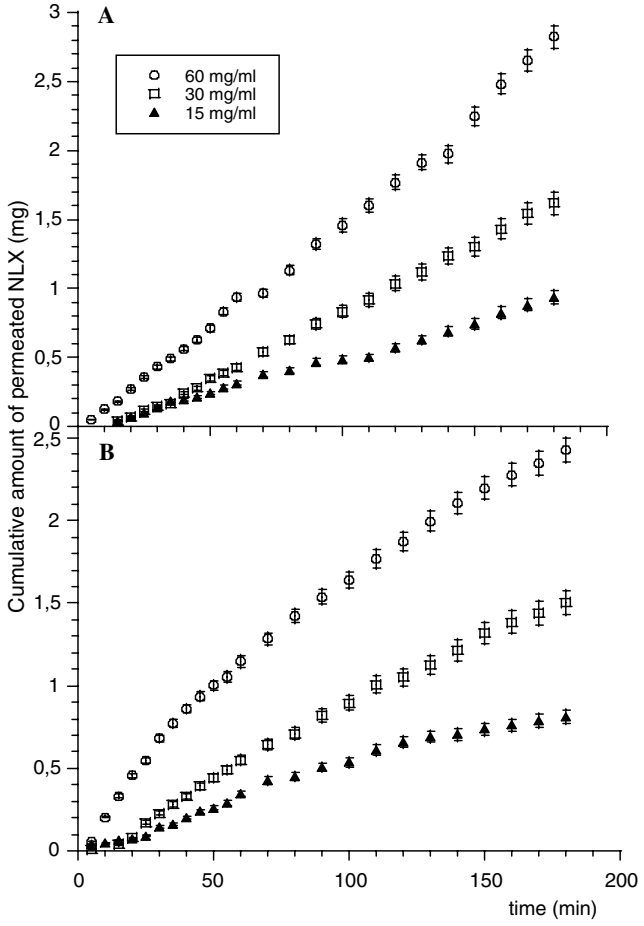

Fig. 2. Plot of cumulative amount-time of NLX permeated across RHO into receiver chamber on simple diffusion of three different initial donor concentration solutions (A) using artificial saliva and (B) using natural human saliva as donor medium. Receptor medium was PBS in all experiments. Values are presented as means ± SD ($n = 6$).

