Investigating the Effect of *Aloe vera* Gel on the Buccal Permeability of Didanosine

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- *Aloe vera* (L.) Burm. F.
- *Aloe barbadensis* Miller
- Asphodelaceae

Abstract

The buccal mucosal route offers several advantages but the delivery of certain drugs can be limited by low membrane permeability. This study investigated the buccal permeability properties of didanosine (ddI) and assessed the potential of *Aloe vera* gel (AVgel) as a novel buccal permeation enhancer. Permeation studies were performed using Franz diffusion cells, and the drug was quantified by UV spectroscopy. Histomorphological evaluations were undertaken using light and transmission electron microscopy. The permeability of ddI was concentration-dependent, and it did not have any adverse effects on the buccal mucosae. A linear relationship ($R^2 = 0.9557$) between the concentrations and flux indicated passive diffusion as the mechanism of drug transport. AVgel at concentrations of 0.25 to 2%w/v enhanced ddI permeability with enhancement ratios from 5.09 (0.25%w/v) to 11.78 (2%w/v) but decreased permeability at 4 and 6%w/v. Ultrastructural analysis of the buccal mucosae treated with phosphate buffer saline pH 7.4 (PBS), ddI/PBS, and ddI/PBS/AVgel 0.5%w/v showed cells with normal plasmalemma, well-developed cristae, and nuclei with regular nuclear envelopes. However, cells from 1, 2, and 6%w/v AVgel-treated mucosae showed irregular nuclear outlines, increased intercellular spacing, and plasmalemma crenulations. This study demonstrates the potential of AVgel as a buccal permeation enhancer for ddI to improve anti-HIV and AIDS therapy.

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

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Introduction

Antiretroviral (ARV) drugs have revolutionized the treatment of HIV (human immunodeficiency virus) infection and AIDS (acquired immune deficiency syndrome) [1], widely acknowledged as being among the most serious public health problems [2]. However, several limitations exist with current ARV drug therapy via the oral route [3,4]. These drugs suffer from low bioavailability due to extensive first pass effects and gastrointestinal degradation. Also, short half-lives necessitate frequent administration of doses, and severe dose-dependent side effects may occur.

Buccal drug delivery, which is the administration of drug from a delivery system (e.g., films, patches, and gels) through the mucosae lining the cheeks of the mouth, has received increased interest as an alternative to the oral route. Drugs administered via the buccal route can bypass enzymatic degradation and hepatic first pass metabolism thereby improving bioavailability [5,6]. It has a high patient acceptability compared to other non-oral routes [7]. Buccal delivery systems offer an attractive approach for pediatrics and for patients with swallowing problems. Buccal delivery of ARV drugs can therefore contribute to overcoming some of their current disadvantages. While the potential of ARV drugs for administration via another non-oral route, namely the transdermal route, has been explored [8,9] their buccal delivery potential remains to be investigated.

The epithelium lining the oral cavity is a barrier to drug permeation. The use of permeation enhancers in many cases is decisive for efficient buccal drug delivery [10,11]. The discovery of new permeation enhancers is essential for optimizing drug delivery via the buccal route. Currently, there is an increasing interest for drug products that either are of natural origin or contain such components [12]. *Aloe vera* (*Aloe barbadensis* Miller) is a succulent plant with strap-shaped

green leaves [12]. For medicinal applications, the aloe latex (or exudate), the aloe gel, and the whole leaf (or whole leaf extract) are the main parts used [13]. The inner pulp of the fresh leaves is used for gel extrusion [14]. The gel is composed mainly of water (> 99%), and the remaining 0.5–1% of solid material comprises several polysaccharides, vitamins, enzymes, lipids, as well as inorganic and small organic compounds [15]. It is recognized as an important medicinal plant that has effective anti-inflammatory, antifungal, and soothing effect on the mucosal lining as well as wound healing properties [16]. While it has recently been shown to be an effective transdermal [17] and intestinal [13] penetration enhancer for various drugs, its applicability for buccal permeation enhancement has not been investigated before. Those studies with AVgel as an enhancer for the intestinal and transdermal routes did not report its histomorphological effects [13,17], which is important for assessing its preliminary suitability. Recently, it has been shown to have the potential to modify drug release profiles in dosage forms [18]. It appears that *Aloe vera* gel, with polysaccharides as a significant component, has the potential unlike several existing penetration enhancers, to also provide multifunctional properties in buccal drug delivery systems. A buccal controlled release product based on *Aloe vera* gel (AVgel) will therefore be an attractive system for the administration of ARV drugs.

