

Theophylline Concentrations in Serum and Saliva after Oral Microcrystalline and Sustained-Release Preparations in Asthmatics

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Theophylline concentrations in saliva have been compared with those in serum in 13 asthmatic patients after single and multiple oral dosage of a microcrystalline theophylline tablet (Nuelin®) and a sustained-release preparation (Nuelin Retard®).

There was wide individual variation in the ratio of serum to saliva concentrations indicating that, even when individual serum to saliva ratios have been established, the use of saliva theophylline measurements alone is unreliable for the routine monitoring of theophylline therapy.

Both preparations produced mean serum theophylline concentrations within the 10–20 mcg/ml range, the sustained-release tablet giving higher morning trough values and smaller trough to peak variation. The preparations were equally and highly bioavailable.

Side effects were reported by three patients, which were the patients with the highest AUC.

The Nuelin Retard preparation appears to provide smooth, sustained serum theophylline concentrations within the therapeutic range and the simple, twice-daily dosage should result in improved patient compliance.

Key words: saliva concentration; serum concentration; sustained-release preparation; theophylline.

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Theophylline preparations have been less used in the Scandinavian countries than in the United States of America. The reason may be the narrow therapeutic interval for theophylline and wide individual variation in the metabolic rate which makes it necessary for the dose to be given four times daily and for the serum concentration to be controlled.

At the same time, in the Scandinavian countries, it has been possible to use beta-stimulant preparations both as tablets and as aerosols.

The appearance of sustained-release theophylline preparations has made it possible to obtain stable theophylline concentrations with 12 hourly dosing intervals (4, 10, 13, 15). At the

same time, several investigators (6, 9, 11) have shown that saliva concentration measurement provides a reliable measure of serum concentration. If this is true then treatment with theophylline drugs would be made much easier.

The purpose of this investigation, therefore, was to compare serum concentrations after a new sustained release preparation of theophylline (Nuelin Retard) with serum concentrations after dosage of a microcrystalline theophylline (Nuelin). The comparison was made after single doses and also under steady-state conditions after multiple dosing.

The bioavailability for the two drugs was compared under steady-state conditions.

At the same time, the saliva theophylline concentrations and the reliability of the ratio for serum and saliva concentrations, under steady-state, were examined.

MATERIAL AND METHODS

Patient selection

Thirteen patients (3 females and 10 males) with a mean age of 41 years (range 19–58 years) were selected for the study (Table 1).

Ten patients were asthmatics and three had asthma with chronic bronchitis; five patients were smokers. With one exception (patient No. 1) all patients admitted into the study showed greater than 20% increase in FEV₁ over the basal value after inhalation of two puffs of terbutaline, isoprenaline or salbutamol.

Pregnant women and patients with hepatic or uncompensated heart disease were excluded.

Medication and dosage

Nuelin Retard tablets containing 175 mg theophylline, formulated as sustained-release preparation and Nuelin tablets containing theophylline 125 mg in a conventional tablet base were provided by Riker Laboratories together with matching placebos.

Table 1
Patient data

Patient trial	Age (years)	Sex M/F	Height (cms)	Weight (kg)	Initial PEFR	% of predicted
1	19	F	168	58	250	48
2	44	M	172	80	350	58
4	38	F	170	65	500	105
5	51	F	153	45	300	69
6	38	M	169	65	250	41
7	38	M	178	106	260	42
8	54	M	172	75	390	66
9	43	M	172	77	500	82
10	30	M	184	95	675	101
11	53	M	177	76	550	91
12	32	M	180	76	480	76
13	34	M	171	68	350	57
14	58	M	174	79	250	42

Restriction of dietary xanthine-containing food and drinks was unnecessary since a specific High Pressure Liquid Chromatographic technique was used to quantify serum and saliva theophylline levels. Throughout the study period intake of other methylxanthine-containing medication or oral beta₂-sympathomimetics was forbidden.

One patient who continued taking a previously prescribed theophylline preparation resulting in a high serum theophylline concentration (28 mcg/ml) associated with side effects was consequently eliminated from the study.

