Department of Pharmaceutical Technology, Medical University of Gdańsk, Poland

Penetration of verapamil hydrochloride in the presence of sodium glycocholate as penetration enhancer through mucous membrane

W. Sawicki

A model buccal drug formulation (BDF) with verapamil hydrochloride (VH) is in the form of a thin elastic disk made of two layers. The aim of this paper was to determine the influence of sodium glycocholate (SG) as a penetration enhancer in a BDF on the *in vitro* penetration rate of VH through pig oesophageal mucosa. It was found that VH penetrates mucosa. In 6 h 10.35 mg/cm^2 of drug diffused from saturated solution in a buffer of pH 5.3 white from diffused only 7.78 mg/cm^2 and 4.68 mg/cm^2 of VH, respectively saturated buffers of pH 6.8 and 7.3. In 6 h approximately 1.3 mg/cm^2 of VH penetrate the mucosa from the control BDF model which does not contain the penetration enhancer. Conversely the content of SG in the model BDF contributes to the increase in drug penetration rate.

1. Introduction

The mucous membrane of the oral cavity may be a favourable site of application for drugs showing a systemic therapeutic effect [1]. There are two fundamental methods to improve buccal drug forms. First, by increasing the drug penetration rate through the mucosa by including an appropriate penetration enhancer in the formulation. Second, by decreasing enzymatic degradation of the drug, for example by including enzyme inhibitors in the formulation [2].

Verapamil hydrochloride (VH) in conventional or slow release oral formulations undergoes extensive first – pass metabolism which results in low bioavailability (usually between 10 and 20%). Achieving a therapeutic effect comparable with intravenous administration of 5 mg of drug requires large oral doses (80–240 mg). Hence VH is a suitable drug for experiments on penetration through buccal mucosa.

The aim of this paper was to determine the influence of sodium glycocholate (SG) as a penetration enhancer in a buccal drug formulation (BDF) model on the penetration rate pf VH through pig oesophageal mucosa. In this study *in vitro* penetration of VH from saturated solutions in phosphate buffers of different pH through pig oesophageal mucosa was evaluated. According to the literature it is evident that this drug, in form of a hydrogel, penetrates hamster cheek pouch mucosa in both ionized and un-ionized form, whereas it penetrates skin mostly in unionized form [3].

Buccal tablets release drug from their whole surface both to the mucosa and, which is undesirable, to the oral cavity. The BDF model developed appears to be a more elegant form. This model of BDF comprises a thin elastic disk made of two layers [4]. A dosing layer is formed by the matrix containing VH which releases the drug only to the mucosa and an outer protective layer prevents possible verapamil release to the oral cavity.

2. Investigations, results and discussion

In the first part of the paper we attempted to establish whether VH, in the ionized or unionized form, can penetrate pig tracheal mucosa. In experiments on hamsters, it has been found that this drug does not the penetrate skin from hydrogel in ionized form [3]. It is possible only for the unionized form.

The use of pig tracheal mucosa in the experiments was because of its very marked similarity in thickness and anatomo-histological structure to human buccal mucosa [5]. The fact that neither is keratinized is also essential. Another advantage of pig mucosa is that its substantial area can be relatively simply prepared in laboratory conditions. All these advantages led to the use of this biological material in experiments concerning penetration of drugs *in vitro*. It was also noticed that beagle dogs have very often been used in *in vivo* experiments concerning drug penetration through oral mucosa [6].

After preparing saturated VH solutions in three phosphate buffers at 37 ± 1 °C, the drug solubility determined in phosphate buffer of pH 5.3, 0.210 g/cm^3, was four times higher than in buffers of pH 6.8 and 7.3, 0.054 g/cm^3 and 0.040 g/cm^3, respectively. Both in this study and in earlier experiments [7], no chemical decomposition of VH in aqueous solutions at 37 °C was found. The decrease of VH solubility with increasing pH is confirmed by experimental results by other authors. For example for buffers of pH 5.0, 6.0, and 7.0 at room temperature, Tenjarla et al. reported VH solubility values of 0.156 g/cm^3, 0.025 g/cm^3 and 0.010 g/cm^3, respectively [8].

