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Bioavailability study of paracetamol tablets in saliva and urine

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SUMMARY

A bioavailability study of two lots of paracetamol tablets was carried out in 5 healthy volunteers, using a crossover aleatory design, and drug monitoring in urine and saliva by high performance liquid chromatography (HPLC). Results were correlated with those obtained in an in vitro dissolution study. Statistical evaluation of bioavailability parameters indicates that the two formulations may be considered bioequivalent, in spite of differences found during early stages of the absorption process, which were preventable according to an in vitro dissolution study.

INTRODUCTION

Paracetamol is a frequently used non salicylate analgesic and antipyretic, which requires a rapid release from the pharmaceutical form to produce an immediate effect. Several authors have reported their results of bioavailability studies carried out with this drug. Some of these studies used plasma (1–3) or saliva (4,5) for drug monitoring, while others were based on urinary excretion data (6–8).

In the present study, we investigated the bioavailability of two lots of paracetamol tablets, using saliva and urine as body fluids. In vivo results were correlated with those obtained in vitro, and have been described in a previous publication (9). If a good correlation is obtained, bioequivalence of pharmaceutical forms of paracetamol may be evaluated in non-blood fluids, with important economic and ethical advantages.

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MATERIALS AND METHODS

Tablets

Paracetamol tablets of 500 mg, developed at Unidad de Producción de Medicamentos, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, were used and identified as Formulation A, while commercial paracetamol tablets of 500 mg, available in the Argentine market, were identified as Formulation B.

In vitro dissolution study

This study was carried out according to USP XXIII, using the paddle method, with a phosphate buffer pH 5.8 and also with HCl 0.1 N, at 50 rpm. The samples were analysed using a UV spectrophotometer at 244 nm. The sampling times were 5, 10, 20, 30, 40, 50 and 60 min.

In vivo study

Five 22–30 year-old healthy female volunteers, with a body weight of 50–69 kg participated in this study.

A single dose of paracetamol (1 tablet of 500 mg after 12 h fasting) was administered, from each one of the formulations assayed, according to a crossover aleatory design, with a washout period of not less than 1 week, between two consecutive administrations.

For drug follow up, saliva samples were collected at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 5, 6 and 7 h after drug ingestion. After centrifugation, the samples were stored in a freezer at –20°C until the moment to be assayed by HPLC.

The amount of paracetamol eliminated from urine of volunteers was also determined, from samples collected at 0.5, 1, 2, 3, 4, 5, 7, 9 and 15 h. Once the volume of urine was measured, a small portion of the urine was frozen until the analysis by HPLC.

Drug analysis

Urine and saliva samples were assayed by HPLC with the following chromatographic conditions: RP18 column 250 mm × 4 mm of internal diameter, and 5 µm of particle size, injection volume 20 µl, temperature 30°C, detection at 254 nm. For urine, mobile phase was water/acetonitrile/triethylamine (99.99:0.01:0.5), adjusted at pH 2.5 with phosphoric acid and the flow rate was set at 1.7 ml/min. For saliva, mobile phase was water/acetonitrile/triethylamine (94:6:0.5) adjusted to pH 2.5 with phosphoric acid and the flow rate was set at 1 ml/min.

Prior to injection into the chromatograph, samples were processed as follows: 1 ml of centrifuged urine was diluted in 10 ml with distilled water. Saliva was centrifuged with $HClO_4$ at 6% P/V in a ratio of 1:0.8, and then 1 ml was diluted in 5 ml of mobile phase.

Prior to analysis, a calibration curve of paracetamol was performed for each body fluid, and was correctly validated. For urine follow-up, linearity of the calibration curve was found in the range 1–8 mg/l. The precision of the system, calculated as the coefficient of variation of 5 injections from one standard was 2.5%. The precision of the method, evaluated according to dispersion of 5 samples was 5.2%, expressed as coefficient of variation.

For saliva samples, a range of linearity between 0.3–20 mg/l was obtained from the calibration curve. The precision of the system was 3.5% and the precision of the method was 4.0%.

Bioavailability parameters

From the profiles of experimental concentration of paracetamol in saliva, as a function of time, the following parameters were obtained: C_{max}, maximum concentration of drug in saliva; T_{max}, time of maximum concentration, $AUC_{(0-7)}$, area under the curve of drug levels in saliva vs time from 0 to 7 h, calculated through the method of trapezoids and MRT, mean residence time, estimated by the ratio between the area under the curve of first statistical moment (AUMC) and the area under the curve (AUC).

