

Observations on 6-MAM (6-Monoacetylmorphine) in Urine

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Abstract

Heroin (diacetylmorphine) is made by acetylation of the 3 and 6 positions of the morphine molecule allowing it to rapidly cross the blood brain barrier. Once taken, it is rapidly de-acetylated in two steps. First, the acetyl group from the 3 position is removed, forming 6-monoacetylmorphine (6-MAM). Second, the acetyl group on the 6 position is hydrolyzed, forming morphine.

Although the major excreted products of heroin metabolism are morphine and morphine glucuronide, detection of heroin use is achieved by monitoring the presence of the intermediate metabolite 6-MAM in urine. The only source of this metabolite is heroin.

The testing logic being that because of its short half-life there would only be minimal amounts of 6-MAM excreted. Workplace testing guidelines set the cut-off for 6-MAM at 10 ng/ml, implying that only 0.5% of the excreted morphine would be 6-MAM. Reviews of our urine samples positive for 6-MAM showed a significant number were above 1000 ng/mL. We attempted to determine the possible reason for the high 6-MAM urine concentrations.

Keywords: Mercury poisoning; Intoxication; 6-Monoacetylmorphine; Urine concentration; Heroin metabolism

Introduction

Heroin (diacetylmorphine) is derived from morphine extracted from the poppy plant (*Papaver somniferum*). The conversion of morphine to heroin is accomplished by the acetylation of morphine at the 3rd and 6th positions of the molecule. The method of acetylation is commonly performed using acetic anhydride, although glacial acetic acid can be used. Thus the diacetyl derivative rapidly crosses the blood brain barrier [1] giving a euphoric effect. Once entering the body by such methods as intravenous injection, smoking or insufflation, heroin is rapidly de-acetylated in two steps. First, the acetyl group from the 3 position is removed, forming 6-monoacetylmorphine (6-MAM). This conversion is estimated to have a half-life of 5 or ten minutes. Second, the acetyl group on the 6 position [2,3] is hydrolyzed, forming morphine. It is generally assumed that the 6-MAM is rapidly formed by enzymatic hydrolysis of heroin, and then is further metabolized at a slower rate to morphine by carboxylesterases. Although the major excreted products of heroin metabolism are morphine and morphine glucuronide, detection of heroin use is achieved by monitoring the presence of the intermediate metabolite 6-MAM in urine. This intermediate is unique to heroin metabolism and is used to identify heroin use. Initial workplace testing guidelines stated that testing for 6-MAM only had to be performed if the concentration of morphine was greater than 2000 ng/ml [4,5]; the logic being that because of its short half-life there would only be minimal amounts of 6-MAM excreted [2]. Workplace testing guidelines set the cut-off for 6-MAM at 10 ng/ml, implying that only 0.5% of the excreted morphine would be 6-MAM.

Reviews of our urine samples positive for 6-MAM showed a significant number were above 1000 ng/mL. Based on previously published studies [6-12], we expected these 6-MAM results to be much

lower than 1000 ng/mL [8]. Possible causes of our high 6-MAM observations include genetic variance, carboxylesterase inhibition, and incomplete synthesis of diacetylmorphine.

Methods

The study was approved by Aspire IRB of Santee California. Patient specimens were referred from pain physician practices and rehabilitation clinics as part of their compliance monitoring programs. A high sensitivity quantitative LC-MS/MS method was created that measured concentrations of 71 drugs and metabolites including morphine and 6-MAM [13].

Results

Figure 1 is a frequency distribution curve of the 6-MAM results displayed as a semi-logarithmic plot [14]. The Y axis displays the observed number of observations for 0.1 log unit. The X axis is the observed urinary concentration of 6-MAM. More than 25% of the concentrations were greater than 1000 ng/mL. Our patient population includes a large number from addiction recovery facilities.

Discussion and Conclusion

More than 25% of the 6-MAM concentrations we observed in this study (N=712) were above 1000 ng/mL (Figure 1). Based on previously published studies such as those of Anderson et al. [15], we expected the great majority of our results to be in the range of 10 to 1000 ng/mL. That study reported observed median values of 328 and 265 ng/mL.

Possible explanations for the unexpected high concentrations of 6-MAM include, esterase inhibitors, genetic variance and the incomplete synthesis of heroin [15].

The conversion of 6-MAM to morphine is performed by Carboxylesterase-2 [16-19]. These esterase enzymes have polymorphic variability. This raises the possibility that the high values were due to inter-individual differences in the metabolic formation of morphine from 6-MAM. This possibility was ruled out by Anderson et al. [15] because they observed great variances in the excreted ratio of 6-MAM to morphine in the same patient.

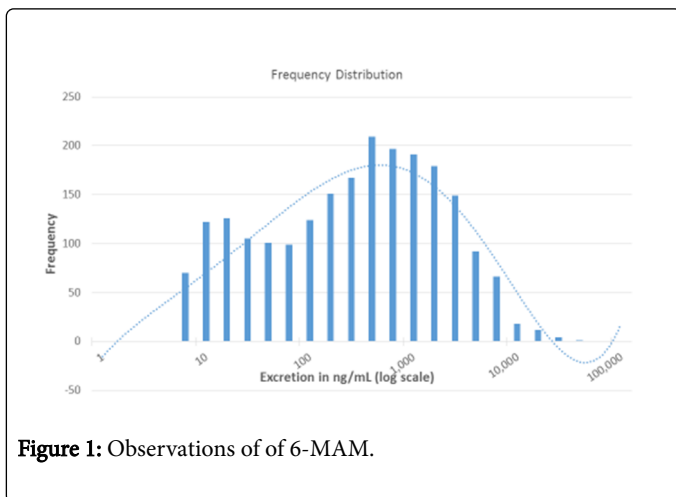


Figure 1: Observations of 6-MAM.

Since polymorphisms cannot explain the high concentrations of 6-MAM with low concentrations of morphine, Anderson et al. hypothesized that a drug-drug inhibition interaction might be responsible. They presented data showing that a derivative of thebaine formed during the heroin production process is a strong inhibitor of the de-acetylation reaction [15].

A third possibility is that the high 6-MAM concentrations are due to the impurity of the administered heroin, namely incomplete acetylation of morphine. Heroin brought into the United States from Mexico can consist of significantly more 6-MAM than heroin that enters into the country from the Mideast. This heroin from Mexico is called "black tar" heroin due to its appearance. Black tar heroin is the result of crude and cheaper synthetic synthesis methods [20]. Our tentative conclusion is that high concentrations of 6-MAM in urine probably reflect high concentrations of 6-MAM in the administered drug and inhibition of its breakdown by carboxylesterase by impurities of a thebaine product made during its synthesis.

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