COMPARISON OF PLASMA AND SALIVA LEVELS OF METOPROLOL AND OXPRENOLOL

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- 1 Plasma and saliva levels of metoprolol and oxprenolol have been compared in two groups of healthy volunteers.
- 2 Mean salivary oxprenolol levels were lower than, but closely related to the corresponding plasma levels ($r=0.93$), the mean ratio of saliva:plasma concentration being 0.42.
- 3 Mean salivary metoprolol concentrations were considerably greater than the corresponding plasma levels and the relationship between the concentrations in the two fluids was less clear.
- 4 The evidence presented suggests that oxprenolol diffuses passively whilst metoprolol is actively secreted into saliva. The mechanism involved in the active process is not known.

Introduction

The excretion of drugs in saliva has been reported in man (Araki, 1951; Borzelleca & Cherrick, 1965) and a correlation between plasma and saliva levels has been shown for a number of compounds in clinical use, including tolbutamide (Matin, Wan & Karam, 1975), paracetamol (Glynn & Bastin, 1973), theophylline (Koysooko, Ellis & Levy, 1974), sulphonamides (Killman & Thaysen, 1955), and antipyrine (Fraser, Mucklow, Murray & Davies, 1976).

It has previously been suggested (Graham & Rowland, 1972) that salivary drug levels may be of use when applied to biopharmaceutical studies in which serial blood sampling is necessary, and that it may prove a more convenient method of biochemical monitoring (Joselon, Ruiz & Goldwater, 1969). It is also hoped that such studies may provide some insight into the mechanism of transfer of drugs across biological membranes. The passage of a drug into saliva has already been shown to be dependent upon its molecular size and the lipid solubility of the unionized form (Amberson & Hober, 1932). Studies with a series of sulphonamides indicate that the transfer is also dependent on the pK_a value of the drug (Killman & Thaysen, 1955) while from other work it is evident that a good relationship exists between salivary drug concentrations and the concentration of drug which remains unbound to plasma proteins (Koysooko *et al.*, 1974). From such studies it would appear that excretion occurs by way of a passive diffusion process.

Recently there has been considerable interest in the bioavailability and pharmacokinetics of β -adrenoceptor blockers and a study was therefore carried out with the aim of investigating the relationship between saliva and plasma concentrations of oxprenolol and metoprolol following a single oral dose. The influence of salivary flow rate on the concentration of drug in saliva has also been assessed in the case of metoprolol since preliminary studies had revealed that saliva metoprolol concentrations were considerably greater than those in the plasma. This suggested the possible existence of an active process of drug excretion into saliva so that salivary flow rate might be expected to modify the actual concentration of drug in saliva. If this is true, then metoprolol concentrations in saliva might be related to the total protein content of saliva or to its α -amylase activity. These two components were therefore estimated in those subjects who were given metoprolol.

Methods

Sampling

Twelve healthy male volunteers aged 18–44 years, receiving no form of medication took part in this study having fasted overnight. Six of the volunteers took 100 mg metoprolol and six took 80 mg oxprenolol in the form of commercially available tablets all from one batch, and in each case 100 ml water was also taken;

a little to help with the tablet and the remainder being used to rinse the mouth after swallowing.

Blood samples (10 ml) were taken by venepuncture immediately before the dose and at 0.5, 1, 1.5, 2, 4, 6, 8 and 12 h post dosing. Each sample was placed into a heparinized tube and centrifuged immediately in order to isolate the plasma.

Saliva samples were collected for 5 min each time a blood sample was taken and the exact volume determined by weighing. In the case of the metoprolol study, further samples were collected at 2.5, 3, 3.5 and 5 h after dosing. The pH values were determined immediately after collection. In the oxprenolol study salivation was stimulated by mouth and tongue movements alone, but in the case of the metoprolol study by chewing on a piece of parafilm.

All samples were stored at -20°C until analysis.

Flow rate study

The influence of different salivary flow rates on metoprolol concentration in the saliva was assessed at 2 and 6 h after dosing. The different flow rates were obtained by using the following stimuli:

- Mouth and tongue movements alone for 5 min.
- Chewing on a piece of parafilm for 5 min.
- 1 drop of 10% citric acid solution on the tongue followed by mouth and tongue movements for 3 min.
- 2 drops 10% citric acid solution on the tongue every minute for 3 min followed by mouth and tongue movements.

These samples were treated as for those collected during the main study.

During the study a light breakfast was given 1.5 h after dosing and a normal lunch after 4.25 h. Subjects rinsed out their mouths thoroughly after each meal.

Analysis of oxprenolol and metoprolol

The concentrations of oxprenolol and metoprolol in plasma and saliva were determined by the method of Degan & Riess, 1976. The procedure consists of solvent extraction of the drug and internal standard (metoprolol and oxprenolol respectively) from the sample, derivatisation with trifluoroacetic anhydride and separation by gas liquid chromatography on a 3% OV 1 column. The method is capable of detecting metoprolol and oxprenolol concentrations of 5 ng/ml and is accurate within $\pm 7\%$ at the 10 ng/ml level and is not affected by the presence of citric acid.

