ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
13 (1995) 1401–1407

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

The secretion of propranolol enantiomers in human saliva: evidence for active transport?

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Received for review 6 April 1995

Abstract

To study the possible transport routes which may lead to the presence of a drug in saliva, the concentration–time curves of the separate enantiomers of propranolol were measured in human saliva and plasma after oral administration of 10 mg of propranolol hydrochloride. Saliva samples were taken with the Salivette® device. Plasma and saliva concentrations of the enantiomers of propranolol were determined by HPLC with fluorescence detection.

The transport of propranolol from plasma to the salivary gland appears to be not stereospecific and not saturable. Therefore, there is no indication that the transport of propranolol to the salivary gland is active. The concentrations of both enantiomers of propranolol in saliva, however, were higher than those of both enantiomers in venous plasma. In the past this phenomenon was interpreted as an indication of active transport, but it could be explained by the fact that salivary concentration more closely reflects the central compartment than that of peripheral venous blood.

Keywords: Propranolol enantiomers; Stereoselective pharmacokinetics; Saliva; Plasma; Salivette®; Active transport; β-Blocking drugs

1. Introduction

In recent years saliva has attracted much attention as a biological specimen for drug monitoring. However, measurements of saliva drug concentrations will usually only be of value if the saliva drug concentration reflects the drug concentration in plasma. To investigate this relationship the aim of the present work was to gain more insight into the possible transport routes which may lead to the detection of a drug in saliva: passive transcellular diffusion; ultrafiltration; and active transport. Important factors that influence this transport are lipid solubility, molecular weight of the drug, flow-rate, pK_a and thereby pH of the saliva, and binding proteins in plasma. The relevant literature has recently been reviewed [1].

In this study propranolol, a β-blocking drug, was used as a model compound to study the mechanisms of transfer of drugs into the saliva. Since the introduction to clinical practice 20 years ago, propranolol, a weak base with a pK_a value of approximately 9.5 [2], has been used as a racemic mixture of the (*R*)- and (*S*)-enantiomers in a fixed 1:1 ratio [3]. The partition coefficient between *n*-octanol and phosphate buffer, determined at 37°C and pH 7.4, is a reliable measure of the lipophilicity of propranolol and is very high (20.2) [4]. The β-blocking drugs are, to a greater or lesser extent, bound to plasma proteins. As basic drugs, they bind not only to albumin but also to α_1 acid

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SSDI 0731-7085(95)01569-8

glycoprotein and lipoproteins. Propranolol is 80–95% bound to plasma proteins [5]. If this high binding capacity is taken into account, the free fraction of propranolol is relatively low and therefore a very low saliva/plasma (S/P) ratio is suspected. However, the S/P ratio was found to be 0.50 [6,7]. Thus, the saliva concentrations were much higher than expected. Therefore, propranolol was chosen as a model compound to investigate if active transport is one of the transfer mechanisms from plasma to saliva.

(S)-propranolol is 100-fold more potent in respect of β-adrenergic blocking activity than its optical antipode [8]. Like many other β-blocking agents, (*R*)- and (*S*)-propranolol do not only differ pharmacodynamically but also pharmacokinetically; in man, metabolism and plasma protein binding of propranolol are stereoselective [9]. Until now, no studies on salivary drug concentrations in man, except one with amphetamine [10], have measured the separate enantiomers. It is not known whether the secretion of enantiomers into the salivary gland is stereospecific. Stereospecific transport might also be an indication of active transport. Other evidence which might discriminate between active and passive transport can be: secretion of a compound into the saliva against a concentration gradient; and the occurrence of saturable processes in the transport. To study these phenomena the concentration–time curves of the separate enantiomers of propranolol were measured in saliva and plasma after a single oral dose of 10 mg of propranolol hydrochloride to volunteers.