The aim of this study was therefore to identify the buccal permeability potential of a model ARV drug, i.e., didanosine (ddI), in the absence and presence of a potential novel buccal permeation enhancer, namely AVgel. In addition, the study also aimed at evaluating the histomorphological effects of ddI and AVgel on the buccal mucosa.

Materials and Methods

Ethical clearance

Ethical approval was obtained from the University of KwaZulu-Natal Animal Ethics Committee in 2008 (001/08/Animal) and renewed annually in 2009 (028/09/Animal), 2010 (029/10/Animal), and 2011 (25/11/Animal).

Materials

Didanosine [ddI; chromatographic purity (HPLC) = 99.4%] was donated by Aspen Pharmacare. AVgel, in dry powder form, was received from the International Aloe Science Council (IASC, 051309, Texas, USA) and was the same sample used in our previously reported study in *Planta Medica* [13]. The ^{1}H-NMR spectrum of the AVgel and the quantities of chemical markers as determined by NMR spectroscopy are available as Supporting Information (**Fig. 1S** and **Table 1S**) and are discussed under the Results section. Disodium hydrogen phosphate, potassium dihydrogen phosphate, and sodium chloride were purchased from Sigma-Aldrich. All other reagents used were of analytical grade.

Methods

Preparation of porcine buccal mucosae: Buccal mucosae harvested from pigs (30–40 kg) (Biomedical Resource Unit, UKZN) sacrificed by LECO euthanasia were appropriately excised; their thickness was 665 ± 72 µm (CV = 8.3%). Fresh buccal mucosae were used for histological evaluations. For buccal permeability studies, the buccal mucosae were snap frozen in liquid nitrogen, stored in a biofreezer (− 85 °C) and used within three months [12].

In vitro permeation: Frozen buccal mucosae were allowed to thaw and equilibrated in phosphate buffer saline pH 7.4 (PBS). Franz diffusion cells (PermeGear, Inc.) with a diffusional area of 0.786 cm^2 were used for permeation experiments. The buccal mucosa was mounted to the diffusional area between the donor and receptor cells and was equilibrated with PBS at 37 °C. The donor compartment contained either varying concentrations of ddI in PBS alone (5, 10, 15, and 20 $mg \cdot mL^{-1}$) or ddI (20 $mg \cdot mL^{-1}$) in the presence of AVgel (0.25, 0.5, 1.0, 2.0, 4.0, and 6.0%w/v). The receptor compartments were filled with PBS. Samples were removed from the receptor compartments at predetermined time intervals and replaced with the same volume of ddI–free PBS. Each experiment was undertaken using a minimum of three replicates. Similar to permeation studies with other drugs [19,20], ddI was quantified by a validated UV spectrophotometry method at a λ_{max} of 250 nm (UV spectrophotometer 1650; Shimadzu).

Permeability data analysis: The cumulative amount of ddI permeated per unit surface area was plotted against time. The steady state flux (J_{ss}) was determined from the linear part of the permeability curve by linear regression analysis (Microsoft Excel 2007). The permeability coefficient (P) was calculated as follows [21]:

$$P = (dQ/dt) / A \times Cd = Jss / Cd \quad (1)$$

Where dQ/dt is the cumulative amount permeated per unit time, A is the diffusion area, and C_d is the drug concentration in the donor compartment. The permeability of ddI was evaluated in the presence of various concentrations of AVgel. The enhancement ratio (ER) was calculated as follows [21]:

$$ER = \frac{\text{Permeability coefficient of drug in the presence of enhancer}}{\text{Permeability coefficient of drug in the absence of enhancer}} \quad (2)$$

Viscosity determination: The viscosities of ddI (20 $mg \cdot mL^{-1}$) only and ddI (20 $mg \cdot mL^{-1}$) in the presence of AVgel (0.25, 0.50, 1.0, 2.0, 4.0, and 6.0%w/v) were determined with a Modular Advanced Rheometer (ThermoHaake MARS Thermo Fischer Scientific), equipped with a titanium cone (C35/1° Ti) set at a sample gap of 0.051 mm and a Thermocontroller (UTC-MARS II). The relationships between the viscosity and shear stress as a function of shear rate were analyzed using HaakeRheoWin, 3.50.0012 software.

Light microscopy and transmission electron microscopy: Fresh buccal mucosa was cut into 1 × 1 × 0.1 cm cross sections. Mucosae were incubated in bottles containing either PBS only, or ddI/PBS (20 $mg \cdot mL^{-1}$), or ddI/PBS (20 $mg \cdot mL^{-1}$)/AVgel in varying concentrations. The bottles were kept in a water bath at 37 °C over six hours. Untreated buccal mucosa was transferred from normal saline into 10% buffered formalin without incubation in PBS and served as the control. Both the control and treated buccal mucosae were fixed in formalin for seven days. They were dehydrated using an ethanol gradient and embedded in paraffin wax. The sections were collected on slides, dried and stained with hematoxylin and eosin (H&E). Semi-thin sections (1 µm) of the epoxy-embedded samples were also obtained and stained with toluidine blue. Sections were examined using a light microscope (Nikon 80i), and bright field images were captured using NIS Elements D software and a camera (Nikon U2).