Salivary total protein estimation

This was carried out according to the method of Biuret, using 200 μl saliva, but it proved necessary to extract with diethyl ether before optical density readings were taken.

Figure 1 Mean plasma (—) and saliva (---) concentrations of (a) oxprenolol (●) and (b) metoprolol (○).

Salivary amylase activity

This was assayed using the Amylochrome® test kit produced by Roche Diagnostics for estimation of amylase activity in plasma and urine. The method is based on the spectrophotometric measurement of a dye produced when the enzyme acts upon a chromogenic substrate.

Each saliva sample was initially diluted 1 in 500 before analysis.

Statistical analysis

The overall effect of the different stimuli on salivary flow rate and on saliva metoprolol concentrations at 2 h and 6 h post dosing was assessed by the analysis of variance. The concentrations were not normally distributed and the differences were therefore analysed using the Friedman two-way analysis of variance by ranks.

Results

The mean plasma and saliva levels of oxprenolol and metoprolol are given in Figure 1 which shows that after oral administration, oxprenolol is present in saliva in lower concentrations than in plasma, whilst salivary metoprolol concentrations are considerably higher than corresponding plasma levels.

Oxprenolol

Figure 2 demonstrates the correlation between plasma and saliva levels of oxprenolol in the six subjects

Figure 2 Scattergram showing correlation between plasma and salivary oxprenolol concentrations. The open circles are the values for one individual.

studied. The coefficient of correlation (r) for the six individuals ranged from 0.86 to 0.98 with a mean value of 0.93. The mean $\pm$ s.e. mean ratio of saliva to plasma oxprenolol concentrations was 0.42 ± 0.05 . The ratio of saliva to plasma concentrations, however, was not constant in any subject.

From a semilogarithmic plot of plasma and saliva oxprenolol concentrations against time (Figure 3), the mean $\pm$ s.e. mean plasma half-life for the decline in plasma concentration between 2 and 8 h was $1.43 \text{ h} \pm 0.12$ which compares favourably with single half-life values previously reported for the drug following oral administration (Brunner, Imhof & Jack, 1975). The mean $\pm$ s.e. mean saliva oxprenolol half-life between these times was $1.45 \text{ h} \pm 0.21$.

Metoprolol

From Figure 1 it is evident that saliva metoprolol concentrations do not follow plasma levels as closely as

Figure 3 Semi-logarithmic plot of the mean plasma (—) and mean saliva (---) oxprenolol concentrations against time.

those of oxprenolol, the correlation coefficient for individuals ranging from 0.57–0.91 and having a mean value of 0.80. The mean $\pm$ s.e. mean plasma metoprolol half-life between 2 and 8 h was 2.10 ± 0.52 . Peak values in both saliva and plasma are reached in 1–1.5 h after drug administration. Subsequently there are secondary peaks in the saliva profile which are not apparent in the plasma profile. From the mean data presented in Figure 1 these secondary peaks would appear to be related to the intake of food, but examination of individual data showed that peak times varied from one subject to another. Thus, there is no

Table 1 Comparison of mean $\pm$ s.e. mean salivary flow rates and metoprolol concentrations obtained in six subjects.

Method of stimulation of salivary flow rate	Salivary flow rates (ml/min)		Salivary metoprolol concentration (ng/ml)	
	2 h	6 h	2 h	6 h
1. Tongue movements only	1.06 ± 0.14	0.90 ± 0.06	280.3 ± 70.7	194.7 ± 75.2
2. Chewing parafilm	$1.85 \pm 0.18^*$	$1.55 \pm 0.09^*$	212.2 ± 30.5 NS	112.0 ± 48.0 NS
3. 1 drop citric acid	$1.65 \pm 0.11^*$	$1.24 \pm 0.10^{**}$	337.2 ± 98.2 NS	113.0 ± 41.3 NS
4. 2 drops citric	$3.18 \pm 0.24^*$	$2.82 \pm 0.23^*$	215.0 ± 49.0 NS	$70.8 \pm 31.2^*$

Significance of difference from method 1 — $^*P < 0.01$, $^{**}P < 0.05$.

clear correlation between saliva and plasma concentrations. Furthermore, the occurrence of secondary peaks did not correspond to changes in pH, flow rate, total protein content or α -amylase activity of the saliva samples.

Results of the flow rate study are presented in Table 1. The analysis of variance between the flow rates achieved by the four different stimuli show that there is a highly significant increase in salivary flow rate produced by chewing on parafilm and by spotting 10% citric acid solution on the tongue when compared to the flow rate obtained using mouth and tongue movements alone. However, analysis of the salivary metoprolol concentrations of various flow rates indicate that there is no significant change in the concentration of drug in saliva with increasing flow rate at either two hours or six hours after dosing, with the exception of the highest flow rate at six hours when the metoprolol concentration was significantly reduced.

