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Research Paper

Investigating paracetamol pharmacokinetics using venous and capillary blood and saliva sampling

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Abstract

Objective The aim of this study was to develop, validate and apply a high performance liquid chromatography (HPLC) assay for analysis of paracetamol, paracetamol glucuronide and paracetamol sulfate in plasma (venous and capillary) and saliva to study paracetamol pharmacokinetics in healthy volunteers.
Methods Samples were prepared using protein precipitation and analysed using reverse phase HPLC with UV detection. This assay was applied to venous and capillary plasma and saliva samples from 20 healthy volunteers after paracetamol 1 g four times daily for three days.
Key findings The HPLC assay for paracetamol and its metabolites was found to be sensitive and selective in plasma and saliva samples over the range 0.05–50 mg/l with an inter- and intraday precision and accuracy within 11.2% and 11.1%, respectively. Mean recoveries for all analytes were > 88%. A study of paracetamol pharmacokinetics in healthy volunteers found close agreement between the sampling matrices for paracetamol and metabolites (metabolites were not detected in saliva). The value for area under the concentration–time curve over the 6 h dosing interval of venous plasma (45.3 ± 12.9 mg/l.h) was significantly higher than that observed for capillary plasma (33.8 ± 12.9 mg/l.h) or saliva (35.1 ± 9.4 mg/l.h; $P > 0.01$).
Conclusions Capillary blood and saliva collection were found to be reliable sampling matrices for the evaluation of paracetamol pharmacokinetics, although paracetamol metabolites were not detected in saliva.

Introduction

Paracetamol is a widely used analgesic and antipyretic medicine used for the relief of mild to moderate pain and fever.[1] Paracetamol metabolism occurs via both oxidation and conjugation pathways, however, at therapeutic doses conjugation with glucuronic and sulfuric acid results in the major primary metabolites paracetamol glucuronide and paracetamol sulfate.[2] When studying paracetamol pharmacokinetics, it is important to assess the pharmacokinetics of these major metabolites and, as such, selection of an appropriate analytical method to measure all three analytes is essential.

Paracetamol concentration has been measured using a variety of methods including colorimetric, spectrophotometric, spectrofluorimetric, capillary electrophoretic, electroanalytical and chromatographic methods, as well as chemiluminescence and immunoassay.[1,2] The high selectivity and sensitivity of high performance liquid chromatography (HPLC) has meant it is has been used widely for the analysis of paracetamol and its metabolites.[1–4]

Paracetamol pharmacokinetic studies have used a number of biological matrices including whole blood, plasma, urine and saliva, and a number of studies have compared concentrations of paracetamol in several of these biological matrices simultaneously.[1,2,5–7] Adithan and Thangam[8] compared saliva and venous serum concentrations in 10 healthy volunteers and found that paracetamol concentrations in saliva were well correlated with those found in serum. However, Smith *et al.*[9] compared serum from venous blood and saliva in 20 healthy volunteers and found that, while there was a significant correlation between serum and saliva plasma concentrations, the agreement between the actual values was poor.

Numerous studies have investigated the comparison between plasma and saliva concentrations. Several studies

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have indicated that contrary to results for other drugs, salivary paracetamol concentrations are consistently higher than those found in plasma, with the difference being particularly pronounced during the first 50 min after administration of the paracetamol, while during the elimination phase observed differences in concentrations were minimal.[7,10]

In a recent study, Mohammed *et al.*[6] compared paracetamol concentrations in blood from venous sampling with capillary blood from finger-prick collection. Those researchers found that while there was a strong correlation between the paired samples, care should be taken when investigating paracetamol pharmacokinetics due to differences in capillary blood and venous blood concentrations observed during the absorption phase.

It has been postulated that the higher paracetamol concentration observed in saliva, when compared with plasma, occur because saliva concentrations of paracetamol reflect those found in arterial blood (as opposed to venous blood).[7] To date, no study has attempted to simultaneously compare the concentrations of paracetamol and its major metabolites in both venous and capillary blood as well as saliva.