For urine samples, the parameters calculated were: E_{15}, maximum amount of paracetamol excreted after 15 h of administration; $(dE/dt)_{max}$, maximum rate of excretion and tmax, time of maximum rate.

Statistical analysis

Mean values of the above mentioned parameters were calculated with the respective standard deviations. Student's *t*-test, analysis of variance (ANOVA) (10), and the confidence interval of 90%, based on the Intervalar Hypothesis Test (11), were used if the values were normally distributed, and the nonparametric Wilcoxon test for not normally distributed parameters (12).

RESULTS AND DISCUSSION

Figure 1 shows the in vitro dissolution profile. There are significant differences ($P < 0.05$) between dissolution percentages at 30 and 60 min, both in buffer medium pH 5.8 and in HCl 0.1 N medium. The mean percentages of dissolution with its typical mean deviation (± SM) at pH 5.8 for Formulation A were 92.88 (± 0.97) and 100.55 (± 0.66), and for Formulation B 29.03 (± 3.71) and 47.71 (± 5.47), while in HCl 0.1 N medium for Formulation A they were 54.63 (± 2.90) and 71.32 (± 6.08), and for B 23.95 (± 4.31) and 39.39 (± 4.29), respectively.

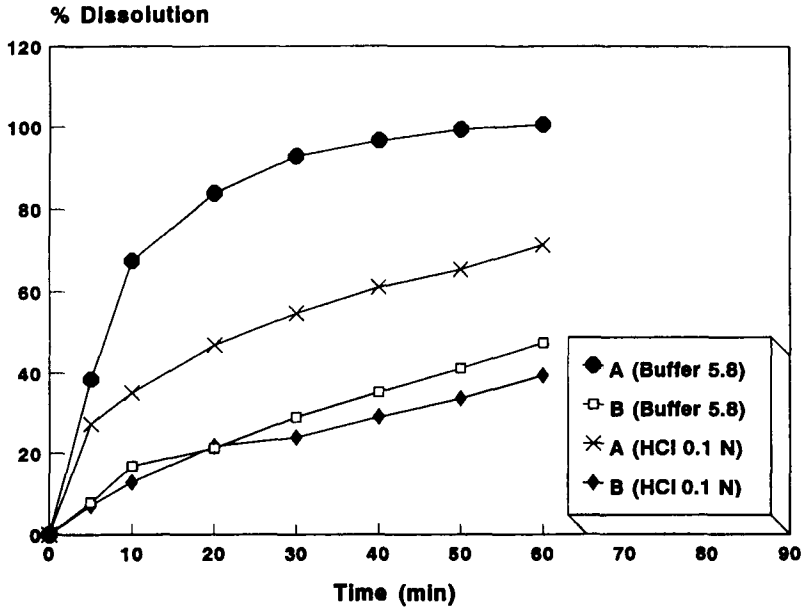

Fig. 1 : In vitro dissolution profiles of paracetamol formulations A and B.

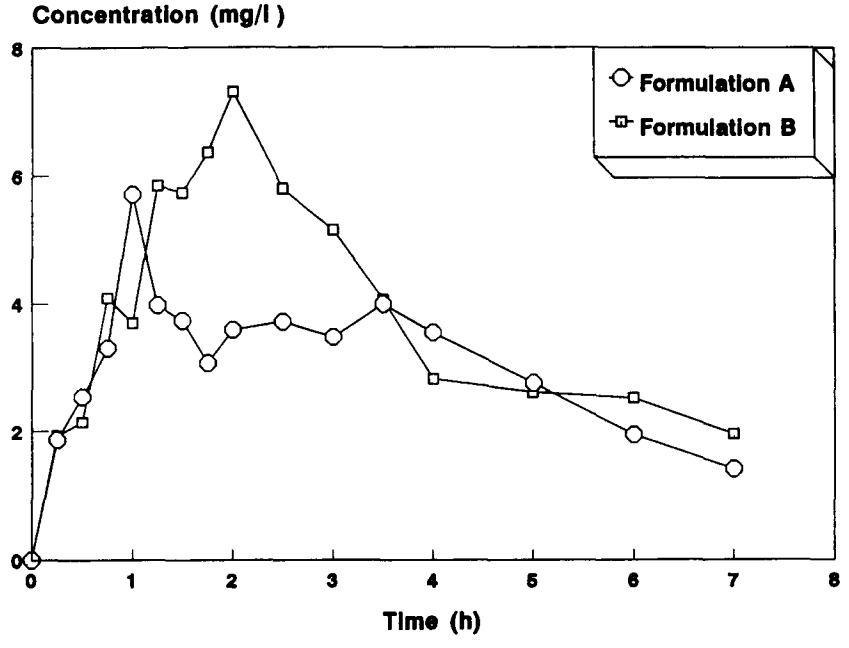

Fig. 2 : Mean profiles of paracetamol saliva concentration in function of time.

