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# Importance of Entero-Salivary Recirculation in Paracetamol Pharmacokinetics

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**ABSTRACT:** The contribution of an entero-salivary recirculation (salivary secretion—swallowed—reabsorption of drug from the gastrointestinal tract) to the values of the pharmacokinetic parameters of paracetamol was studied in a two-way crossover design. Five healthy volunteers took a tablet of Paracetamol (500 mg) in two occasions separated by a washout period. The difference between the two treatments consisted of saliva that was allowed or not to be swallowed during the 4 h of study. No statistically significant differences were found in the values of the pharmacokinetic parameters between treatments.

The half-life time calculated from salivary levels was similar to the values previously reported by other authors. The percent of the oral dose excreted in saliva during 4 h of study was very low (0.1%). Secondary peaks appeared in 8 of 10 profiles. The lack of influence of salivary secretion on the pharmacokinetic parameters of Paracetamol and the low percent secreted in this fluid suggests that entero-salivary recirculation is a possible physiological phenomenon undergoing after oral administration, but it is not one of the principal phenomenon that defines the pharmacokinetic of the drug. We confirm that working with salivary samples in pharmacokinetic studies of paracetamol is a useful tool. Copyright © 2002 John Wiley & Sons, Ltd.

**Key words:** paracetamol; salivary secretion; entero-salivary circulation

## Introduction

For almost four decades, paracetamol has been extensively studied to find a correlation between plasma and salivary levels in man [1–6]. During the absorption process the drug first enters into the arterial circulatory system including the salivary glands [3]. Then, a good correlation between salivary levels and free drug of the arterial blood that irrigates salivary glands is found. Previous work suggested that pharmacokinetic studies could be carried out in saliva bearing in mind some physiological and technical considerations [5,7–9]. It is evident that working with a non-invasive technique of sample collection has many advantages for the volunteers and investigators that participate in the study.

Proposed mechanism responsible for the existence of secondary peaks in the plasmatic profiles after oral administration of paracetamol to man and rats include enterohepatic recirculation [10–12] and variable gastric emptying [13–15].

The fraction of the orally administrated dose of paracetamol secreted in saliva and afterwards swallowed could be available in the gastrointestinal tract (GI) for a new absorption (entero-salivary recirculation).

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Generally, a secondary peak in the plasmatic profile appears when around 20% of the dose was previously absorbed. The quantity of drug secreted in saliva and swallowed could be reabsorbed giving a new peak in the salivary profile or at least contribute to the genesis of it. Then, secretion of drug in saliva would be reflecting the absorption process.

Saliva is more sensitive than venous blood samples for absorption studies in man [10]. For this and because of technical reasons we chose saliva to search for the importance of entero-salivary recirculation (absorption—salivary secretion—swallow—reabsorption of drug from the GI) in the pharmacokinetics of paracetamol in man. We studied the percent of drug secreted in saliva during 4 h of study and the influence of this phenomenon on the values of the pharmacokinetic parameters and shapes of salivary profiles after oral administration of paracetamol (500 mg) in man.

## Materials and Methods

### *Study design*

Five healthy volunteers (3 female, 2 male) who were 26–29 years old (mean: 27 years) and weighed 55–85 kg (mean: 64.4 kg) took part in the study. Their good health was previously established. Participants were non-smokers taking no other medications during the study. Drinks or beverages containing xantines or alcohol were not allowed.

All the subjects gave written consent after being advised of the nature and risks of the study. Approval to conduct the study was obtained from Ethical Committee formed by professors of the Faculty of Pharmacy and Biochemistry, University of Buenos Aires.

Subjects who had fasted overnight, the following morning they brushed their teeth without toothpaste and rinsed their mouth with water that was discarded. The first sample (time zero of the salivary profile) was taken at 8.00 a.m. by spitting directly into sterile pots (non-stimulated saliva). Afterwards, each volunteer took one tablet of paracetamol 500 mg (Tylenol®, McNeil Consumer Healthcare) with 250 ml of water in two occasions separated by 48 h washout period according to a two-way crossover design (treatments 1 and 2). The differences between the two treatments were based on the sampling schedule and whether saliva was allowed or not to be swallowed (treatments 2 and 1, respectively).