The aim of this study was to develop and validate a HPLC assay for the simultaneous quantification of paracetamol and its major metabolites in venous and capillary plasma and saliva. This assay was then used to simultaneously investigate the pharmacokinetics of paracetamol and its major metabolites in these biological matrices in 20 healthy volunteers.

Materials and Methods

Materials

Paracetamol (Sigma Aldrich, St Louis, MO, USA), paracetamol sulfate (Sigma Aldrich), paracetamol glucuronide (Sigma Aldrich), 3-acetamidophenol (Sigma Aldrich), potassium dihydrogen orthophosphate (Ajax Finechem, Taren Point, NSW, Australia), perchloric acid (Sigma Aldrich), phosphoric acid (Univar Australia Pty. Ltd, Ingleburn, NSW, Australia), THF (Ajax Finechem) and isopropanol (Sigma Aldrich) were purchased and all were of analytical grade or above. Water was purified with a Milli-Q system. Analyte-free plasma samples were sourced from the blood bank at Concord Repatriation General Hospital and analyte-free saliva was obtained from healthy volunteers. Both drug-free plasma and saliva were analysed to identify potential co-eluting chromatographic peaks as part of the HPLC analysis.

Assay

Samples were analysed using a Shimadzu HPLC system consisting of a SCL-10AVP system controller, SPD-10AVP diode array UV detector (detecting eluent at 254 nm), SIL-10ADVP autoinjector and LC-10ATVP pump set to 0.8 ml/min. Mobile phase consisted of potassium dihydrogen orthophosphate (0.1 M)–isopropanol–tetrahydrofuran (THF) (100 : 1.5 : 0.1, v/v/v, pH 3.7, adjusted with orthophosphoric acid), which was filtered and sonicated before being pumped through an octadecylsilane HPLC column (Grace, Baulkham Hills, NSW, Australia; 4.6 mm × 25 cm × 5 μm). Analytical run time was 60 min and retention times for paracetamol glucuronide, paracetamol sulfate and paracetamol were 8.7, 20.8 and 28.5 min, respectively. An internal standard method was used with 3-acetamidophenol as the standard, which eluted at 49.5 min.

Sample preparation

Standard samples and quality control (QC) samples were thawed and test samples were aliquoted (100 μl venous plasma and saliva versus 20 μl capillary plasma). Each sample was then diluted with Milli-Q water (80 and 16 μl) and internal standard (3-acetaminophen, 10 mg/l) was added (10 and 2 μl) followed by perchloric acid (10 and 2 μl). Samples were vortex mixed before being centrifuged (16 100*g* for 5 min) and were then injected into the column (50 and 20 μl).

Assay validation

The assay performance was investigated using replicate analyses of calibration curves of standard samples at 0.05, 0.5, 5 and 50 mg/l for each analyte analysed twice a day on three consecutive days for each biological matrix. QC samples at concentrations of 0.2, 2 and 20 mg/l for each analyte were positioned randomly throughout the standards and run in triplicate. Peak heights were measured and the ratio of analyte peak height: internal standard peak height was calculated. Least squares linear regression analysis was used to prepare calibration curves and calculate slope, y-intercept and linearity, reported as coefficient of determination (r^2). QC samples were used to calculate the intra- and interday accuracy and precision of the method. Accuracy was reported as the back calculated concentration divided by the nominal concentration of the QC samples, while precision was expressed as the coefficient of variation (CV%) of the replicate samples. Percentage recovery was calculated by the running of replicate standards diluted in Milli-Q water during one calibration curve for comparison with the replicate biological samples. The lower limit of quantification (LLOQ; 0.05 mg/l) was validated by further analysis of six replicate samples. A run was considered invalid if greater than one QC sample exceeded the accepted error (15% or 20% for LLOQ).