Patients were allowed to use their pressurized beta₂-sympathomimetic aerosols as 'rescue' treatment when necessary. The patients were instructed to record on a diary card, the number of aerosol puffs taken. Established chronic therapy with corticosteroids or sodium cromoglycate was maintained at the dosage being given at the start of the trial.

Design

At least 24 h before the start of the study and during the 48 h washout period between the two phases, the intake of all theophylline-containing oral drugs was discontinued.

The trial was a two part, crossover study using a double-blind technique. The patients were randomly allocated to receive either conventional Nuelin or Nuelin Retard first and crossed over to receive the alternative treatment in the next consecutive week.

Phase I. On the first day of phase I the patient attended the hospital laboratory for pulmonary function testing. At 8.30 a.m. basal lung function data were obtained and blood and saliva samples were taken for assay of theophylline.

At 9.00 a.m. on the first day test medication of either Nuelin Retard 350 mg or Nuelin 250 mg was taken with 150–200 ml of water.

Lung function was repeated at 1, 2, 3 and 4.5 h after dosing. Blood and saliva samples were taken at 1, 2 and 4.5 h after dosing.

A tablet of either Nuelin Retard, placebo or Nuelin 125 mg was given at 13.30 and again at 1800 h.

Nuelin $\pm$ Nuelin Retard

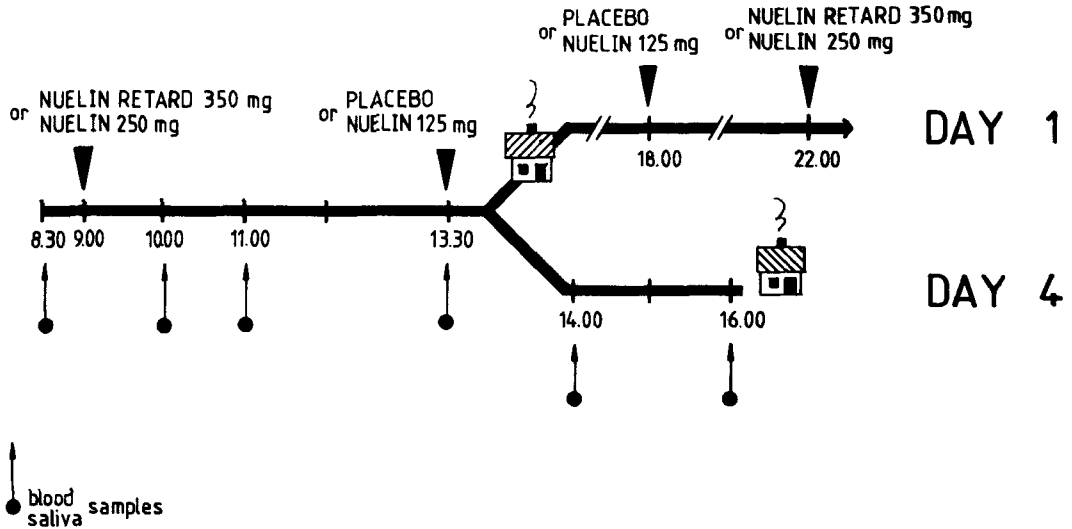


Fig. 1. Trial design.

At 22.00 h a further test dose of 350 mg Nuelin Retard, or Nuelin 250 mg was given. (See Fig. 1).

Dosage of both drugs was maintained at home at these times throughout Days 2 and 3.

After leaving hospital on the afternoon of Day 1 and for the whole of Days 2 and 3, all the patients measured their own peak expiratory flow rate (PEFR) at the following times and recorded the measurements on a specially prepared diary card: 8.30–9.00, 11.00, 13.00, 15.00, 17.45–18.00, 21.45–22.00.

The use of additional treatment by sympathomimetic aerosols, as a 'rescue' procedure, was also recorded on the diary card.

