ELSEVIER

International Journal of Pharmaceutics 238 (2002) 181–189

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Pharmacokinetics of verapamil and its metabolite norverapamil from a buccal drug formulation

Wiesław Sawicki *, Stanisław Janicki

Department of Pharmaceutical Technology, Medical University of Gdańsk, ul. Hallera 107, 80-416 Gdańsk, Poland

Received 26 January 2001; received in revised form 29 January 2002; accepted 13 February 2002

Abstract

Pharmacokinetics of verapamil and its metabolite norverapamil, from buccal drug formulation administered in a dose 20 mg in relation to conventional tablets of verapamil 40 mg, used in medical practice, was determined. Buccal formulation has previously been designed as an alternative form of dosing verapamil. Bioavailability was determined by a crossover method in 12 healthy volunteers. Drug concentration in plasma was determined by means of HPLC with a fluorescence detector. For buccal formulation the average values of C_{max} and $AUC_{0-24\ h}$ for verapamil were much higher than for the reference Staveran tablets and amounted to 51.28 and 320.23 ng/ml h, respectively. However, for norverapamil the corresponding values for buccal formulation were much lower than for a conventional tablet. It has been demonstrated that the proposed buccal verapamil dosing ensures different metabolism of the drug as compared to tablets. Better parameters of bioavailability of verapamil from buccal formulation of twice a smaller dose than that in the tablet, prove that this new drug might be form more effective clinically than the conventional one.

Keywords: Verapamil; Norverapamil; Buccal; Bioavailability; Pharmacokinetics

1. Introduction

Verapamil belongs to a drug group of calcium channel antagonists. It is currently employed in the treatment of hypertension, arrhythmia and angina pectoris (Kirsten et al., 1998). Verapamil has at least 12 known metabolites in humans, and one metabolite, norverapamil, is particularly interesting because it is only produced in significant amounts after oral administration of verapamil (Gupta et al., 1996a). Norverapamil has been shown to have additive effects to verapamil, and is one tenth as active as verapamil in altering atrioventricular nodal conduction (Johnson et al., 1991).

The therapeutic range of verapamil in serum varies from 20 to 500 ng/ml depending on the drug form used (Buzinkaiova et al., 1995). To reach such a concentration the oral dose of verapamil should be twelve times higher than the intravenous one (Liu et al., 1992).

* Corresponding author. Tel.: +48-58-349-3184; fax: +48-58-349-3190.

E-mail address: wsawicki@farmacja.amg.gda.pl (W. Sawicki).

0378-5173/02/$ - see front matter
PII: S0378-5173(02)00069-8

Verapamil is available in the form of conventional tablets in doses of 40, 80, 120, 160 mg and as slow release tablets in doses of 120, 180, and 240 mg. The oral absorption of the drug from these forms is 90% but its bioavailability approaches only 10–20%, due to a large first-pass effect mainly in the liver (Jankowski et al., 1991; Elkheshen, 1998).

The buccal mucosa may be a more favourable site of absorption of verapamil than the digestive tract (Nagai and Machida, 1993; Junginger, 1991). Non-keratinized and strongly supplied with blood buccal mucosa, with a dense capillary vessel network, it constitutes a relatively large drug absorption area. Drug can thus reach the systemic circulation directly through capillary vessels, bypassing the first-pass metabolism in the intestines and liver or avoiding inactivation in the stomach (Harris and Robinson, 1992). That in turn contributes to higher bioavailability parameters after administration of a smaller dose of the drug than in conventional tablets.

The aim of this paper is to determine pharmacokinetics of verapamil and its metabolite norverapamil from administered as buccal drug formulation in a dose of 20 mg. The new drug forms were designed and prepared at the Department of Pharmaceutical Technology, Medical University of Gdansk (Sawicki and Janicki, 1995), to provide a more effective way of releasing the drug as compared to conventional oral tablets which are commonly used in medical practice.

