The time-dependant post-mortem redistribution of antipsychotic drugs

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The post mortem redistribution of ten commonly prescribed antipsychotic drugs (APs) was investigated. Femoral blood was collected from 273 cases at admission to mortuary (AD) and at post-mortem (PM). The PM samples were collected at various times up to nine days after admission and the sample pairs analysed using LC–MS/MS. The drugs included in this study were 9OH-risperidone (paliperidone), amisulpride, chlorpromazine, clozapine, haloperidol, olanzapine, promethazine, quetiapine, risperidone, and zuclopenthixol. Haloperidol, quetiapine and risperidone showed minimal changes between AD and PM specimens, whereas the majority of drugs showed significant changes between the sample pairs collected at different time points post mortem (p < 0.01) in addition to an average concentration change greater than the uncertainty of measurement of the applied method. Average increases in blood concentrations after admission to the mortuary ranged up to 112% (chlorpromazine and olanzapine) but also decreases up to −43% (9OH-risperidone) were seen. There were large standard deviations between sample pairs and substantial day-to-day unpredictable changes that highlight the difficulty in the interpretation of drug concentrations post-mortem. Based on the presented data, we recommend that specimens for toxicological analysis should to be taken as soon as possible after admission of a deceased person to the mortuary in order to minimise the effects of the PM interval on the drug concentration in blood.

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1. Introduction

Post mortem redistribution (PMR) is a well-recognised but under-explored phenomenon that complicates the interpretation of drug concentrations in medicolegal death investigations. It is believed to occur by diffusion of drug from tissue-bound stores at higher concentrations adjacent to blood vessels into blood after death, therefore increasing blood concentrations post-mortem [1]. The two main factors that appear to influence the PMR of a drug are sampling site and time of sampling relative to the time of death. Peripheral blood is regarded as more suitable for post-mortem drug testing because of its distance from central organs and the gastrointestinal tract [2,3].

PMR has been most associated with a large volume of distribution (Vd) >3 L/kg and a high degree of lipophilicity [2,4–7]. Basic drugs are considered to be more susceptible to PMR as their ionised fraction increases with the mainly aqueous content of cells as they become more acidic post-mortem. During post-mortem lysis of cells basic drugs diffuse more easily into hydrophilic body fluids, which can potentially cause increases in drug concentrations in blood [8]. Since antipsychotic drugs (APs) are basic and generally lipophilic with a large Vd (Table 1) they are likely to be susceptible to PMR, however, this has not been studied in detail.

Currently published data on the PMR for APs has been obtained from animal studies, targeting one or a few analytes [9–11], or from human tissue distribution studies in post-mortem cases [12–19]. These studies focused predominantly on the impact of sampling site on a post-mortem drug concentration, rather than the influence of the post-mortem time interval (PMI). This is probably due to the difficulty in obtaining relevant specimens for testing and ethical restrictions on human experimentation on deceased persons.

Since an autopsy is unlikely to be carried out immediately following admission of a body to a mortuary, a PMI of a few to several days is common increasing the likelihood of substantial post-mortem changes in concentrations. The Victorian Institute of Forensic Medicine (VIFM) is able to obtain a peripheral blood specimen on admission to the mortuary as part of its ability to conduct preliminary examinations prior to a coroner’s order on whether an autopsy should be conducted. The order to conduct an autopsy can take several days. This allows an opportunity to compare the blood concentrations on admission and the subsequent concentrations...
Table 1
Volumes of distribution \( \left(V_d\right) \), protein binding \( \left(F_p\right) \), and lipophilicity \( \left(\log P\right) \) values for APs of interest.

<table>
<thead>
<tr>
<th>Drug</th>
<th>( V_d )</th>
<th>( F_p )</th>
<th>( \log P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amisulpride</td>
<td>13–16</td>
<td>0.17</td>
<td>1.5</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>10–35</td>
<td>0.98</td>
<td>5.18</td>
</tr>
<tr>
<td>Clozapine</td>
<td>2–7</td>
<td>0.95</td>
<td>3.67</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>18–30</td>
<td>0.9</td>
<td>3.7</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>10–20</td>
<td>0.93</td>
<td>2.65</td>
</tr>
<tr>
<td>Promethazine</td>
<td>13</td>
<td>0.93</td>
<td>4.52</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>8–12</td>
<td>0.83</td>
<td>2.93</td>
</tr>
<tr>
<td>Risperidone</td>
<td>0.7–2.1</td>
<td>0.9</td>
<td>3.27</td>
</tr>
<tr>
<td>9OH-riperidone</td>
<td>U/K</td>
<td>U/K</td>
<td>2.3</td>
</tr>
<tr>
<td>Zuclopenthixol</td>
<td>15–20</td>
<td>0.98</td>
<td>4.46</td>
</tr>
</tbody>
</table>

\( V_d \) and \( F_p \) are obtained from Baselt [29], \( \log P \) values are calculated using ALGOPS 2.1. U/K: unknown.

from blood taken at autopsy, in order to study the effect of time on the PMR.