Clinical study

Ethics approval for this study was granted by the Sydney South West Area Health Service Human Research Ethics Committee, Concord Repatriation General Hospital. Twenty

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healthy volunteers were recruited. Participants were excluded if they were under 18 years of age, pregnant or breastfeeding, had a history of liver, kidney or gum disease or a history of alcohol abuse. Volunteers were asked to take two 500 mg paracetamol tablets (Panamax; Sanofi-Aventis, Macquarie Park, NSW, Australia) four times daily for three days. A steady-state paracetamol dosing protocol was utilised to reflect the clinical use of the drug. On the fourth day volunteers attended the hospital having fasted since the previous night and were provided with a standard breakfast. Half an hour following consumption of breakfast each volunteer had a cannula inserted into the antecubital vein and a systemic venous blood sample (9 ml), a sample of finger-prick blood (100 μl collected into an microcentrifuge tube using a Unistik 2 capillary blood sampling device) and a sample of unstimulated saliva were collected. Volunteers then received a final dose of two 500 mg paracetamol tablets and samples of each biological matrix were collected at 0.5, 1, 2, 4 and 6 h following drug administration.

All blood samples were centrifuged at 16 100*g* for 10 min immediately following sampling and plasma was harvested. All samples were stored at −20°C until analysis. Comparisons of paracetamol and metabolite concentrations observed in each biological matrix were assessed by calculation of Pearson's correlation coefficient. The area under the concentration–time curve over the 6 h dosing interval ($AUC_{0\text{-}\tau}$) at steady-state was calculated using the linear trapezoidal rule, where τ is the duration of the dosing interval at steady-state (6 h). The maximum concentration (C_{max}) for paracetamol and its major metabolites at steady-state was determined by observation. The distribution of pharmacokinetic parameters was assessed for using the Kolmogorov-Smirnov test. One-way analysis of variance with Bonferroni post-hoc correction was used to compare pharmacokinetic parameters between matrices. All statistical analysis was conducted using PASW statistics software version 18.0 (IBM SPSS, Chicago, Illinois, USA, 2009) and significance was considered at the $P < 0.05$ level.

Results

HPLC assay performance

The assay was shown to be selective for both paracetamol and its major metabolites in all matrices. No co-eluting endogenous chromatographic peaks were observed across six samples each of drug-free plasma and saliva. The assay was found to be linear over the concentration range 0.05–50 mg/l for all analytes in both saliva and plasma. Inter- and intraday precision and accuracy for all standards and QC samples were within acceptable ranges (i.e. within 15% of nominal values and 20% at LLOQ). Details of the intra- and interday precision and accuracy for plasma and saliva assays are presented in Tables 1 and 2. The recovery of paracetamol and its sulfate and glucuronide conjugates was calculated to be 88.4 ± 3.6%, 88.4 ± 5.0% and 88.7 ± 6.9%, respectively. The recovery of the internal standard was calculated to be 91.1 ± 5.3%. These results indicated that the assays for plasma and saliva were selective, linear, accurate and precise over the concentration range 0.05–50 mg/l for paracetamol and its major metabolites.

Paracetamol pharmacokinetics

Twenty participants completed the study and no adverse effects were observed. The participants (14 female) had a mean (±SD) age of 35.6 ± 13.0 years with a mean total body weight of 75.9 ± 19.8 kg and height of 1.64 ± 0.09 m.

The mean concentration–time profiles for paracetamol in venous plasma, capillary plasma and saliva are illustrated in Figure 1. Figure 2 presents the mean concentration–time data for the metabolites in capillary and venous plasma ($n = 20$). These results indicated that the concentrations of paracetamol and its metabolites presented similar temporal patterns across biological matrices. Peak paracetamol concentration was observed at 1 h following administration of paracetamol in all matrices, with the highest peak concentration, in this particular individual, being observed in saliva followed by venous plasma with the lowest concentration observed in capillary plasma. The collective results confirmed the good agreement in the temporal pattern of paracetamol concentrations across biological matrices; in particular, in the first hour following paracetamol administration, venous plasma had the highest observed paracetamol concentrations and capillary plasma the lowest concentrations. Metabolites were not detected at quantifiable concentrations in saliva; however, peak concentration of metabolites was similar between venous and capillary plasma, with peak concentrations occurring later in capillary plasma than venous plasma.

The concentration–time profiles for paracetamol sulfate and paracetamol glucuronide in venous and capillary plasma were in close agreement at all time points.

The correlations between paracetamol concentrations in saliva and venous plasma and capillary plasma and venous plasma were investigated. These results indicated that saliva ($r^2 = 0.64$, $P < 0.001$) and capillary plasma ($r^2 = 0.64$, $P < 0.001$) paracetamol concentrations were reliable predictors of venous plasma paracetamol concentrations.