2. Methods

2.1. Drug formulations to be examined

The employed buccal drug formulations have a form of a thin elastic disc made of two layers (Sawicki and Janicki, 1995). A dosing layer is formed by the matrix containing verapamil and is designed to release the drug only to the mucosa. An outer protective layer of a greater diameter and thickness is to prevent verapamil release to the oral cavity.

Composition of the dosing layer is: verapamil hydrochloride (Sigma, St. Louis, USA) 0.02 g; Povidone K-30 (Fluka Chemie, Buchs, Germany) 0.076 g; glycerol (P.O.Ch., Gliwice, Poland) 0.0173 g; polyoxyethylene alkyl ethers (Brij 96) (Aldrich, Milwaukee, USA) 0.0199. Diameter of the form is 10 mm; thickness is 0.38 mm. Composition of the protective layer is: Povidone K-30 0.152 g; glycerol 0.035 g. Diameter is 12.5 mm, thickness 0.5 mm.

Conventional tablets Staveran 40 mg (Polpharma S.A., Starogard Gdański, Poland) were used as the reference drug.

2.2. In vitro drug release testing

The measurements of verapamil release rate from the buccal drug formulation and Staveran tablets were performed using the Ph Eur apparatus Pharma Test Model PTWS-3, (Pharma Test, Hainburg, Germany). The concentration of verapamil in samples was determined spectrophotometrically at 278 nm (Sawicki and Janicki, 1995).

The measurement of the verapamil release rate from the matrix of dosing layer in buccal drug formulation was carried out by a dynamic method similar to the dissolution method employing the basket apparatus. The rotating basket was replaced with a disc to which the drug-loaded matrix was attached. The release study was carried out in 50 ml of phosphate buffer of pH 6.8 (37 ± 0.5 °C). The disc rotated at 60 rpm and the buffer was changed every 10 min.

Paddle apparatus according to Ph Eur was used in testing the release of verapamil from Staveran tablets. Beakers were filled with 250 ml of the solution of hydrochloric acid (0.1 mol/l) in the temperature of 37 ± 0.5 °C

The results obtained are presented in Fig. 1.

2.3. In vivo study

The bioavailability studies were carried out in a group of 12 Caucasian volunteers, six men and six women, aged 20–38 (26.2 ± 5.82), weighing from 50 to 94 kg (70.67 ± 13.57 kg); height ranging from 155 to 185 cm (171.75 ± 0.16 cm). The study had the approval of the Ethics Committee of the Medical University of Gdańsk. The volunteers were admitted to the study after detailed medical

and laboratory examinations (morphology, ionogram, ALAT, AspAT, creatinine, general examination of urine). The main inclusion criteria were the absence of heart, liver, renal and digestive tract diseases. All the women were in the first half of their menstrual cycle and had negative results from a pregnancy test (Clearview test, Unipath, Bedford, UK). None of the volunteers took other drugs during the previous 4 weeks neither took part in other clinical examinations during 3 months preceding the study. The volunteers received written information about the aim of the study and informed consent was obtained from each of them. They started the study after an overnight fast. The crossover test was conducted 7 days after administering a single dose of the buccal drug formulation (20 mg) and Staveran (40 mg). The study began on 5th December 1998. During all the stages of the test the volunteers stayed for 24 h in the Clinical Pharmacology ward of the Child Health Center Monument Institute in Warsaw. They were examined by a cardiologist. They received their first meal (standardized breakfast) 2 h after the experiment. Blood was taken for analysis from the elbow vein before drug administration and then 0.25, 0.5,1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after administration of the drugs tested. Plasma was separated at approximately 0.5 h after collection and stored at -20 °C until analysis. Plasma levels of verapamil and norverapamil were determined employing the modified high-performance liquid chromatographic method with fluorescence detection (Sawicki, 2001). Absolute extraction yield for verapamil and norverapamil was 92.12 and 89.58%, respectively at 3.03 and 1.62% variation coefficients (CV), showing stability in the whole range of calibration curve. The internal standard, propranolol, was extracted with yield of 82.5% on average (CV = 3.72%, $n = 34$). The precision of the method was determined as the inner and interseries reproducibility. The variation coefficient for verapamil varied from 3.55 to 5.12%, and for norverapamil from 3.64 to 9.68% for the lowest added concentration of 5 ng/ml in the interseries examination. The detection limit of verapamil and norverapamil was experimentally established at 1 ng of verapamil standard and 4 ng of norverapamil standard with the addition of 50 ng of the internal standard. The determined detection limit of verapamil was 0.92 ng/ml and indication limit was 3.08 ng/ml, which corresponded to the concentration of 1.23 ng/ml of the plasma sample. For norverapamil these values were 0.3, 1 and 0.4 ng/ml of the plasma sample, respectively.

