Correlation of paired toxic plasma and saliva paracetamol concentrations following deliberate self-poisoning with paracetamol

Jessamine H. Soderstrom,¹ Daniel M. Fatovich,² Christine Mandelt,³ Sam Vasikaran,⁴ David L. McCoubrie,¹ Frank F. Daly¹ & Sally A. Burrows⁵

¹Clinical Toxicologist, Emergency Physician, Royal Perth Hospital, ²Royal Perth Hospital, University of Western Australia and the Centre for Clinical Research in Emergency Medicine, Western Australian Institute for Medical Research, ³PathWest, Royal Perth Hospital, ⁴University of Western Australia and ⁵School of Medicine and Pharmacology and Royal Perth Hospital, University of Western Australia, Perth, WA, Australia

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Paracetamol is commonly used in deliberate self poisoning (DSP) and this requires blood sampling to refine risk assessment. If saliva concentrations agreed with plasma concentrations, then this could support the development of non-invasive testing. Our pilot work supports this hypothesis, but was largely confined to nontoxic concentrations.

WHAT THIS STUDY ADDS

- We found agreement between the indications for treatment of paracetamol DSP based on plasma and saliva paracetamol concentrations. Saliva may hold promise as a non-invasive method to risk stratify paracetamol poisoning.

Correspondence

Professor Daniel M. Fatovich MBBS
FACEM, Department of Emergency
Medicine, Royal Perth Hospital, Box X2213
GPO, Perth, WA 6847, Australia.
Tel.: +61 8 9224 2244
Fax: +61 8 9224 7045
E-mail: daniel.fatovich@health.wa.gov.au

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AIMS

Paracetamol is commonly used in deliberate self poisoning (DSP) and requires blood sampling to refine risk assessment. We aimed to test the agreement between plasma and saliva paracetamol concentrations in the toxic range in DSP.

METHODS

Contemporaneous paired plasma and saliva paracetamol concentrations were measured. Saliva was collected using a Sarstedt Salivette® device and the concentration was measured using a colorimetric method.

RESULTS

Fifty-six patients (44, 78% female) median age 26 years (IQR 20–41) were enrolled. The median reported paracetamol ingestion was 10 g (IQR 6–14). Specimens were collected at a median of 4 h (IQR 4–5.3) post ingestion. The median plasma and saliva paracetamol concentrations were 29 mg l⁻¹ (IQR 8–110) and 38 mg l⁻¹ (IQR 10–105) respectively [mean difference 8 mg l⁻¹, 95% confidence interval (CI) 2, 14]. Lin's concordance correlation was 0.97 (95% CI 0.96, 0.98). There were 15 patients who were treated with N-acetylcysteine. Their median reported paracetamol ingestion was 14 g (IQR 10–23) and samples were collected at a median of 4 h post ingestion. The median plasma and saliva paracetamol concentrations were 167 mg l⁻¹ (IQR 110–200) and 170 mg l⁻¹ (IQR 103–210) respectively (mean difference 15 mg l⁻¹, 95% CI –4, 35). Lin's concordance correlation was 0.94 (95% CI 0.88, 0.99). No patient needing treatment would have been missed using saliva concentrations only.

CONCLUSIONS

The agreement between the indications for treatment of paracetamol DSP based on plasma and saliva paracetamol concentrations extends into the toxic range, but with slightly lower agreement. Saliva may hold promise as a non-invasive method to risk stratify paracetamol poisoning.

Introduction

Paracetamol is a common pharmaceutical agent used in deliberate self poisoning [1]. In this setting plasma paracetamol concentrations are used for refining risk assessment with the use of the modified Rumack–Matthew nomogram [2, 3].