On Day 4 patients returned to the hospital laboratory and the morning dose of either Nuelin Retard 350 mg or conventional Nuelin 250 mg was taken at 09.00 h. Patients undergoing the Nuelin test regime then received 125 mg conventional Nuelin at 13.00 h whilst those on Nuelin Retard were given placebo tablets at the same time. PEFR and blood and saliva samples were taken at exactly the same time as for Day 1 with the addition of further blood and saliva samples at 14.00 and 16.00 h. An additional PEFR was recorded at 15.00 h.

Wash-out period. The last two daily doses of Nuelin and the last dose of Nuelin Retard were discontinued on Day 4: dosage was discontinued for Days 5, 6 and 7, which were used as a wash-out period between the two parts of the cross-over study.

Phase II. Phase II, using the alternative drug treatment, was started after the wash-out period, and the procedure followed was exactly the same as for phase I.

Blood and saliva sampling

Blood was taken into a dry glass tube, the serum separated by centrifugation and deep frozen until the concentration measurements were made. In the same way, saliva was collected in dry glass tubes. Saliva secretion was stimulated with five drops of lemon juice. The first portion of saliva, after stimulation, was discarded.

Theophylline levels in serum and saliva were assayed quantitatively using a specific High Pressure Liquid Chromatographic technique.

The HPLC analysis was performed using a solvent delivery system (Model 6000 A, Waters

Associates, Milford, Mass. 01757), a loop injector (Model U6K, Waters Associates), and a dual channel absorbance detector for monitoring of the eluent at both 254 nm and 280 nm (Model 440, Waters Associates). The absorbance detector was linked to a dual channel chart recorder (Omni-Scribe, Houston Instruments, Austin Texas 78753). The simultaneous monitoring at two different wavelengths was used to detect, by calculating the peak ratios, possible interference from other substances with the theophylline determination.

The column was a 30 cm $\times$ 4 mm i.d. reversed phase column (μ -Bondapak C₁₈, Waters Associates). The procedure used for the analysis was as described by Orcutt et al. (12) except that the composition of the mobile phase was CH₃CN/acetate buffer 0.01 M, pH 4.0, 8/92 and that the internal standard (Beta-hydroxyethyltheophylline, 36.7 mg/l) was dissolved in CH₃CN/acetate buffer 0.02 M, pH 4.0, 8/42. The flow rate was 4 ml/min.

Area under curve (AUC) was calculated using Simson's integration equation and calculated on a Texas Instrument program.

Pulmonary function

Peak expiratory flow rate (PEFR) was measured on the laboratory days and at home with the

same Wright Mini Peak Flow Meter.

Two measurements of PEFR were taken and the highest result was recorded.

RESULTS

Nuelin

On Day 1 the mean ($\pm$ s.d.) peak value was 9.36 ($\pm$ 3.41) mcg/ml at 10.00 h. On Day 4 the mean ($\pm$ s.d.) peak value was 14.57 ($\pm$ 4.8) mcg/ml also at 10.00 h, – that is within 1 h after tablet administration (Table 2 and Fig. 2).

The mean morning, steady-state trough value at 09.00 h on Day 4 was 7.47 ($\pm$ 4.0) mcg/ml, serum theophylline.

The lowest mean steady-state serum theophylline level after the first dose of Nuelin was still within the accepted therapeutic range at a value of 11.15 mcg/ml.

As expected, individual steady-state values varied as shown in Table 2; however, 13 patients had achieved steady-state levels greater than 10 mcg/ml, nine greater than 12 mcg/ml, and six greater than 15 mcg/ml.

