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Enhanced *in vitro* transbuccal drug delivery of ondansetron HCl

Longsheng Hu[a], Bassam B. Damaj[b], Richard Martin[b], Bozena B. Michniak-Kohn[a,c,*]

[a] Ernest Mario School of Pharmacy, Rutgers-The State University of New Jersey, Piscataway, NJ 08854, United States
[b] NexMed, Inc., 6330 Nancy Ridge Drive, Suite 103, San Diego, CA 92121, United States
[c] New Jersey Center for Biomaterials, Rutgers-The State University of New Jersey, Piscataway, NJ 08854, United States

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ABSTRACT

The effect of chemical enhancers and iontophoresis on the *in vitro* transbuccal delivery of 0.5% ondansetron HCl (ODAN HCl) was investigated using porcine buccal tissue. The chemical enhancers used were dodecyl 2-(*N*,*N*-dimethyl amino) propionate (DDAIP), its HCl salt dodecyl-2-(*N*,*N*-dimethylamino) propionate hydrochloride (DDAIP HCl), *N*-(4-bromobenzoyl)-*S*,*S*-dimethyliminosulfurane (Br-iminosulfurane), and azone. This study demonstrated that anodal iontophoresis at 0.1, 0.2 and 0.3 mA current intensity significantly increased transbuccal delivery of ODAN HCl 3.3-fold, 5.2-fold and 7.1-fold respectively, compared to control. DDAIP HCl provided significantly higher transbuccal delivery of ODAN HCl than did DDAIP, azone and Br-iminosulfurane. It was found that DDAIP HCl in water significantly enhanced drug permeability (920 $\mu g/cm^2$) compared to DDAIP HCl in propylene glycol (PG) (490 $\mu g/cm^2$) during 24 h. It was also found that 5% (w/v) DDAIP HCl in water alone provided higher permeation flux (29.3 $\mu g/cm^2/h$) than iontophoresis alone at 0.3 mA (22.8 $\mu g/cm^2/h$) during the same 8 h treatment. A light microscopy study showed that treatment with chemical enhancers and iontophoresis did not cause major morphological changes in the buccal tissue. EpiOral™ MTS cytotoxicity studies demonstrated that DDAIP HCl at less than 5% (w/v) in water did not have significant detrimental effects on the cells.

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1. Introduction

Ondansetron hydrochloride (ODAN HCl) is a selective blocking agent of the serotonin 5-HT_3 receptor that is used to prevent post-operative nausea and vomiting (http://www.webmd.com/drugs, Ondansetron HCl). It is the active ingredient of ZOFRAN® Orally Disintegrating Tablets (Glaxo Wellcome SmithKline) as the dihydrate, the racemic form of ondansetron—(±) 1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl) methyl]-4H-carbazol-4-one, monohydrochloride, dihydrate (Fig. 1).

The empirical formula is $C_{18}H_{19}N_3O{\cdot}HCl{\cdot}2H_2O$ with a molecular weight of 365.9. While the tablet or injectable dosage form of ODAN HCl is clinically proven to be effective, patients have to endure either painful injection or the side effects associated with gastrointestinal (GI) absorption. Therefore, it is desirable to develop an alternative approach to promoting patients' compliance and reduce the effects of GI absorption and the issues with oral administration with accompanying nausea and vomiting.

Buccal drug delivery has been considered as an alternative route to oral dosing of compounds with significant first-pass effect of the liver that can metabolize drugs. Some of the advantages of the buccal route are: (a) avoidance of hepatic first-pass metabolism; (b) a more rapid onset of drug action; and (c) improved patient compliance. The major challenges of buccal administration of drugs with systemic effect are small absorption area and the barrier properties of the buccal mucosa, which implies low drug bioavailability (Senel and Hincal, 2001). ODAN HCl buccal delivery systems have rarely been reported in the literature. A buccal adhesive tablet containing ODAN HCl was developed and evaluated by Hassan et al. (2009). The tablets were prepared using carbopol 934, sodium alginate, sodium carboxymethylcellulose, and hydroxypropylmethylcellulose as mucoadhsive polymers to impart mucoadhesion and ethyl cellulose to act as an impermeable backing layer. Their *in vitro* release and permeation study results through bovine buccal mucosa demonstrated that both the drug and device were found to be stable in natural human saliva and the buccal adhesive tablet dosage form could be an alternative route to circumvent the hepatic first-pass metabolism and improve the bioavailability of ODAN HCl. Mashru et al. studied the effect of pH on buccal delivery of ODAN HCl. They found that the permeability coefficient and the partition coefficient of ODAN HCl increased with increasing pH (Mashru et al., 2005). To enhance

* Corresponding author at: Ernest Mario School of Pharmacy, Rutgers-The State University of New Jersey, Piscataway, NJ 08854, United States. Tel.: +1 732 445 3589; fax: +1 732 445 5006.
E-mail address: michniak@biology.rutgers.edu (B.B. Michniak-Kohn).

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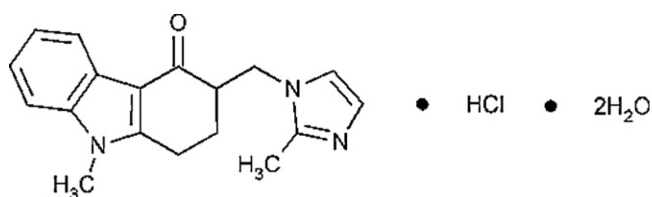

Fig. 1. Chemical structure of ODAN HCl dihydrate.

permeability of buccal tissues, strategies such as the use of iontophoresis or chemical enhancers have been investigated (Veuillez et al., 2001). Giannola et al. (2007) reported that iontophoresis at 2 mA/cm^2 enhanced permeation of naltrexone hydrochloride in a tablet formulation through buccal mucosa by 2-fold using human saliva as medium. Jacobsen (2001) investigated the impact of current density on the in vitro buccal delivery of atenolol HCl using a three-chamber permeation cell. Their study found that as current density increased from 0.1, 0.2, 0.3, to 0.4 mA/cm^2 increased enhancement ratios of 6, 18, 36, and 58, respectively, were recorded. Light microscopy of hematoxylin–eosin stained sections of porcine buccal mucosa demonstrated that 8 h iontophoretic treatment showed some disordering of the outer epithelial cell layers, but no major morphological changes were observed. Their study concluded that it was feasible to use iontophoretic approach to enhance and control the rate of transbuccal drug delivery. Chemical enhancers have been extensively studied for promoting transbuccal delivery of many drugs such as morphine sulfate (Senel and Hincal, 2001), insulin (Morishita et al., 2001), and ergotamine (Tsutsumi et al., 2002), etc. In general, classes of chemical enhancers for buccal drug delivery include surfactants (sodium lauryl sulfate, sodium dodecyl sulfate, etc.), bile salts (sodium glycocholate, sodium fusidate, etc.), fatty acids (oleic acid, lauric acid, etc.), inclusion complexes (cyclodextrins), chelators (ethylene diamine tetra acetic acid, citric acid), polymers (chitosan), azone, cod liver oil extracts and lysalbinic acid (Hassan et al., 2010). However, depending on concentrations applied, these enhancers can potentially cause swelling, irritation, lipid extraction, or ulceration of the tissue (Veuillez et al., 2001). There is still no chemical enhancer approved for use in a commercially available buccal drug formulation. Therefore, the effort to search for more efficient and non-toxic enhancers is continuing.