Under treatment 1, saliva samples were collected over 15-min intervals throughout a 4-h period after the oral administration. Saliva was not swallowed during the time sample. For treatment 2, saliva samples were taken between 6.5 and 8.5 min of each 15-min interval throughout a 4-h period. Saliva was swallowed during the study except while taking the samples.

Samples were stored in freezer ($-40^{\circ}$C) until paracetamol quantification.

### *Analysis of samples by HPLC*

Saliva was analyzed by HPLC after validating a modified method of Goicoechea *et al.* [16].

Saliva was thawed and centrifuged at 10 000 rpm for 10 min. The supernatant (sample) was separated to determine paracetamol concentration.

To 250 µl of sample, 250 µl of acetonitrile/$H_3PO_4$ 2.6% were added, vortex mixed for 2 min, centrifuged at 10 000 rpm for 20 min and separated the supernatant for direct injection.

The chromatographic system consisted of a Spectra System P200 pump, Thermo Separation sample injector, UV-100 Spectra Series ultraviolet detector set at 248 nm. The analytical column ($250 \times 4.6\,mm^2$) was a RP-C18, 5 µm (Microsorb MV, Varian) maintained at room temperature. The injected volume was 20 µl. The mobile phase consisted of 0.1 M potassium phosphate monobasic/methanol/acetic acid (83:16:4.9) delivered at a flow rate of 1 ml/min. The chromatographic data were calculated with the computer program PC1000.

The linear range was 0.1–40 µg/ml and inter- and intra-day coefficients of variation were both $<5\%$. The recovery percentage for paracetamol in saliva was 100, 98 and 94% for 0.2, 5 and 20 µg/ml, respectively.

### *Statistical analysis*

The following pharmacokinetic parameters were calculated from paracetamol salivary levels

obtained under the two treatments using the non-linear regression program TOPFIT 2.0 (18): total area under the saliva concentration–time profile from zero to infinity (AUC), half-life time ($T_{1/2}$), apparent volume of distribution ($V_z/F$) and clearance (Cl/$F$). Values of Cl and $V_z$ are expressed referring to the bioavailability ($F$). The AUC was calculated by the linear trapezoidal rule extrapolating to infinity the last experimental point. The maximum salivary concentration ($C_{max}$) and time for the peak ($T_{max}$) were determined by visual inspection of the individual salivary profiles.

The Wilcoxon signed rank sum test (non-parametric test for paired samples) was used to compare the values of the parameters between models. The significance level of the test was set to 0.05. Values are reported as medians $\pm$ interquartile range.

## Results

Table 1 shows the individual and median values of the pharmacokinetic parameters obtained under the two treatments. No statistically significant differences were observed for AUC, $V_z/F$, Cl/$F$, $T_{1/2}$ or $C_{max}$ comparing between treatments according to Wilcoxon $T$-test. The median AUC (interquartile range) value for treatment 1 was 22.4 [19.8–25.4] μg h/ml, whereas for treatment 2 it was 25.1 [21.0–31.5] μg h/ml. The mean volume of saliva excreted throughout the 4-h period during treatment 1 was $118.5 \pm 55.9$ ml, expressed as mean $\pm$ standard deviation. The mean percents of drug excreted in saliva were $0.105 \pm 0.054\%$ and $0.02 \pm 0.004\%$ for treatments 1 and 2. The difference between the former percents is the amount of drug in the saliva that was swallowed. Even though the former difference is statistically significant ($p < 0.05$), the quantity of drug swallowed is too small to show a difference in the values of the AUC between treatments.

The median values obtained for the half-life time (interquartile range) were 1.6 [1.46–1.76 h] and 1.75 [1.75–1.86 h] for treatments 1 and 2, respectively. The finding of essentially identical saliva paracetamol elimination half-life is in agreement with the previously reported by Kamali *et al*. [4,8,9].