2. Experimental

2.1. Materials and reagents

(*R*, *S*)-propranolol hydrochloride and their separate enantiomers were kindly donated by ICI Holland (Ridderkerk, The Netherlands), and (*S*)-alprenolol hydrochloride was kindly donated by AB Hässle (Mölndal, Sweden). Standard solutions of propranolol hydrochloride and the separate enantiomers were prepared (0.1, 1.0, or 10.0 mg l^{-1} in methanol) and kept at 4°C.

2.2. Study design

Nine health volunteers (one male and eight females) aged 20–30 years and free of medication took part in this study. Volunteers were prevented from participating if they were pregnant or lactating. All procedures and treatments were conducted under the supervision of an experienced physician. The study was approved by an ethics committee and informed written consent was obtained from all subjects.

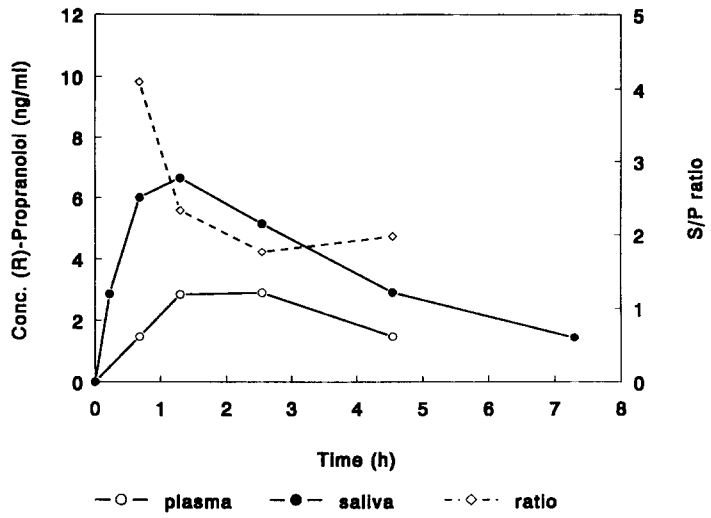
Each of the volunteers took a gelatin capsule containing 10 mg of propranolol hydrochloride (Inderal®) orally. The capsule was administered with 100 ml of water. Four hours after the capsule had been taken a standard meal was provided. In order to facilitate convenient blood sampling, a catheter was placed into the forearm. Before ingestion of the capsule, samples of saliva and venous blood were obtained for assay blanks. Blood (10 ml) and saliva samples (2–3 ml) were subsequently taken $\pm$15 min, $\pm$30 min, $\pm$1 h, $\pm$2 h, $\pm$4 h, and $\pm$8 h after administration of the drug. Blood samples were taken in heparinized collection tubes and centrifuged at 1200*g* for 10 min. Saliva samples were collected using the Salivette® device. Plasma and saliva samples were stored at −20°C until assayed.

2.3. Analysis

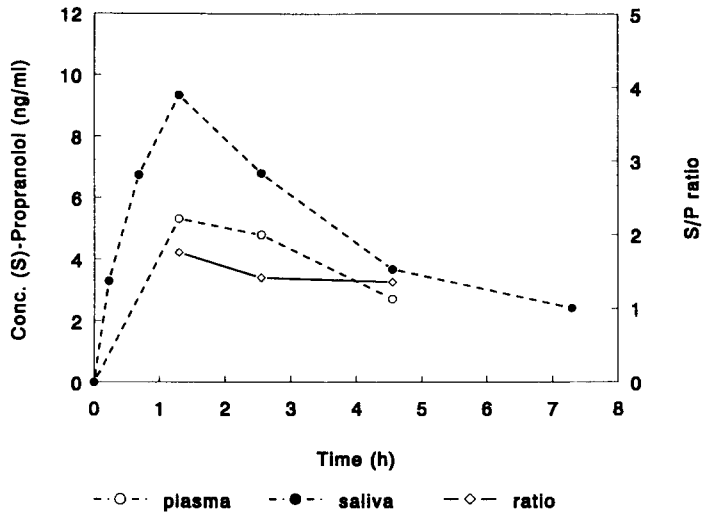
Plasma and saliva concentrations of the enantiomers of propranolol were determined by HPLC with fluorescence detection, using a 250 × 4.6 mm i.d. column packed with Chiralcel OD-H from Daicel Industries (JT Baker Chemicals, Deventer, The Netherlands), according to a validated method [11]. The peak-heights of each enantiomer were divided by those of the internal standard and the ratios were used for quantitation.