The mean area under the serum concentration curve (AUC) was 77.73 ($\pm$ 27.68) mcg/ml $\times$ 6h. The mean trough to peak variation of serum-theophylline at steady state was 7.68

Table 2

Mean theophylline concentrations ($\pm$ s.d.) in serum and serum: saliva ratios after administration of a total daily dose of 750 mg Nuelin or 700 Nuelin Retard adjusted to equivalent of 750 mg dose. Serum AUC (mcg/ml $\times$ 6h) on Day 4

Time	Day 1				Day 4			
	Nuelin		Nuelin Retard		Nuelin		Nuelin Retard	
	Serum	Serum to saliva ratio	Serum	Serum to saliva ratio	Serum	Serum to saliva ratio	Serum	Serum to saliva ratio
0900	1.86 $\pm$ 1.45	1.93 $\pm$ 1.08	1.66 $\pm$ 0.99	1.18 $\pm$ 0.58	7.47 $\pm$ 4.05	1.93 $\pm$ 0.87	10.19 $\pm$ 4.37	2.00 $\pm$ 0.47
1000	9.35 $\pm$ 3.41	1.71 $\pm$ 0.44	3.70 $\pm$ 1.84	1.81 $\pm$ 0.41	14.57 $\pm$ 4.89	1.91 $\pm$ 0.59	11.53 $\pm$ 5.13	2.01 $\pm$ 0.53
1100	8.77 $\pm$ 2.35	1.79 $\pm$ 0.36	5.18 $\pm$ 2.37	1.83 $\pm$ 0.34	14.35 $\pm$ 4.82	1.92 $\pm$ 0.51	11.90 $\pm$ 5.50	1.82 $\pm$ 0.31
1300	7.12 $\pm$ 1.80	1.80 $\pm$ 0.30	7.94 $\pm$ 3.78	1.94 $\pm$ 0.69	11.15 $\pm$ 4.74	1.75 $\pm$ 0.65	12.65 $\pm$ 5.56	1.84 $\pm$ 0.30
1400					13.38 $\pm$ 4.75	1.79 $\pm$ 0.32	12.57 $\pm$ 5.10	1.86 $\pm$ 0.36
1600					12.60 $\pm$ 4.52	1.87 $\pm$ 0.48	11.70 $\pm$ 5.69	1.93 $\pm$ 0.51
AUC					77.73 $\pm$ 27.68		72.33 $\pm$ 33.01	

