



Research paper

Evaluation of galantamine transbuccal absorption by reconstituted human oral epithelium and porcine tissue as buccal mucosa models: Part I

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ABSTRACT

Over the last decade, interest in delivering drugs through buccal mucosa has increased. As a major limitation in buccal drug delivery could be the low permeability of the epithelium, the aim of this study was to evaluate the aptitude of galantamine, useful in Alzheimer's disease, to penetrate the buccal mucosa. The evaluation of the ability of galantamine to permeate through the buccal epithelium was investigated using two permeation models. Firstly, in vitro permeation experiments were carried out using reconstituted human oral non-keratinised epithelium and Transwell diffusion cells system. Results were validated by ex vivo experiments using porcine buccal mucosa as membrane and Franz type diffusion cells as permeation model. The entity of buccal permeation was expressed in terms of drug flux (J_s) and permeability coefficients (K_p). Data collected by in vitro and ex vivo experiments were in agreement and suggested that buccal mucosa does not block diffusion of galantamine. The effects of drug application on histology of tissue specimens used in every experiment were also studied: no sign of flogosis and no significant cytological or architectural changes were highlighted.

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1. Introduction

Alzheimer's disease is characterized by progressive decline in memory with impairment of at least one other cognitive function; the median survival for affected patients is approximately 8 years from the onset of symptoms [1,2]. Degradation of cholinergic neurons in the cerebral cortex and other areas of the brain – resulting in cholinergic transmission and acetylcholine levels deficit – is considered the primary cause of cognitive decline. Consequently, research strategies have focused on cholinergic transmission enhancement [3]. To date, the most successful pharmacologic strategy has been the inhibition of acetylcholinesterase, the enzyme responsible of acetylcholine catabolism in the synaptic cleft [1]. Galantamine is a cholinesterase inhibitor that differs from other medications of the same class since it has also a modulating effect on nicotinic receptors which enhance the drug effects [4,5]. It has been suggested that this dual mode of action could produce a beneficial cascade of neurotransmitters, possibly affecting the serotonergic

and gamma-aminobutyric acid systems. Efficacy of galantamine on the cognitive, functional and behavioral symptoms of dementia in Alzheimer's, has been demonstrated in several large-scale clinical trials [6]. At present, galantamine is available in the market as either tablets or oral solutions, and two daily oral administrations are required [2]. Even if oral administration is convenient for most patients suffering from mental disorder, it is extremely difficult to follow scheduled dosage. So, an alternative way of galantamine administration could be helpful for the success of the therapy.

Due to its relative permeability, the buccal mucosa offers good opportunity for local/systemic pharmacological actions. Buccal delivery specifically refers to the drug administration through the mucosal membrane lining of the inner cheek [7–10]. This mucosa is richly vascularized, accessible for the administration and removal of a dosage form; moreover, drug delivery has a high patient acceptability compared to other non-oral routes. Drug administration on buccal mucosa could be helpful in adhering to a correct dosage regimen for Alzheimer's patients.

Following application of actives on buccal tissue, therapeutic efficacy mainly depends on the ability of drugs to permeate through the tissue fast enough, providing the required plasma concentrations. To establish the aptitude of drugs to permeate buccal mucosa, tissues from various animals have been used as models for human mucosa as well as cells grown in culture [11–14]. Porcine buccal mucosa is one of the most used in ex vivo studies; on the other hand, stratified

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cultured TR146 cell layers (the so-called reconstituted human oral epithelium) are analogous to normal human buccal epithelium and could be used in *in vitro* studies [15,16].

Aim of this study was to establish the aptitude of galantamine to penetrate the buccal mucosa using porcine buccal mucosa and reconstituted human oral non-keratinized epithelium as membrane models. Since topical application of drug could damage the structure of the biological tissue, we observed also the effects of galantamine local administration on the histology of buccal tissue.

2. Materials and methods

2.1. Materials

Galantamine hydrobromide, USP grade, was purchased from Biodar (Yavne, Israel), 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT) and all components of buffer solutions were purchased 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 [17].