2.4. Pharmacokinetic calculations and statistical analysis

The kinetic parameters were determined by fitting the individual enantiomeric data points by least-squares regression analysis using the software package MW/Pharm (Medi\Ware BV, Groningen, The Netherlands). Enantiomeric kinetic parameters fitted best with a single-compartment model. The area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal rule after extrapo-



(a)



(b)

Fig. 1. Saliva and plasma concentrations of (*R*)-propranolol (a) and (*S*)-propranolol (b), and the saliva/plasma (S/P) curve from one subject receiving a single gelatin capsule of 10 mg of propranolol hydrochloride.

lation to infinity. Statistical comparisons were made by the Wilcoxon matched-pairs signed-ranks test. Data are tabulated as means ± SEM. Statistical significance was accepted when $p < 0.05$.

3. Results

The data obtained from four female volunteers were not used in the kinetical evaluation; the concentration–time curves of two volunteers showed severe contamination of the saliva samples taken in the first hour after administration, (*R*)-propranolol could not be detected in the saliva samples of one volunteer, and the saliva data from another volunteer could not be fitted because (*R*)- and (*S*)-propranolol were only present in two saliva samples. The saliva and plasma concentration–time curves of (*R*)- and (*S*)-propranolol, and the saliva/plasma (S/P) curve after administration of a single oral dose of 10 mg of (*R*, *S*)-propranolol hydrochloride to one volunteer are depicted in Fig. 1. Following absorption, plasma enantiomeric propranolol concentrations declined monoexponentially; hence, enantiomeric kinetic parameters were estimated from the plasma concentration–time and saliva concentration–time profiles, and are summarized in Table 1. The times for the concentration of both enantiomers to reach a maximum were approximately 1.55 h and 0.89 h for plasma and saliva, respectively. The concentrations of the (*S*)-enantiomer in all nine volunteers were higher than those of the (*R*)-enantiomer in

Table 1
Individual and mean kinetic parameters [a] for propranolol enantiomers in five subjects

Subject	AUC h mg l^{-1}	Cl $l\,h^{-1}$	V_d l	$t_{1/2}$ h	t_{max} h	C_{max} μg ml^{-1}
(*R*)-propranolol in plasma						
1	12.93	0.23	0.35	1.05	1.87	3.15
2	8.91	0.34	0.78	1.60	1.60	2.46
3	8.07	0.37	1.30	2.43	1.60	2.07
4	5.18	0.58	2.69	3.22	1.70	0.88
5	8.24	0.36	1.58	3.01	1.06	1.82
Mean	8.67	0.38	1.34	2.26	1.56	2.08
SD	2.78	0.13	0.89	0.93	0.30	0.84
(*S*)-propranolol in plasma						
1	26.18	0.11	0.34	2.05	1.61	5.44
2	16.24	0.18	0.43	1.60	1.73	4.13
3	17.34	0.17	0.60	2.41	1.61	4.46
4	8.85	0.34	1.37	2.80	1.68	1.68
5	14.80	0.20	0.74	2.54	1.05	3.86
Mean	16.68	0.21	0.70	2.28	1.54	3.91
SD	6.24	0.08	0.41	0.47	0.28	1.38
p value [b]	0.04	0.04	0.04	0.69	0.69	0.04
(*R*)-propranolol in saliva						
1	32.96	0.09	0.34	2.56	1.13	6.64
2	11.41	0.26	0.06	0.16	0.28	18.04
3	5.98	0.50	0.48	0.67	1.24	2.33
4	2.70	1.11	1.10	0.68	1.36	1.30
5	16.18	0.19	0.09	0.35	0.37	24.56
Mean	13.85	0.43	0.41	0.88	0.87	10.57
SD	11.86	0.41	0.42	0.96	0.51	10.26
(*S*)-propranolol in saliva						
1	46.43	0.06	0.28	2.98	1.22	8.20
2	11.56	0.26	0.15	0.40	0.20	14.06
3	14.07	0.21	0.25	0.80	1.28	4.46
4	4.50	0.67	0.61	0.63	1.23	2.39
5	13.51	0.22	0.09	0.28	0.57	12.28
Mean	18.01	0.29	0.27	1.02	0.90	8.28
SD	16.34	0.23	0.20	1.12	0.49	4.97
p value [b]	0.22	0.22	0.22	0.22	0.69	0.69