Calculation of pharmacokinetic parameters was done provided employing Topfit version 2.0 (Fisher Verlag, Stuttgart, Germany, 1993).

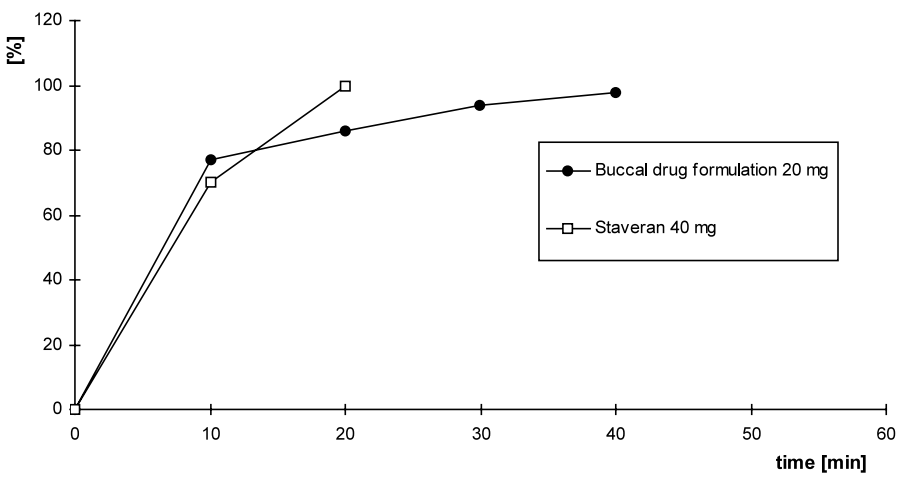

Fig. 1. In vitro verapamil release from buccal drug formulation 20 mg and Staveran tablet 40 mg ($n = 6$).

Table 1
Mean plasma concentrations of verapamil and bioavailability parameters in volunteers after administration of buccal drug formulation and Staveran tablets

Parameter Time (h)	Buccal drug formulation Mean plasma of verapamil concentrations (ng/ml)	Staveran	Analysis of variance
0.25	9.33 ± 5.30	4.44 ± 2.79	IS
0.50	27.30 ± 14.23	12.82 ± 2.61	IS
1.00	46.38 ± 16.67	31.67 ± 7.35	IS
1.50	51.28 ± 11.44	27.45 ± 8.88	IS
2.00	38.43 ± 11.40	24.29 ± 8.97	IS
3.00	32.01 ± 10.41	21.12 ± 6.01	IS
4.00	22.95 ± 6.21	17.51 ± 6.26	IS
6.00	15.56 ± 5.25	12.06 ± 6.28	NS
8.00	12.69 ± 4.39	7.76 ± 2.66	IS
12.00	8.64 ± 3.27	5.21 ± 3.15	IS
24.00	4.21 ± 1.86	2.19 ± 1.14	IS
C_{max} (ng/ml)	51.28 ± 11.44	31.67 ± 7.35	IS
t_{max} (h)	1.50	1.00	
$AUC_{0 \rightarrow 24h}$ (ng/ml h)	320.23	203.30	
$AUC_{0\ h \rightarrow \infty}$ (ng/ml h)	364.85	224.22	
EBA (%) from $AUC_{0\ h \rightarrow \infty}$	325.44	100.00	

IS, statistically significant; NS, statistically insignificant; ($P < 0.05$).