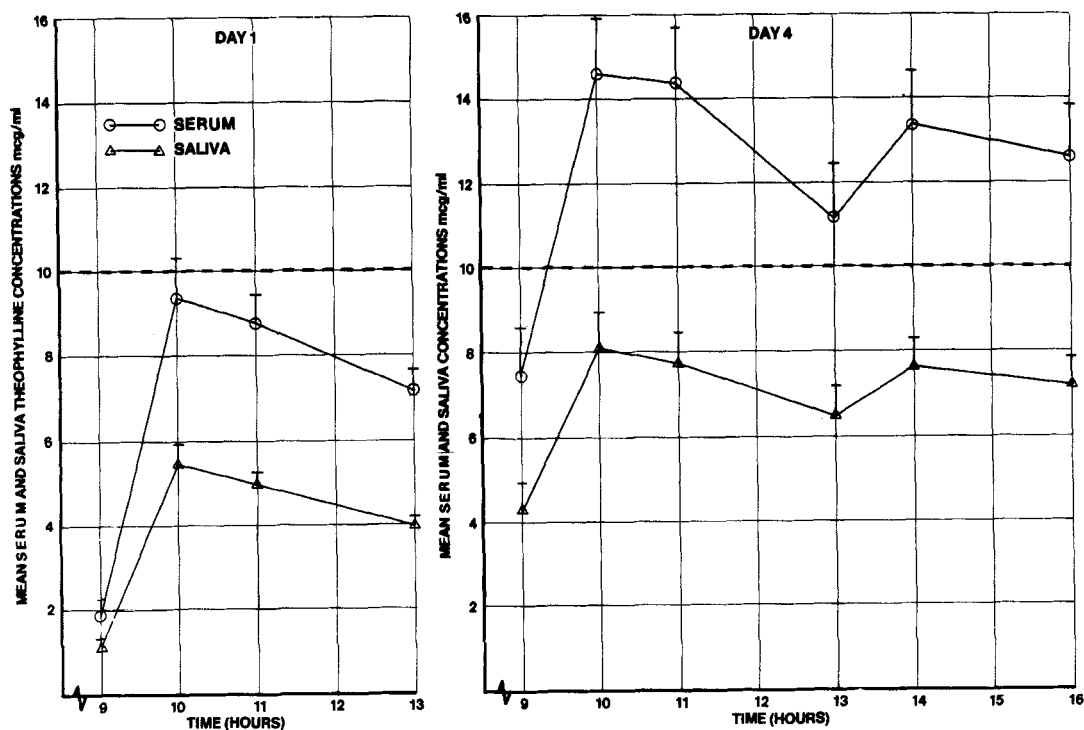


Fig. 2. Mean serum and saliva theophylline concentrations, mcg/ml of patients taking Nuelin 750 mg per day, on Days 1 and 4. Doses at 0900, 1330, 1800, 2200 h.

mcg/ml and the mean time to peak value was 1.23 h.

Nuelin Retard

On Day 1 the mean ($\pm$ s.d.) peak value achieved was $7.94 (\pm 3.7)$ mcg/ml at 13.00 h. On Day 4 the mean ($\pm$ s.d.) peak value was $12.65 (\pm 5.5)$ mcg/ml at 13.00 h, i.e. within 4 h of tablet administration.

At 14.00 h, 5 h after administration, the mean plasma value was virtually identical to the peak (12.57 mcg/ml). The mean morning steady-state trough value at 09.00 h on Day 4 was 10.19 ± 4.3 mcg/ml.

After Nuelin Retard the mean plasma theophylline level remained virtually constant over the measured time interval from 09.00 to 16.00 h.

Again, as expected, individual steady-state values varied as shown in Table 2. Fourteen patients achieved steady-state levels greater than

10 mcg/ml, six greater than 12 mcg/ml and three patients had levels greater than 15 mcg/ml. Two patients, (Nos. 6 and 12) had serum levels less than 10 mcg/ml and both of these were moderate smokers.

The mean AUC was $72.33 (\pm 33.01)$ mcg/ml $\times$ 6h. The mean trough to peak variation of serum-theophylline at steady state was 3.42 mcg/ml and the mean time to peak was 3.3 h.

Saliva theophylline assay

Saliva specimens (taken concurrently with plasma specimens) were quantitatively assayed and the mean values are displayed graphically in Figs. 2 and 3.

The correlation on Day 4 between theophylline concentration in serum and saliva samples is shown in Figs. 4 and 5.

Mean serum/saliva ratios and s.d. for Nuelin and Nuelin Retard are shown in Table 2.