2. Methods

2.1. Case selection

Cases were selected in which both an admission to mortuary blood specimen (AD) and a post-mortem peripheral blood specimen (PM) taken at autopsy had been collected and showed the presence of at least one AP drug during routine toxicological testing. Only cases in which the investigation by the coroner was completed were included in this study.

Several exclusion criteria were applied. Cases that contained insufficient sample volume following routine toxicological analysis and subsequent long-term storage of 2 mL of specimen were excluded as were suspicious death cases. Additionally, all cases where the time interval between death and sampling of the AD sample was greater than 24 h were excluded from the study. Cases were also excluded where the circumstances of the death indicated significant trauma prior to death. In these instances, the integrity of the blood vessels was likely to have been compromised. Samples in this study that showed signs of decomposition (visually evaluated) were also excluded.

A total of 273 cases (546 paired specimens) were selected that showed the presence of at least one AP and matched the criteria described above. A total of ten APs were detected in these cases including 9OH-riperidone (paliperidone), amisulpride, chlorpromazine, clozapine, haloperidol, olanzapine, promethazine, quetiapine, risperidone, and zuclopenthixol.

2.2. Ethical review process

Ethics approval was granted by the Ethical Review Committee of the VIFM (Reference number: EC 5/2011).

2.3. Analysis of specimens

All specimens were analysed using a previously published validated tandem LC–MS method using three transitions per drug [20]. A matrix-matched freshly spiked seven-point calibration curve was extracted with every assay and used to calculate the respective concentrations of the drugs. Quality control (QC) samples were run after every ten samples. The assay was only accepted if all QC’s were within 20% of the target concentration. All 273 sample pairs were re-analysed despite some of them having had the AD specimen or the PM specimen tested during routine toxicological analysis. This was done in order to minimise differences in drug concentration potentially caused by different analysis times due to instability of compounds. The following formula was used to evaluate the change in concentration \( \% \) between AD and PM sample:

\[
\text{Conc (PM)} - \text{Conc (AD)} 
= 100 - \Delta \text{Conc} \%
\]

where Conc = concentration.

If \( \Delta \text{Conc} \% > 0 \) an increase in concentration was observed between AD and PM sample, if \( \Delta \text{Conc} \% < 0 \) a decrease in concentration was observed between AD and PM sample.

2.4. Statistical evaluation

All AD specimens were compared with their respective PM sample using a two-tailed Wilcoxon Matched-Pairs Rank-Sum Test, with samples grouped according to the AP. This non-parametric test was chosen to evaluate the results, as normal distribution cannot be assumed for the sample set. The two-tailed approach was chosen, as concentration changes in any direction needed to be considered. Significance values were only evaluated for individual PMI where six or more sample pairs were available, as the Wilcoxon Matched-Pairs Rank-Sum Test requires at least six matched pairs to be significantly different before assuming significant differences within a group of pairs. Subsequently, individually paired cases and their concentration change at defined time points post-mortem were combined in a group and used to evaluate a trend over time. The p-value was reported for all cases containing one drug. If there were six or more sample results for any given PMI, the significance value was provided for the individual PMI, in addition to the group value (Supplement 1). Additionally, n-values, the mean and standard deviation for each PMI are reported.

3. Results and discussion

In order to evaluate the post-mortem drug concentration changes of each drug, several factors have been taken into consideration. In addition to the statistical evaluation, the average concentration change over the investigated PMI has been determined in order to make the data comparable with the outcomes of previous studies (Table 2). As inaccuracies (RSD) caused by the analytical method used in this study have proven to be under 20% for all drugs with the exception of olanzapine (OLZ) (which was excluded from method validation due to its instability [20]), concentration changes greater than 40% (2RSDs) were considered likely to be caused by reasons other than method inaccuracy. Additionally, the drug concentration change on every day of the PMI has been determined along with the standard deviation, giving more detailed information on the change over time (Supplement 1).

The majority of drugs showed significant changes between AD and PM specimens \( p < 0.01 \) in addition to an average concentration change greater than 40%. Average increases in blood concentrations after admission to the mortuary ranged up to 112% (chlorpromazine and olanzapine) but also decreases up to –43% were observed (9OH-riperidone).