Samples for transmission electron microscopy (TEM) were incubated as described above. They were cut into pieces not exceeding 0.5 mm^3 and fixed for 24 hours (4 °C) using Karnovsky's fixa-

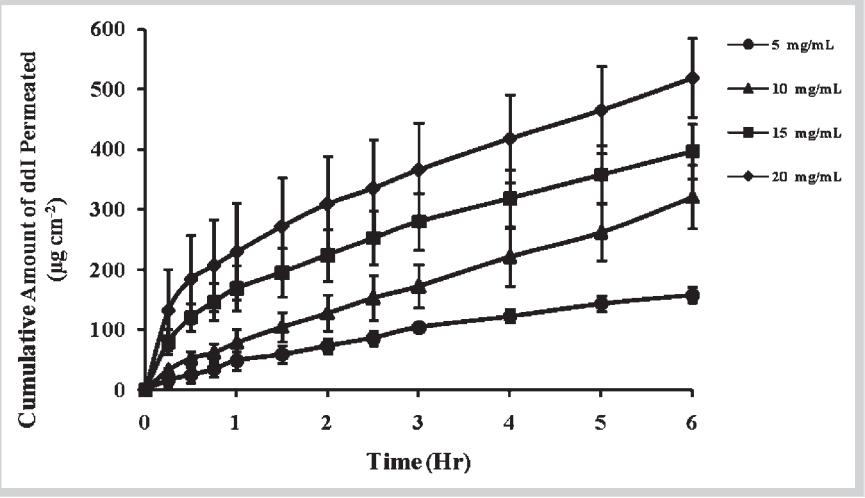

Fig. 1 Cumulative amount of ddI permeated per unit surface area vs. time profiles observed for ddI donor concentrations (mean values ± SD; n ≥ 3).

Table 1 Effect of ddI donor concentration on its permeability parameters.

Donor concentration of ddI (mg·mL^{-1})	Cumulative amount of ddI permeated (µg·cm^{-2})	Linear equation ($y = mx + c$)	Correlation coefficient (R^2)	Flux (Jss) (µg·cm^{-2}·hr^{-1})	Permeability coefficient (P) × 10^{-2} (cm·hr^{-1})
5	158.15 ± 13.17	$Q = 25.94_t + 15.65$	0.97	25.94 ± 1.35	0.52 ± 0.03
10	321.08 ± 52.82	$Q = 49.85_t + 22.23$	0.99	49.85 ± 8.99	0.49 ± 0.09
15	397.03 ± 46.01	$Q = 57.35_t + 85.14$	0.92	57.35 ± 5.88	0.38 ± 0.04
20	456.89 ± 57.11	$Q = 71.57_t + 128.70$	0.89	71.57 ± 3.12	0.36 ± 0.02

tive [22] buffered to pH 7.2, processed and embedded in epoxy resin using standard protocols. Ultrathin sections (90 nm) were cut, contrasted with uranyl acetate and lead citrate and viewed with a transmission electron microscope (JEOL 1010).

All experiments were performed using a minimum of three replicates.

Statistical analysis

The results, expressed as mean ± standard deviation (SD), were analyzed using one-way ANOVA followed by the Mann-Whitney test using GraphPad Prism® (Graph Pad Software, Inc., version 3). Differences were considered significant at $p < 0.05$.

Supporting information

The chemical composition of the AVgel and its ^{1}H-NMR spectrum are available as Supporting Information.

Results and Discussion

The permeability potential of ddI in the absence of an enhancer was initially investigated. **Fig. 1** shows the cumulative amount of ddI permeated at different donor concentrations. The flux values increased with an increase in ddI concentration and ranged from 25.94 ± 1.35 µg·cm^{-2}·hr^{-1} to 71.57 ± 3.12 µg·cm^{-2}·hr^{-1} (**Table 1**). There was a significant difference ($p = 0.001$) between all concentrations except between the flux values of 15 mg·mL^{-1}and 20 mg·mL^{-1} ddI, which were not significant ($p = 0.302$).

A linear relationship ($R^2 = 0.9557$) between the flux and ddI concentrations was obtained (**Fig. 2**), indicating passive diffusion as the main mechanism of ddI transport across the buccal mucosa [23,24]. Didanosine is hydrophilic, and its passive diffusion should favor the paracellular pathway [25,26].