In this study, iontophoresis and chemical enhancers were evaluated separately as well as in combination in order to promote transbuccal delivery of ODAN HCl. Porcine buccal tissue was selected as a model for human buccal mucosa because the porcine epithelium of buccal tissue is similar to human and is non-keratinized and contains both neutral and polar lipids which are the major barriers to permeation (Squier et al., 1991). Porcine mucosa was much more accessible than human buccal mucosa. There are essentially no differences between human and pig buccal mucosa in terms of structure, composition, and permeability (Lesch and Squier, 1989; Collins et al., 1981; de Vries et al., 1991; Wertz, 1991; Wertz and Squier, 1996). The thickness of the buccal mucosa from human and pig was 730 ± 134 μm (N = 8) and 666 ± 98 μm (N = 64) (Nielsen and Rassing, 2000). Lesch and Squier (1989) indicated that permeability constants to tritium-labeled water obtained for human tissues (skin and buccal) were not significantly different from those of the pig. In an *in vitro* permeation study, Nielsen and Rassing (2000) reported that mannitol and testosterone were less permeable through porcine buccal tissue than human buccal tissue, but their permeability values were not statistically different. This further confirms that pig tissue is a valid model for human.

Chemical enhancers: DDAIP and its HCl salt DDAIP HCl, and Br-iminosulfurane were investigated for their abilities to enhance transbuccal delivery of ODAN HCl with and without the use of iontophoresis. 1-Dodecylazacycloheptan-2-one (Azone), a derivative of caprolactam was used as a control enhancer. This is a hydrophobic substance specifically developed as a skin penetration enhancer and has been used to promote the oral mucosal absorption of salicylic acid (Kurosaki et al., 1989). Amino acid alanine based DDAIP and its HCl salt DDAIP HCl at ≤5% have low toxicity profiles and are biodegradable (Pfister et al., 2006). They were previously reported to effectively enhance the transdermal delivery of alprostadil, ketoprofen, ondansetron, miconazole (Fujii et al., 2002), indomethacin, clonidine and hydrocortisone (Büyüktimkin et al., 1993).

Biodegradable Br-iminosulfurane, a low toxic aromatic *S*,*S*-dimethyliminosulfurane derivative, was reported to be an effective enhancer for transdermal delivery of hydrocortisone (Song et al., 2005). However, the effects of these enhancers have not been studied on transbuccal drug delivery.

The aim of the present work was to develop enhanced *in vitro* transbuccal delivery systems of ondansetron HCl using chemical enhancers: DDAIP, DDAIP HCl and Br-iminosulfurane and iontophoresis separately as well as in combination.

2. Materials and methods

2.1. Materials

ODAN HCl dihydrate was obtained from Polymed, Inc. (Houston, TX, USA). DDAIP and DDAIP HCl were provided by NexMed (San Diego, CA, USA). Azone and Br-iminosulfurane were synthesized at New Jersey Center for Biomaterials, Rutgers-The State University of New Jersey (Piscataway, NJ, USA). Silver wire, propylene glycol (PG) (ReagentPlus®, 99%) and citric acid were purchased from Sigma Aldrich (Saint Louis, MO, USA). Phosphate buffer saline tablets were purchased from MP Biomedicals (LLC, Solon, OH, USA). Cellulose gum (CMC) was provided by TIC Gums (Belcamp, MD, USA). Tissue-Tek® compound was purchased from Sakura Finetek USA, Inc. (Torrance, CA, USA). Formalin 10% was purchased from Fisher Scientific, Inc. (Agawam, MA, USA). EpiOral™ Tissue (ORL-202) was purchased from MatTek Corporation (Ashland, MA, USA). MTS - CellTiter 96® AQueous One Solution Reagent was purchased from Promega Corp. (Madison, WI, USA). Porcine buccal tissue was originally obtained from Barton's Farms and Biologicals (Great Meadows, NJ, USA).

2.2. Preparation of ondansetron HCl gel formulation

Nonionized cellulose gum (CMC) 1% (w/v) was uniformly dispersed in deionized water to obtain a gel. Then 0.5% (w/v) ODAN HCl was added into CMC gel together with 0.01% citric acid to form a 0.5% ODAN HCl gel with pH = 4.

2.3. Buccal tissue preparation

Buccal mucosa samples with underlying connective tissue were surgically removed from the pig cheek area and stored under—30 °C for future use. Prior to use, the samples were thawed at room temperature for at least 3 h. Then the underlying connective tissue was removed using a scalpel blade and the remaining buccal mucosa was then carefully trimmed using surgical scissors to a thickness of about 300–400 μm. The buccal tissues were placed in phosphate buffered saline (PBS) with pH = 7.5 for 1 h prior to use.

2.4. Preparation of Ag and AgCl electrodes

Pure silver (Ag) wire with 0.5 mm in diameter was used as the anodal electrode. An AgCl electrode was prepared by dipping silver chloride powder coated silver wire and a pure silver wire into 0.1 N HCl solution, and connecting them to a power source 3 mA for 12 h.

The purple layer coated silver wire—AgCl electrode was later used as a cathodal electrode in the iontophoretic experiment.

2.5. Enhancer solution preparation

All enhancer solutions were prepared at 5% w/v or 2.5% w/v. The DDAIP HCl solutions were prepared in either water or propylene glycol (PG). The Br-iminosulfurane, DDAIP and azone solutions were prepared in PG only due to their low aqueous solubilities.

2.6. In vitro transbuccal permeation study

Franz diffusion cells (PermeGear, PA, USA) were used for all *in vitro* permeation studies using buccal tissue under varying conditions: passive (control), 1.0 h enhancer pretreatment, 8.0 h iontophoresis (0.1, 0.2 and 0.3 mA), and combined treatment of 1.0 h enhancer pretreatment and 8.0 h iontophoresis at 0.3 mA, and then passive only up to 24 h. All experiments were performed at 37 °C.

For the passive permeation study, Franz cell receptor compartment was filled with PBS solution and stirred at 600 rpm. The buccal tissue was placed in between the donor and receptor compartments with the side of connective tissue facing the donor compartment. The available diffusion area was 0.64 cm^2. 0.3 ml of the ODAN HCl gel was added into the donor compartment at the beginning of the experiment. At different time points (0.0, 0.5, 1.0, 3.0, 5.0, 8.0, 12, 20.0, 24.0 h), 300 μl sample was withdrawn from receptor compartment for HPLC analysis and immediately replaced with 300 μl of PBS (pH = 7.5) (Diaz del Consuelo et al., 2005; Jacobsen, 2001; Kulkarni et al., 2010; Senel and Hincal, 2001).

For enhancer pretreatment, the same procedures described above for passive permeation were followed except that the buccal tissue was pretreated for 1 h by adding 30 μl of chemical enhancer solution on top of buccal tissue in the donor compartment prior to the application of 0.3 ml ODAN HCl gel.