To obtain an estimate for within-patient va-

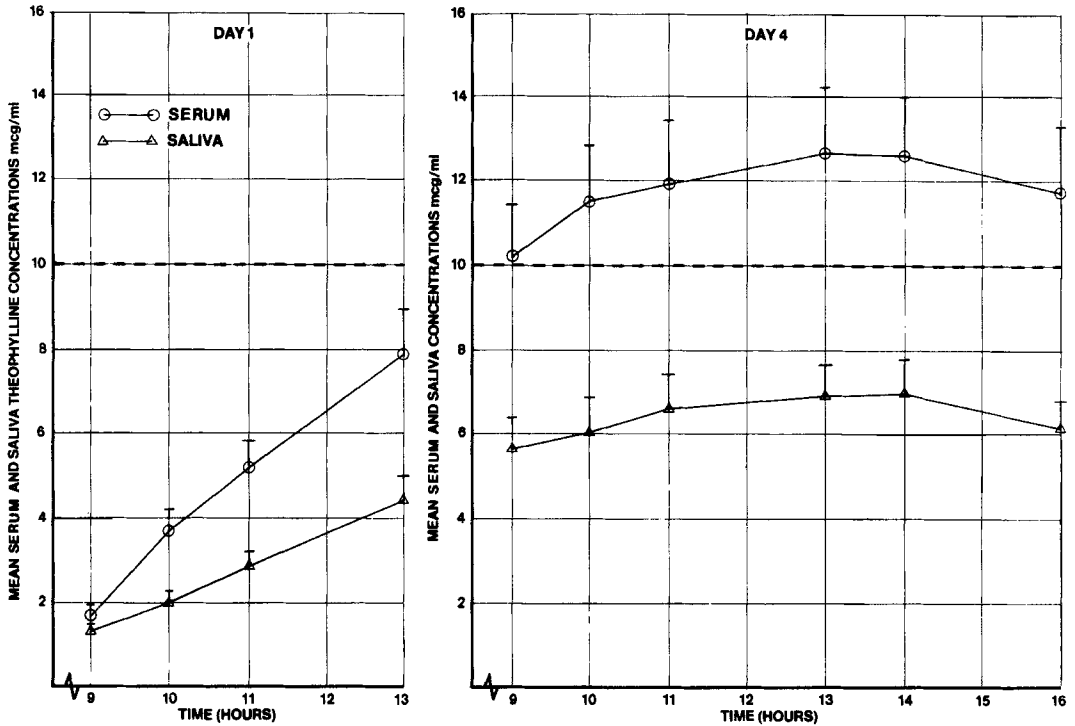


Fig. 3. Mean serum and saliva theophylline concentrations in mcg/ml of patients taking Nuelin Retard 700 mg per day, on Days 1 and 4. Values adjusted to 750 per day. Doses at 0900 and 2200 h.

riation a one-way analysis of variance of the individual serum/saliva ratio for Day 4, for Nuelin and Nuelin Retard, was made (1). The residual mean square for Nuelin serum/saliva ratio was 0.0989 with an s.d. of 0.32 and for Nuelin Retard serum/saliva ratio had a residual mean square of 0.1526 with an s.d. of 0.39. The 95% confidence interval was ± 0.64 for Nuelin and ± 0.68 for Nuelin Retard.

Pulmonary function

Pulmonary function of the patients was measured on visits to the laboratory on Days 1 and 4. Patients measured and recorded their PEFR values at home, on the second half of Day 1 and throughout Days 2 and 3.

Patients were selected for the study on the basis of a minimal reversibility recorded as a 20% increase in PEFR in response to a standardised bronchodilator aerosol. However, when the study actually began, many of the patients were at or close to their predicted values (Table 1).

Unfortunately, many of the patients had misunderstood the instructions regarding the use of their 'rescue' beta-adrenergic stimulant aerosols and on examination of their diary cards it is clear that patients had continued to use these aerosols frequently as *routine* treatment. A number of patients also took doses from their aerosols even on the laboratory days.

For these reasons, the pulmonary function results obtained cannot be considered valid and are not presented in this report.

Side effects

Side effects were reported by three patients, Nos. 4, 5 and 7: nausea, vomiting, dizziness or headache. These were the three patients with the highest AUC on Day 4. Thus the side effects only occurred in relation to higher than average serum theophylline values, and throughout the study the side effects were never sufficiently severe that any patient found it necessary to stop taking the tablets.