3. Results and discussion

Verapamil is a drug which undergoes a marked first pass effect. Administered as conventional drug formulations as well as the slow releasing forms it undergoes a presystemic elimination before distribution in to systemic circulation (Harder et al., 1991). In available drug forms verapamil occurs as a racemate, a mixture of two enantiomers, R-(+) and S-(−). The enantiomers have various pharmacokinetic properties and hence differ in bioavailability and pharmacological activity (Wainer and Granvil, 1993). Metabolism of verapamil is stereoselective (Robinson and Mehvar, 1996). S-(−) verapamil is a subject to approximately double first-pass effect as compared to R-(+). In spite of that S-(−) enantiomer exerts approximately seven times a stronger haemodynamic and dromotropic effect (Lalka et al., 1993).

It was noted that frequent administration of the drug to people with healthy livers results in an increase of verapamil bioavailability due to the so-called saturable first-pass effect. That is the cause of nonlinear pharmacokinetics of the drug (Gupta et al., 1996b).

The main task of this paper was to work out an alternative effective way of releasing verapamil from a properly designed buccal drug formulation, decreasing at the same time the dose in relation to conventional tablets used in medical practice. Brij 96, which was included in the buccal drug formulation, plays the role of a penetration enhancer. Earlier experiments showed that it caused the greatest increase of the in vitro penetration of verapamil from a buccal drug formulation model through pig oesophageal mucosa (Sawicki and Janicki, 1998). The data in the literature (O'Hagan, 1990) indicate that the enhancing mechanisms, of surfactants is primarily due to a combination of two mechanisms, i.e. the enhancement of membrane fluidity and the opening of epithelial junctions.

The average content of verapamil in the buccal drug formulation was $0.021 \pm 3.5\%$ whereas twice as much was in the reference drug ($0.041 \pm 6.5\%$). Results of the release rate determination of verapamil from the tested drugs (Fig. 1) were as observed for the conventional drug forms. Both the buccal drug formulation and Staveran released all the contained verapamil in 30–40 min.

Table 2
Mean plasma concentrations of norverapamil and bioavailability parameters in volunteers after administration of buccal drug formulation and Staveran tablets

Parameter Time (h)	Buccal drug formulation Mean plasma of norverapamil concentrations (ng/ml)	Staveran	Analysis of variance
0.25	1.33 ± 0.54	3.93 ± 2.54	IS
0.50	2.23 ± 0.90	11.73 ± 3.19	IS
1.00	3.05 ± 0.87	27.35 ± 5.54	IS
1.50	3.60 ± 0.95	24.53 ± 6.02	IS
2.00	3.98 ± 1.07	21.63 ± 7.55	IS
3.00	4.28 ± 1.03	21.23 ± 7.56	IS
4.00	4.87 ± 1.58	15.42 ± 4.29	IS
6.00	5.03 ± 1.94	10.01 ± 4.74	IS
8.00	4.61 ± 2.07	5.95 ± 2.43	NS
12.00	3.31 ± 1.59	3.88 ± 1.86	NS
24.00	1.36 ± 0.72	1.77 ± 1.13	NS
C_{max} (ng/ml)	5.03 ± 1.94	27.35 ± 5.54	IS
t_{max} (h)	6.00	1.00	
$AUC_{0 \to 24\ h}$ (ng/ml h)	77.59	171.43	
$AUC_{0\ h \to \infty}$ (ng/ml h)	97.33	185.68	
EBA (%) from $AUC_{0\ h \to \infty}$	104.84	100.00	

IS, statistically significant; NS, statistically insignificant; ($P < 0.05$).