For iontophoresis, Phoresor II Auto (Model PM 850) provided 0.1, 0.2 and 0.3 mA for 8 h of treatment. The anodal electrode (Ag) was placed in the gel formulation in the donor compartment about 2 mm above the buccal tissue membrane. The cathode (AgCl) was inserted into the receptor compartment. After 8 h, iontophoresis was discontinued and then the passive-only permeation experiment continued for 16 h. The sampling method and time points were the same as for passive and chemical enhancer pretreatment permeation experiments.

2.7. Quantification of ondansetron HCl

The concentrations of ODAN HCl in the receptor compartment were analyzed by HPLC. The system consisted of an Agilent HP 1100 series pump, a VWD detector and Agilent ChemStation for LC. A C18 column (150 × 4.6 mm C18 (2) 100 A Luna 5 μm, Phenomenex) with a guard column was used at 25 °C. The mobile phase consisted of methanol and PBS (pH = 7.5) at 65:35 (Zheng et al., 2002). The flow rate was 1.0 ml/min and the drug was detected at 310 nm. The injection volume was 20 μl. The linear range was 5.36–107.2 μg/ml (r = 0.9994). The detection limit was 0.107 μg/ml and daily RSD ≤ ± 3.0%.

2.8. Data analysis for permeation study

The steady state flux at time t (J, $\mu g\,cm^{-2}$) was calculated from the slope of the linear portion of the profile of cumulative drug amounts permeated vs. time. The cumulative drug amount in the receptor compartment after 8 h and 24 h was defined as Q_8 and Q_{24} ($\mu g\,cm^{-2}$), respectively. The enhancement ratio (ER) for flux was calculated as follows:

$$ER = \frac{\text{Flux for treated buccal tissue with enhancer or iontophoresis or their combination}}{\text{Flux for untreated buccal tissue}}$$

Results were presented as mean ± standard deviation (S.D.) (n) where n represented the number of replicates. Data analysis of flux was performed for treated tissue against control by the unpaired Student's t-test. ANOVA was used to compare fluxes among different treated tissues. A probability of less than 5% ($p < 0.05$) was considered significant.

2.9. Histology of tissues

The morphological changes in both untreated and treated buccal tissues were evaluated using light microscopy. Buccal membrane samples were sectioned carefully and fixed in 10% buffered formalin for 1-day at room temperature. Tissue samples were successively dehydrated with 50%, 75%, 95% and 100% alcohol for 1 h each. This was followed by immersing in xylene at least three times, and finally embedding in Tissue-Tek O.C.T. compound under dry ice. Using a microtome (Leica Model CM 1850, Leica Microsystems, Inc. Bannockburn, IL, USA), 7 μm thin slices were prepared and then stained with Mayer's Harris Hematoxylin and Eosin Y (H&E). The stained slices were examined under a Nikon Eclipse E 800 light microscope (Micro Optics, Cedar Knolls, NJ, USA) at 40×. A Nikon Digital Camera (Model DXM 1200) was used to capture images. Images were processed by SPOT ™ Imaging Software, Version 5.0 (Diagnostic Instrument, Inc., Sterling Heights, MI, USA).

2.10. Buccal tissue cytotoxicity study

MTS 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) assay was used to evaluate enhancer cytotoxicity in buccal tissues. MTS assay is based on the ability of a mitochondrial dehydrogenase enzyme derived from viable cells to cleave the tetrazolium rings and form purple color formazan crystals that are largely impermeable to cell membranes, thus resulting in their accumulation within healthy cells (Promega Corp., 2009). The number of surviving cells is directly proportional to the level of the formazan. The color can then be quantified at 490 nm using Microplate Power Wave X Scanning Spectrophotometer (Bio-TEK Instruments, Inc., Winooski, VT, USA).

EpiOral™ tissue (ORL-200) from MatTek Corp. was used and this is a multilayered tissue mainly composed of an organized basal layer and multiple non-cornified layers analogous to native human buccal tissue. A 24-well plate containing ORL-200 (cell culture inserts) was stored in the refrigerator (4 °C) prior to use. Under sterile forceps, the cell culture inserts were transferred into four 6-well plates containing pre-warmed assay medium (37 °C). The 6-well plates containing the tissue samples were then placed in a humidified 37 °C and 5% CO_2 incubator for 1 h prior to dosing. Tissues were exposed to 20, 60, and 240 min of enhancer solution dosed in duplicate. Two inserts were left untreated to serve as a Negative Control (sterilized water) and another two inserts served as a Positive Control (1% Triton X-100—a nonionic surfactant, polyethylene glycol p-(1,1,3,3-tetramethylbutyl)-phenyl ether). Exposure time for the Positive and Negative Controls was 60 min as per EpiOral 200 Protocol from MatTek Corp. (MatTek, 2009). After 1 h incubation, the assay media was removed from the wells and replaced with 0.9 ml of pre-warmed fresh media, then 40 μl of 1:1 diluted enhancer solutions in sterilized water were added into the cell culture inserts atop the EpiOral™ tissue. 40 μl of sterilized water as negative control and 100 μl of 1% Triton-100 as positive control were added in separate wells. Then the well plates containing the

Table 1
Effect of current on transbuccal delivery of ODAN HCl at Stage I[a].

Iontophoresis (mA)	Flux (μg/cm²/h)	Q_8 (μg/cm²)	ER
Control	3.2 ± 0.7	25.5 ± 5.1	1.0
0.1	10.6 ± 4.5[b]	83.3 ± 33.5	3.3
0.2	16.5 ± 6.5[b]	132.7 ± 50.1	5.2
0.3	22.8 ± 4.6[b]	190.4 ± 42.7	7.1

[a] Data are presented as means ± S.D. ($4 \leq N \leq 5$).
[b] Statistically significantly higher than control at $p < 0.05$ (Student's *t*-test).

dosed EpiOral™ tissues were returned to the incubator for 20, 60, and 240 min. After the exposures, each tissue insert was gently removed, rinsed with PBS solution at least twice and transferred into a 24-well plate containing premixed MTS solution (ratio of MTS reagent: assay medium = 1:4). The 24-well plate was then returned to 37 °C, 5% CO_2 incubator for 3 h. After this, 100 μl of the reacted MTS solutions from each well was pipetted into a marked 96-well microtiter plate for spectrophotometer reading (SPR) at 490 nm using Microplate Power Wave X Scanning Spectrophotometer (Bio-TEK Instruments, Inc., Winooski, VT, USA). 100 μl of assay medium was used as a blank. The EpiOral tissue % viability at each of the dosed concentrations was calculated using the following formula:

$$\%\text{Viability} = 100 \times \frac{\text{SPR for Treated Sample}}{\text{SPR for Negative Control}}$$

Dose response curve was established using a semi-log scale to plot % viability (linear y axis) vs. the dosing time (log × axis). ET-50 value—the time required for the % viability of EpiOral™ tissue to fall to 50 was obtained through interpolation. All the SPR were deducted from blank readings for viability and ET-50 value final calculations.