Table 2
Number of sample pairs per drug \( n \), average concentration change including range \( \% \), the investigated PMI \( \) (time) \( \) [days] and \( p \)-value \( p \) for the studied antipsychotic drugs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>( n )</th>
<th>Mean ( \Delta \text{Conc} % ) [min, max]</th>
<th>Time [days]</th>
<th>Significance ( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amisulpride</td>
<td>11</td>
<td>57 [43, 84]</td>
<td>2–8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>17</td>
<td>112 [25, 216]</td>
<td>1–9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Clozapine</td>
<td>15</td>
<td>41 [16, 74]</td>
<td>2–6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>18</td>
<td>2 [30, 40]</td>
<td>1–9</td>
<td>0.83</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>95</td>
<td>112 [17, 234]</td>
<td>1–9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Promethazine</td>
<td>22</td>
<td>63 [13, 174]</td>
<td>1–7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>57</td>
<td>25 [16, 38]</td>
<td>1–7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Risperidone</td>
<td>33</td>
<td>–15 [–36, 12]</td>
<td>2–7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>9OH-riperidone</td>
<td>35</td>
<td>–43 [–68, –26]</td>
<td>2–8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Zuclopenthixol</td>
<td>15</td>
<td>62 [28, 146]</td>
<td>1–7</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Bold: mean \( \Delta \text{Conc} > 2 \text{RSD} (\geq 40\% \)) and \( p < 0.01 \).
90H-risperidone was the only analyte which showed a reduction in drug concentration over all time points (35 sample pairs from cases with PMI ranging from 1–8 days, \( p < 0.01 \)), with an average loss of 43%. 90H-risperidone is the main-metabolite of the atypical AP risperidone and is also available in some countries as paliperidone. It is formed by cytochrome (CYP) P450 enzymes, specifically CYP2D6, and is likely to contribute to the in vivo effects of risperidone [21]. In a clinical setting risperidone is rapidly metabolised and concentrations have been shown to be generally lower than 90H-risperidone [22]. Hence, 90H-risperidone is commonly measured in addition to risperidone, providing an indication, wherever possible, of prior risperidone ingestion in cases where risperidone can no longer be detected. With protein binding estimated at \( \sim 77 \% \) in human plasma and a partition coefficient (log \( P \)) of 2.3, 90H-risperidone is less lipophilic than its parent compound, risperidone (protein binding = 90\%, log \( P = 3.0 \)) [23]. Consequently, 90H-risperidone should be less likely than risperidone to distribute into organs and fatty tissue after death. However, results of this study showed losses of more than 65% after a PMI of eight days, with a loss in concentration of approximately 40% after four days. The significant losses of 90H-risperidone over the time frame examined are interesting, considering a previous study investigating the stability of 90H-risperidone in-spiked whole blood samples did not reveal any significant losses over ten weeks of storage at \( 4^\circ \mathrm{C} \) and \( -60^\circ \mathrm{C} \) [20]. However, whole blood samples in the stability study were preserved with 200 mg sodium fluoride and 30 mg potassium oxalate, which is likely to improve the stability of 90H-risperidone.

Risperidone was the only analyte in which the concentrations decreased although a slight increase occurred at day seven (Fig. 1). Risperidone showed an average loss of \( 15 \% \) over the investigated PMI (\( p < 0.01 \)). Interestingly, Rodda et al. reported that the heart to femoral ratio of 90H-risperidone reflected that of risperidone [19]. This observation combined with the results of this study for the post-mortem drug concentration changes of risperidone and 90H-risperidone, emphasise that despite sharing a similar heart to femoral ratio, drugs may undergo different patterns of post-mortem changes over time. Furthermore, risperidone shows the smallest \( V_d \) of all investigated drugs (0.7–2.1 L/kg), suggesting that it is not likely to be susceptible to significant PMR in the first few days; a response that is supported by our data in the early PMI period. As the average concentration change was less than 40\%, the PMR of risperidone was considered not significant as it is unlikely to materially affect the interpretation of its likely effects.

Only two drugs (chlorpromazine and olanzapine) showed consistent increases in concentration over the nine days PMI (\( p < 0.01 \)). These increases were generally greater than the uncertainty of measurement. Chlorpromazine showed an increase in concentration over time, with an average increase of \( \sim 112 \% \) over a PMI of nine days. This is consistent with reported heart/femoral blood ratios ranging from 1.57 (1.0–2.7; unknown sample size) up to 2.0 (0.8–7.2, \( n = 6 \)) and even 4.0 (1.0–8.0, \( n = 5 \)) [24] that have been reported in the literature, suggesting that chlorpromazine is subject to substantial PMR. This raises doubt over what can be said of blood concentrations that could be caused by redistribution since these changes could be mistaken for drug misuse and toxicity [25]. Olanzapine concentrations increased on average \( \sim 112 \% \) over the investigated PMI of nine days (\( \sim 100 \% \) increase after four days) suggesting that this drug is highly susceptible to PMR. While the SDs of olanzapine were large (seven out of nine were greater than 30\%), this was not entirely unexpected due to the large case to case variation. The drug is also known to be inherently unstable blood [20] and it is likely that larger increases occurred but some drug was lost to degradation. While the analysis of Horak and Jenkins [13] found the PMR of olanzapine to be “minimal” with a heart to femoral ratio of 1.24, this is supported by previous case studies, where the heart to femoral blood ratio of olanzapine has been reported to range from 1.1 to 1.4 [17,26]. However, the observed variability in the detection of olanzapine highlights the limited value of single case studies as large variations in detection are likely to give misleading results. With a total of 95 sample pairs analysed in this study, olanzapine is highly likely to undergo PMR over time, however the true extent of PMR cannot be determined due to its instability.