Natural human saliva (pH 6.8) was obtained from a healthy donor without any conditioning habits, i.e. smoking, alcohol, coffee, drinking or any other further habit able to alter its composition; it 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 were 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 behavior 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. Galantamine permeability studies

2.2.1.1. *In vitro* permeation of galantamine throughout reconstituted human oral epithelium. Galantamine permeation was investigated *in vitro* by measuring drug fluxes throughout TR146 cell layers, derived from a human neck metastasis originating from a buccal carcinoma, cultured on permeable polycarbonate inserts. Mucosal specimens were supplied by Skinethic Laboratories (Nice, France). Upon arrival, the bags containing the inserts with cultured cell layers were opened under sterile airflow. Each insert (area 0.5 cm²) containing the epithelial tissue (100 μm thick) was taken out and any remaining agarose that adhered to the insert's walls was rapidly removed by gentle blotting on sterile filter paper and placed in culture dishes filled with maintenance medium (Skinethic). The culture dishes were placed overnight in the incubator at 37 °C, 5% CO_2 and saturated humidity. Before testing, the maintenance medium was changed by adding fresh medium. The permeability of galantamine was performed using filter-grown TR146 cell aged 12 days old. The experiments were maintained 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.

Accurately measured amounts of drug solution (galantamine 1.5 mg in 0.5 ml of simulated saliva) were applied to the apical side of the cell layers. To avoid cell stress, PBS (pH 7.4, 25 ml) was used as acceptor fluid. The acceptor solution was stirred by means of a magnetic stirrer to avoid formation of stagnant boundary layers next to the membrane surface. An insert in which simulated saliva was applied to the apical side of the cell layer was used as control. At regular intervals, samples (0.5 ml) 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 fluid. The galantamine transferred from the donor to the acceptor compartment was monitored spectrophotometrically by measuring the drug that reached the acceptor fluid. The integrity of the TR146 cell tissue was monitored after each permeability study (see Sections 2.2.4 and 2.2.5). Results are reported as means $\pm$ SD of six different experiments in which inserts of a single time production batch were used ($P < 0.05$).

2.2.1.2. *Ex vivo* permeation of galantamine throughout porcine buccal mucosa. The permeation kinetic throughout the porcine buccal mucosa was evaluated using Franz type diffusion cells. Mucosal specimens (kindly supplying by Pig Farm, Pioppo, Palermo) were obtained from tissue removed from two freshly slaughtered domestic pigs. After sampling, all specimens were immediately placed in a refrigerated transport box and transferred to the laboratory within 1 h. Excesses of connective and adipose tissue were trimmed away until 0.8 ± 0.1 mm thick slides were obtained. Some specimens were used fresh; the remaining specimens were stored at -40 °C for periods up to six months. The frozen specimens were equilibrated in PBS, (pH 7.4) for 1 h at room temperature to thaw completely before starting experiments. To avoid damage of the epithelial surface, the mucosal samples were carefully cut to obtain suitable disks. These sections of mucosa were then mounted in the flow-through cell. Tissue disks were equilibrated for 1 h at 37 °C [Polimix EH 2 bath equipped with a constant-rate adjustable stirrer RECO® S5 (Kinematica, Switzerland)] adding PBS in both the donor and the acceptor compartment. This step was followed by the removal of PBS from the compartments.

In the donor compartment was then placed drug solution (3 mg of galantamine in 1.0 ml of simulated saliva or human saliva), in the acceptor compartment was placed PBS (26 ml). The acceptor solution was stirred by means of a magnetic stirrer. At regular time intervals, samples (0.5 ml) were withdrawn from the acceptor compartment. To avoid saturation phenomena and maintain the “sink” conditions, the sample volume taken out was replaced by fresh fluid. Each experiment was carried out for six hours. The integrity of the mucosal tissue was monitored after each permeability study (see Section 2.2.5). Results are reported as means $\pm$ SD of six different experiments in which inserts of a single time production batch were used ($P < 0.05$).

2.2.2. Drug assay

The cumulative amount permeated through reconstituted human oral epithelium and porcine buccal mucosa was calculated from the galantamine concentration in the acceptor medium and plotted as a function of time. Each experiment was performed six times using six different culture cell inserts of one single time production batch and six different samples of porcine buccal mucosa. Each data point on the plot represents the mean of the recorded values ($P < 0.05$).

In all experiments, the drug transferred from the donor to the acceptor compartment was monitored spectrophotometrically (UV/vis Shimadzu mod. 1700 Pharmaspec instrument) by measuring the amount that reached the acceptor fluid using the appropriate calibration curve and blank ($\lambda_{\text{max}} = 288.2$ nm, $E_{1\%} = 0.084$ in

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