Table 3
Mean pharmacokinetics parameters of verapamil in volunteers after administration of buccal drug formulation and Staveran tablets

Parameter	Buccal drug formulation	Staveran	Analysis of variance
K_a (h^{-1})	2.50 ± 1.40	2.20 ± 0.95	NS
$t_{0,5a}$ (h)	0.36 ± 0.19	0.36 ± 0.12	NS
K_{el} (h^{-1})	0.10 ± 0.02	0.12 ± 0.02	NS
$t_{0,5el}$ (h)	7.12 ± 1.21	6.17 ± 1.23	NS
Cl (ml/min)	866.56 ± 304.94	3311.39 ± 996.79	IS
V_d (l)	570.87 ± 192.17	1698.46 ± 375.77	IS

IS, statistically significant; NS, statistically insignificant; ($P < 0.05$).

Table 4
Mean pharmacokinetics parameters of norverapamil in volunteers after administration of buccal drug formulation and Staveran tablets

Parameter	Buccal drug formulation	Staveran	Analysis of variance
K_a (h^{-1})	0.77 ± 0.92	2.27 ± 1.10	IS
$t_{0,5a}$ (h)	1.55 ± 0.82	0.39 ± 0.22	IS
K_{el} (h^{-1})	0.08 ± 0.02	0.13 ± 0.03	IS
$t_{0,5el}$ (h)	9.85 ± 2.96	5.73 ± 1.14	IS
Cl (ml/min)	3778.27 ± 1093.89	3981.62 ± 1231.45	NS
V_d (l)	3185.78 ± 1309.27	1890.33 ± 425.39	IS

IS, statistically significant; NS, statistically insignificant; ($P < 0.05$).

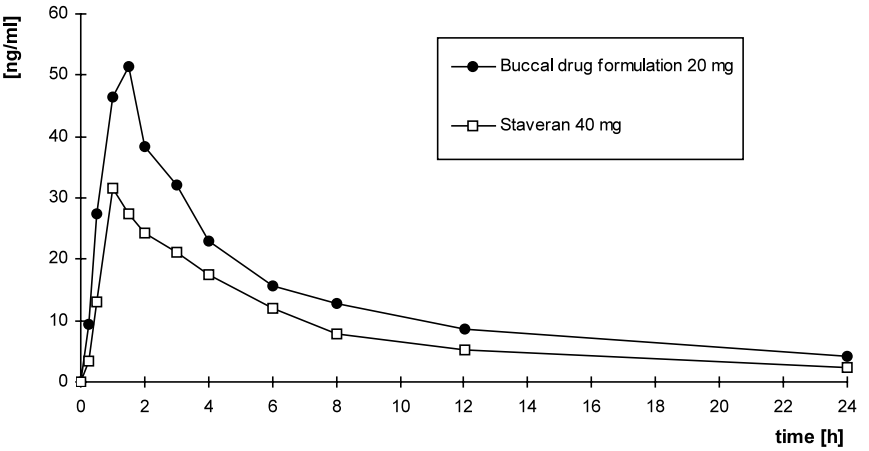

Fig. 2. Mean plasma concentrations of verapamil in 12 volunteers after administration of buccal drug formulation 20 mg and Staveran tablet 40 mg.