Clozapine and promethazine showed the most significant increases in the first three days of the PMI. The largest increase in concentration occurred at four days for clozapine (>70\%) and three days for promethazine (>170\%). As the drug concentration decreased from this point onwards, drug results obtained after a longer PMI (four days onwards) intriguingly appear to be more likely to represent drug concentrations at the time of admission of a deceased person. Both drugs appear to undergo a pattern of increase in drug concentration followed by decrease, causing large inter-day concentration differences (Fig. 1). Flanagan et al. investigated the PMR of clozapine in the domestic pig [9]. Two pigs were administered with a single dose of 10 mg/kg of clozapine. After death, blood was taken from a peripheral vein at different time points over a 24 h period. Interestingly, both pigs showed an increase in blood concentration initially, followed by a decrease. Clozapine was no longer detectable in one of the pigs after 24 h. Consequently, the observed pattern of post-mortem behaviour of clozapine is supported by the results of our study.

Amisulpride and zuclopenthixol showed slower increases in concentration with the largest increase reached after four (amisulpride, >80\%) and five days (zuclopenthixol, >145\%), averaging 57\% and 62\%, respectively.

The two remaining drugs (in addition to risperidone) appeared to have undergone only minor post-mortem changes.

Quetiapine showed an average concentration change of 25\% over the investigated PMI (seven days). Following a tissue distribution study in 2000, Anderson et al. concluded that quetiapine was likely to undergo PMR [18]; this finding was also supported by Parker & McIntyre in 2005, who reported a heart to femoral ratio of 1.4, suggesting some propensity for PMR [27]. However, our results highlight that different conclusions may be reached depending on the method since death. With an average increase of less than 40\% over seven days of PMI, the concentration change is within the inaccuracy of the method and also would not materially affect any interpretations made.

Haloperidol was the only drug included in this study where post-mortem concentration changes were statistically not significant over the whole time frame average concentration change being only 2\% (Table 2). The only published study investigating the PMR of haloperidol is a tissue distribution study in the rat which showed an increase six hours after death [10]. No additional blood samples were collected after this time, making conclusions regarding a longer PMI difficult.

There were limitations to this study. The PM sample was taken during the autopsy process, therefore the possibility of contamination through collection of non-femoral blood, urine, faeces, serous fluid that has leaked from the chest cavity or stomach contents cannot be fully excluded. Furthermore, despite having excluded putrefied samples, a previous study has shown that even non-decomposed samples can result in altered extraction efficiencies and variable matrix effects compared with ante-mortem blood samples [28]. These outcomes suggest that variations are likely to be even higher if the sample group is not controlled. Another drawback is the unpredictability of the change in drug concentration that may have occurred in the time frame between death and taking of the AD sample.
Fig. 1. Concentration change between AD and PM specimen over the PMI displaying twice the RSD (—; ΔConc, change in concentration; *, mean ΔConc < 2RSD. Please note that the scales are set to different ranges.
4. Conclusions

In conclusion, the majority of drugs showed significant changes between AD and PM specimens (p < 0.01) in addition to a average concentration change greater than the uncertainty of measurement of the applied method. Haloperidol, quetiapine and risperidone did not show concentration changes greater than the extent of the uncertainty of measurement, therefore their risk to undergo significant postmortem redistribution was considered low. The outcomes of this study highlight the limitations of reporting postmortem concentration changes. While average values reported in this study can give an indication of whether or not a drug is subject to PMR, the analysis of samples collected over various days of the PMI has shown that individual variations between different time points of the PMI are can be significant. In addition to large standard deviations, this complicates the interpretation of post-mortem drug results, especially when a long or unknown time frame has passed between death and sampling of a specimen for toxicological analysis. Specimens for toxicological analysis need to be taken as soon as possible after admission of a deceased person to the mortuary. However, the large variations in reported results highlight that speculation concerning the magnitude of a post-mortem drug concentration change are impractical. It is more important to be aware of the variability of the change that is likely to occur.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.forsciint.2012.05.028.

References