All the experiments on *in vitro* penetration of VH through mucosa were carried out in special diffusion cells made of plexiglas and designed in the Department of Pharmaceutical Technology, Medical University of Gdańsk. The cells are characterized by a relatively large diffusion area of 3.14 cm^2, almost twice as large as in the experiments of Liu et al. [3]. The tightness of sealing the chambers allowed for shaking the donor and acceptor solutions in water bath. It was not necessary to install magnetic stirrers inside the diffusion cell chambers.

The mean amounts of VH which penetrated mucosa from saturated solutions in phosphate buffers of pH 5.3, 6.8, and 7.3 to an acceptor solution (phosphate buffer pH 6.8) are demonstrated in Fig. 1. It was found that VH penetrates mucosa. In 6 h 10.35 mg/cm^2 of drug diffused from the saturated solution in pH 7.3 buffer. However, only 7.78 mg/cm^2 and 4.68 mg/cm^2 of VH diffused from saturated buffers of pH 6.8 and 5.3, respectively. Assuming that in a saturated solution of VH in phosphate buffer at pH 5.3 the dissociation constant, pKa of the drug is 8.9, it can be inferred that the drug is almost totally ionized [9]. It may be concluded that VH can penetrate pig tracheal mucosa in ionized form. It cannot be stated that penetration of VH, not only from saturated solutions in phosphate buffer but most importantly from BDF, occurs only in the ionized form. VH can also penetrate in the union-

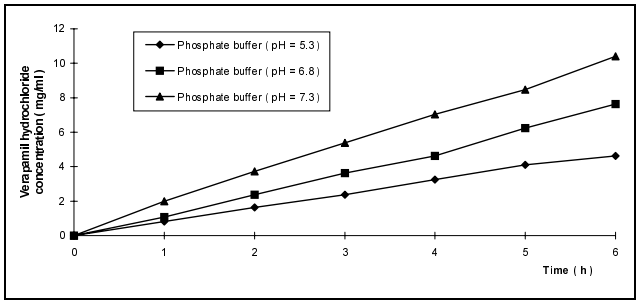

Fig. 1: *In vitro* penetration of verapamil hydrochloride from saturated solutions in phosphate buffers through the porcine oesophageal mucosa

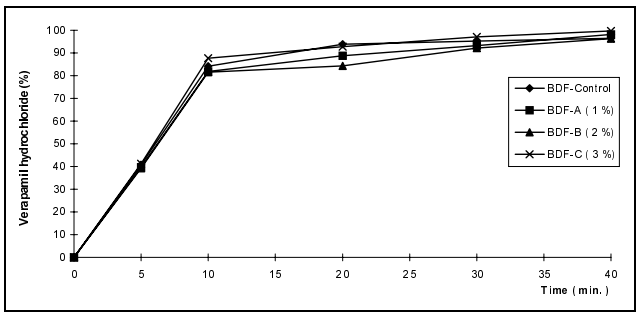

Fig. 2: Influence of the amount of sodium glycocholate on verapamil hydrochloride release from buccal drug formulations A, B, C

ized form. Drug ionization state may depend on pH at the surface of the mucosa which is not necessarily the same as the pH within the BDF.

At the next stage of biopharmaceutical evaluation of VH the release rate of drug from the model BDF and the magnitude of VH penetration from the model BDF through pig oesophageal mucosa was determined in relation to the amount of penetration enhancer. It was found that a content of SG from 1–3% in formulae A, B and C of the BDF model (Tables 1, 2) does not substantially change the release rate. All the formulations release approximately 90% of the drug in 40 min (Fig. 2).