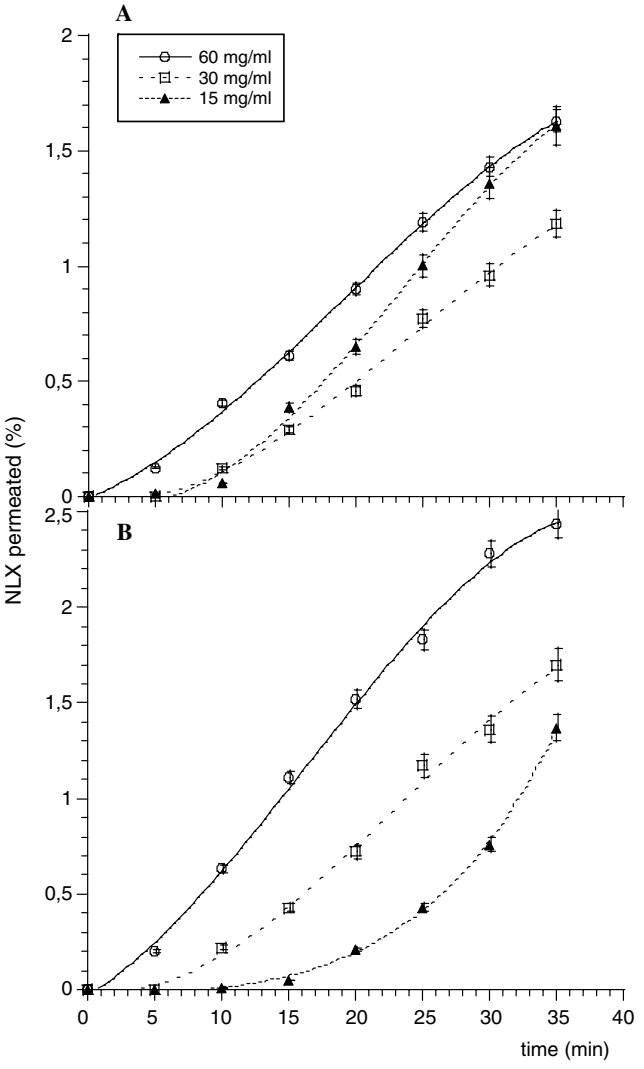

Fig. 3. Plot of the effect of initial NLX concentration on lag time during simple diffusion experiments across RHO, using, respectively: (A) artificial saliva and (B) natural human saliva as donor medium. Receptor medium was PBS in all experiments. The time lag was more evident when the permeated amount of NLX was reported as percentage of the administered dose versus time. Values are presented as means ± SD ($n = 6$).

Flux data, attained at the steady state, showed that increasing drug concentration from 15 to 60 mg/ml in artificial saliva resulted in about 3-fold increase in the flux, whereas in natural human saliva the increase was about 2-fold. Therefore, the steady-state permeability coefficients (K_p) decreased as the NLX concentrations in the donor were increased (Table 1). The increase of concentration gradient between the donor and acceptor compartment can determine high membrane accumulation of NLX. To confirm this hypothesis, at the end-time of the experiments, the inserts were washed with water until all traces of external NLX were removed. The cell layers were then treated with a $FeCl_3$ solution to underscore the drug eventually entrapped into the tissue. In chemical analysis, the use of colour tests is very common. Many substances when brought into contact with various chemical reagents give definite colours. In particular, substances bearing phenolic functional groups, as does NLX, react with $FeCl_3$ and form coloured complexes. In our hands, a green-blue colour developed, thus indicating that tissue contained effective drug concentration. The colour intensity increased when the concentration gradient was raised.

In buccal administration, drug permeation is often facilitated by means of enhancers; thus we investigated the effects of co-administration of chemical penetration enhancers on the rate of mucosal NLX penetration. Among different classes of penetration promoters, we chose sodium dehydrocholate (NaDHC), EDTA disodium salt (NaEDTA) and trisodium citrate dihydrate (TNaC). Since the enhancing effect is dependent on the enhancer concentration, especially for bile salts, three different concentrations were used (0.1%, 0.5% and 1%). At these concentrations no significant differences were observed in the permeation rate of NLX through the membrane using both artificial and natural human saliva. The chosen enhancers of the three classes gave the same results.

NLX is a hydrophilic molecule and is electrically charged at physiological pH ($pK_a = 8.1$ at 37 °C) [22].

Table 1
Calculated steady-state flux values, permeability coefficients and enhancement ratios obtained in studies on permeation of NLX through RHO

Diffusion	Current density (mA)	Donor medium	NLX concentration (mg/ml)	J (mg/cm^2 h^{-1})	K_p (cm/h)	ER
Simple		Artificial saliva	15	0.66 ± 0.01	0.044	
			30	1.19 ± 0.02	0.040	
			60	1.82 ± 0.03	0.030	
		Natural human saliva	15	0.69 ± 0.01	0.046	
			30	1.13 ± 0.02	0.038	
			60	1.59 ± 0.03	0.025	
Enhanced by iontophoresis	0.5	Artificial saliva	30	1.21 ± 0.02	0.040	1
	1		30	1.81 ± 0.03	0.060	1.5
	2		30	3.74 ± 0.06	0.125	3.1
	0.5	Natural human saliva	30	1.15 ± 0.02	0.038	1
	1		30	3.21 ± 0.05	0.107	2.8
	2		30	5.60 ± 0.09	0.187	4.9

Values are presented as means ± SD ($n = 6$). Donor phase was artificial or natural human saliva, receptor phase was PBS.

These physicochemical properties make this drug a suitable compound for iontophoretic movement through biological membranes. In view of that, we investigated the NLX permeation in presence of electric field. The application of a current density of 0.5 mA/cm^2 was not sufficient to appreciably enhance permeation, whereas a current density of 1 mA/cm^2 or more determines a good improvement. Flux may be divided into the contributions of passage through the lipid matrix and through aqueous pores of the mucosal membrane [23]; iontophoresis enhances only the aqueous pathway. Accordingly, we attributed the permeability improvement to the increase of NLX movements in the membrane aqueous domain.