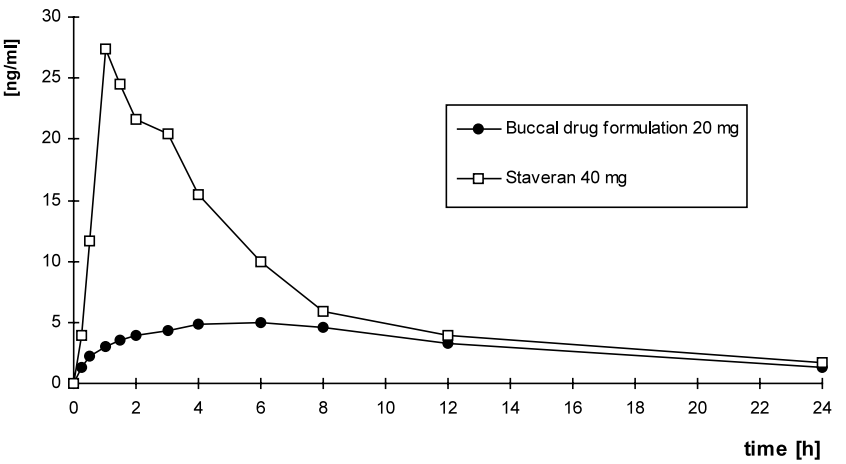

Fig. 3. Mean plasma concentrations of norverapamil in 12 volunteers after administration of buccal drug formulation 20 mg and Staveran tablet 40 mg.

Tables 1–4 shows average verapamil and norverapamil concentrations in volunteers' plasma along with the pharmacokinetic parameters. The tables present also statistical evaluation of the differences between the buccal drug formulation and Staveran tablets calculated by ANOVA. Figs. 2 and 3 illustrate the profiles of average verapamil and norverapamil concentration in plasma. A modified analytical method used for determination of verapamil and norverapamil in plasma was characterized by good working parameters confirmed by validation and quality control carried out in the course of application.

During the clinical tests, after being properly instructed, volunteers stuck a polymer buccal drug formulation disc to the upper part of the buccal pocket mucosa. The formulation, unlike Staveran, has not been washed down with 100 ml of water. The buccal drug formulation adhered easily and did not detach in the course of the experiment. Only one out of ten volunteers described the presence of buccal drug formulation in the oral cavity as perceptible and disturbing while

speaking. No volunteer felt any trace of its presence in the oral cavity about 1–1.5 h after administering buccal drug formulation, which proves its total biodegradation.

Maximum plasma concentration (C_{max}) of verapamil after administration of the buccal drug formulation and after Staveran occurred at a similar time (t_{max}) 1.5 and 1 h, respectively, and amounted to 51.28 ng/ml for buccal drug formulation and 31.67 ng/ml for Staveran (Table 1; Fig. 2). The values are definitely more favourable for buccal drug formulation, especially as that formulation contains twice a smaller dose than Staveran. A consequence of a higher C_{max} value is a considerably larger area under curve ($AUC_{0-24\ h}$), which is 320. A total of 23 ng/ml h for buccal drug formulation and 203.30 ng/m h for Staveran, respectively (Table 1). In vitro release rates, which are similar for both drugs, rationalize the similar average values of the absorption rate constant for verapamil (K_a) amounting to $2.5 \pm 1.4\ h^{-1}$ for buccal drug formulation and $2.2 \pm 0.95\ h^{-1}$ for Staveran (Fig. 1; Table 3). Due to large differences in the values of C_{max} and $AUC_{0-24\ h}$ the tested drugs differ also with reference to the clearance (Cl) and the volume of distribution (V_d). These parameters are 866.6 ± 304.9 l and 3311.4 ± 996.8 ml/min, respectively, for buccal drug formulation and 570.9 ± 192.2 l and 1698.5 ± 375.8 ml/min, respectively, for Staveran (Table 3).

One of the first studies on buccal dosing of verapamil were reported by Asthana et al. (1984). They prepared a 40 mg conventional tablet containing 20 mg of the drug. Unfortunately this form of drug did not contain any adjuvant substance of a mucoadhesive character, which made the sticking of the tablet to buccal mucosa impossible. Thus seven volunteers had to made the efforts to keep the tablet in the buccal pocket until it disintegrated completely (about 24 min). The average value of C_{max} and t_{max} was similar to buccal drug formulation. However, the value of $AUC_{0-\infty\ h}$ was more than two times smaller than for buccal drug formulation, despite of an indentical dose.