3. Results and discussion

3.1. Effect of current on transbuccal delivery of ODAN HCl

Anodal iontophoresis at 0.1, 0.2, and 0.3 mA was applied to buccal tissue for 8 h and then discontinued to allow passive permeation of drug for another 16 h. The flux, cumulative amount of drug permeated and ER are shown in Tables 1 and 2 for Stage I (0–8 h) and Stage II (8–24 h). Iontophoresis (0.1, 0.2 and 0.3 mA) provided significantly higher flux of ODAN HCl when compared to control (untreated) ($p < 0.05$). The transbuccal flux linearly increased as current increased from 0.1 to 0.3 mA (Fig. 2). Fig. 3 shows cumulative drug amount permeated from 0 to 24 h. It indicates that the enhancement effect of iontophoresis was significant not only during the 8 h of treatment but throughout the 24 h of the study. Furthermore, the enhancement ratio increased as current increased at Stage I. The enhancement ratio at Stage II leveled off but was still significantly higher than that of control.

Table 2
Effect of current on transbuccal delivery of ODAN HCl at Stage II[a].

Iontophoresis (mA)	Flux (μg/cm²/h)	Q_{24} (μg/cm²)	ER
Control	4.9 ± 1.1	104.7 ± 22.8	1.0
0.1	13.7 ± 4.3[b]	296.9 ± 90.1	2.8
0.2	12.7 ± 5.3[b]	337.4 ± 130.5	2.6
0.3	11.9 ± 2.3[b]	380.4 ± 68.1	2.4

[a] Data are presented as means ± S.D. ($4 \leq N \leq 5$).
[b] Statistically significantly higher than control at $p < 0.05$ (Student's *t*-test).

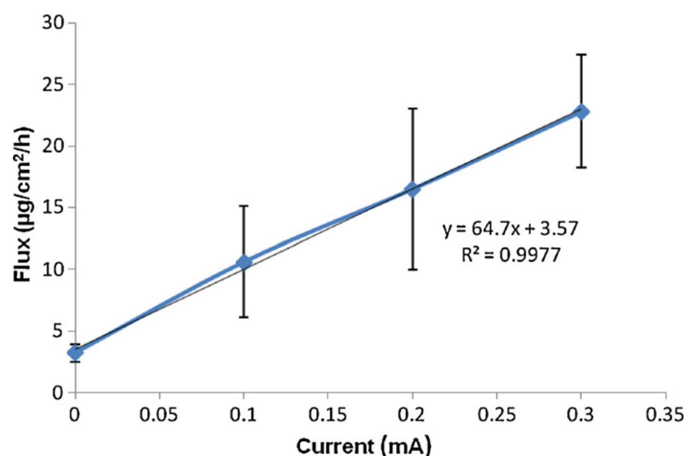

Fig. 2. Effect of current on flux of transbuccal delivery of ODAN HCl. Data are presented as means ± S.D. ($4 \leq N \leq 5$).

Table 3
Effect of chemical enhancers on transbuccal delivery of ODAN HCl at Stage I[a].

Chemical enhancers	Flux (μg/cm²/h)	Q_8 (μg/cm²)	ER
Control	3.2 ± 0.7	25.5 ± 5.1	1.0
Propylene glycol (PG)	10.7 ± 2.6[b]	83.4 ± 19.3	3.3
2.5% Azone in PG	11.3 ± 2.9[b]	88.7 ± 23.1	3.5
5.0% DDAIP in PG	5.1 ± 1.1	41.5 ± 8.1	1.6
5.0% DDAIP HCL in water	29.3 ± 8.0[c]	231.2 ± 62.7	9.2
5.0% DDAIP HCL in PG	12.4 ± 7.0[b]	100.7 ± 56.4	3.9
5.0% Br-iminosulfurane in PG	9.2 ± 3.6[b]	73.1 ± 27.8	2.9

[a] Data are presented as means ± S.D. ($N = 4$).
[b] Statistically significantly higher than control at $p < 0.05$ (Student's *t*-test).
[c] Statistically significantly higher than the other enhancer treated and control at $p < 0.05$ (ANOVA).

3.2. Effect of chemical enhancers on transbuccal delivery of ODAN HCl

Azone in PG, DDAIP HCl in water, DDAIP HCl in PG, DDAIP in PG, Br-iminosulfurane in PG or PG alone was applied (30 μl) to the buccal tissue for 1 h prior to the permeation experiment. After the 1 h enhancer pretreatment, 0.3 ml of 0.5% ODAN HCl gel formulation was applied. Samples were taken at different time points from 0 to 24 h. Tables 3 and 4 compare flux and ER of passive transport of ODAN HCl through enhancer pretreated and untreated (control) tissues. The passive flux of ODAN HCl was significantly greater in all enhancer treated tissues in comparison to control ($p < 0.05$). DDAIP HCl in water resulted in significantly higher flux and ER than did DDAIP in PG, Azone in PG and Br-iminosulfurane in PG ($p < 0.05$). Fig. 4 shows the cumulative amount of ODAN HCl permeated through tissue from 0 to 24 h. It shows that compared to control, the enhancement effect of chemical enhancers was sig-

Table 4
Effect of chemical enhancers on transbuccal delivery of ODAN HCl at Stage II[a].

Chemical enhancers	Flux (μg/cm²/h)	Q_{24} (μg/cm²)	ER
Control	4.9 ± 1.1	104.7 ± 22.8	1.0
PG	10.9 ± 0.8[b]	257.1 ± 31.9	2.2
2.5% Azone in PG	15.8 ± 3.1[b]	340.7 ± 70.0	3.2
5.0% DDAIP in PG	11.3 ± 0.8[b]	221.0 ± 15.6	2.3
5.0% DDAIP HCl in water	41.6 ± 7.6[c]	920.3 ± 169.1	8.5
5.0% DDAIP HCl in PG	24.5 ± 3.8[b]	490.8 ± 107.2	5.0
5.0% Br-iminosulfurane in PG	14.8 ± 4.1[b]	309.5 ± 83.1	3.0

[a] Data are presented as means ± S.D. ($N = 4$).
[b] Statistically significantly higher than control at $p < 0.05$ (Student's *t*-test).
[c] Statistically significantly higher than the other enhancer treated and control at $p < 0.05$ (ANOVA).

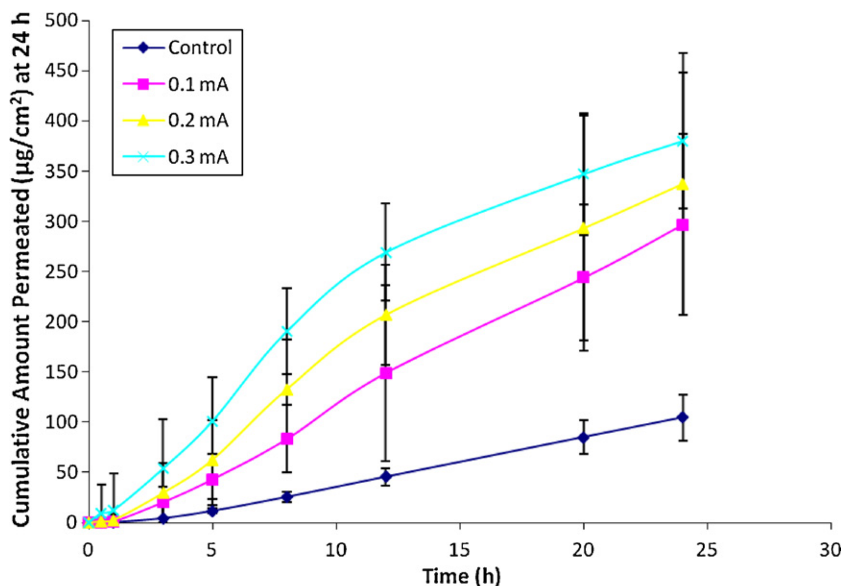

Fig. 3. Effect of iontophoretic current on cumulative amount of ODAN HCl permeated through porcine buccal tissue at 24 h. Data are presented as means ± S.D. ($4 \le N \le 5$).