Table 1: Composition of hydrogel (%) used for the verapamil hydrochloride matrix dosing layer in the model buccal drug formulation

Substance	Formulations			
	A	B	C	Control
Verapamil hydrochloride	2.00	2.00	2.00	2.00
PVP K-30	6.87	6.87	6.87	6.87
Glycerol	1.72	1.72	1.72	1.72
Sodium glycocholate	0.11	0.22	0.33	–
Ethanol 95 °C	13.75	13.75	13.75	13.75
Purified water	75.55	75.44	75.33	75.66

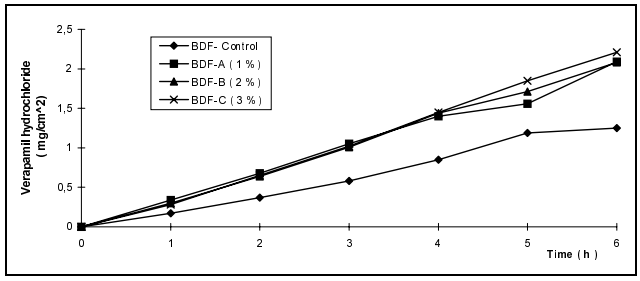

Fig. 3: Influence of the amount of sodium glycocholate on verapamil hydrochloride penetration from buccal drug formulations A, B, C through the mucous membrane of the pig oesophagus

In the study of VH penetration, phosphate buffer of pH 6.8 was used as the acceptor solution. Some authors recommend the use of Krebs buffer saturated with a mixture of 95% O_2 and 5% CO_2 in this type of research in order to supply the mucosa with oxygen [10].

Experiments for a given model BDF formulation, with different enhancer concentrations, and the corresponding control models, were in each case conducted on oesophageal mucosa of the same animal. Such a procedure allowed for the elimination of possible differences in membrane thickness in particular animals and in preparation technique. All the BDF model formulations prepared adhered easily to mucosa and did not come off during the experiment. It was found that in 6 h approximately 1.3 mg/cm^2 of VH penetrate mucosa from the control BDF model which does not contain the penetration enhancer. On the other hand the content of SG in the BDF model contributes to an increase in the drug penetration rate (Fig. 3). The largest value was obtained for a BDF model containing 3% of penetration enhancer. For this model approximately 2.21 mg/cm^2 of drug penetrated in 6 h. It is noteworthy that increasing the concentration of penetration enhancer from 1% to 3% does not significantly affect the penetration rate. In setting the level of penetration enhancer in the BDF, the experience of other researchers was used [11, 12]. Mucosal irritation which could be a result of too high a concentration of SG in the BDF was not found. In the course of further study it would be interesting to establish the minimum SG concentration in a BDF which would ensure increase penetration of VH.

The BDF model evaluated in the form of a polymer disc shows some advantages compared with a buccal tablet form. Being flexible and not very thick, it can be less

Table 2: Characteristics of the model buccal drug formulation (BDF)

Formulation	Layer of the model BDF	Mean thickness (μm)	Diameter (mm)	Verapamil hydrochloride (%)	PVP K-30 (%)	Glycerol (%)	Sodium glycocholate (%)
A	matrix dosing	395.0	30.0	19.4	63.5	16.1	1.0
	protective	650.0	40.0	–	83.5	17.0	–
B	matrix dosing	400.0	30.0	19.4	62.5	16.1	2.0
	protective	650.0	40.0	–	83.0	17.0	–
C	matrix dosing	410.0	30.0	19.4	61.5	16.1	3.0
	protective	650.0	40.0	–	83.0	17.0	–
Control	matrix dosing	410.0	30.0	19.4	64.5	16.1	–
	protective	650.0	40.0	–	83.0	17.0	–

perceptible than a tablet in the oral cavity. There is also less likelihood of its detachment from the mucosa during meals. However, the main advantage of the buccal formulation model is that the matrix allows dosing of the drug exlusively to the mucosa and the protective layer prevents possible passage of the drug to the stomach with saliva. Polymer PVP K-30 which was used for the BDF model appeared to be suitable despite having only average mucoadhesive properties as demonstrated by Ponchel et al. [13].