Table 1. Pharmacokinetic parameters obtained from salivary levels of Paracetamol for two treatments: (1) without swallowing saliva and spitting all the study in sterile pots and (2) swallowing saliva during the study except when collecting the samples

| Subject | *AUC* (μg h/ml) | $T_{1/2}$ (h) | *Cl/F (ml/min)* | $V_z/F$ (l) | $C_{max}$[a] (μg/ml) |
|---|---|---|---|---|---|
| **Treatment** 1 | | | | | |
| 1 | 19.8 | 1.76 | 420 | 64.0 | 5.0 |
| 2 | 16.4 | 1.6 | 508 | 70.1 | 6.1 |
| 3 | 30.2 | 1.82 | 276 | 43.6 | 16.3 |
| 4 | 22.4 | 1.42 | 372 | 45.6 | 6.8 |
| 5 | 25.4 | 1.43 | 329 | 40.7 | 14.8 |
| **Median** | 22.4 | 1.6 | 372 | 45.6 | 6.8 |
| 1° quartile | 19.8 | 1.43 | 329 | 43.6 | 6.1 |
| 3° quartile | 25.4 | 1.76 | 420 | 64.0 | 14.8 |
| **Treatment 2** | | | | | |
| 1 | 20.6 | 1.75 | 405 | 61.3 | 9.1 |
| 2 | 21 | 1.75 | 396 | 60.1 | 11.7 |
| 3 | 31.5 | 1.71 | 264 | 39.2 | 16.1 |
| 4 | 25.1 | 1.86 | 332 | 53.3 | 10.1 |
| 5 | 32.7 | 2.07 | 255 | 45.7 | 8.4 |
| **Median** | 25.1 | 1.75 | 332 | 53.3 | 10.1 |
| 1° quartile | 21 | 1.75 | 264 | 45.7 | 9.1 |
| 3° quartile | 31.5 | 1.86 | 396 | 60.1 | 11.7 |

[a] $C_{max}$ is obtained by means of visual inspection of the concentration–time profiles.
AUC: area under the saliva concentration–time curve extrapolated to infinity.
Cl: clearance.
$V_z$: apparent volume of distribution.
$C_{max}$: maximum drug concentration observed from salivary profile.
$T_{1/2}$: half-life time.
$F$: bioavailability.

The median salivary concentration-time profiles under treatments 1 and 2 for 5 subjects are represented in Figure 1(A) and 1(B), respectively. Data are presented as medians with the interquartile ranges shown as vertical lines. Secondary peaks appear in the individual profiles (not shown) of 4 of 5 volunteers under treatment 1 but these peaks are occluded in the median profile. The same happened for treatment 2 and its median profile.

Considering the median values of $T_{max}$: 0.63 [0.625–1.125] h and 0.64 [0.38–0.64] h (treatments 1 and 2) no significant difference between treatments was found according to Wilcoxon $T$-test.