[a] Abbreviations: AUC = area under the concentration–time curve from time zero to infinity; Cl = total body clearance; V_d = volume of distribution; $t_{1/2}$ = elimination half-time; t_{max} = time of peak concentration; C_{max} = peak concentration.
[b] (R) compared with (S) enantiomer.

both plasma and saliva. Statistical significance between the enantiomeric kinetic parameters for plasma could be demonstrated for AUC, total body clearance (Cl), volume of distribution (V_d) and C_{max}, but not for $t_{1/2}$ and t_{max}. For saliva no significant differences between the enantiomers were observed. Most pharmacokinetic parameters in saliva showed greater inter-individual variation than in plasma.

In the nine volunteers the saliva concentrations of both enantiomers were consistently found to be higher than both enantiomers in plasma in the first 2 h, except in one volunteer. Ratios of saliva to plasma propranolol concentrations during the absorption phase were generally higher than with the passage of time (Fig. 2), and this difference was significant for (*R*)-, (*S*)- and total ((*R*) + (*S*))-propranolol. The S/P ratio of total propranolol declined from 1.49 ± 0.22 in the first 2 h to 0.98 ± 0.21 from 2 to 4 h. The total ((*R*) + (*S*))-propranolol saliva concentrations of all volunteers significantly and linearly correlates with the plasma concentrations in both time-intervals ($p < 0.05$) (Fig. 3).

There was no significant difference in (*S*)/(*R*) ratios in both plasma and saliva during the absorption phase as well as after absorption. During the absorption phase, plasma (*S*)/(*R*) ratios ranged from 1.56 to 2.05 with a mean of 1.74 (Table 2). Saliva (*S*)/(*R*) ratios were similar and ranged from 1.40 to 2.20 with a mean of

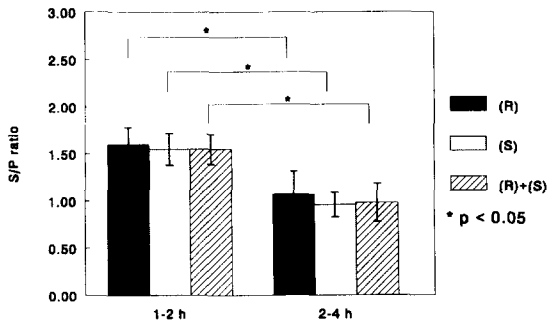


Fig. 2. Saliva to plasma (S/P) ratios ± SEM of the separate enantiomers and racemic propranolol at 1–2 h ($n = 9$) and at 2–4 h ($n = 5$) after intake.

1.69. In four volunteers the (*R*)-enantiomer was not detectable in saliva after 2 h. The mean values are based on the samples where both plasma and saliva (*S*)/(*R*) ratios were measured.

4. Discussion

Although much has been learned in the past decade about secretion of drugs into saliva, little is known about stereoselective secretion. The objective of this study was to investigate if the transport of propranolol into the saliva is active. Stereospecific transport might be an indication of active transport.