Fig. 4 shows the amount of drug permeated versus time under iontophoresis with constant current of 1 mA/cm^2 at different drug concentrations in artificial saliva. The

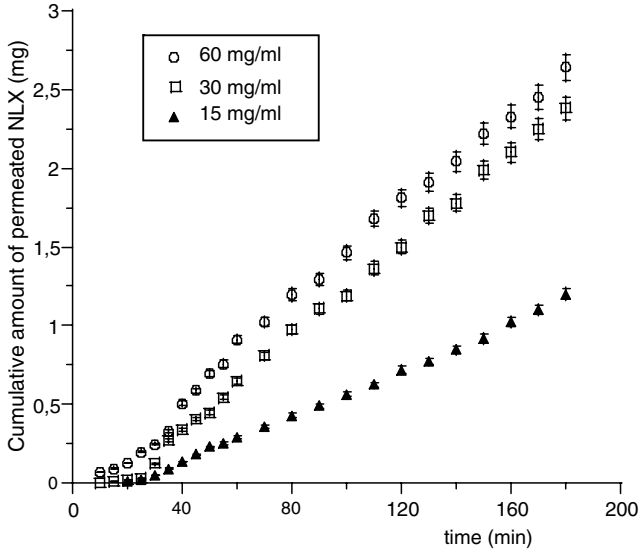

Fig. 4. Plot of cumulative amount of NLX permeated through RHO during iontophoresis of three different concentration solutions versus time using: artificial saliva as donor medium, 1 mA/cm^2 current density and PBS as receptor phase. Values are presented as means ± SD ($n = 6$).

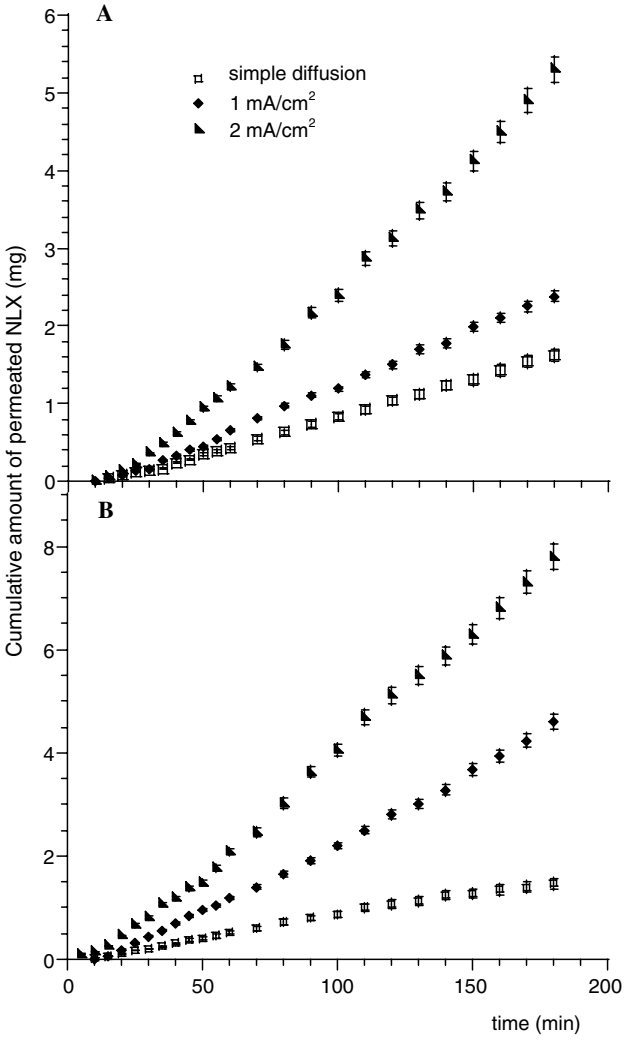

Fig. 5. Plot of the effect of current density on delivery rate through RHO after administration of a 30 mg/ml NLX solution, using, respectively: (A) artificial saliva and (B) natural human saliva as donor medium. Receptor medium was PBS in all experiments.Values are presented as means ± SD ($n = 6$).

steady-state fluxes and permeability coefficients are listed in Table 1. From details reported in Fig. 5, it is evident that the increase in electric field determines significant growing in the amount of drug permeated both using artificial and natural human saliva. The ratio of the permeability coefficient of NLX under iontophoretic conditions to the value under passive diffusion is the enhancement ratio (ER) and is listed in Table 1.