nificant throughout the 24 h of the study. It is of interest to note that DDAIP HCl in water exhibited significantly higher permeability than DDAIP HCl in PG ($p < 0.05$), indicating that PG actually acted as a penetration "retardant" when used as a vehicle for DDAIP HCl. Permeation routes of buccal mucosa have been reported to be transcellular (intracellular, passing through the cell) or paracellular (intercellular, passing around the cell) depending on physicochemical properties of the drug (Junginger and Verhoef, 1999; Song et al., 2004; Squier and Wertz, 1993). The enhancement differences among the four enhancers may be due to their different properties and mechanisms of action. Azone is a hydrophobic enhancer which is reported to increase lipid fluidity and enhances only intercellular drug diffusion (Barry, 1987). Hydrophobic enhancer Br-iminosulfurane is believed to be more effective in enhancing hydrophobic drug permeation through lipid membranes (Song et al., 2005). Wolka et al. (2004) suggested that DDAIP enhances drug transport by interacting with the polar region of the phospholipid bilayer and also by increasing the motional freedom of lipid hydrocarbon chains in the cell membrane, indicating DDAIP mechanism of enhancement of uptake was primarily through paracellular pathway.

Buccal tissue is non-keratinized, lacks the organized intercellular lipid lamellae and contains large amount of polar lipids (Squier et al., 1991). The hydrophilic nature of the lipids allows more interaction with hydrophilic compounds, i.e. paracellular route is more likely to be the predominant route for the absorption of a hydrophilic drug. Therefore, hydrophilic DDAIP HCl was more potent in enhancing transbuccal delivery of ODAN HCl through intercellular (paracellular) pathway than hydrophobic enhancers azone, DDAIP and Br-iminosulfurane. Furthermore, it was also interesting to find that DDAIP HCl pretreatment alone provided significantly higher enhancement of transbuccal delivery of ODAN HCl than iontophoresis at 0.3 mA during the first 8 h and the following 16 h of the study ($p < 0.05$). It is most probable that iontophoresis enhanced transbuccal drug delivery through disordering of the outer epithelial cell layers, i.e. more of the paracellular route (Jacobsen, 2001) similar to disrupting porcine stratum corneum and increasing intercellular space for enhancing transdermal drug

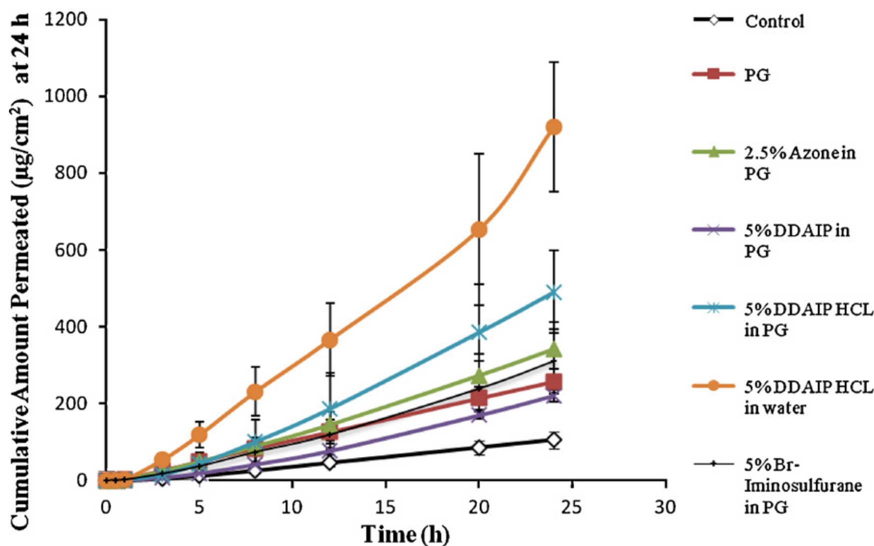

Fig. 4. Effect of chemical enhancers on cumulative amounts of ODAN HCl permeated through porcine buccal tissue at 24 h. Data are presented as means ± S.D. ($N = 4$).

Table 5
Effect of combined treatment of current and chemical enhancers on transbuccal delivery of ODAN HCl at Stage I[a].

Treatment	Flux (μg/cm²/h)	Q_8 (μg/cm²)	ER
Control	3.2 ± 0.7	25.5 ± 5.1	1.0
0.3 mA	22.8 ± 4.6[b]	190.4 ± 42.7	7.1
PG+0.3 mA	19.7 ± 1.2[b]	133.9 ± 10.8	4.6
2.5% Azone in PG+0.3 mA	34.1 ± 6.0[b]	267.9 ± 42.2	10.7
5.0% DDAIP in PG+0.3 mA	23.5 ± 1.6[b]	196.3 ± 9.1	7.3
5.0% DDAIP HCl+0.3 mA in water	43.0 ± 14.6[b]	336.7 ± 110.7	13.4
5.0% DDAIP HCl in PG+0.3 mA	26.1 ± 4.2[b]	210.8 ± 52.8	8.2
5.0% Br-iminosulfurane in PG+0.3 mA	24.0 ± 3.6[b]	188.6 ± 25.1	7.5

[a] Data are presented as means ± S.D. ($3 \le N \le 5$).
[b] Statistically significantly higher than control at $p < 0.05$ (Student's *t*-test).

Table 6
Effect of combined treatment of current and chemical enhancers on transbuccal delivery of ODAN HCl at Stage II[a].

Treatment	Flux (μg/cm²/h)	Q_{24} (μg/cm²)	ER
Control	4.9 ± 1.1	104.7 ± 22.8	1.0
0.3 mA	11.9 ± 2.3[b]	380.4 ± 68.1	2.4
PG+0.3 mA	10.7 ± 1.5[b]	306.9 ± 15.0	2.0
2.5% Azone in PG+0.3 mA	15.1 ± 0.5[b]	520.9 ± 52.7	3.1
5.0% DDAIP in PG+0.3 mA	12.5 ± 3.1[b]	405.0 ± 46.2	2.6
5.0% DDAIP HCl+0.3 mA in water	30.2 ± 7.7[b]	833.5 ± 214.4	6.2
5.0% DDAIP HCl in PG+0.3 mA	20.5 ± 5.2[b]	538.8 ± 131.4	4.2
5.0% Br-iminosulfurane in PG+0.3 mA	13.0 ± 2.5[b]	405.3 ± 22.7	2.7

[a] Data are presented as means ± S.D. ($3 \le N \le 5$).
[b] Statistically significantly higher than control at $p < 0.05$ (Student's *t*-test).

delivery (Bhatia et al., 1997; Singh et al., 1998). Table 4 shows that using 5% DDAIP HCl in water treatment, transbuccal delivery of ODAN HCl (Q_{24}) could reach 920.3 (μg/cm²) within 24 h, i.e. potentially when a small patch of 10 cm² containing only 0.5% ODAN HCl is used, this particular enhanced drug delivery system could deliver 9.2 mg/day into blood circulation through buccal route.

