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Salivary and Plasma Concentrations of Nifedipine after a Single Oral Dose in Healthy Volunteers

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Abstract: Salivary and plasma nifedipine concentrations were measured in 6 healthy volunteers. A correlation of 0.965 ($p<0.001$) was observed between salivary and plasma nifedipine concentrations. The level of significance of maximum concentration (Cmax) between plasma and saliva was statistically significant ($p< 0.001$). Identical salivary and plasma nifedipine time to peak drug levels (t_{max}) was established. Peak salivary Concentration (C_{max}) ranged from 59.57 to 63.90 ng while peak plasma concentrations ranged from 35.95 to 39.40 ng. Results suggest salivary nifedipine concentration to be of value in bioavailability and pharmacokinetic studies.

Key words: Sailvary, plasma, nifedipine, concentration, volunteers

INTRODUCTION

Nifedipine is an active substance belonging to the 1.4-dihydropyridine class of compounds which are of great importance within the cardiovascular field as calcium channel blockers. It is strongly lipophylic, slightly non-polar, practically insoluble in water and only slightly soluble in intestinal fluids (Kozjek *et al*., 1987).

Many drugs can with advantage be measured in saliva. Concentrations in saliva sometimes correlate well with concentrations in plasma. Examples are phenytoin, phenobarbitone, primidone, lithium and antipyrine (Schmid and Kupferberg, 1975; Croth *et al*., 1974; Mucklow *et al*., 1978; Welch *et al*., 1975).

Saliva is easy to obtain without the trauma of venepuncture, and its collection requires less technical expertise.

The strong lipothylicity of nifedipine led us to carry out this research to elucidate the relationship between salivary and plasma nifedipine concentration levels.

EXPERIMENTAL

All sample handling and extraction steps were carried out under red light to prevent light degradation of nifedipine.

MATERIALS

Adalat retard tablets containing 20 mg nifedipine, production series Number BL 711 was supplied by the manufacturer, Bayer, Leverkusen, West Germany. Methanol, Sodium acetate, Acetic acid, Dichloromethane and 4-Dimethylaminobenzaldehyde were all obtained from B.D.H. Chemicals, Poole, England. All solvents were glass distilled.

All glassware used during the extraction procedure was cleaned with chromic acid, washed with distilled water and oven dried.

INSTRUMENTATION

High performance liquid chromatography. The HPLC Model used is Waters 204 equipped with 441 model U.V. detector fitted with 254 m filter, model U6K septum less injector and SE 120 model recorder was used. The column used for the chromatographic separation was a μ-Bondapak radial pak catridge, 15 cm ×8.0 mm I.D. packed with 10 μm reversed phase C18-support, Waters part Number 85721, (Waters Reversed Associates, Inc., Milford). The mobile phase consisted of methanol: 0.01M sodium acetate (55:45), pH adjusted to 5.2 with 0.2 M sodium acetate solution. This was used at a flow rate of 2.0 mL min^{-1}.

METHODS

Six healthy male volunteers aged 21-30 years (mean 24 + 3) and body weights 54-70 kg (mean 60 + 3) took part in the study. Prior to enrolment into the study, the volunteers had to submit a written informed consent. None of the subjects had received any medication for a period of 2 weeks prior to the study. Beverages

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containing alcohol and caffeine were not permitted during the study. On the day of each study, and indwelling cannula with a heparin lock was inserted into the antecubital vein of the volunteer. Basal measurements were performed after a minimal stablilization of 15 min on a couch. Each subject was given a single tablet containing 20 mg of nifedipine (Adalat) with 100 mL of water after overnight fasting. They remained on the couch and continued to fast for a period of 4 h. Blood samples (5 mL) were withdrawn prior to the dose and at 0.5, 1, 2, 3, 4, 6, 8 and 12 h after the dose. The samples were collected in heparinized tubes, centrifuged for 10 m, at 1500 g and the plasma was harvested into glass tubes wrapped with aluminium foil, and kept at -20°C until analysis. Salivary samples (5 mL) were also collected after stimulation with a rubber band at the same times as the plasma was collected. The salivary samples were collected in wrapped plain plastic containers, centrifuged at 3000 rpm for 10 m and harvested into plastic tubes wrapped with aluminium foil and stored at -20°C until assayed.

Preparation of samples for HPLC analysis: Plasma (0.5 mL) was placed in a 15 mL centrifuge tube to which 500 ng; 4-dimethylaminobenzaldehyde (internal standard) had been added. Sample was deproteinized with acetic acid (0.5M and extracted with 5 mL dichloromethane, whirl-mixed on an autovortex mixer for 15 s and centrifuged for 15 m. The aqueous layer was removed using an append-of pipette, the organic extract was transferred to a clean 15 mL centrifuge tubes and evaporated to dryness under a stream of nitrogen at 40°C. The dry residue was dissolved in 100 mL of methanol and 15 mL aliquot was injected into the HPLC.