Xiang et al. [3] highlighted the promising potential of zalcitabine (ddC), the only other ARV reported to date for buccal delivery. They reported a flux of 13.42 ± 6.35 µg·cm^{-2}·hr^{-1}for ddC at 20 mg·mL^{-1} which is lower than the flux of ddI (71.57 ± 3.12 µg·cm^{-2}·hr^{-1}). Several drugs with similar and lower flux values have been reported as having the potential for improving drug therapy via the buccal route [23,24,27]. ddI may therefore be regarded as having the potential for improving HIV and AIDS drug therapy when administered by the buccal route.

The AVgel employed in this study to investigate its effect on ddI permeation was the same as used by Chen et al. [13] to study its effects on intestinal drug permeability. The ^{1}H-NMR spectrum of the AVgel is shown in **Fig. 1S** and the quantities of chemical markers as determined by NMR spectroscopy in **Table 1S**. The results indicate that the AVgel material contained all the essential markers especially aloverose.

The buccal permeability of ddI in the presence of AVgel (**Fig. 3**) was investigated. The flux of ddI in the absence of AVgel was 71.57 ± 3.12 µg·cm^{-2}·hr^{-1}. It increased significantly ($p < 0.001$) with an increase in AVgel concentration up to 2%w/v (**Table 2**), which demonstrated the highest permeability coefficient of 3.3×10^{-2} cm·hr^{-1} and an enhancement ratio (ER) of 11.78, thereby confirming for the first time the buccal permeation enhancement property of AVgel.

The permeation enhancing potential of AVgel from 0.25 to 2.0%w/v may have a similar mechanism to those proposed for other polysaccharides reported as permeation enhancers [28]. Polysaccharides such as chitosan are known to demonstrate mucoadhesivity, which causes prolonged drug retention on mucosae. It has been proposed that chitosan enhances buccal perme-

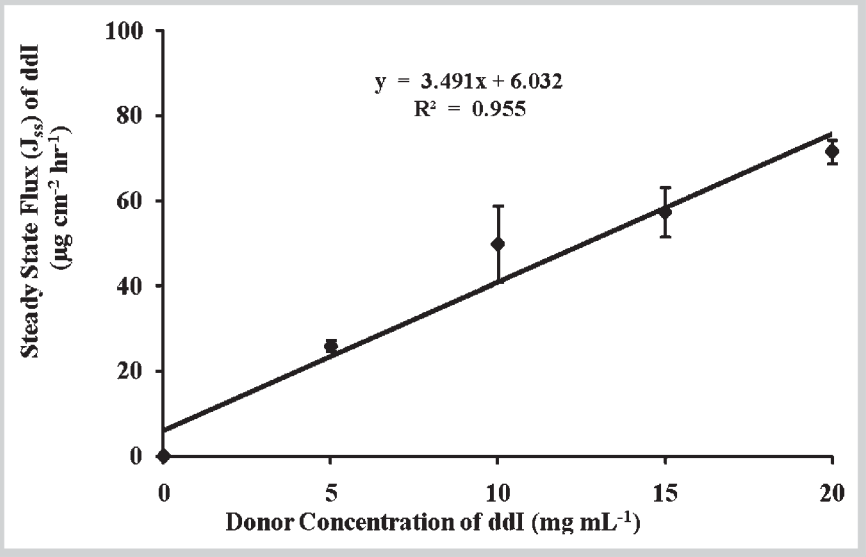

Fig. 2 Effect of donor concentration on the steady state flux of ddI at pH 7.4 (mean values ± SD; n ≥ 3).

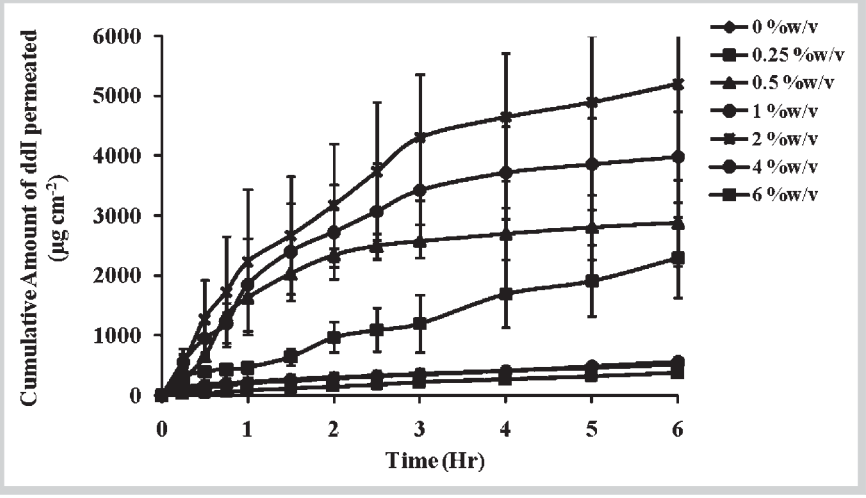

Fig. 3 Cumulative amount of ddI permeated per unit surface area vs. time profiles observed for AVgel concentrations (mean values ± SD; n ≥ 3).