Such sorption enhancers as the bile acid derivatives SG, sodium deoxycholate or sodium taurodihydrofusidan, have most often been used to increase absorption of proteins and peptides [11]. Wolany et al. proved the possibility of increasing octreotide penetration from Sandostatin® with sodium glycocholate enhancers and with the addition of disodium versenate through dog oral cavity mucosa [12]. Also de Vries confirmed the increase of octreotide peptide penetration through pig tracheal mucosa from the "Haftring" dosage system in the presence of SG [14]. This author also evaluated the depth of peptide penetration in mucosa by dividing it into layers by means of a microtome. Ishida et al. also used SG as enhancer in a buccal tablet with insulin. In this tablet the adhesion layer was formed by a mixture of hydroxypropylocellulose and Carbopol [15]. The bioavailability of insulin from tablets determined in beagle dogs was only 0,5% with respect to intravenous injection.

The probable mechanism of action of bile acid derivatives or compounds complexing calcium ions depends on the destruction of desmosome integrity and basic membranes of mucosa. It may also be similar to that of sodium doxyglycocholate described by Hoogstraate et al. [16]. According to this author the mechanism of action probably involves the following steps: doxyglycocholate diffuses into the buccal epithelium via the intercellular route, where doxyglycocholate preferentially solubilizes polar intercellular lipids by forming mixed micelles and thereby changing the organization of the intercellular domains such that the intercellular transport of hydrophilic compounds could be increased.

3. Experimental

3.1. Materials

Verapamil hydrochloride (VH), (Orion, Finland), polyvinylpyrrolidone (PVP) K-30 – Kollidon K-30 (BASF, United States), glycerol (P.O.Ch. Gliwice, Poland), sodium glycocholate (SG), (SIGMA, United States), KH_2PO_4 (Fluka AG, Switzerland), Na_2HPO_4 (Fluka AG, Switzerland), $NaH_2PO_4 \times 2\,H_2O$ (Fluka AG, Switzerland), NaOH (POCh Gliwice, Poland). All other chemicals were of analytical grade.

3.2. Equipment

Ultrospec III, spectrophotometer (Pharmacia LKB Biochrom, England), Apparatus specified in the USP 23 (apparatus 1), (Pharma Test, PTWS III, Germany).

3.3. Methods

3.3.1. Determination of VH solubility in phosphate buffers of pH 5.3, 6,8, and 7,3

Phosphate buffers consisting of (pH 5.3) – 97.5 parts 9.078 g 1/15 M KH_2PO_4 in 1 l H_2O + 2.5 parts 11.188 g 1/15 M Na_2HPO_4 in 1 l H_2O, (pH 6.8) – 40 parts 25.5 g $NaH_2PO_4 \times 2\,H_2O$ in 1 l H_2O + 60 parts 18.5 g Na_2HPO_4 in 1 lH_2O, (pH 7.3) – 50 parts 6.8 g KH_2PO_4 in 1 l H_2O + 50 parts 1.52 g NaOH in 1 l H_2O, were prepared.

VH solubility in the buffers was characterrised by shaking an excess of the drug with the relevant solvent at a strictly controlled temperature. To achieve this 200 ml conical flasks with ground in stoppers, containing 30 g of VH and 150 ml of the buffer, were placed in a shaker – assisted water bath at 37 ± 1 °C and shaken for 24 h. 5 ml of saturated solutions were collected with a pipette with filtration using a G-4 filter in 37 °C. The amount of VH in the solution was determined spectrophotometrically at 278 nm.

