Postmortem Redistribution of Morphine and Morphine-3-Glucuronide in Rabbit Models
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Abstract

Opioid overdose is a major cause of premature death all over the world. Deaths related to morphine and heroin abuse were more than any listed drugs therefore, the interpretation of toxicological results of these drugs is important to reach the right decision regarding the cause of death. Postmortem redistribution is known to influence the blood and tissues concentrations of various drugs. A rabbit model was used to elucidate this phenomenon for heroin and its metabolites morphine and morphine-3-glucuronide (M3G). Nine male New Zealand white rabbits were anaesthetised and administered 1mg/kg of diamorphine via the left auricular vein. One hour after the injections, the rabbits were sacrificed. The concentration of morphine and M3G were determined in femoral blood, cardiac blood, vitreous humour, and various tissues (liver, cardiac muscle, bone marrow, lungs, kidneys, abdominal fat and subcutaneous fat). Rabbits were placed into three groups. Group one (n=3) autopsied immediately, group 2 (n=3) autopsied 30 minutes after death and group 3 (n=3) autopsied 24 hours after death. Samples were analysed for morphine and M3G by a validated liquid chromatography mass spectrometry (LC-MS-MS). Antemortem blood morphine and M3G concentrations were determined in femoral blood, cardiac blood, vitreous humour, and various tissues (liver, cardiac muscle, bone marrow, lungs, kidneys, abdominal fat and subcutaneous fat).

Introduction

Opioid overdose is a major cause of premature death all over the world and the deaths related to morphine and heroin abuse were more than any listed drugs. The interpretations of toxicological results of these drugs are important to reach the right decision regarding the cause of death and to help avoid potential miscarriages of justice. Postmortem redistribution is known to influence the blood and tissues concentrations of various drugs. Numerous previous studies were carried out to determine the PMR of morphine and its metabolites in human and animals [1-4]. The aim of this study was to look at the phenomena of PMR of heroin and its metabolites morphine and M3G using the rabbit as a model and we need to see if the rabbit model fits with the human data.

Methods

Specimen
- Nine New Zealand white rabbits (Harlan) with weight between (3.4-4.7kg), mean 4.26±0.38 kg.
- Rabbits were divided in to three groups and samples were collected as soon as after sacrifice (n=3) and collected again after 30 minutes (n=3) and collected later on after 24 hours (n=3).
- The experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986.

Experimental Procedure
- The rabbits were anaesthetised by inhalation of 3-5% isoflurane with oxygen, anaesthesia was maintained throughout the procedure.
- Following anaesthetisation rabbits were administered 1mg/kg of diamorphine (heroin) prepared as 2mg/ml stock in saline and injected via the Left auricular vein.
- 1 hour after administration a ‘time of death’ blood sample was taken via the right auricular vein. Immediately following the ‘time of death’ sample the rabbit was euthanized using (15 % w/v RP Potassium Chloride) and the death was confirmed by the lack of heart beat.
- Following Death the rabbit was placed in a supine position and kept at room temperature and then samples were collected.

Extraction and LC-MS-MS analysis
- 150μl of the sample were diluted with 450μl of blank horse plasma.
- 50μl of internal standard (M3d and M3dG3) were added to each sample.
- 1000μl of 0.5 ammonium carbonate solution was added to each sample and vortex.
- Then, plasma solid phase extraction was performed and the sample were analysed by LC-MS-MS.

Results

- Antemortem blood morphine and M3G concentrations were similar across all the rabbits.
- There was increase in the concentration of morphine in cardiac blood and slight increase in vitreous humour over time.
- There was decrease in the concentration of morphine in the muscle and lung over time.

Conclusions

- Antemortem blood morphine and M3G concentrations were similar across all the rabbits.
- Morphine PMR results in rabbits were similar to that in human and therefore, could be used as a model to show the PMR of morphine in human.
- Morphine and M3G concentration found to be changed with respect to time and site of sampling.
- Cardiac blood is considered as a good sample to show the concentration for morphine at the time of death.
- Cardiac blood is considered as a good sample to show the concentration of M3G at the time of death.
- There was a correlation between the concentration of morphine in femoral blood bone marrow and heart muscle over time.

References

Table 1: Antemortem M3G concentrations in different samples

<table>
<thead>
<tr>
<th>Group</th>
<th>Rabbit 1</th>
<th>Rabbit 2</th>
<th>Rabbit 3</th>
<th>Mean</th>
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<tr>
<td>M3G (μg/kg)</td>
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</tbody>
</table>

Figure 1: Morphine (mean & 95% CI) over time concentration in different samples.

Figure 2: M3G (mean & 95% CI) over time concentration in different samples.