In volunteers, who took a conventional tablet, Securon 40 mg, administered sublingually in a crushed form the first-pass effect was much more limited as compared to the traditional, oral administration of the drug (John et al., 1992). In results, after sublingual administration of Securon a stronger pharmacodynamic effect was observed. A higher $K_a = 3.32\ h^{-1}$ value caused a faster appearance of the drug in the blood ($t_{max} = 0.58$ h), ensuring average values $C_{max} = 59.2$ ng/ml and $AUC_{0-8\ h} = 310$ ng/ml h. After normal administration of the tablet these parameters were as follows: $K_a = 1.93\ h^{-1}$, $t_{max} = 1.13$ h, $C_{max} = 48.58$ ng/ml and $AUC_{0-8\ h} = 234$ ng/ml h. Bioavailability parameters after sublingual administration of the drug with those for the buccal drug formulation at twice a smaller dose, are similar. The observation favours sublingual administration. It is worth stressing that according to both cited papers the volunteers who kept a conventional tablet buccally and those keeping it crushed under the tongue, felt a bitter, unpleasant taste of verapamil all the time. The buccal drug formulation made by us has important advantage in that the outer protective layer, which is a component of the disc, prevents verapamil penetration towards the lumen of the oesophagus. None of the volunteers complained of a bitter taste. Bioadhesivity of buccal drug formulation restricts the possibility of swallowing it.

The results of comparative determination of pharmacokinetic parameters of a verapamil metabolite, norverapamil, from buccal drug formulation and Staveran are also very interesting. It has been reported in the literature (Gupta et al., 1996a) that after administration of oral drug formulations of verapamil, its metabolite, norverapamil reaches similar and sometimes higher concentrations in plasma than the parent drug. The results for buccal drug formulation (Tables 2 and 4; Fig. 3), presented in this paper, do not confirm it. The average value of $C_{max} = 5.03$ ng/ml for norverapamil determined after administration of buccal verapamil formulations, was five times lower than after Staveran ($C_{max} = 27.35$ ng/ml). Also $AUC_{0-24\ h}$ was much lower for buccal formulation in respect to Staveran and amounted to 77.59 versus 171.43 ng/ml h. Comparing the data presented in Tables 1–4 and Figs. 2 and 3 one has to conclude that whereas for Staveran tablets average values of C_{max} for verapamil and norverapamil are similar and reach 31.67 and

27.35 ng/ml, respectively, for buccal drug formulation the concentration of verapamil is ten times higher than that of norverapamil. The absorption of norverapamil from buccal drug formulation ($K_a = 0.77\ h^{-1}$) is much slower than absorption of verapamil. Similarly, the values of clearance (Cl = 3778.27 ml/min) and distribution volume (V_d = 3185,78 l) of norverapamil are several times higher than corresponding parameters of verapamil.

The above data indicate that verapamil released from a buccal drug formulation to buccal mucosa is quickly absorbed into the blood stream and undergoes metabolism in the liver to only a small extent.

More favourable bioavailability parameters of verapamil from a buccal formulation compared to standard Staveran tablets containing twice the dose of drug is clear evidence that the buccal delivery from the system designed in our laboratory is promising.

Acknowledgements

The authors wish to express their deep gratitude to Dr M. Migdal the coordinator of the Clinical Pharmacology Unit of the Child Health Center Monument Institute, Warsaw, Poland for organizing and supervising the clinical phase of the experiments.

References

Asthana, O.P., Woodcock, B.G., Wenchel, M., Fromming, K.H., Schwabe, L., Rietbrock, N., 1984. Verapamil disposition and effect on PQ-intervals after buccal, oral and intarvenous administration. Arzneim.-Forsch./Drug Res. 34, 498–502.

Buzinkaiova, T., Skacani, I., Netriova, J., 1995. Quantitative assay of verapamil in drugs and serum by capillary isotachophoresis. Pharmazie 50, 799–800.

Elkheshen, S.A., 1998. Bioadhesive matrix as controlled release dosage form for verapamil hydrochloride. Pharm. Ind. 60, 555–559.