3.3. Effect of combined treatment of chemical enhancers and iontophoresis on transbuccal delivery of ODAN HCl

Azone in PG, DDAIP HCl in water, DDAIP HCl in PG, DDAIP in PG, Br-iminosulfurane in PG and vehicle PG was applied (30 μl) to the top of buccal tissue for 1 h prior to the anodal iontophoretic permeation experiment. After 1 h enhancer pretreatment, 0.3 ml of 0.5% ODAN HCl gel formulation was applied to the top of buccal tissues, and then 0.3 mA iontophoresis was applied for 8 h. At the end of 8 h of 0.3 mA iontophoresis treatment, iontophoresis was ceased to allow passive permeation to continue for another 16 h. Samples were taken at different time points from 0 to 24 h. Tables 5 and 6 show that combined treatment of enhancer with iontophoresis provided no synergistic only additive enhancement effect in the case of azone, DDAIP and Br-iminosulfurane. The combined treatments did provide significant higher permeability than that of control ($p < 0.05$) and the combination of DDAIP HCl in water and iontophoresis (0.3 mA) was the most effective treatment in enhancing transbuccal delivery of ODAN HCl (Fig. 5). However, with DDAIP HCl in water pretreatment, the flux (30.2 μg/cm²/h) from the combined treatment was much less than the sum of the fluxes of DDAIP HCl in water (41.6 μg/cm²/h) and iontophoresis (11.9 μg/cm²/h) during the 24 h of the study. The same trend was recorded for DDAIP HCl in PG. It can be explained by the fact that DDAIP HCl—the salt form of DDAIP contained ions that competed with ODAN HCl for iontophoresis, thus the enhancement effect of iontophoresis was reduced.

In addition, buccal tissues have an epithelial layer as the barrier that limits the passive permeation of ionized drugs such as ODAN HCl—the control. The cumulative amounts calculated for the control were so small that there were almost no differences among the different samples. However, when the permeation of ODAN HCl through buccal tissue was enhanced, the cumulative amounts calculated were higher (as expected). This immediately resulted in more noticeable variations among buccal tissue samples and hence the standard deviations of the cumulative amounts calculated also increased.

Pig and human buccal mucosa are similar in terms of structure, composition and permeability (Lesch and Squier, 1989; Collins

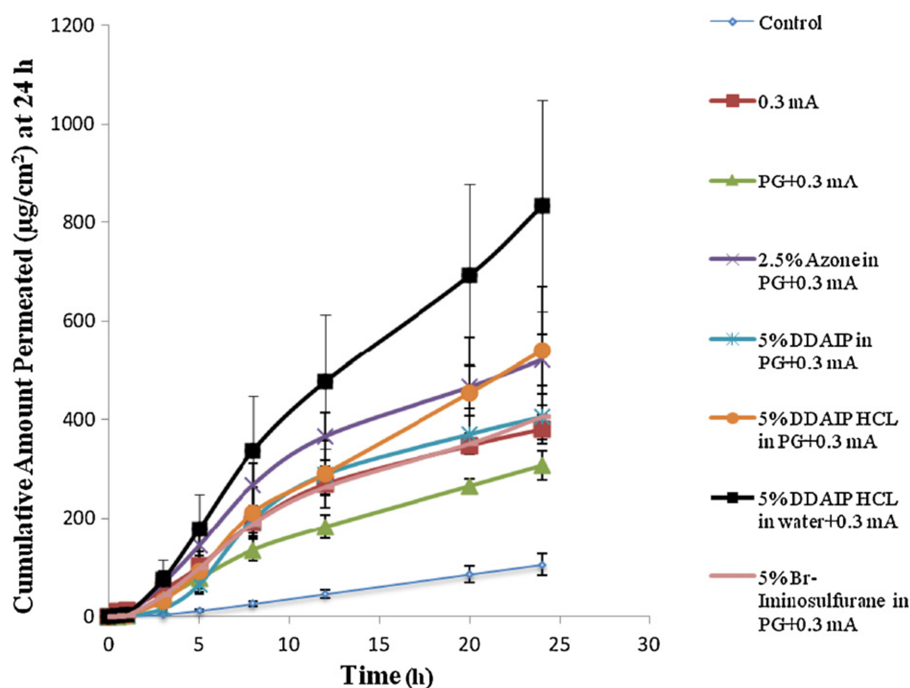

Fig. 5. Effect of combined treatment of iontophoresis with chemical enhancers on ODAN HCl permeation through porcine buccal tissue at 24 h. Data are presented as means ± S.D. ($3 \le N \le 5$).

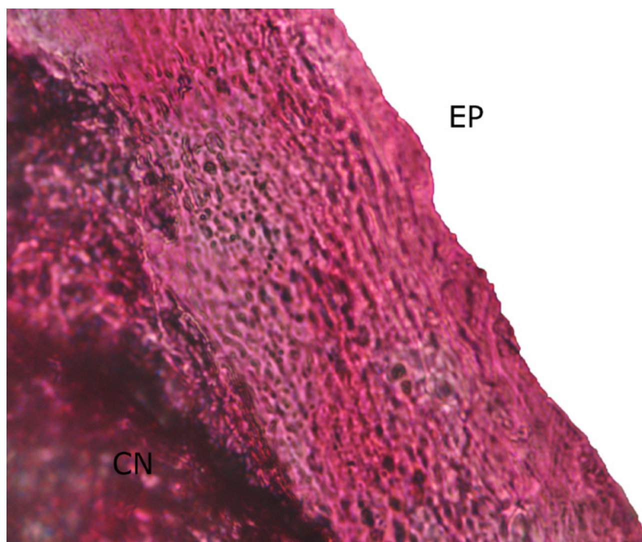

Fig. 6. Untreated porcine buccal tissue (EP, epithelium; CN, connective tissue).

et al., 1981; de Vries et al., 1991; Wertz, 1991; Wertz and Squier, 1996). This *in vitro* permability study allows many variables such as flushing action of saliva or the ingestion of foods encountered *in vivo* to be controlled. When employing in vivo buccal iontophoretic drug delivery, the donor compartment can be placed on the buccal mucosa and receptor compartment can optionally be placed anywhere on the body, i.e. on buccal mucosa or on skin (Jacobsen, 2001). Saliva dilution, enzyme degradation and irritation on human mucosa in vivo can potentially affect transbuccal drug delivery. However, advanced buccal bioadhesive drug delivery systems including patch, tablet, semisolids/liquids and particulates (Smart, 2005) may address these issues in the near future.