Worth of note, the flux in natural human saliva is 1.8-fold greater than that observed in artificial saliva, applying a current density of 1 mA/cm^2. On the other hand, applying a current density of 2 mA/cm^2 the flux in natural human saliva is 1.5-fold greater than that observed in artificial saliva. Since natural saliva in addition to inorganic salts contains also several protein components that might alter the movement of solutes in electric fields, we attributed the increase of flux to the composition of natural saliva even if in this work we used saliva obtained from just one person.

The drug itself and the application of current could have some effects on the structure of the biological tissue, so we observed their histological effects on RHO. Overall, the three types of negative controls, blank controls, permeation controls and iontophoresis controls, showed not-keratinised squamous-cell epithelia composed by an average of five cellular layers. No significant cytological or architectural changes were highlighted but some defects were found in almost all the samples in the form of maturational defects of the intermediate layers and mild loss of nuclei polarity. Negative controls subjected to iontophoresis in absence of drug also showed an irregular distribution of the layers closer to the polycarbonate substrate due to a partial cell detachment (Fig. 6). Samples submitted only to NLX passive diffusion, even at highest concentration used, showed a uniform cellular swelling in the absence of other significant changes in cell morphology or tissue structure (Fig. 7). In details, cells appeared vacuolated due to the presence of intracytoplasmic material, which is, likely, ascribable to accumulated NLX. We confirmed

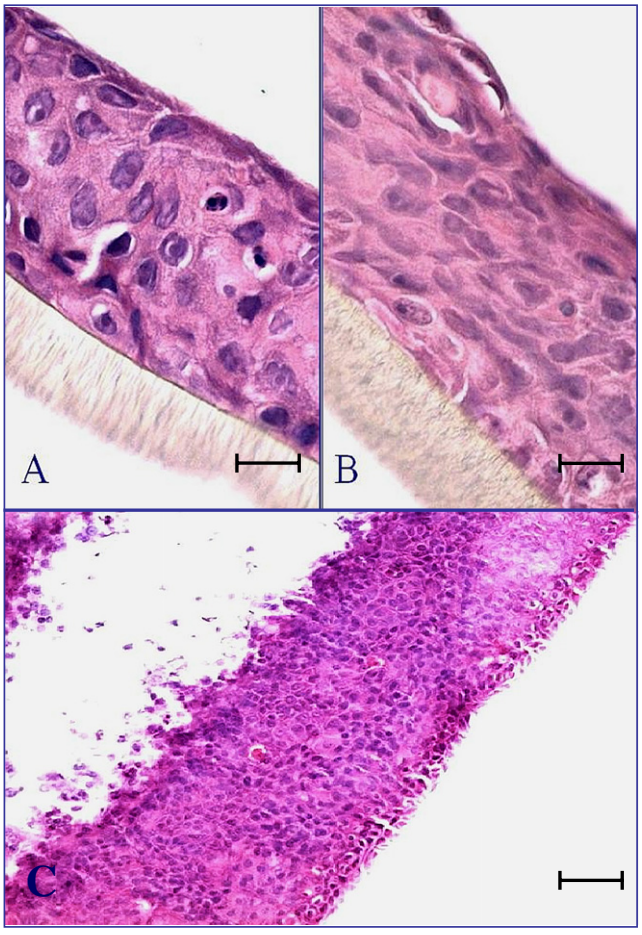

Fig. 6. Microphotographs of formalin-fixed, paraffin-embedded cross-sections of RHO: (A) control untreated (bar 250 μm), (B) control treated with saliva (bar 250 μm), (C) control subjected to iontophoresis (bar 100 μm). For all controls no significant alterations were observed although some defects were evidenced. Nevertheless, a peculiar disarray of the cell layers closer to the polycarbonate membrane was observed for controls subjected to iontophoresis.