The pharmacokinetic parameters of paracetamol and its metabolites in each biological matrix are summarised in Table 3. Paracetamol *AUC* and C_{max} data were found to be normally distributed ($P \geq 0.05$). A one-way analysis of variance with Bonferroni post-hoc correction indicated that the only significant differences between the matrices occurred for the *AUC* for paracetamol with the *AUC* for venous plasma being significantly higher than either the saliva ($P = 0.025$) or capillary plasma *AUC* ($P = 0.010$) (Table 3). Otherwise, these

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Table 1 Intraday precision and accuracy in plasma and saliva

Plasma						
	Paracetamol		Paracetamol glucuronide		Paracetamol sulfate	
Nominal concn (mg/l)	%CV	Difference from nominal concn (%)	%CV	Difference from nominal concn (%)	%CV	Difference from nominal concn (%)
Standards						
0.05	9.1	9.2	10.4	6.5	11.1	6.0
0.5	6.5	2.7	4.5	2.6	4.5	5.2
5	2.2	1.4	3.3	2.8	3.4	1.9
50	3.9	1.7	4.3	2.5	5.8	1.6
Quality controls						
0.2	5.4	10.2	5.9	5.7	5.5	1.1
2	4.1	6.3	6.3	3.7	6.5	5.3
20	4.2	2.2	4.6	3.2	5.4	1.8
Saliva						
	Paracetamol		Paracetamol glucuronide		Paracetamol sulfate	
Nominal concn (mg/l)	%CV	Difference from nominal concn (%)	%CV	Difference from nominal concn (%)	%CV	Difference from nominal concn (%)
Standards						
0.05	6.3	5.2	5.7	5.1	7.8	6.8
0.5	4.2	2.6	4.4	2.5	4.4	3.2
5	2.9	1.7	3.5	2.1	2.4	2.2
50	1.5	1.5	1.2	0.9	1.8	1.7
Quality controls						
0.2	5.5	4.9	5.1	3.7	3.3	2.5
2	3.2	3.5	2.8	1.4	3.1	2.0
20	2.1	2.5	2.6	1.4	3.2	1.4

n = 2.

results showed close agreement in pharmacokinetic parameters observed among different matrices.

Discussion

Numerous studies have investigated paracetamol pharmacokinetics in a range of populations however, the majority of studies thus far have selected plasma or serum from venous blood as the matrix of choice for these assessments.[11–14] Due to the invasive nature and the more restrictive clinical setting required for venous blood sampling, some studies have investigated the potential use of other biological matrices for the investigation of paracetamol pharmacokinetics including urine, capillary blood and saliva.[1,2,6,9,15] No single study however, has simultaneously investigated the pharmacokinetics of paracetamol and its major metabolites paracetamol sulfate and paracetamol glucuronide in venous blood, capillary blood and saliva. These less invasive sampling methods have the advantage of being able to be used outside of a controlled clinical setting, as well as in special patient populations (e.g. paediatric and geriatric patients) where traditional sampling techniques may be complicated by clinical and ethical issues.

This study reports the development and validation of a valid, sensitive and selective HPLC assay method for the measurement of the concentration of paracetamol and its major metabolites in plasma and saliva. This method was then used to investigate the pharmacokinetics of paracetamol using venous plasma, capillary plasma and saliva sampling in a cohort of healthy volunteers.

The developed chromatographic assay was shown to be sufficiently valid as the accuracy and precision of the inter- and intraday paracetamol, paracetamol glucuronide and paracetamol sulfate concentrations were less than 11.2% for all plasma and saliva concentrations. These results were in agreement with several other studies.[1,2] Analyte recovery were similar in both matrices and similar recoveries were also seen in other similar studies.[11] The concentration–time profile of paracetamol and its metabolites in plasma and saliva were in agreement with previous studies.[1–3,10]

This study found a significant correlation between the concentration of paracetamol observed in venous plasma and those observed in both saliva and capillary plasma, which is in agreement with other studies which have found correlations between venous plasma and saliva paracetamol concentrations.[15,16] Results of this study indicated that despite not