Discussion

The two β -adrenoceptor blockers studied have very similar physical characteristics in that their pKa values and lipid solubilities are comparable. They differ, however, in their binding to plasma proteins, 78% oxprenolol being protein bound compared with only 12% in the case of metoprolol (Riess, Brechbuhler & Theobald, personal communication; Johansson, Appelgren, Borg & Elofsson, 1974).

In the present study a good correlation has been established between plasma and saliva levels of oxprenolol and although saliva to plasma oxprenolol concentration ratios vary, the mean value of 0.42 is close to the predicted value for a passive diffusion process and using the formula of Fraser *et al.* (1976):

$$R = \frac{1 + 10(pK_a - pH_s)}{1 + 10(pK_a - pH_p)} \times \frac{f_p}{f_s}$$

where R is the ratio of saliva and plasma drug concentrations, pH_p and pH_s are the pH values and f_p and f_s are the fractions of unbound drug in the plasma and saliva respectively. pK_a refers to the pK_a of the drug. In the case of oxprenolol the pK_a is 9.5, the value of f_s is 1 and f_p is about 0.22. During the study the mean pH of saliva was 7.26 ± 0.04 (s.e. mean) so that R would be expected to have a value of 0.3.

For metoprolol, application of the above formula would predict a saliva to plasma metoprolol concentration ratio of about 1.2. From this study, however, it is obvious that this ratio is greatly exceeded at each assessment time during the study. It would seem, therefore, that an active process of metoprolol transfer exists, although this has been shown not to be related to salivary flow rate, total protein content or α -amylase activity. Since this latter

parameter mainly reflects the secreting activity of the parotid gland, it appears that the active process suggested for transport of the drug into saliva, is not dependent on the parotid secretory activity.

An alternative explanation for the initial high salivary metoprolol level is contamination during the process of ingestion. This is unlikely since film coated tablets were used and care was taken to wash out the mouth with water after they were swallowed. Furthermore the peak concentrations in saliva were not attained until 1–1.5 h after ingestion and the earlier values were not unduly high.

In conclusion it would seem that oxprenolol is transferred passively into saliva and that mean salivary concentrations may provide useful pharmacokinetic data although in one individual, saliva levels cannot be used to predict the corresponding blood levels. Metoprolol differs in that it is apparently transferred from plasma into saliva by an active process. Further work is required to clarify the mechanism of this process and to investigate the reasons for the occurrence of secondary peaks in the metoprolol profiles in saliva.

References

- AMBERSON, W.R. & HOBBER, R. (1932). The permeability of mammalian salivary glands to organic non-electrolytes. *J. cell comp. Physiol.*, **2**, 201–221.
- ARAKI, Y. (1951). Nitrogenous substances in saliva. 2. Urea and rhodan. *Jap. J. Physiol.*, **2**, 255–259.
- BORZELLECA, J.F. & CHERRICK, H.M. (1965). Excretion of drugs in saliva. *Antibiotics. J. Oral Ther. Pharmac.*, **2**, 180–187.
- BRUNNER, L., IMHOF, P. & JACK, D. (1975). Relationship between plasma concentrations and cardiovascular effects of oral oxprenolol in man. *Eur. J. clin. Pharmac.*, **8**, 3–9.
- DEGEN, P.H. & RIESS, W. (1976). Simplified method for the determination of oxprenolol and other beta blockers by gas liquid chromatography. *J. Chromatogr.*, **121**, 72–75.
- FRASER, H.S., MUCKLOW, J.C., MURRAY, S. & DAVIES, D.S. (1976). Assessment of antipyrine kinetics by measurement in saliva. *Br. J. clin. Pharmac.*, **3**, 321–325.
- GLYNN, J.P. & BASTAIN, W. (1973). Salivary excretion of paracetamol in man. *J. Pharm. Pharmac.*, **25**, 420–421.
- GRAHAM, G. & ROWLAND, M. (1972). Application of salivary salicylate data to biopharmaceutical studies of salicylates. *J. pharm. Sci.*, **61**, 1219–1222.
- JOHANSSON, K.A., APPELGREN, C., BORG, K.O. & ELOFSSON, R. (1974). Binding of two adrenergic beta-receptor antagonists, alprenolol and H 9326, to human serum proteins. *Acta Pharm. Suec.*, **11**, 333–346.
- JOSELOM, M.M., RUIZ, R. & GOLDWATER, L.J. (1969). The use of salivary (parotid) fluid in biochemical monitoring. *Am. Ind. Hyg. Assoc. J.*, **30**, 77–82.

- KILLMAN, S.A. & THAYSEN, J.H. (1955). The permeability of the human parotid gland to a series of sulphonamide compounds, para-aminohippurate and insulin. *Scand. J. clin. lab. Invest.*, **7**, 86–91.
- KOYSOOKO, M.S., ELLIS, F. & LEVY, G. (1974). Relationship between theophylline concentration in plasma and saliva of man. *Clin. Pharmac. Ther.*, **15**, 454–460.
- MATIN, S.B., WAN, S.H. & KARAM, J.H. (1975). Pharmacokinetics of tolbutamide: Prediction by concentration in saliva. *Clin. Pharmac. Ther.*, **16**, 1052–1058.

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