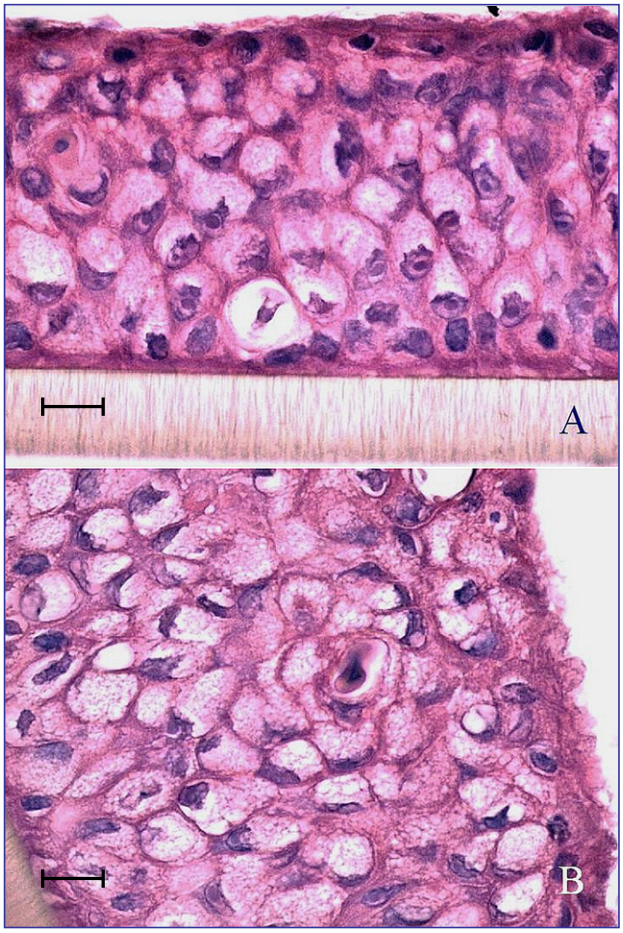

Fig. 7. Microphotographs of formalin-fixed, paraffin-embedded cross-sections of RHO after NLX simple diffusion: (A) sample treated with a 15 mg/ml NLX saliva solution, (B) sample treated with a 60 mg/ml NLX saliva solution (bar 100 μm). Whole thickness intracellular swelling in the setting of well-preserved tissue architecture was observed for both samples.

this suggestion by $FeCl_3$ reaction (Fig. 8). The application of iontophoresis caused cytoarchitectural changes consisting of cellular detachment and disarray involving the side of the sample adjacent to the polycarbonate substrate (Fig. 9). Cytopathic effects represented by nuclear pycnosis or swelling, diffuse signs of abrupt keratinisation and loss of cellular alignment were associated with an increase in current density applied (Fig. 10). Moreover, no severe cytopathic effects were observed in samples treated with NLX with or without the application of iontophoresis; on the contrary intense cytopathic changes with apoptotic figures and signs of abrupt keratinisation were found in samples treated with the potent cytotoxic drug 5-FU used as control.

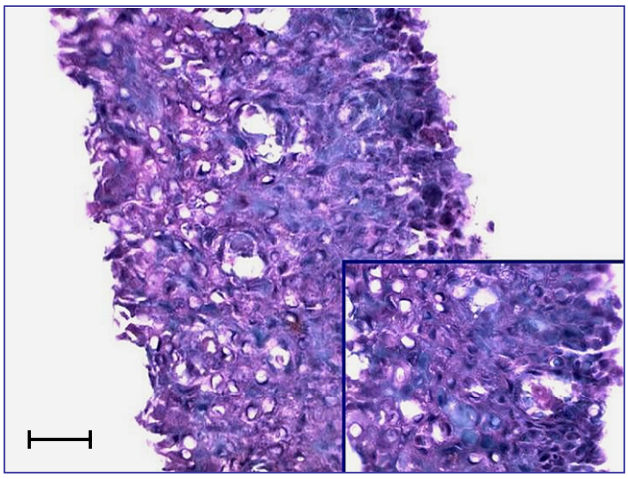

Fig. 8. Microphotograph of formalin-fixed, paraffin-embedded cross-sections of RHO treated with an iron chloride solution after NLX simple diffusion (bar 125 μm). Staining confirmed the presence of intracellular NLX in the form of cytoplasmatic vacuoles (inset, magnification 100×).

