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Salivary secretion of chlorpropamide in man.

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Abstract

The presence of chlorpropamide in saliva was established by chromatographic method. Plasma and saliva chlorpropamide were measured by high performance liquid chromatographic (HPLC) method [1] in six healthy volunteers who took a single dose each after an overnight fast.
Salivary levels of the drug were consistently and significantly lower than those in plasma, but the linear regression analysis revealed good correlation ($r = 96 \pm 0.05$) between saliva (S) and plasma (P) concentrations. S/P ratio varied from (.01 - .05). The mean S/P ratio was 0.33 ± 0.004. The mean time to peak plasma saliva concentration (tmax) were 4.66 + 1.03h and 4.33 + l.h respectively; the mean peak Plasma/saliva, concentration (Cmax) were $30.06 \pm 2.34\ \mu gml^{-1}$ and $1.46 \pm 0.31\ \mu gml^{-1}$ respectively.
The mean plasma pharmacokinetic values except absorption half life (t ½ ab), absorption rate constant (ka), tmax, were significantly ($P<0.05$) different from the saliva pharmacokinetic values.

Key words: *Chlorpropamide;* plasma; saliva; pharmacokinetics

Introduction

Recently there has been considerable interest in the estimation of drug salivary levels for a number of compounds in clinical use including paracetamol [2], theophylline [3], tolbutamide [4], chloroquine [5], proguanil [6], carbamazepine, phenytoin and Phenobarbital [7], concentrations of certain drugs in saliva has been shown to provide a reliable index of their concentration in plasma [7,8]. The pharmacokinetics of chlorpropamide in plasma have been reported [9,10,11]; the information on its availability in saliva was in a study of the relationship between the saliva and plasma concentration (S/P ratio of some weakly acidic and basic drugs [8]. No detailed information has been published on salivary secretion of chlorproamide. This work is therefore designed to demonstrate the presence of chlorpropamide in saliva, to determine its levels and its pharmacokinetics in saliva after a single oral dose.

Materials and methods

Chlorpropamide (Diabenese® bought in zaria, Nigeria, chlorpropamide standard powder was a gift from Pfizer International, New York, U.S.A. Tolbutamide powder used as internal standard was also a gift from Boechringer Mannheim GMBH, West Germany. All the chemicals were analytical grade obtained from British Drug Houses (BDH) chemical Limited, Poole. England. The liquid chromalography used was waters model 204. liquid chromatograph fitted with model 510 reciprocrating pumps. UV model 441 detector and U6K septumless universal injector. A Bondapak C18 reversed phase column (10mm) was used for analysis. Solvent system consisted of methanol: 0.2% acetic acid (3:2) at pH 6.7.

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This was delivered at 0.8ml/min. The pH of saliva was determined on a KENT pH electronic meter model 7060.2.1

Clinical procedure

The subjects were six healthy male volunteers from 25 to 30 years (mean + S.D = 28.50 years) and weighing from 60-70kg (mean + SD = 65.33 + 2.52kg), None of them had any history of cardiac, hepatic, renal or gastrointestinal disease. All have normal body biochemistry and heamatological profiles. They were non-smokers, they were instructed to abstain from any drug two weeks before and during the study. Their informed consent was obtained after detailed explanation to them. The study was approved by the ethnical committee of the Ahmadu Bcllo Teaching Hospital. Zaria Nigeria.

After an overnight fast, the subjects swallowed a single oral 250mg dose of chlorpropamide tablet (Diabenese®, with 150ml of water. Venous blood (5ml) was then withdrawn through an indwelling cannula just before and at 0.5,

1, 2, 4, 6, 8, 12, 36, 48, 72 144 and 168h after dosing. Blood was collected into heparinized tubes. Saliva samples were obtained simultaneously (after they have rinsed their mouths) by direct spitting into plastic bottles. The saliva was stimulated using glass beads and samples were collected over a period of 2min. All samples were centrifuged at 2000g for 10 min to remove mucoid sediment and to obtain plasma from blood samples. Samples so treated were stored at -20^{o}C pending analysis.