There have been several studies showing agreement between plasma and saliva paracetamol concentrations in healthy volunteers and in patients taking therapeutic amounts of paracetamol [4–12]. We reported a pilot study in 2008 that found agreement between paired plasma and saliva paracetamol concentrations in patients presenting with deliberate self poisoning (DSP). There was agreement between the indications for treatment with N-acetyl cysteine (NAC) based on plasma and saliva concentrations. However, this was a small study with only three patients with concentrations $>100 \text{ mg l}^{-1}$ and no patient had concentrations $>150 \text{ mg l}^{-1}$. Only one required NAC [9].

The aim of this study was to determine the agreement between plasma and saliva paracetamol concentrations in the toxic range in DSP. Toxic range was defined as an elevated paracetamol concentration requiring treatment with NAC [3]. The secondary aim was to assess the utility of a saliva collection device.

Methods

This study was conducted in the Emergency Department (ED) of the Royal Perth Hospital. This is an adult tertiary referral university hospital with an annual census of 64 000 with an admission rate of 45%. A Clinical Toxicology Service is based within the ED. The study was approved by the Royal Perth Hospital Ethics Committee (EC 2008/178). It was a convenience study, primarily dependent upon the presence of research nurses. All patients were enrolled prospectively and written informed consent was obtained.

All patients presenting to the ED with deliberate paracetamol ingestion were eligible to participate. Exclusion criteria were altered mental state, persistent vomiting, trauma to the oral cavity and delayed presentation ($>12 \text{ h}$ post ingestion). Suitable patients were identified by either the medical staff or the research nurses in the ED.

Patients had blood sampling according to routine clinical practice. In addition, contemporaneous collection of saliva was performed within 5 min of the blood sample using the Sarstedt Salivette® device. The absorbent plug within the device was chewed for 2 min to collect the saliva and then placed in the test tube provided. There was no mouth washing or medications to stimulate saliva [9]. Both samples were placed in a specimen collection bag and sent to the laboratory immediately after collection. Clinical staff were blinded to the results of the saliva concentration and all patients received standard care. A pre-formatted data sheet was used to collect data, including

patient demographics, reported paracetamol dose, time of ingestion, co-ingestants and problems with saliva collection.

Plasma and saliva paracetamol assays

Paracetamol is 15–21% bound to proteins at 280 mg l^{-1} [13]. It is a weak acid with a pK_a 9.5. The saliva : plasma ratio is independent in drugs that have a $\text{pK}_a > 8.5$ and the saliva : plasma ratio is equal to the unbound fraction of the drug in plasma at $\text{pK}_a > 8.5$ [14]. This means that there should be good correlation between plasma and saliva paracetamol concentrations in healthy subjects [5, 15].

Paracetamol is unionized at physiological pH and other chemical properties of the drug suggest that it is mainly transferred into the saliva by passive diffusion i.e. down a concentration gradient. Passive diffusion is influenced by the concentration gradient, the surface area over which the transfer occurs, the thickness of the membrane and a diffusion constant that pertains to the physico-chemical properties of the drug [14]. Saliva can contain traces of protein due to contamination from blood or gingival fluid and this may provide an additional route for the entry of drugs into saliva [14].

Salivary paracetamol may be dependent on salivary flow rate [7]. The Salivette® we used did not contain citrate so it did not stimulate salivary flow. The device absorbs the saliva into the cotton dental roll after chewing. The advantage of this is that it absorbs and collects a large volume of saliva (approximately 1.5 ml) in a short time rather than collecting a mixture of saliva and gingival crevicular fluid [7, 14].

The plasma and saliva samples were analyzed upon arrival at the laboratory using an enzymatic/colorimetric assay on the Abbott Architect C16000®. The assay measures total paracetamol. Aryl acylamidase cleaves the amide bond of the paracetamol leaving p-aminophenol and acetate. The p-aminophenol is reacted with 8-hydroxyquinoline-5-sulphonic acid in the presence of manganese ions to form a coloured compound. There is an increased absorbance at 615 nm, which is directly proportional to the concentration of the paracetamol in the sample [16]. The assay range was very similar for both assays ($3.0\text{--}377.6 \text{ mg l}^{-1}$).