The systemic clearance of a drug comprises metabolic clearance and renal excretion. First consider hepatic metabolism. From the higher concentration of the (*S*)-enantiomer than of the (*R*)-enantiomer it can be concluded that plasma clearance of propranolol, predominantly by metabolism, is clearly stereoselective with preferential removal of the (*R*)-enantiomer from the circulation. This result is in agreement with the findings of Walle et al. [9]. Although numerous metabolites are found in man, all metabolic products can be attributed to three primary pathways, i.e. glucuronidation (17%), side-chain oxidation (41%) and ring oxidation (42%) [3]. The higher clearance of (*R*)- compared to (*S*)-propranolol was solely due to a 2.5-fold greater clearance of the (*R*)- compared to the (*S*)-enantiomer through ring oxidation. The clearance through both glucuronidation and side chain oxidation was identical for the (*R*)- and (*S*)-enantiomers. The reason for the enantiomeric differences in propranolol clearance appears to involve differences in the catalytic activities of one or more cytochrome P-450 isoenzyme(s) involved in the ring oxidation of (*R*)- and (*S*)-propranolol [9]. The same enzyme is responsible for the large inter-individual variation in the stereoselectivity as a result of the difference in the expression of this enzyme due to genetic predisposition.

The salivary secretion of a drug has been compared with the renal excretion [12,13]. Few studies have determined whether stereoselective renal excretion of drugs occurs. Renal excretion comprises three processes: glomerular filtration; tubular secretion; and tubular reab-

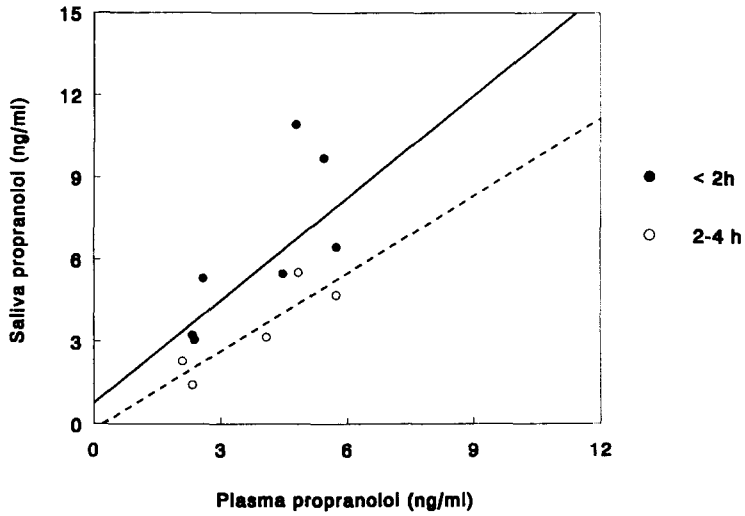


Fig. 3. Correlation between total saliva and plasma propranolol concentrations in nine volunteers at two time-intervals after administration of 10 mg of propranolol hydrochloride. Regression line of time-interval <2 h: $Y = 0.76 + 1.25X$ (——), SE of estimate = 1.57, SE of intercept = 1.81, SE of slope = 1.25, correlation coefficient = 0.82. Regression line of time-interval 2–4 h: $Y = -0.19 + 0.95X$ (– – –), SE of estimate = 0.90, SE of intercept = 1.16, SE of slope = 0.29, correlation coefficient = 0.89.

Table 2
Enantiomeric (*S*)/(*R*) ratios of propranolol in plasma and saliva at different time-intervals after absorption calculated from the non-fitted data

	1–2 h		2–4 h		>4 h	
	Plasma	Saliva	Plasma	Saliva	Plasma	Saliva
Mean [a]	1.74	1.69	1.90	1.69	1.75	1.48
SEM	0.11	0.11	0.15	0.21	0.09	0.49
n	9	9	5	5	2	2

[a] Mean values are based solely on those samples where both plasma and saliva (*S*)/(*R*) ratios were measured.

sorption. Of these, tubular secretion and reabsorption, which can involve saturable carrier-mediated processes, may be stereoselective. Enantioselectivity in the renal active excretory processes of a number of amine drugs, for instance pindolol [14] and metoprolol [15], has been found [16]. Whether propranolol metabolites are excreted stereospecifically by the kidney is not known.