Drug analysis

Chlorpropamide in plasma and saliva was determined by HPLC method [1], pooled blank and test plasma and saliva samples were extracted with dichloromethane under acidic condition (pH3). The aqueous layer was aspirated and the organic layer was evaporated to dryness under a stream of nitrogen at 40o^{C}. The residue was reconstituted in methanol and 10ml injected into the I1PLC column. The UV spectrum was also ran on a recording spectrophotometer (Pye-Unicam. SP8 100).

Standard Curve was constructed by plotting the peak height ratios of chlorpropamide to the internal standard tolbutamide against the drug concentration in the standard. The standard curve has a correlation of 0.999. The concentration of the drug in the samples were then determined using the peak height of each sample to the internal standard (tolbutamide) with reference to the standard curve. Coefficient of variation (CV) of assay at three different concentrations of plasma and saliva for five samples at each level for within - assay and day-to-day assay was generally not more than 3%.

Pharmacokinetics analysis.

Analysis of the results were carried out by using an itenerated computer program (residual) kinemp 1 for least squares regression in a non-model-dependent fashion. The initial concentration Co, lag time, and elimination half lives were obtained through residual plot, they were fed into the computer together with the plasma-time levels of the volunteers to calculate other standard parameters.

Results were expressed as mean + S.D. They were analyzed for statistical significance by students t - test for paired data. The differences between plasma and saliva results were by analysis of variance (ANOVA). $P < 0.05$ being considered significant in each case.

Result and discussion

The ultra violet (UV) spectrum of the extract of the test saliva was identified with the UV spetrum of chlorpropamide standard solution with absorption maximum at 232nm. The extract of the test saliva also gave a single peak with retention time of 8min when injected into the HPLC (fig. 1). This corresponds to the retention time of standard chlorpropamidc powder. The retention time of the internal standard (Tolbutamide) was 1 min. the pharmacokinetics of chlorpropamidc obtained in plasma as shown in Table 1 compared well with the reported pharmacokinetics of chlorpropamide [9.10, 11]. The plasma and saliva concentration - time profiles for the six healthy subjects are shown in fig 2. The salivary concentrations and the Cmax observed were lower than those of plasma.

The concentration of most drugs in saliva corresponds to the free unbound plasma drug concentrations, and this is a more meaningful value for considerations of pharmacological activity or toxicity than both bound and unbound drug [5], since chlorpropamide is about 95% bound to plasma protein [9,10], at any point in time, only about 5% of the plasma drug concentration will be free (unbound form) to traverse salivary gland. The lowered salivary concentration of chlorpropamide led to the significant ($P<0.05$) differences observed in the Cmax and elimination parameters. The occurrence of tmax at about the same time in saliva and plasma indicated that equilibrium is reached rapidly between them, in line with posti's hypothesis that saliva should be regarded as an integral part of the central compartment [12] rather than a 'deep' pharmacokinetic compartment as suggested by [13], it also explained the mean saliva/plasma (S/P) ratio of less than unity (.01 - .05) obtained at each starting point in this study. However, a correlation ($r = 0.93$) was observed to exist between saliva and plasma levels of chlorpropamide. This is a reflection of the advantage of collection of stimulated saliva which has a narrow pH range around the value of 7.0 whereas the pH of unstimulated saliva showed a large variability [8]. A correlation ($r = 0.54$) of S/chlorpropamidc was observed in a study of relationship between the saliva and plasma concentration (S/P) ratio [8]. The authors collected single sample venous blood and saliva from diabetic patients on regular chlorpropamide therapy ranging from 100-500 j/gm daily, and measured the drug concentration using gas liquid chromatograph (GLC) method. They envisaged errors due to non standardization of salivary flow rate and pH. The use of a more sensitive technique (HPLC) in this study may also contribute to the observed improved correlation of S/P ratio of chlorpropamide. Saliva can be obtained by a non invasive technique and the concentration of most drugs in saliva correspond to the free or pharmacologically active form of the drug in plasma, the monitoring of drug concentration in saliva should be encouraged and more works should be done in monitoring the salivary concentration of chlorpropamide in steady state. Having established the presence of chlorpropamide in saliva together with its correlation and pharmacokinetics, Estimation of chlorpropamide concentration in saliva of patients suffering from chlorpropamide overdose may assist the clinician to decide further treatment without the discomfort required for vcnipunctures.