Figure 2 shows the mean curve of salivary levels of paracetamol in 5 volunteers, after administration of both formulations (A and B). Except in one subject, saliva level values obtained at 30 min (C_{30}) and at 60 min (C_{60}) were higher in Formulation A (Table I), correlating with results found in vitro.

Table II shows the values for T_{max}, C_{max}, $AUC_{(0-7)}$, and MRT, for each subject and for each formulation with their mean values and standard deviations.

An analysis of variance was made for values of C_{max}, AUC and MRT, and no significant differences were found ($P > 0.05$) between treatments. The values

Table I : Saliva levels at 30 and 60 min for formulations A and B

Subjects	*C (30 min) (mg/l)*		*C (60 min) (mg/l)*	
	A	*B*	*A*	*B*
1	1.9	1.41	3.83	3.06
2	0.62	4.15	2.72	6.29
3	3.62	1.06	6.71	1.59
4	3.42	1.85	5.58	3.8
5	3.17	1.96	2.13	2.1
Mean	2.55	2.08	4.19	3.36
s (n–)	1.27	1.2	1.92	1.84

for T_{max}, on the other hand, shows significant differences according to the Wilcoxon test ($P < 0.05$). This finding may indicate a retardation in Formulation B to reach C_{max}, correlating with results in vitro. Although the absorption process had a rapid beginning in Formulation A, the further absorption rate seems to be similar, for the two formulations, since MRT showed no significant difference.

Limits for the confidence interval of AUC, based on the intervalar hypothesis test were 0.79–1.36. Taking into account the tolerance region between 0.80–1.25, and the patient's safety when this analgesic is used, this interval can be accepted as indicative of bioquivalence of the two formulations.

Figure 3 shows the mean profiles of cumulative urinary excretion for both formulations of paracetamol, and Figure 4 the average urinary elimination rate as a function of time.

Table III shows the values for E_{15}, $(dE/dt)_{max}$ and t_{max} for each subject and for each formulation, with mean values and standard deviation.

The results of Student's *t*-test used for amounts of paracetamol excreted during the early time (0.5, 1 and 2 h), and for urinary elimination rate during the first hour, show significant differences between treatments ($P < 0.05$), indicating that urine follow up allows for observation of differences in the initial absorption rate, as was also possible in the saliva and in the in vitro study.

Table II : Bioavailability parameters for formulations A and B from saliva levels

Subjects	T_{max} *(h)*		C_{max} *(mg/l)*		$AUC_{(0-7)}$ *(mg.h/l)*		*MRT (h)*	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*
1	1.25	3	4.09	5.47	20.79	20.66	3.4	3.5
2	1.5	2	4.88	9.51	17.44	25	3	2.3
3	1	2.5	6.71	8.55	22	21.9	2.9	3.3
4	0.7	1.25	10.7	4.6	24.5	19.2	3.2	3.5
5	0.75	1.5	3.74	10.94	19.6	24.18	3.5	3
Mean	1.04	2.05	6.02	7.81	20.87	22.18	3.2	3.12
s (n–1)	0.33	0.72	2.85	2.69	2.63	2.4	0.25	0.5

Table III : Bioavailability parameters for formulations A and B from urine levels

Subjects	E_{15} *(mg)*		*dE/dt (mg/h)*		t_{max} *(h)*	
	A	*B*	*A*	*B*	*A*	*B*
1	23.48	17.76	2.79	2.9	5	4
2	12.15	12.05	1.69	1.8	4	6
3	23.31	14.66	3.55	1.61	3	7
4	13.93	14.38	3.86	3.29	2	3
5	12.59	12.28	1.76	2.92	4	2
Mean	17.09	14.22	2.73	2.5	3.6	4.4
s (n–1)	5.79	2.3	0.99	0.74	1.14	2.07