The Salivette® devices were centrifuged at $3800 \text{ rev min}^{-1}$ (14 200 g) for 8 min. The container with the absorbent plug was then discarded and an aliquot of the sample was transferred into a labelled sample cup for analysis. The minimum sample volume required was 125 μl and the analysis time was 10 min.

The intended use of the Abbott Multigent paracetamol assay is for the quantitative determination of paracetamol in human serum or plasma on the Architect cSystem [16]. It has not been validated by Abbott for the quantification of paracetamol in saliva. There is no information on the intra-individual test assay variability for serum or plasma but there are performance characteristics that state within run,

between run, between day and a total standard deviation (SD) and % coefficient of variation (CV) for three concentrations of commercial controls [16]. These controls were assayed in duplicate twice a day for 20 days and each run was separated by at least 2 h. The results were well within the acceptance criteria of $CV \leq 5\%$ for samples $\geq 18 \text{ mg l}^{-1}$ or $\leq 1.25 \text{ SD}$ from $\geq 3 \text{ mg l}^{-1}$ to $< 18 \text{ mg l}^{-1}$. This approach was validated in our pilot study, which confirmed the accuracy and precision of the assay by spiking studies [17]. This showed (i) mean recovery (paracetamol concentration $37.5\text{--}525 \text{ mg l}^{-1}$) 100.01% ($+0.02 \text{ SD}$), (ii) precision of repeated assay over 24 h period $CV 4\%$ and (iii) lower limit of detection 0.9 mg l^{-1} .

Statistical analysis

Data were recorded and analyzed using SPSS (PASW Statistics 18, Chicago, USA) and Stata 11 (StataCorp. 2009, Stata Statistical Software: Release 11, College Station, TX: StataCorp LP). Standard descriptives were obtained and medians were used to report skewed data. Agreement between plasma and saliva concentrations was assessed by Lin's concordance correlation and a Bland–Altman plot. The Bland–Altman plot is obtained by calculating the mean of and the difference between each pair of readings and plotting the n differences against their corresponding means. If the 95% limits of agreement around the mean difference correspond to differences that are not clinically important and most of the points lie within these limits, then the two methods can be used interchangeably [18]. While investigators often use the Pearson correlation coefficient to compare methods of measurement, this cannot detect systematic biases, only random error [19]. Lin's concordance correlation modifies the Pearson correlation coefficient which assesses the closeness of the data about the line of best fit in the scatter plot by taking into account how far the line of best fit is from the 45° line through the origin [18]. These plots graphically illustrate the departure from the line of perfect agreement.

A power calculation found that 15 patients with a toxic paracetamol concentration were needed to detect an intraclass correlation of 0.9 (excellent agreement) to be significantly different from 0.6 (moderate agreement). This sample size has a power of 89%, with $\alpha 0.05$. However, we also enrolled patients with nontoxic paracetamol concentrations, in order to confirm the findings from our pilot study. The number of nontoxic patients recruited was determined by the time it took to recruit the 15 patients with toxic plasma paracetamol concentrations.

Results

Fifty-six patients were enrolled between April 2009 and October 2010. There were 86 patients who were missed, but they were similar to those who were enrolled for age, gender and plasma paracetamol concentration. Nine

patients were excluded from analysis, eight due to insufficient saliva and one due to a spurious result. This patient had a plasma concentration of 151 mg l^{-1} and a saliva concentration of 1270 mg l^{-1} . This was considered to be secondary to either paracetamol residue or regurgitated gastric contents into the mouth, resulting in the abnormally high saliva paracetamol concentrations. Of the eight patients excluded due to insufficient saliva samples, six had co-ingested medications possessing anticholinergic properties including antihistamines (doxylamine and promethazine) and antipsychotics (quetiapine). In total, there were 18 patients who co-ingested medications with anticholinergic properties (13 antihistamines, five psychotropics).