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Table 2 Interday precision and accuracy in plasma and saliva

Plasma						
	Paracetamol		Paracetamol glucuronide		Paracetamol sulfate	
Nominal concn (mg/l)	%CV	Difference from nominal concn (%)	%CV	Difference from nominal concn (%)	%CV	Difference from nominal concn (%)
Standards						
0.05	7.2	5.5	7.0	1.0	8.2	1.0
0.5	5.2	1.8	2.6	2.6	3.9	4.2
5	1.5	0.5	2.3	1.9	2.3	0.8
50	2.2	0.5	3.3	0.2	1.5	0.5
Quality controls						
0.2	6.2	6.5	4.8	2.7	2.1	0.3
2	3.9	3.3	4.8	0.7	5.8	2.8
20	3.8	2.0	3.6	1.1	4.1	0.2
Saliva						
	Paracetamol		Paracetamol glucuronide		Paracetamol sulfate	
Nominal concn (mg/l)	%CV	Difference from nominal concn (%)	%CV	Difference from nominal concn (%)	%CV	Difference from nominal concn (%)
Standards						
0.05	5.2	4.2	8.2	6.8	11.2	8.0
0.5	3.6	1.6	2.4	1.6	2.4	3.2
5	1.3	0.9	2.1	1.4	2.1	1.8
50	0.4	0.3	1.1	0.6	1.4	0.7
Quality controls						
0.2	5.6	5.5	5.1	3.7	2.0	1.9
2	2.5	2.3	2.8	1.4	3.2	1.8
20	1.2	0.8	2.6	1.4	4.9	0.9

n = 2.

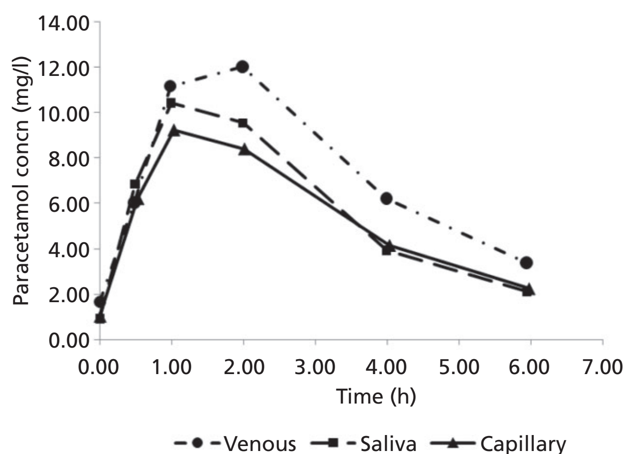

Figure 1 Mean paracetamol concentration–time profile of subjects in venous plasma, saliva and capillary plasma, *n* = 20.

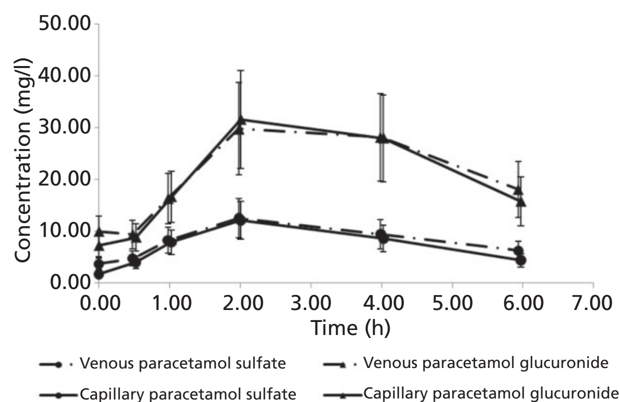

Figure 2 Mean paracetamol sulfate and paracetamol glucuronide concentration–time profiles of subjects in venous plasma and capillary plasma, *n* = 20.

reaching significance at individual time points, the correlation between matrices appeared to be strongest in the first hour following paracetamol administration, that is, during the absorption phase of paracetamol metabolism as opposed to the elimination phase. This result differed from that of Mohammed *et al.*[6] who compared paracetamol levels in whole capillary and venous blood samples from 12 healthy male volunteers, and Kamali *et al.*[16] who compared venous plasma and saliva samples from 10 healthy volunteers of