Concentration of AVgel (%w/v)	Correlation coefficient (R^2)	Flux (Jss) ($\mu g \cdot cm^{-2} \cdot hr^{-1}$)*	Permeability coefficient (P) × 10^{-2} ($cm \cdot hr^{-1}$)	Enhancement ratio (ER)
0.0	0.89	71.57 ± 3.12[a]	0.36 ± 0.02	1
0.25	0.99	364.69 ± 92.59	1.82 ± 0.46	5.09
0.5	0.89	613.69 ± 292.49[b]	3.07 ± 1.46	8.58
1.0	0.85	650.07 ± 164.41[b]	3.25 ± 0.82	9.08
2.0	0.88	842.73 ± 129.24[b]	4.21 ± 0.65	11.78
4.0	0.95	83.95 ± 11.71[c]	0.42 ± 0.06	1.17
6.0	0.99	62.02 ± 5.41[c]	0.31 ± 0.03	0.87

Table 2 Effect of AVgel concentration on the permeability parameters of ddI.

* [(a vs. b; $p < 0.05$), (a vs. c; $p > 0.05$)]; [a] flux of the control; [b] statistically significant higher than control (ANOVA); [c] statistically non-significant compared to control (ANOVA)

ability by interactions with the epithelial barrier that may weaken it, partially dismantling the extracellular matrix structure and intercellular joint. Since the major components of AVgel are polysaccharides [18], a similar mechanism may apply. Also, AVgel is cationic, and its possible ionic interaction with sialic acid residues on the buccal mucosae could alter membrane permeability [25,28].

Further increases in AVgel to 4.0 and 6.0%w/v led to a decrease in flux to 83.95 ± 9.24 and 62.06 ± 5.58 $\mu g \cdot cm^{-2} \cdot hr^{-1}$, respectively. Although there is a 10-fold reduction in the flux between 2 and 4%w/v AVgel, the flux at 4 and 6%w/v is reduced to a value which is statistically similar to the flux in the absence of AVgel (**Table 3**). The decrease may be attributed to a higher viscosity of AVgel at higher concentrations that can increase resistance to drug diffusion and hinder drug movement [18,29]. Increasing the concentration of AVgel in the ddI/PBS/AVgel formulations led to an increased viscosity of the formulations (**Fig. 4**) and displayed a linear correlation ($R^2 = 0.972$). The viscosity of AVgel at 6.0%w/v

Concentration of AVgel (% w/v)	Viscosity (η) (mPa)	Percentage increase in viscosity (%)
0	0.84 ± 0.00	0
0.25	0.94 ± 0.05	12.19
0.5	1.05 ± 0.01	25.14
1	1.22 ± 0.04	45.51
2	1.86 ± 0.52	121.89
4	2.21 ± 0.14	163.38
6	2.84 ± 0.11	238.72

Table 3 Effect of AVgel concentration on the viscosity of ddI/PBS/AVgel formulations.

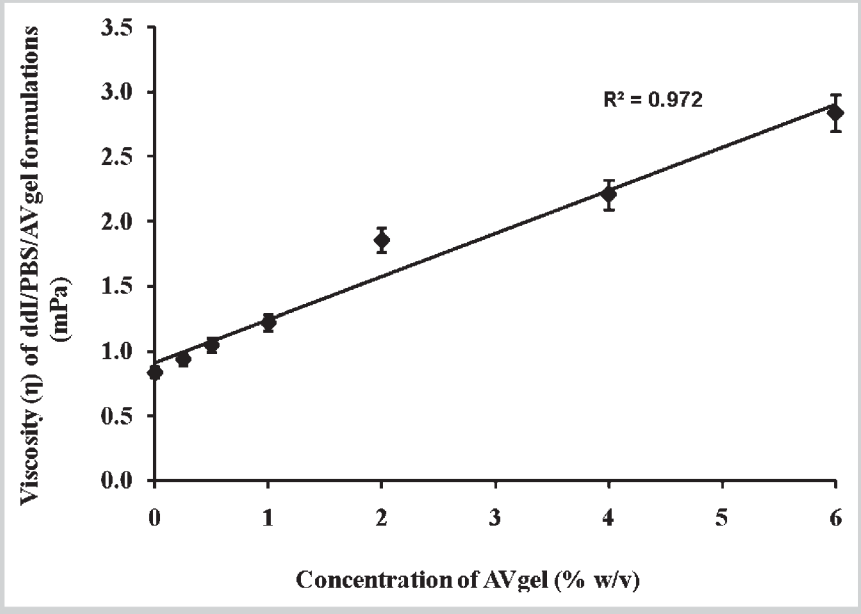

Fig. 4 Effect of AVgel concentration on the viscosity of ddI/PBS/AVgel formulations (mean values ± SD; n = 3).