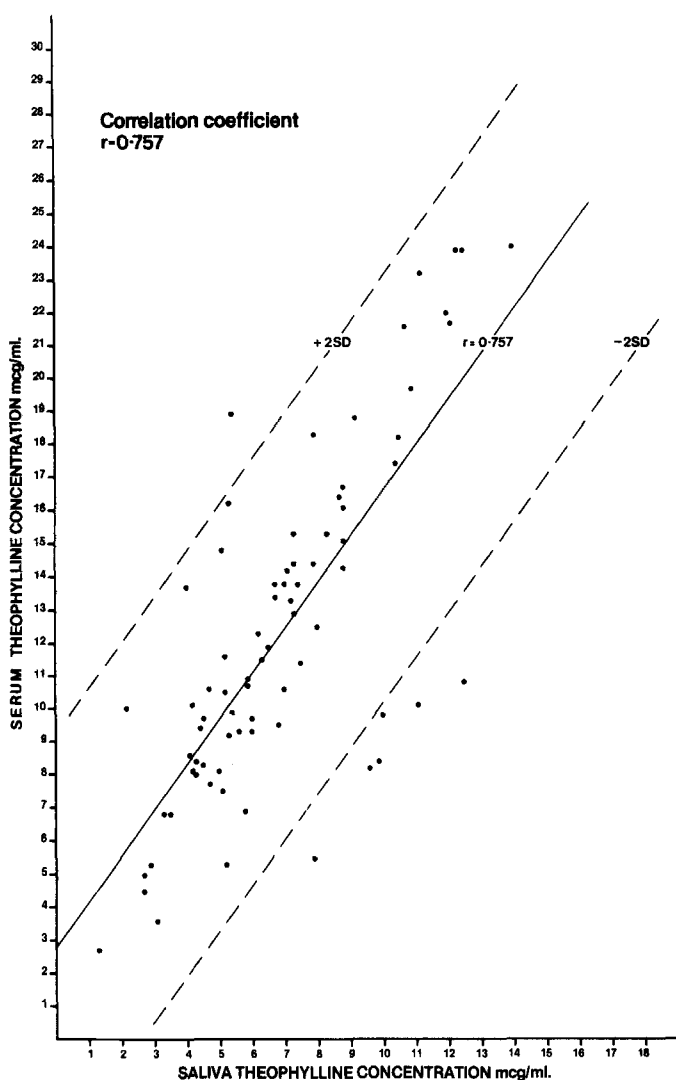


Fig. 4. Nuelin correlation of serum + saliva concentrations.

DISCUSSION

In this comparison of Nuelin and Nuelin Retard in 13 patients with asthma, or asthma with bronchitis, both treatments produced steady-state serum plasma theophylline values within the therapeutic range of 10–20 mcg/ml.

Nuelin Retard produced a significantly higher morning trough value than Nuelin ($P < 0.002$, paired t-test) and the steady-state serum level was maintained at an almost constant value for 7 h after the morning tablet dose.

The relatively slow development of peak serum and saliva concentrations and lack of large trough to peak variation after Nuelin Retard (mean trough to peak variation for Nuelin Retard was 3.42 mcg/ml, for Nuelin 7.68 mcg/ml, $P = 0.001$, paired t-test) confirm the satisfactory sustained-release characteristics of the preparation.

Theophylline was given in the same dose to all patients regardless of their weight and this may be the reason why there were wide differences in the serum concentrations of the patients. In