The remaining 47 patients were predominantly female (37, 78%), with a median age 26 years (IQR 20–41). The median reported dose of paracetamol ingested was 10 g (IQR 6–14). Specimens were collected at a median time of 4 h (IQR 4–5.3) after ingestion. Figure 1 displays the scatterplot of the concentrations. The median plasma and saliva paracetamol concentrations were 29 mg l^{-1} (IQR 8–110) and 38 mg l^{-1} (IQR 10–105) respectively. Lin's concordance correlation was 0.97 [95% confidence interval (CI) 0.96, 0.98]. The mean difference between plasma and saliva paracetamol concentrations was 8 mg l^{-1} (95% CI 2, 14).

Figure 2 displays the Bland–Altman plot for all patients. Most of the scatter in the plot is within the 95% limits of agreement (-49.6 ± 34) with the observed average agreement being close to the line of perfect average agreement. In 14 of the 47 patients (29.8% 95% CI 17.8, 45.1) the plasma concentration exceeded the saliva concentration. This compares with two out of 29 in our previous study (6.9% 95% CI 0.8, 22.8). There were no false negative saliva paracetamol concentrations. Thirteen patients had plasma paracetamol concentrations $> 100 \text{ mg l}^{-1}$ and of these nine (69%) patients had plasma concentrations $> 150 \text{ mg l}^{-1}$.

Patients requiring treatment with NAC

There were 15 (32%) patients who required treatment with NAC. Of these patients, the median reported ingested dose of paracetamol was 14 g (IQR 10–23). Specimens were collected at a median time of 4 h (IQR 3.2–4.7) post ingestion. Figure 3 displays the scatterplot of the concentrations. The median plasma and saliva concentrations in this group of patients with toxic concentrations of paracetamol requiring treatment with NAC were 167 mg l^{-1} (IQR 110–200) and 170 mg l^{-1} (IQR 103–210) respectively. Lin's concordance correlation was 0.94 (95% CI 0.88, 0.99). The mean difference was 15 mg l^{-1} (95% CI -4 , 35). The Bland–Altman plot (Figure 4) revealed most were within the 95% limits of agreement with a value of -20.2 ± 12 . In seven (47%, 95% CI 22.3, 72.6) of these 15 patients, the plasma concentration exceeded the saliva concentration. There was complete agreement between the indications for treatment in all of the patients based on the plasma and saliva concentrations.

Figure 1

Scatterplot of plasma paracetamol concentrations and saliva paracetamol concentrations for all patients. Lin's concordance correlation = 0.97 (95% CI 0.96, 0.98). Reduced major axis regression (RMA) minimizes the sum of the product of x and y deviations (as opposed to the sum of the squared vertical deviations as in ordinary least squares). RMA yields a symmetric line regardless of which variable is defined as y and which is defined as x. reduced major axis (—); line of perfect concordance (---)

Figure 2

Bland–Altman plot for all patients. observed average agreement (—); 95% limits of agreement (---)

Saliva collection

There were problems with saliva collection in seven (12%, 95% CI 5.6, 24.7) of our patients using the Salivette® device, compared with 19% (95% CI 6, 42) in our previous study. In patients where there was difficulty collecting saliva, five (71%, 95% CI 30, 95) had co-ingested other medications which had anticholinergic effects, possibly resulting in

reduced saliva secretion. The laboratory staff reported that the Salivette® device was superior to use compared with our previous method (in SPIT 1) of spitting saliva into a specimen container. It was faster to process, being cleaner and safer for the laboratory staff to handle. The Salivette® device also resulted in a much cleaner sample after centrifugation.