Saliva (0.5 mL) to which 1000 ng, 4-dimethylaminobenzaldehyde had been added was also extracted with 5 mL dichloromethane and treated as described above.

Preparation of standards for HPLC: Control plasma samples (0.5 mL) were spiked with 10, 20, 30, 40 and 50 mg nifedipine and with 500 ng 4-dimethlaminobenzaldehyde as internal standard. Control saliva samples (0.5 mL) were also spiked with 25, 50, 75, 100, 150 and 200 mg nifedipine and with 1000 ng 4-dimethylaminobenzaldehyde as internal standard. The samples were processed as described above. Standard curve was constructed by plotting the peak height ratio of nifedipine to the internal standard against the drug concentration in each standard. The level of the drug in an unknown sample (plasma or saliva) was derived from this curve.

RESULTS AND DISCUSSION

Linear calibration curves with good correlation coefficients (r = 0.9996 and 0.999) of nifedipine was obtained for saliva and plasma samples respectively. Nifedipine was detected in both plasma and saliva samples of all the six subjects within the 12 h of sampling. The HPLC chromatograms of the extracted saliva and plasma samples at the time of maximum concentration gave peaks corresponding to retention time of reference nifedipine samples of 9.4 m (Retention time of internal standard, 4-dimethylaminobenzaldehyde is 5.8 m). This peak was absent from the blank plasma and saliva extract which was collected before the ingestion of nifedipine tablet.

There were differences as well as similarities in the individual plasma and saliva concentration levels. Table 1 shows the mean nifedipine plasma and saliva concentration levels.

Pharmacokinetic parameters derived from the mean plasma and salivary concentrations data are shown in Table 2.

Following oral administration of 20 mg nifedipine tablets, Peak plasma Concentrations (C_{max}) ranged from 35.95 to 39.40 ng. this finding is in agreement with the results of Kleinbloesem *et al.* (1984) and Kozjek *et al.* (1987). The peak salivary Concentration (C_{max}) ranged from 59.51 to 63.90 ng. The mean maximal plasma and saliva levels (C_{max}) were attained at 2.33 ± 0.47 h.

Table 1: Mean plasma and saliva nifedipine concentration in healthy volunteers following oral administration of 20 mg nifedipine

	Concentration (ng mL^{-1})±SD	
Time (h)	Plasma	Saliva
0.5	16.54±1.09	22.29±1.97
1.0	27.73±1.36	27.05±6.61
2.0	37.28±1.83	59.50±2.45
3.0	36.53±0.40	55.66±10.33
4.0	28.50±1.21	49.13±7.41
6.0	20.04±0.72	37.50±4.52
12.0	6.34±1.38	13.62±2.37

Table 2: Mean (±SD) pharmacokinetic parameters of nifedipine derived from the Mean Plasma and Saliva Concentration data after a single 20 mg oral dose

Parameter	Plasma	Saliva
Lag time (h)	0.13±0.037	0.17±0.08
Absorption half-life $t_{1/2}$ (α) (h)	0.71±0.077	1.05±0.58
Absorption rate constant Ka (h^{-1})	0.96±0.14	0.86±0.38
Maximum concentration C_{max} (ng mL^{-1})	37.78±1.21	61.69±34
Time to peak t_{max} (h)	2.33±0.47	2.33±0.47
Elimination half-life $t_{1/2}$ (β) (h)	3.23±0.33	3.44±0.75
Elimination rate constant Ke (h^{-1})	0.22±0.02	0.21±0.45
Area under the curve:		
AUC (0-12 h) ng/mL/h	235.73±6.65	401.29±25.37
AUC (0-∞) ng/mL/h	265.93±13.67	469.35±41.98
Volume of distribution (L)	349.56±20.64	21.64±37.48
Clearance (L h^{-1})	75.40±3.69	43.42±3.58

S.D. = Standard Deviation

The level of significance of C_{max} between plasma and saliva were statistically significant ($p<0.001$). There was good correlation between plasma saliva nifedipine levels ($r = 0.965$). Similarly, identical saliva and plasma nifedipine time to peak drug levels (t_{max}) was established in this study. Ogunbona and Oluwatodimu (1986) established similar result in the study that equilibrium is reached rapidly between plasma and saliva in line with Posti's hypothesis that saliva should be regarded as an integral part of the central compartment (Posti, 1985) rather than a deep pharmacokinetic compartments as suggested by Galeazzi *et al.* (1976).

Consistently, higher concentrations of nifedipine in saliva relative to those in plasma was observed in this study. The higher salivary concentration is attributed to the high lipohylicity and lipid solubility properties of nifedipine. It is suggested from this study that salivary concentration levels of drugs that are highly lipophylic and lipid soluble could be used to estimate their pharmacokinetic and bioavailability data.

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