(2.84 mPa) was almost three times (up to 240%) higher than that at 0.25%w/v (0.94 mPa) (**Table 3**). The viscosities of AVgel at 4.0 and 6.0%w/v may have been high enough to impede the buccal permeability enhancing potential of AVgel. Similar trends, with an initial increase in flux with an increase in enhancer concentrations (propylene glycol) but resultant flux decreases with further increases have been reported in another study [30], although possible reasons were not investigated.

The ER of ddI increased approximately 12-fold with AVgel 2.0%w/v but decreased to 0.87-fold with AVgel 6.0%w/v (**Table 2**). The ER values in this study are within the range of previous studies using AVgel at similar concentrations for other routes. The ER for colchicine through porcine skin was 11.2 (AVgel 3.0%w/v) [17] while that of insulin through the intestinal epithelial monolayer was 2.31 (2.5%w/v AVgel) [13]. A higher ER at a slightly lower concentration of 2%w/v is reported for the buccal mucosa in this study. One explanation is that the buccal mucosa is more permeable than skin. Also, insulin in the previous study is a larger molecule and may not permeate to a similar extent as ddI. The ER values of other buccal enhancers were found comparable to those observed with AVgel in this study. Other chemical enhancers such as sodium glycodeoxycholate (ER = 32), menthol (ER = 2.02), and sodium glycolate (ER = 9) have been reported as effective enhancers for buccal delivery [3,21].

While permeation enhancing effects of substances are extensively reported, their effects on buccal mucosa morphology are limited [3,31]. Since buccal delivery involves retention of a delivery system on the mucosae, an assessment of histological effects of a drug and or enhancer/s to evaluate their suitability is essential.

Histomorphological effects of the control/untreated and the treated porcine buccal mucosae (PBS alone and ddI/PBS in the absence or presence of AVgel) were assessed. The morphology of pig buccal mucosa has been described previously, and it closely resembles human buccal epithelium [32,33]. In the control group, the buccal epithelium resembled that of a normal non-keratinized stratified squamous layer (**Fig. 5a**). Basal cells appeared oval and darkly stained in H&E (**Fig. 5b**) and toluidine blue (**Fig. 5c**) sections, reflecting their greater mitotic activity. The middle region showed large polygonal cells, and superficial cells showed desquamation (**Fig. 5d**). Basal cells were nucleated while some of the superficial cells were anucleate. The basal cell layer represents the germinal tissue from which new cells are produced and should form the focus of such studies. Damage to superficial layers can be rectified by renewed growth from the germinal layer, but chronic or severe damage to the basal cell layer is probably irreversible [34]. The appearance of the control, PBS, and ddI/PBS samples in H&E (**Fig. 5a–b**) and toluidine blue (**Fig. 5c–e**), respectively, were similar suggesting no influence of PBS or ddI (either alone or in combination) on tissue morphology. Therefore, ddI at the highest concentration had no adverse effects on the buccal mucosae.

The buccal mucosa upon treatment with ddI/PBS in combination with AVgel was examined. The addition of 0.5%w/v AVgel led to an increase in intercellular spaces and darker staining of the cytoplasm, resembling the structure of control samples (**Fig. 5f**).