The present results indicate that there was no stereoselectivity in the saliva secretion of the enantiomers of propranolol because the S/P ratios of both enantiomers and the (*S*)/(*R*) ratios of propranolol in both plasma and saliva did not differ significantly. Previous studies [17–19] indicated that mean (*S*)-propranolol plasma levels were higher than those of the corresponding (*R*)-enantiomer when (*R*, *S*)-propranolol had been administered orally. The (*S*)/(*R*) plasma concentration ratio appeared to be dose-dependent with higher doses producing a lower degree of stereoselectivity. The calculated values of the pharmacokinetic parameters (Table 1) showed the same pattern for (*S*)- and (*R*)-propranolol as described earlier [5]. Other evidence for active transport might be the occurrence of saturable processes in the transport. From the results of the present work using an individual dose of 10 mg there were no indications of saturable processes. This dosing was chosen because establishment of a substantial clinical effect in the volunteers was not wanted and therefore the lowest possible dose was applied. However, it might be possible that the saturation process can only be observed when higher doses are administered. Hence, there was a weak linear but significant correlation between saliva concentrations and plasma concentrations. Besides, the saliva concentrations were higher than the venous plasma concentrations in the first 2 h, and thereafter equal to the plasma concentrations. This is in contrast with earlier findings [6,7,20]. However, these investigators did not clearly explain the way saliva was collected. The method of saliva sampling is very important. For instance, if Parafilm® is used to stimulate salivation, it was shown in vitro that Parafilm® absorbs 20% of the propranolol when shaking pieces of Parafilm® at pH 7.0 for 3 min [21]. Therefore, this material should not be used to stimulate salivary flow for drug measurements. In this way the measured saliva concentrations are lower than the real concentrations. The Salivette® device also absorbs a significant amount of propranolol [11]. However, the results obtained in this way can be corrected for this loss because the calibration curves can be prepared with the Salivette®. With Parafilm® this is difficult to achieve.

How can the observation that the concentrations of propranolol are greater in the first 2 h in saliva than in plasma be explained? Many authors still refer to the equation originally developed by Rasmussen describing the S/P ratios of many compounds [22]. However, in this equation only passive diffusion transport is taken into account. It is assumed that the diffusion of drugs between plasma and saliva is passive and rapid. However, when the salivary glands remove only a small fraction of the drug presented to them through their blood supply, this transport becomes dependent on the salivary flow. Besides, saliva and plasma values could reflect different compartments [23]. After the uptake of an orally administered substance from the intestine, the arterial blood initially has a higher concentration than the venous blood. If absorption is complete and the substance is not metabolized in a particular organ, the situation is reversed because the substance rediffuses into the blood. This is seen in the results. The S/P ratio declines with the passage of time. Other studies have observed the same phenomenon that the S/P ratio was higher during the absorption phase than with the passage of time [10,13,23].

5. Conclusions

The transport of propranolol from plasma to the salivary gland is not stereospecific and not saturable. Therefore, it can be concluded that there is no indication that the transport of propranolol to the salivary gland is active. The concentration of both enantiomers of propranolol in saliva was higher than that of both enantiomers in plasma. This phenomenon could be explained by the fact that both values reflect different compartments, namely the saliva concentration more closely reflects the cellular concentration in organs of the central compartment than that of peripheral venous blood.

Acknowledgement

This study is part of the central research program "Toxicology and Bioanalysis" of GUIDE (Groningen Utrecht Institute of Drug Exploration), The Netherlands.

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