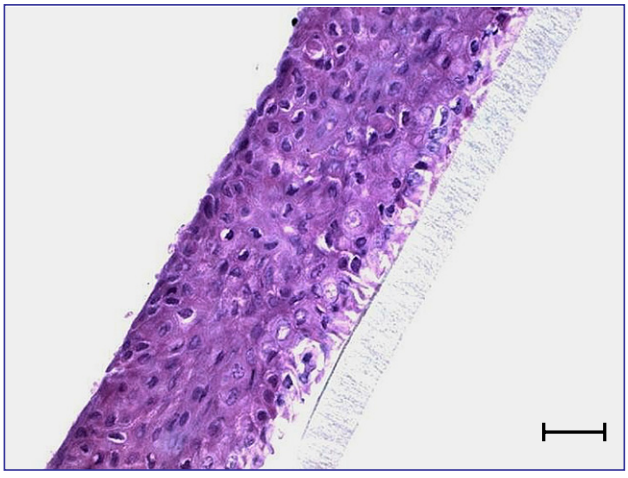

Fig. 9. Microphotograph of formalin-fixed, paraffin-embedded cross-sections of RHO subjected to enhanced permeation of NLX on application of 0.5 mA/cm^2 current density (bar 400 μm). Samples showed disarray of the cellular layers proximal to the polycarbonate membrane and cellular detachment.

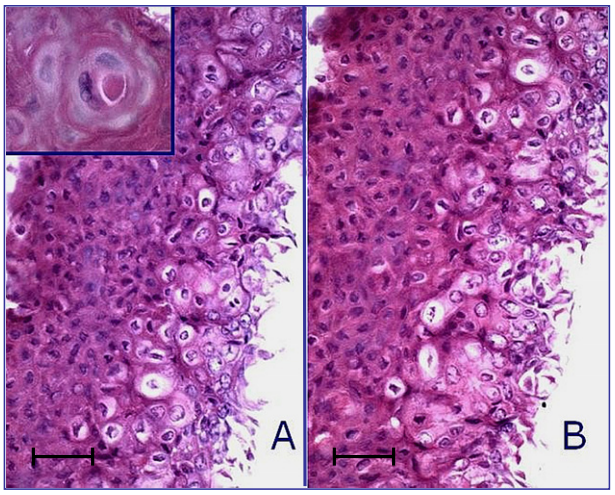

Fig. 10. Microphotographs of formalin-fixed, paraffin-embedded cross-sections of RHO exposed to permeation of NLX enhanced by iontophoresis on application of a current density of 2 mA/cm^2. Signs of prominent cytoarchitectural changes, consisting of nuclear pycnosis or swelling and abrupt keratinisation (inset, magnification 100×), were associated with the increase in current density applied in different regions (A and B, bar 250 μm) of the same sample.

In conclusion, the data demonstrated that RHO does not restrain permeation of NLX. The drug passively crosses the membrane, whereas the application of electric fields promotes drug diffusion. Both fluxes and permeability coefficients, in artificial or natural human saliva, increase significantly when the applied current density grows.

It has been suggested that NLX blood concentrations of at least 1 ng/ml are necessary for sufficient receptor blockade after challenge doses of up to 25 mg intravenous heroin [24]. On the basis of flux values reported in Table 1 we could assume that clinical application of NLX buccal delivery is feasible.

Histologically, cytoarchitectural changes, up to nuclear pycnosis or cellular swelling, appeared as a consequence of growing of current density, although our findings are limited to the range of current 0.5–2 mA/cm^2.

Since RHO has architecture and permeability similar to buccal mucosa we can hypothesize that it could represent an alternative site of administration of NLX.

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