3.4. Histological study

A histological study was performed to evaluate the integrity of treated and untreated porcine tissues using standard H&E methodology. Treated tissues included those following 0.3 mA iontophoresis for 8 h and combined treatment of 0.3 mA iontophoresis for 8 h plus 1 h enhancer pretreatment: DDAIP HCl in water and DDAIP HCl in PG. Light micrographs (40×) (Fig. 6) show the morphology of treated and untreated buccal tissues. Compared to untreated (Fig. 6), no major morphological changes were observed after 0.5% ODAN HCl passive permeation (Fig. 7), 0.3 mA for 8 h (Fig. 8), 0.3 mA for 8 h + 5% DDAIP HCl in water treatment (Fig. 9), and 0.3 mA for 8 h + 5% DDAIP HCl in PG treatment (Fig. 10). 10% Oleic acid in PG pretreatment was used as a positive control since it was reported to cause detachment of keratinocytes in stratum corneum of skin (Wang et al., 2003). Thus a similar approach was taken and 10% Oleic acid in PG pretreatment was used as a positive control and integrity of the treated tissue was recorded. The micrograph showed significant damage in the buccal epithelial layers—the white arrow pointed area (Fig. 11).

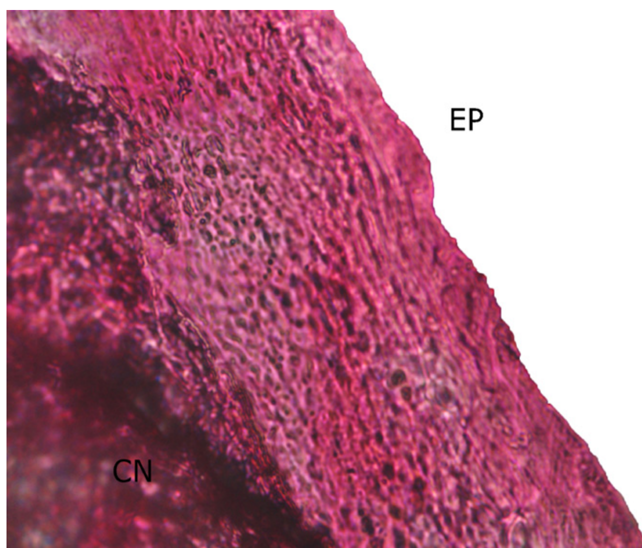

Fig. 7. 0.5% ODAN HCl passive permeation (EP, epithelium; CN, connective tissue).

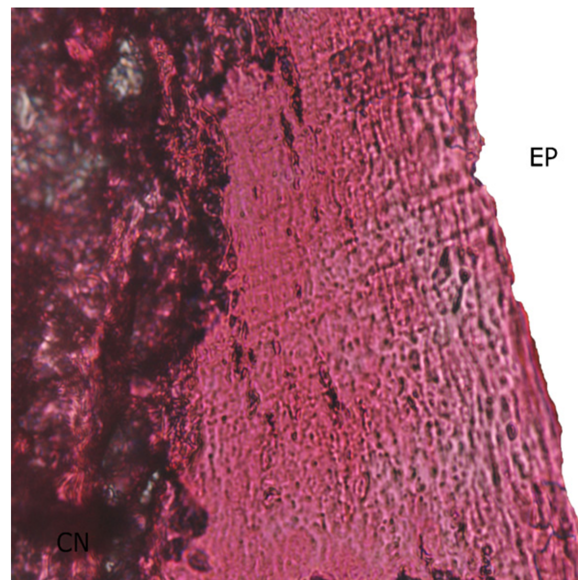

Fig. 8. Iontophoresis 0.3 mA for 8 h (EP, epithelium; CN, connective tissue).

3.5. EpiOral™ cytotoxicity study

Cytotoxicity evaluation (MTS assay) was conducted using EpiOral™ tissue in duplicate using 5% DDAIP HCl in water—the best

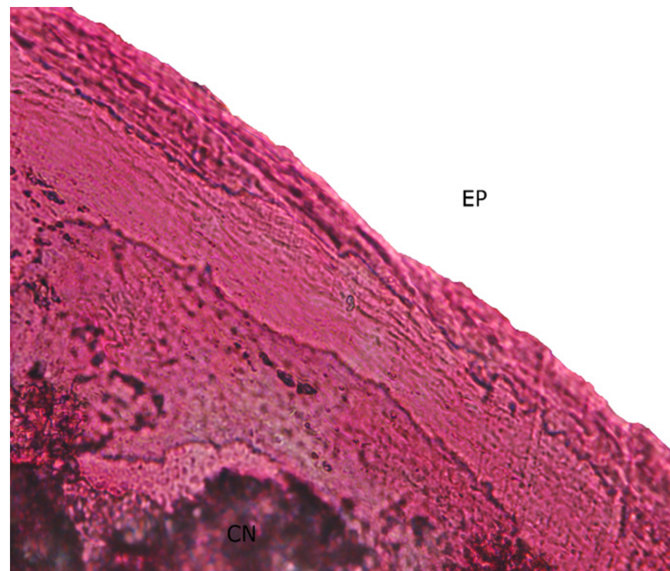

Fig. 9. Combined treatment of iontophoresis 0.3 mA for 8 h + 5% DDAIP HCl in water 1 h pretreatment (EP, epithelium; CN, connective tissue).

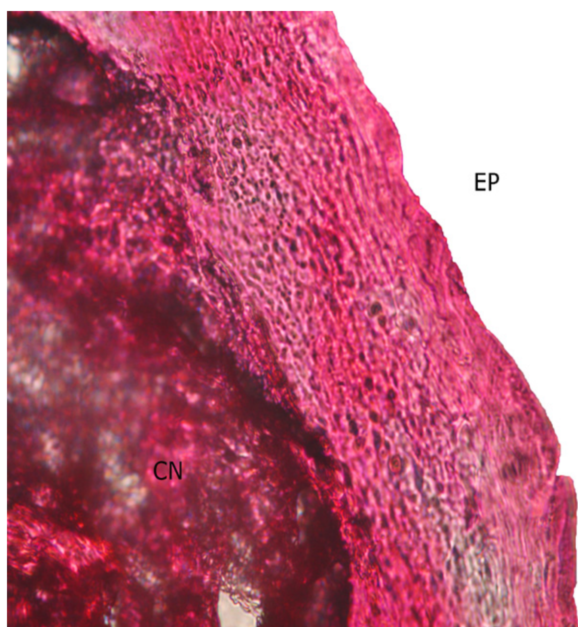

Fig. 10. Combined treatment of iontophoresis 0.3 mA for 8 h + 5% DDAIP HCl in PG 1 h pretreatment (EP, epithelium; CN, connective tissue.)