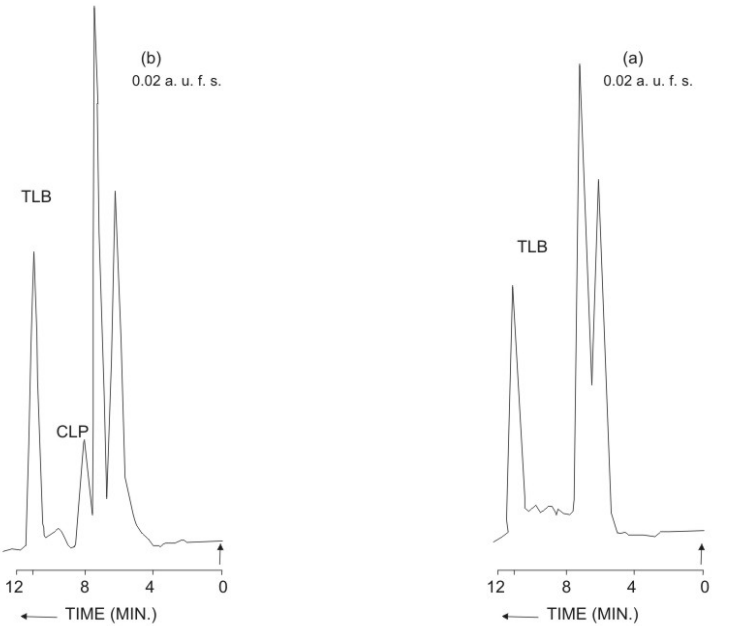

Fig. 1 High -performance liquid chromatograms of (a) an extract of blank saliva containing the internal standard (Tolbutamide) (TLB), and (b) an extract of test saliva sample obtained from a volunteer following an oral administration of 250mg chlorpropamide (CLP) tablet.

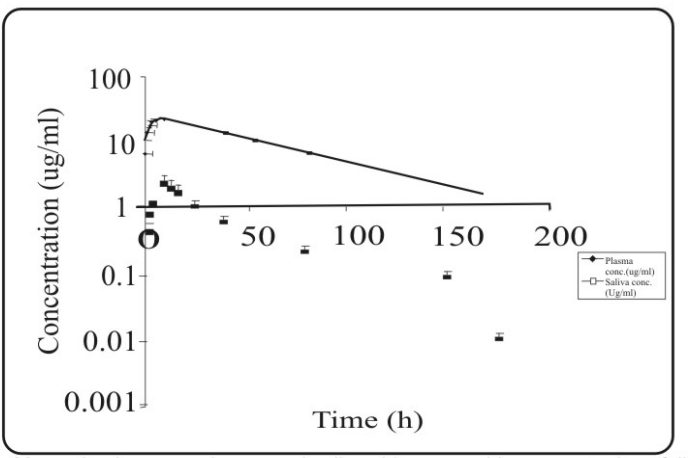

Fig. 2: Mean simultaneous plasma and saliva chlorpropamide concentrations following oral administration of 250 mg of the drug to 6 volunteers

Table 1: Some pharmacokinetic parameters obtained from saliva and plasma in six subjects following oral administration of 250mg chlorpropamide tablets.

Volunteer	Cmax (ug/ml)		tmax (h)		ab t v^h)		t % B (h)	
	Saliva	Plasma	Saliva	Plasma	Saliva	Plasma	Saliva	Plasma
I	1.53	31.79	6	4	1.35	0.75	16.19	37.00
II	1.25	27.73	4	4	1.35	0.44	29.49	43.69
III	2.04	32.36	4	2	1.16	0.33	18.76	49.23
IV	1.21	26.61	4	6	0.65	0.78	26.40	42.15
V	1.51	30.53	6	6	1.31	0.98	20.83	44.40
VI	1.25	31.39	4	4	0.61	0.74	16.98	37.87
*' Mean	1.46	30.06	4.66	4.33	1.07	0.67	21.44	52.39
,	0.31	2.34	1.03	1.50	0.35	0.24	5.37	4.51

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