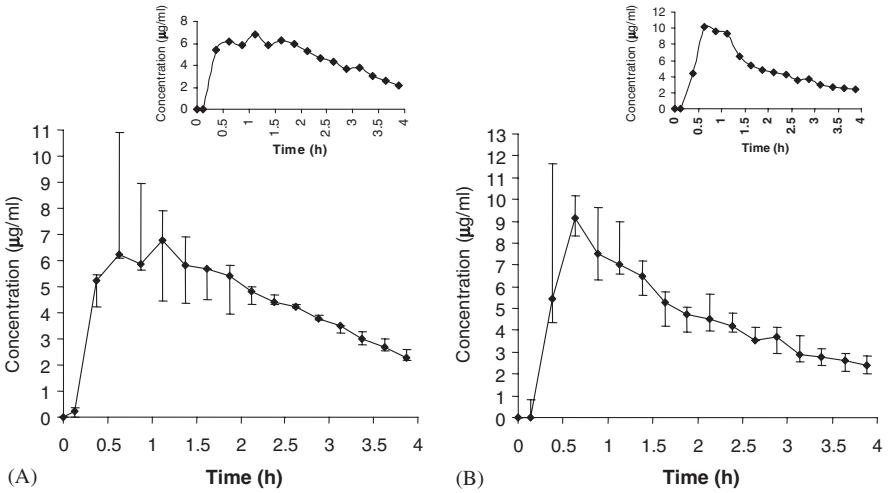


Figure 1. Salivary profiles after (A) treatment 1 and (B) treatment 2 from 5 subjects that received an oral dose of paracetamol (500 mg tablet). Data are expressed as medians ± interquartile range. Inserts show representative salivary profiles from subject 4 after both treatments

## Discussion

Phenomena postulated to explain the existence of secondary peaks in the plasmatic profiles after oral administration of paracetamol in man include enterohepatic recirculation. After oral administration, a fraction of the dose is absorbed from the GI reaching the systemic circulation. The principal metabolites formed in the liver are the conjugates of paracetamol with glucuronic and sulfuric acids eliminated by biliary and renal excretions [11,17,18]. The conjugates are stored in the gallbladder until some stimulus evokes its liberation. When they reach the GI, the drug is reabsorbed probably after hydrolysis of the conjugates. A new peak in the profile would be observed if the quantity reabsorbed is at least 20% of the administrated dose.

We observed secondary peaks in 8 of 10 salivary profiles considering both treatments. Subjects 1, 4 and 5 showed secondary peaks in their salivary profiles under treatments 1 and 2. Subjects 2 and 3 showed secondary peaks only under treatments 1 and 2, respectively. Then, secondary peaks appeared independently from the fact that drug secreted in saliva had been swallowed or not.

When a fraction of the orally administrated dose of paracetamol is absorbed in the GI, it reaches a closed system including the blood that irrigates the salivary glands. Paracetamol is a drug that rapidly equilibrates between saliva and plasma. Except during the absorption phase, the ratio $S/P$ (saliva to plasma concentration ratio of paracetamol at the corresponding times) is around 1. Thus, the salivary levels of paracetamol reflect the kinetic aspect of the drug in plasma. The median values for $Cl/F$ found for treatments 1 and 2 were 5.78 and 5.15 ml/min kg, respectively. The value reported in literature calculated from plasmatic levels is 5.15 ml/min kg, considering an average weight of 70 kg [19]. For $V_z/F$ we found 0.71 and 0.83 l/kg for treatments 1 and 2, respectively. In literature it is reported 1.1 l/kg, value calculated from plasma concentrations. The pharmacokinetic parameters values obtained from salivary levels are really closed to those previously reported calculated from plasma levels. Then, working with a non-invasive technique would enable to study some of pharmacokinetic aspects of drugs with similar characteristics to paracetamol.

An amount of the absorbed drug is secreted into saliva and as it can be swallowed it would be available in the GI to be reabsorbed. This quantity of reabsorbed drug can produce itself or contribute to the existence of secondary peaks

 

if it is a considerable amount of drug. Moreover, modifications in the pharmacokinetic parameters of the drug can also result because of this amount of drug salivary secreted and possible reabsorbed (entero-salivary recirculation). In the present work, we intended to evaluate the influence of salivary secretion on the calculus of the kinetic parameters of paracetamol. We compared the percents of drug secreted in saliva when subjects were allowed or not to swallow the saliva during the study and the differences in the values of the pharmacokinetic parameters between treatments. Then, if any significant difference could be found in these parameters they could be thought as a result of the drug swallowed with the saliva.

The percents of the administrated dose of paracetamol secreted in saliva under both treatments were very low (around 0.1 and 0.02% for treatments 1 and 2, respectively). No significant differences were found in the kinetic parameters comparing between treatments.

Therefore, for all the results previously discussed we suggest that the entero-salivary circulation is not a physiological phenomenon of relevance in the pharmacokinetic of the drug.

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