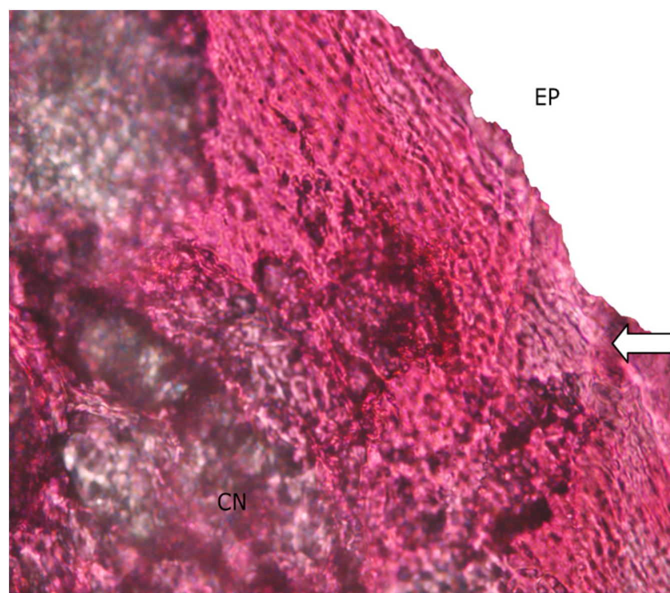

Fig. 11. Combined treatment of iontophoresis 0.3 mA for 8 h + 10% oleic acid in PG 1 h pretreatment (EP, epithelium; CN, connective tissue; white area, damaged area).

performing chemical enhancer from this study. Sterilized water treated tissue was used as negative control and 1% Triton-100 treated tissue as positive control. At the end of the experiments, cell viability was evaluated by measuring the mitochondrial dehydrogenase activities according to the MTS assay (Promega Corp., 2009). The mean optical density (OD) of the untreated control tissues was set to represent 100% of viability (MTS test, $N = 2$, OD = 0.999) and the results were quantified as percentage of the negative controls. (Fig. 12) demonstrated that DDAIP HCl treatment in a concentration range of 0.05% to 5% in water for 4 h did not reduce the viability of EpiOral^{TM} tissue compared to water—the negative control, and viability (100%) of 5% DDAIP HCl in water treated EpiOral tissue was significantly higher than that (49%) of positive control. The DDAIP HCl in water dose response curve obtained from MTS EpiOral^{TM} tissue (Fig. 13) indicated that ET-50 value of 5% DDAIP HCl in water was greater than 1000 min, significantly more than the 49 min for the positive control, indicating that at concentrations up to 5% in water, DDAIP HCl is potentially safe to use for transbuccal drug delivery. Furthermore, Pfister et al. (2006) conducted a two year rat skin study and their results demonstrated that DDAIP and DDAIP HCl have a low acute toxicity (mouse and rat oral LD50 > 5.0 g/kg) and are rapidly metabolized to *n*-dodecanol and *N*,*N*-dimethylalanine by esterase enzymes in skin, plasma and microsomes, are well tolerated on skin of rats and mice at $\leq$5% in repeat dose studies, were negative for sensitization, genotoxicity, reproductive effects and carcinogenicity. One would assume that these agents have low toxicity potential especially when given in low doses to buccal tissues. This of course, can be achieved by the iontophoretic plus enhancer approach that lowers the dose of enhancer. Therefore, DDAIP and DDAIP HCl may be potentially safe for chronic use.

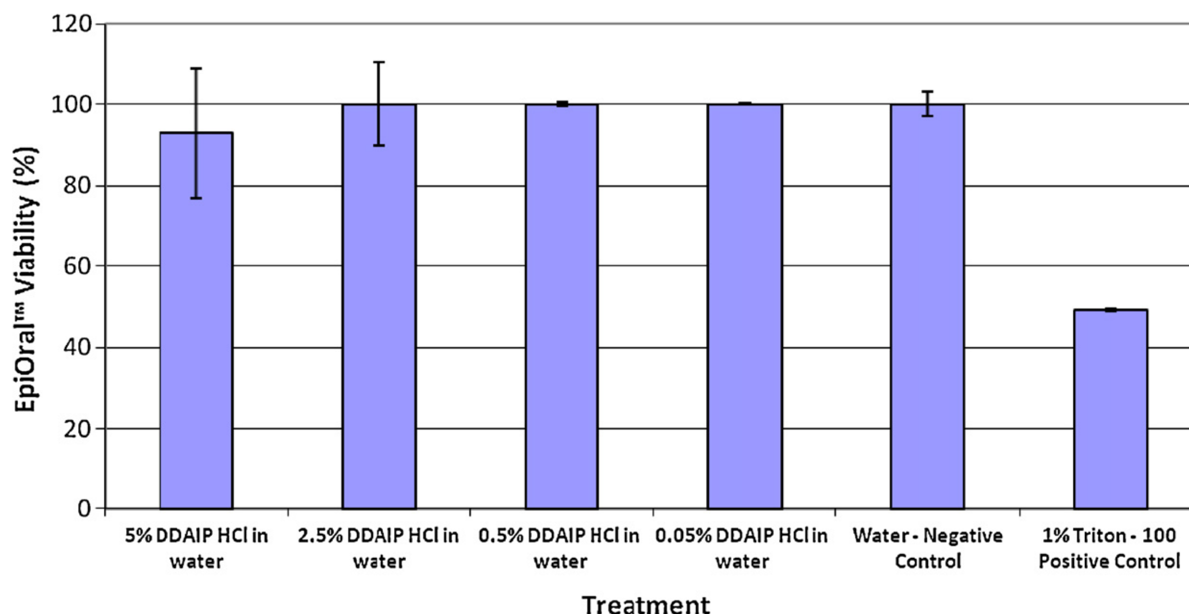

Fig. 12. EpiOral^{TM} tissue viability (%) of different treatments for 4 h. Data are presented as means ± S.D. ($N = 2$).

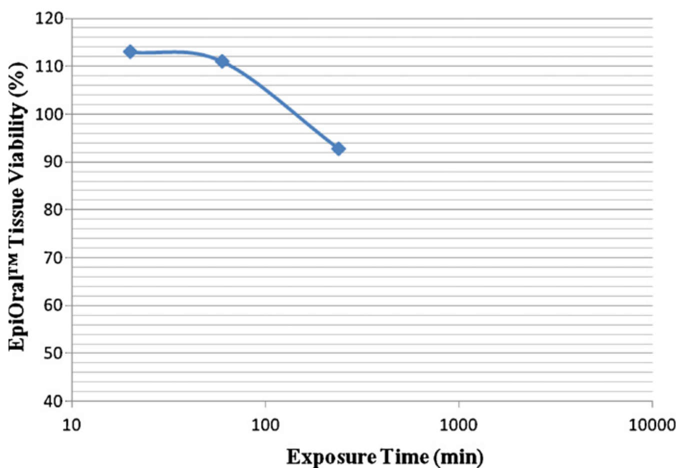

Fig. 13. ET value of 5% DDAIP HCl in water in a dose response curve from EpiOral™ tissue ($N = 2$).

4. Conclusions

It was concluded that both iontophoresis (0.1, 0.2, 0.3 mA) or DDAIP HCl pretreatment can provide significantly higher permeability for ODAN HCl across porcine buccal tissues compared to control ($p < 0.05$) while azone, DDAIP and Br-iminosulfurane were only marginally effective. The combination of iontophoresis and chemical enhancers resulted in no synergistic enhancement effects. 5% DDAIP HCl in water produced no major morphological changes in porcine buccal tissue and was the most effective enhancer/vehicle formulation for transbuccal delivery of ODAN HCl.

Acknowledgments

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