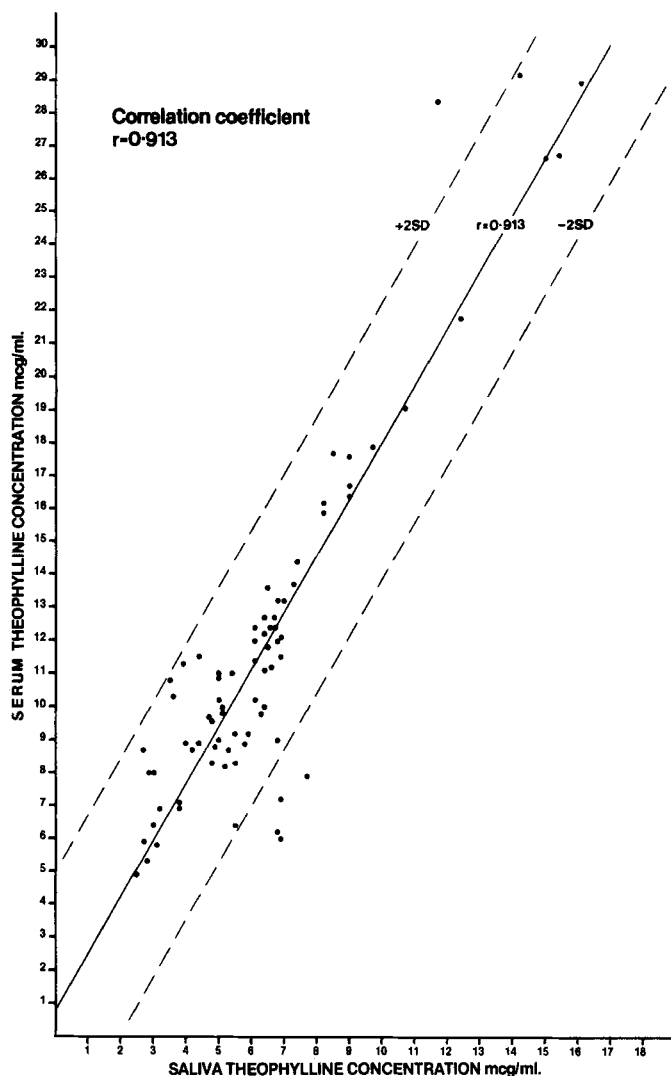


Fig. 5. Nuelin Retard correlation of serum + saliva concentrations.

calculating theophylline dosage, by body weight, it is advisable to correct for obesity (5). The two patients with serum levels greater than 20 mcg/ml were patient No. 5 (45 kg) who received 16.7 mg/kg and patient No. 7 (106 kg) who received 10 mg/kg. This latter patient may be characterised as a 'slow-metaboliser'.

If the area under the serum concentration curve is correlated with theophylline dosage in mg/kg, and patient No. 7 is excluded, then there is still a poor correlation, (correlation coefficient is 0.65), which indicates large individual patient

differences in the metabolism of theophylline.

The bioavailability of Nuelin has been previously shown to be 100% (8). In this investigation the bioavailability for Nuelin and Nuelin Retard was compared under steady-state conditions between 09.00 and 15.00 h. The mean AUC for Nuelin was found to be 78 mcg/ml $\times$ 6 h and for Nuelin Retard 72 mcg/ml $\times$ 6 h. This difference is not significant ($P = 0.22$, paired t-test). That is, the bioavailability of Nuelin Retard, relative to that of Nuelin is $99\% \pm 18\%$ at the 95% confidence level.

Serum concentration at 09.00 h on Day 1 in Phase I and Phase II are 'blank values'. Within-patient variation here was greater (max 3.96 mcg/ml) than has been shown earlier (9). Much of the variation is possibly due to differences in intake of xanthine-containing foods or drinks by the individual patients, and it has been shown (14) that these dietary xanthines are metabolised into theophylline.

In this study a wide, within-patient variation in serum/saliva theophylline ratio was found. The observed variation means that for a patient whose ratio has been previously shown to be 2.0 it can only be said, on the basis of a new saliva concentration, that the true predicted serum theophylline concentration (95% confidence limits) may be between 14 and 26 mcg/ml for Nuelin and between 12 and 28 mcg/ml for Nuelin Retard.

Possible explanations for the variation are: –

- a) The saliva may have been affected by the lemon juice even though the first portion of saliva was rejected.
- b) Changes in pH may have influenced the ratio between the protein-bound and unbound part of theophylline in the serum (16), in addition, saliva concentration is proportional to the unbound theophylline fraction.
- c) The correlation between serum and saliva theophylline concentrations is not fully described by a linear equation (2).

The variation observed is of the same order or less than previously found by several authors (2, 3, 7) and rather more than that shown by others (6, 9, 11). This variation in serum/saliva concentration ratio indicates that, even after having established the plasma/saliva ratio for an individual patient, the use of saliva theophylline measurements alone is unreliable for the routine monitoring of theophylline treatment.

CONCLUSIONS

Nuelin Retard and Nuelin produced similar theophylline concentrations within the therapeutic range and the preparations were equally bioavailable.

The morning trough values, 12 h after Nuelin Retard were significantly greater than those

after Nuelin and trough to peak variation was significantly less for the sustained release preparation.

The Nuelin Retard preparation appears to provide smooth, sustained, serum theophylline concentrations within the therapeutic range and the simple, twice-daily dosage should result in improved patient compliance.

Saliva theophylline measurements alone are unreliable for monitoring theophylline therapy.

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