Gupta, S.K., Atkinson, L., Theeuwes, F., Wong, P., Gilbert, P.J., Longstreth, J., 1996a. Pharmacokinetics of verapamil from an osmotic system with delayed onset. Eur. J. Pharm. Biopharm. 42, 74–81.

Gupta, S.K., Hwang, S., Atkinson, L., Longstreth, J., 1996b. Simultaneous first-order and capacity-limited elimination kinetics after oral administration of verapamil. J. Clin. Pharmacol. 36, 25–34.

Harder, S., Thurmann, P., Siewert, M., Blume, H., Huber, T.H., Rietbrock, N., 1991. Pharmacodynamic profile of verapamil in relation to absolute bioavailability: investigations with a conventional and controlled release formulation. J Cardiovasc. Pharmacol. 17, 207–212.

Harris, D., Robinson, J.R., 1992. Drug delivery via the mucous membranes of the oral cavity. J. Pharm. Sci. 81, 1–9.

Jankowski, A., Marzec, A., Lamparczyk, H., 1991. Comparative bioavailability of verapamil from rapidly absorbed and slow release preparations. J. Pharm. Biomed. Anal. 10, 1101–1103.

John, D.N., Fort, S., Lewis, M.J., Luscombe, D.K., 1992. Pharmacokinetics and pharmacodynamics of verapamil following sublingual and oral administration to healthy volunteers. Br. J. Clin. Pharmacol. 33, 623–627.

Johnson, K.E., Balderston, S.M., Pieper, J.A., Mann, D.E., Reiter, M.J., 1991. Electrophysiologic effects of verapamil metabolites in the isolated heart. J. Cardiovasc. Pharmacol. 17, 830–837.

Junginger, H.E., 1991. Mucoadhesive hydrogels. Pharm. Ind. 11, 1056–1065.

Kirsten, R., Nelson, K., Kirsten, D., Heintz, B., 1998. Clinical pharmacokinetics of vasodilators. Clin. Pharmacokinet. 34, 457–482.

Liu, X.Y., Takayama, K., Machida, Y., Nagai, T., 1992. Permeability of verapamil hydrochloride through keratinized buccal mucosa in comparison with abdominal skin and its enhancement with d-limonene and Azone. S.T.P. Pharma Sci. 2, 247–252.

Nagai, T., Machida, Y., 1993. Buccal delivery systems using hydrogels. Adv. Drug Deliv. Rev. 11, 179–191.

O'Hagan, D.T., 1990. Absorption of peptides and proteins from the respiratory tract and the potential for development of locally administered vaccine. Crit. Rev. Ther. Drug Carrier Syst. 7, 35–97.

Robinson, M.A., Mehvar, R., 1996. Enantioselective distribution of verapamil and norverapamil into human and rat erythrocytes: the role of plasma protein binding. Biopharm. Drug Dispos. 17, 577–587.

Lalka, D., Griffith, R., Cronenberger, C., 1993. The hepatic first-pass metabolism of problematic drugs. J. Clin. Pharmacol. 33, 657–669.

Sawicki, W., 2001. A validated method for determination of verapamil and norverapamil in human plasma. J. Pharm. Biomed. Anal. 25, 689–694.

Sawicki, W., Janicki, S., 1995. Model podpoliczkowej postaci leku z chlorowodorkiem werapamilu. Farm. Pol. 51, 599–603.

Sawicki, W., Janicki, S., 1998. Influence of polyoxyethylene-10-oleylether on in vitro verapamil hydrochloride pene-

tration through mucous membrane from a model buccal drug formulation. S.T.P Pharma Sci. 8, 107–111.
Wainer, I.W., Granvil, C.P., 1993. Stereoselective separations of chiral anticancer drugs and their application to pharmacodynamic and pharmacokinetic studies. Ther. Drug Monit. 15, 570–574.