Figure 3

Scatterplot of plasma paracetamol concentrations and saliva paracetamol concentrations for patients requiring treatment with N-acetylcysteine. Lin's concordance correlation = 0.94 (95% CI 0.88, 0.99). Reduced major axis regression (RMA) minimizes the sum of the product of x and y deviations (as opposed to the sum of the squared vertical deviations as in ordinary least squares). RMA yields a symmetric line regardless of which variable is defined as y and which is defined as x. reduced major axis (—); line of perfect concordance (---)

Figure 4

Bland-Altman plot for patients treated with N-acetylcysteine. observed average agreement (—); 95% limits of agreement (---)

Discussion

There was agreement between toxic plasma and saliva paracetamol concentrations in keeping with the findings from our pilot study and the other non-ED studies [7–9]. This study demonstrates that the agreement extends to toxic concentrations of paracetamol. If treatment with NAC

was based only on saliva concentrations, there would have been no patients who were treated unnecessarily, or any that missed out on necessary treatment

Paracetamol concentrations are used to refine risk assessment for paracetamol DSP. For patients presenting within 8 h of acute paracetamol ingestion, only a single timed paracetamol concentration is required [3]. Isolated

paracetamol concentrations are more difficult to interpret in patients with a delayed presentation >15 h and supratherapeutic ingestions where other investigations including aminotransferases and coagulation profile are required.

While this study provides evidence of agreement between plasma and saliva paracetamol concentrations, the clinical utility of this result is uncertain. It does, however, develop the hypothesis that saliva paracetamol concentrations may be a useful clinical alternative to plasma concentrations. This could obviate the need for venisection in some patients. The potential for this approach within the paediatric population is of particular interest, but further evaluation is required [20, 21]. Our results would also support the development of a quantitative point of care test. This would be particularly useful in a remote setting with limited laboratory support.

The Salivette® device costs US\$ 0.58 per device and this approach is preferable to spitting into a sample jar that was used in our first study. The laboratory staff also found processing the saliva samples using the Salivette® device to be superior. We note that saliva concentrations in our pilot study were reliably higher than the plasma concentrations, but this was not so prominent in the current study. Whether or not this is related to the use of this device is unknown.

Limitations

This was a small study although it was adequately powered to confirm agreement between plasma and saliva paracetamol concentrations extended to the toxic range. A larger study would be required to define the clinical utility and safety of saliva concentrations. The challenge is with concentrations close to the toxic range and their reliability in terms of making treatment decisions. The mean difference between the plasma and saliva concentrations in the toxic range was 15 mg l⁻¹ with the saliva concentrations averaging higher. However, in almost half our toxic group, the plasma concentration exceeded the saliva concentration. This variability could be problematic. By way of example, if the saliva paracetamol concentration was 150 mg l⁻¹, the plasma concentration could be 135 mg l⁻¹. This would mean that the patient would have been treated unnecessarily, based on current guidelines. The utility of the saliva assay may be in cohorting patients into two groups: those with low concentrations requiring no further investigation and those with potential for toxicity requiring accurate refinement with the plasma assay.

The co-ingestion of medications, especially those with anticholinergic effects, increased problems with saliva collection in about one in seven patients, so use of saliva will not always be possible. Kamali *et al.* showed that administering pirenzepine (anticholinergic agent) to healthy volunteers prior to taking a therapeutic dose of paracetamol resulted in a significant increase in the saliva:plasma paracetamol ratios, thought to be secondary to decreased

saliva flow [22]. The regurgitation of stomach contents and residue in the mouth may also skew the results. Studies reporting saliva paracetamol concentrations utilized a number of biochemical assays or analyzers. The assay used in this study is commonly available. Validation studies are required for alternative proprietary assays.

In conclusion, the agreement between the indications for treatment of paracetamol DSP based on plasma and saliva paracetamol concentrations extends to the toxic range, but with slightly lower agreement. This is congruent with the findings of our pilot study and supports the development of a point of care test. Saliva may hold promise as a non-invasive method to risk stratify paracetamol poisoning.

Competing Interests

All authors declare no competing interests, except Frank Daly, who received an honorarium from GlaxoSmithKline to develop guidelines for the management of paracetamol overdose in 2006.

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