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Table 3 Pharmacokinetic parameters for paracetamol and its sulfate and glucuronide conjugates

	Paracetamol		Paracetamol sulfate		Paracetamol glucuronide	
	$AUC_{(0-\tau)}$ (mg/l.h)	C_{max} (mg/L)	$AUC_{(0-\tau)}$ (mg/l.h)	C_{max} (mg/l)	$AUC_{(0-\tau)}$ (mg/l.h)	C_{max} (mg/l)
Venous plasma	45.3 ± 12.9	14.8 ± 5.0	53.3 ± 20.8	13.1 ± 5.2	136.9 ± 54.4	33.6 ± 14.2
Capillary plasma	33.8 ± 12.9*	13.3 ± 6.1	47.5 ± 15.6	12.8 ± 3.6	136.1 ± 49.9	34.6 ± 13.8
Saliva	35.1 ± 9.4[a]	13.5 ± 5.2	ND	ND	ND	ND

$n = 20$. Figures expressed as mean ± SD. $AUC_{(0-\tau)}$, area under the concentration–time curve over the 6 h dosing interval; C_{max}, maximum observed concentration. *Capillary plasma vs venous plasma, $P = 0.010$. [a]Saliva vs venous plasma, $P = 0.025$. ND, not detected.

either sex. Both of those studies found that paracetamol concentrations differed most in the first hour with minimal or no significant differences at one hour post-paracetamol administration or later. Mohammed *et al*.[6] however, did not collect samples past one hour and when samples were collected at 15 min after paracetamol administration half of the subjects were not able to provide both capillary and venous blood samples. This may have led to some inconsistency at the lower time point. A possible limitation of this study is the testing of only one time point between administration and one hour post-administration. Testing at further time points during this period may have been useful in assessing this differing result.

Further to these results it was observed that paracetamol concentrations in venous plasma resulted in an increase in the time to C_{max} along with a significantly higher *AUC* for paracetamol than for either capillary plasma or saliva (which were not significantly different from one another). Previous studies have shown similar results, with Mohammed *et al*.[6] finding that paracetamol concentration was initially higher for capillary (finger-prick) blood than for venous blood, which took considerably longer to reach C_{max}. This was thought to be a result of the arteriovenous difference between the samples with capillary blood consisting of a mix of venous, arterial and capillary blood, which facilitates rapid transportation of the drug across capillary walls during the distribution phase of metabolism resulting in an initially higher paracetamol concentration in capillary blood than venous blood with venous blood reaching higher concentrations at a later time.[6]

There were no significant differences in concentrations of either metabolite between venous and capillary plasma at any individual time point and no significant differences in pharmacokinetic parameters (*AUC* or C_{max}). This indicated that capillary blood sampling was equally effective in assessment of these metabolites as venous blood sampling.

Comparison of the results of many studies of paracetamol pharmacokinetics can be difficult as studies vary widely with regard to the protocols employed including paracetamol dose regimen, sampling site, sample storage, sample preparation, assay methods etc. For example, while one study selected a cohort of healthy volunteers and assessed whole blood with HPLC, another study investigated patients in overdose using saliva samples and colorimetry.[6,15] These significant variations in methodology make comparing the results of the different studies reported in the literature challenging. To confirm the findings of this study and conclusively compare the results with those reported in the literature a larger subject cohort is required.

Conclusions

A sensitive and selective HPLC assay for paracetamol and its major metabolites was developed and validated. This assay was used to assess the pharmacokinetics of paracetamol and its metabolites in venous plasma, capillary plasma and saliva in a cohort of 20 healthy volunteers. This study found that while the metabolites of paracetamol were not detected in saliva, saliva paracetamol levels were comparable with venous plasma levels. Concentrations of paracetamol and its metabolites in capillary plasma were also comparable with concentrations in venous plasma. The less invasive nature of both saliva and capillary plasma sampling makes them potentially viable alternative sampling means for future studies, particularly in vulnerable populations such as geriatric and paediatric cohorts.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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