3.3.2. Preparing the buccal drug formulation (BDF) model with VH

On the basis of previously conducted experiments and literature data [11, 17] the mucoadhesive polymer PVP K-30 was selected. SG and glycerol played the roles of penetration enhancer and plasticizer, respectively. The PVP K-30 in the form of a aqueous solution together with VH, SG and glycerol, were mde into an homogenous gel. The composition of the hydrogel used to prepare the matrix layer containing VH is given in Table 1. The polymer was gradually added to the specified amount of water in a beaker and mixed with a magnetic stirrer (approximately 150 rpm). The resultant hydrogel served as a solvent for the drug, penetration enhancer, and the plasticizer, which were all successively added to the beaker. The stirring was continued for 15 min. 30.0 g portions of the hydrogels were poured out on single glass Petri dishes of 7 cm diameter, and dried for 48 h at room temperature; they formed plastic films, out of which circular matrices could be cut. The Petri dishes had previously been coated with silicone by immersion in a 1% silicone emulsion for 5 min and drying in the oven for 20 min at 300 °C. The outside protective layers of the model BDF were formed similarly as 4 cm discs. 55.0 g portions of the hydrogels containing no VH were poured out on Petri plates. Both the dosing layer and the protective layer were then wetted with a small amount of water and joined together by mechanical pressure. The characteristics of the resulting model drug formulations are given in Table 2. Each buccal drug formulation model contained 60 mg VH on average. A control model containing no enhancer was also prepared.

3.3.3. In vitro release tests

The measurement of the release rate of VH from the matrix dosing layer in the model BDF was carried out by a dynamic method. It was similar to the dissolution method of USP 23 apparatus No. 1. The rotating basket was replaced with a disc to which the drug – loaded matrix was attached.

The release test was carried out in 50 ml of phosphate buffer of pH 6.8 at 37 ± 1 °C. The disc rotated at 60 rpm and the buffer was changed every 10 min. The amount of VH in the solution was determined spectrophotometrically at 278 nm. The results obtained are presented in Fig. 2.

3.3.4. Determination of in vitro penetration of VH from saturated solutions in phosphate buffers of pH 5.3; 6.8; and 7.3 and from the model BDF through porcine oesophageal mucosa

Porcine oesophagi were excised immediately after the slaughter of the animals (70 to 80 kg in body mass) and transported in containers at 4 °C. After rinsing with pH 6.8 phosphate buffer, a 10 cm longitudinal cut was made in each oesophagus with a scalpel. The mucosa was subsequently dissected and appeared to be 800 to 850 μm thick on average. The mucosa obtained was divided into pieces of approximately 12 cm^2 and stored in glass containers in a phosphate buffer of pH 6.8 at 4 °C.

The experimental measurements of the rate of VH penetration through mucosa from saturated solutions in phosphate buffers and from BDF models containing 1%, 2% and 3% of SG were conducted in special diffusion cells [4]. 8 ml of saturated solution of VH in phosphate buffer of appropriate pH were introduced into the diffusion cell chamber. Phosphate buffer with appropriate pH was used as acceptor solution, in the other chamber, by sealed and separated with pig oesophageal mucosa. The cells were placed in a shaker – assisted water bath. Every hour, the entire volume of the buffer was replaced with fresh buffer. Three 1 ml samples were collected from the buffer and the VH content spectrophotometrically determined. In order to get rid of proteins and other compounds which could interfere with VH, samples were gravity filtered using a membrane filter of 0.2 μm pore diameter.

The mean amounts of VH determined in the acceptor solution are presented in Fig. 1. The experiments on penetration of VH through the mucosa from buccal drug formulations A, B, C into the model BDF were carried out in the diffusion cell described above. In these experiments a model BDF with diameters of dosing matrix and protective layer 30 and 40 mm respectively was additionally placed on the surface of the mucosa. The amounts of VH determined in the acceptor solution for particular BDF formulations and for the control model not containing the penetration enhancer are presented in Fig. 3.

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Dr. W. Sawicki
Department of Pharmaceutical Technology
Medical University of Gdańsk
Gen. J. Hallera av. 107
80-416 Gdańsk
Poland
wsawicki@farmacja.amg.gda.pl