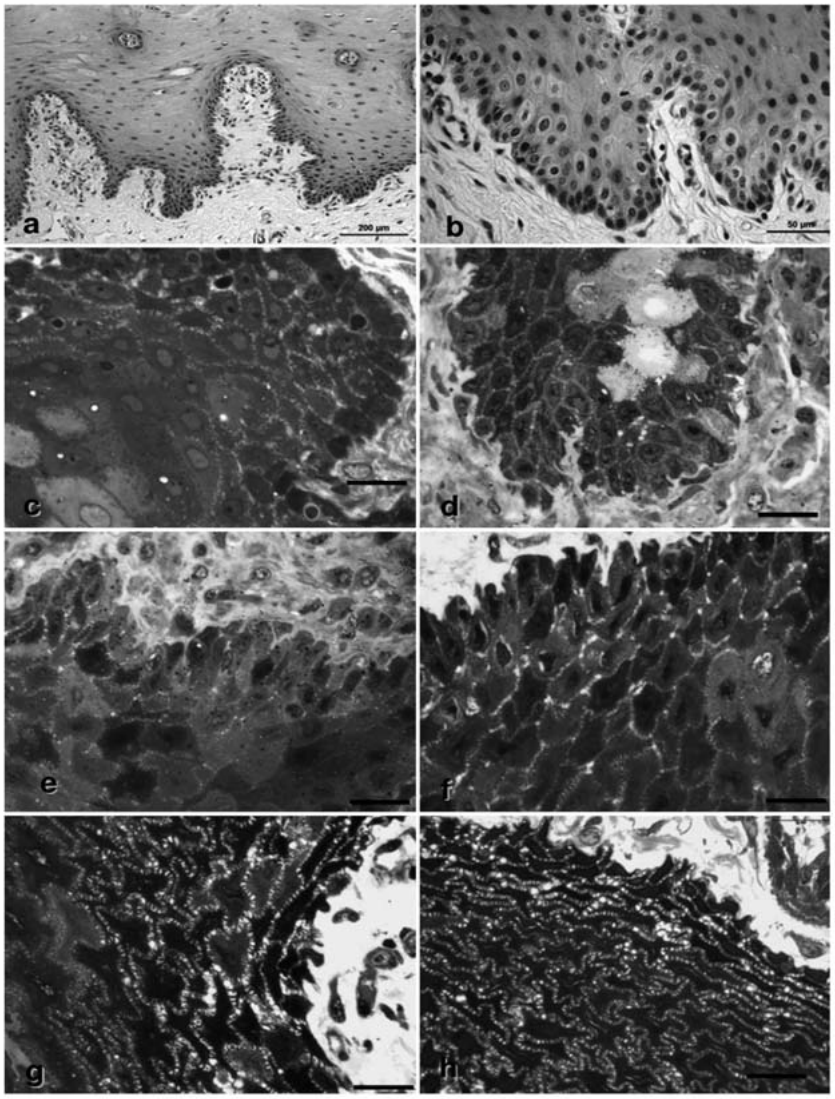

Fig. 5 Microphotographs of the control and treated buccal mucosal sections for light microscopy (LM) stained with H&E: **a** control/untreated, **b** ddI/PBS; and with toluidine blue: **c** control/untreated, **d** PBS, **e** ddI/PBS, **f** ddI/PBS/AVgel 0.5%w/v, **g** ddI/PBS/AVgel 1.0%w/v, **h** ddI/PBS/AVgel 6.0%w/v.

However, an increased AVgel concentration to 1%w/v showed a marked increase in intercellular spaces and distortion of cellular outlines (**Fig. 5g**). Cells appeared irregular and crenulated compared to controls. This was accentuated in 6%w/v AVgel samples where extreme compaction of cells in the basal region was observed (**Fig. 5h**). Although not shown, cells from the middle and superficial layers also appeared severely damaged. Furthermore, the epithelial surface and basal lamina of the mucosa in the H&E sections of the control, PBS alone, ddI/PBS, and ddI/PBS/AVgel 0.5%w/v still appeared intact after six hours, but extensive disordering of this cell layer was observed in toluidine blue sections of the ddI/PBS/AVgel 6%w/v. This disorder increased towards the epithelial surface and may be due to the higher concentration effect of AVgel on the buccal mucosa.

The ultrastructure of buccal mucosae was evaluated. The control buccal mucosae showed short profiles of endoplasmic reticulum, an abundance of ribosomes, and regular nuclei with evenly-dispersed chromatin (**Fig. 6a**). Mitochondria appeared dense with well-developed cristae suggesting normal cellular activity (**Fig. 6b**). Intercellular spaces were small, and clearly defined desmosomes between attachment plaques in neighboring cells were observed (**Fig. 6c**). PBS and ddI/PBS treated mucosae showed a similar ultrastructure to the saline control, confirming trends observed at light microscope level. Cells from 0.5%w/v AVgel samples also showed signs of active cellular metabolism and regular nuclear outlines (**Fig. 6d**). While electron translucent clearings within the mitochondria were occasionally observed, cellular damage was not evident (**Fig. 6e**). However, increasing AVgel concentration to 1%w/v led to cellular damage evident by irregular nuclear outlines, peripheral distribution of visibly-compacted chromatin, electron-lucent mitochondria containing little internal detail, and distended endoplasmic reticulum profiles (**Fig. 6f**). Increased intercellular spacing and crenulation of the plasmalemma also became evident (**Fig. 6g**). Further increases in AVgel concentrations to 2 and 6%w/v led to disruption of basal cell layers, severe cellular compaction, and larger intercellular spaces (**Fig. 6h** and **6i**).