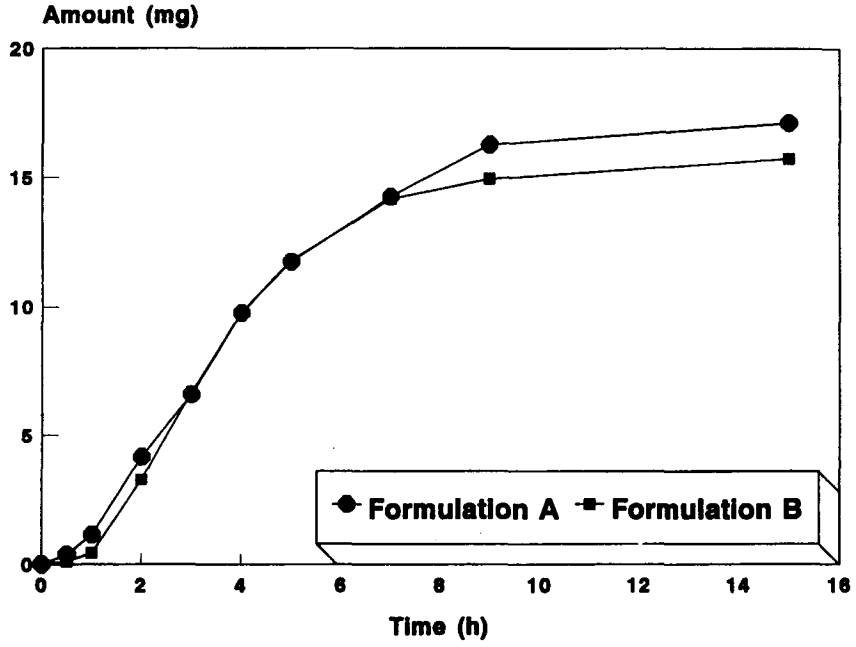

Fig. 3 : Mean profiles of paracetamol urinary excretion.

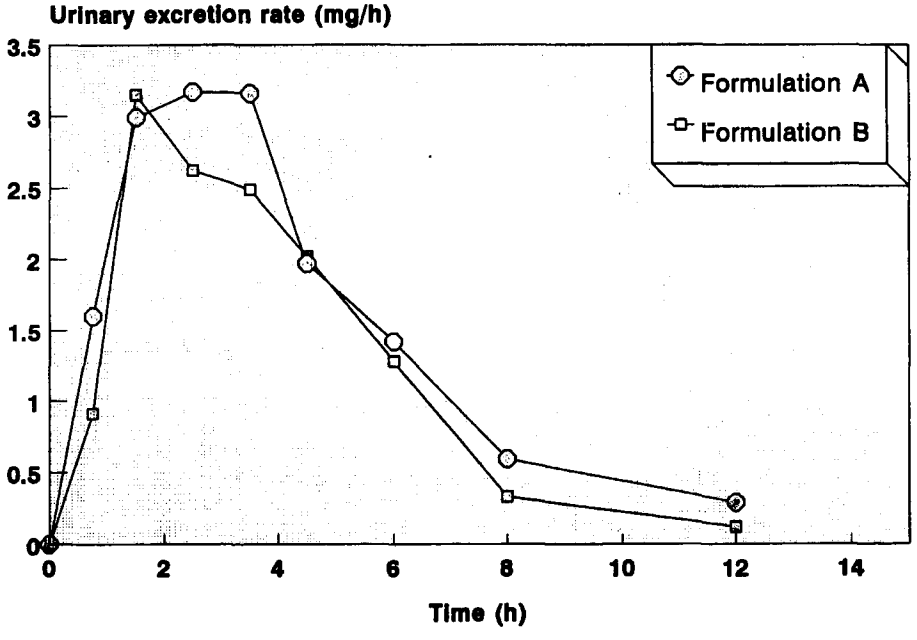

Fig. 4 : Average urinary elimination rate, as a function of time.

CONCLUSIONS

1.Bioequivalence studies of paracetamol tablets can be carried out using drug levels in saliva and urine.

2.Both formulations studied can be considered bioequivalent, although formulation A showed a more rapid initial rate of absorption than formulation B. These results were observed in saliva and in urine, and were predicted from the in vitro dissolution assays.

3.The results obtained with saliva partially correlated with those found in vitro and in urine.

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