Histomorphological evaluations showed that AVgel caused adverse effects on the mucosa at higher concentrations of 1, 2, and 6%w/v. Since the buccal mucosa was not adversely affected at lower concentration of 0.5%w/v, AVgel may therefore be considered as a safe permeation enhancer up to this concentration. At 0.5%w/v, AVgel showed an ER of 5.09 which is still higher than several other reported enhancers [3,23,27].

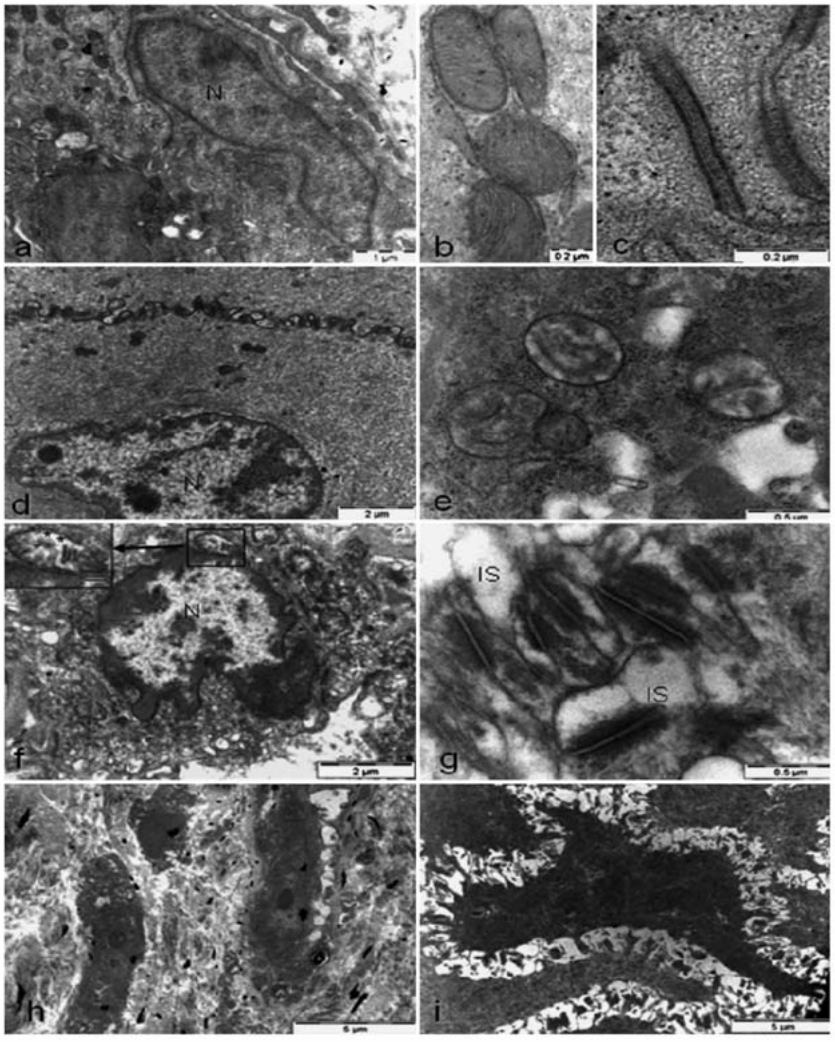

Fig. 6 Microphotographs of the control and treated ultra-thin buccal mucosa sections for transmission electron microscopy (TEM): **a** control/untreated, **b** PBS, **c** ddI/PBS, **d–e** ddI/PBS/AVgel 0.5%w/v, **f–g** ddI/PBS/AVgel 1.0%w/v, **h** ddI/PBS/AVgel 2.0%w/v, **i** ddI/PBS/AVgel 6.0%w/v.

The study has shown that ddI can permeate the buccal mucosa without adversely affecting its morphology. AVgel at concentrations up to 2%w/v was identified as an effective buccal permeation enhancer for ddI. Based on the findings it is proposed that AVgel be used in concentrations at or lower than 0.5%w/v due to adverse mucosal effects at higher concentrations. Histomorphological evaluations therefore proved useful in correlating the permeation enhancing properties of AVgel with its effects on the buccal mucosa. The results confirm the potential of developing a buccal drug delivery system containing ddI and AVgel as an enhancer for improving drug therapy.

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Conflict of Interest

The authors declare that there are no conflicts of interest for this study. The authors alone are responsible for the design, content, and writing of this paper.

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