In Vitro Formation of Acetylmorphine from Morphine and Aspirin in Gastric Contents and Water.

Claire K. Kaspar, BS[1]; John F. Wyman, PhD[1]; Cassandra E. Cushman[2]; Leon Glass, PhD[3]; Eric Lavins, BS[1]; Thomas P. Gilson, MD[1].

[1]Cuyahoga County Regional Forensic Science Laboratory, Toxicology Department, Cuyahoga County Medical Examiner's Office, Cleveland, OH. [2]West Virginia University, Morgantown, WV, [3]Ethos Laboratories, Fairfield, OH.

Abstract

After attending this presentation, attendees will understand that it is possible to form acetylmorphine *in vitro* by incubating human gastric contents or deionized (DI) water with morphine and aspirin. This presentation will impact the forensic science community by suggesting that detection of 6-acetylmorphine (6-AM) may no longer solely be an indicator of heroin use. Forensic toxicologists across the world have considered detection of 6-AM to be definitive evidence of heroin use. 6-AM was detected in an 85 year old female with a history of a witnessed arrest in bed at a nursing home. The decedent was under hospice care for failure to thrive, and had a history of multiple strokes, syncope, hyperkalemia, osteoporosis, anemia and osteomyelitis. There was no history of illicit drug use and the decedent was prescribed morphine sulfate elixir (Roxanol®). Whether she was taking aspirin (acetylsalicylic acid) was not recorded and salicylates were not detected by colorimetry. The manner of death was natural and the cause of death was ruled as bronchopneumonia due to hypertensive atherosclerotic cardiovascular disease with remote myocardial and cerebral infarcts.

What about the 6-AM? Is it possible that an individual may be taking morphine (pain management) and aspirin (anticoagulant), and the aspirin may acetylate morphine to produce acetylmorphine? In the present study, the possibility of formation of acetylmorphine when morphine is mixed in solution with aspirin was investigated.

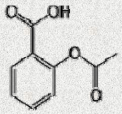
Two opioid negative, postmortem gastric specimens were selected for this study, along with morphine sulfate-extended release (ER) tablets (15 mg) and coated-aspirin tablets (325 mg). Morphine and aspirin tablets were placed into 50 mL samples of the two separate gastric specimens, as well as deionized water. The three morphine/aspirin solutions were incubated at 37° C for increasing lengths of time. A separate experiment was run in gastric contents using 15 mg morphine sulfate powder in lieu of morphine extended release tablets. One milliliter aliquots were taken from all samples at 10 minute intervals up to 1 hour, and then at 90 minutes, 2 hours and ultimately 26 hours. Aliquots were extracted using a previously published UCT solid phase opiate procedure, and analyzed by GC/MS in SIM mode. Acetylmorphine was detected in all of the samples containing morphine and aspirin in combination. Levels of acetylmorphine were greater in gastric contents than in DI water during the same incubation period. After 120 minutes, the 6-AM concentrations for the samples containing aspirin and an ER tablet were 21 ng/mL and 25 ng/mL in the gastric solutions, compared to 7 ng/mL in water. After 26 hours at room temperature the gastric concentrations were 124 ng/mL and 121 ng/mL, and in water 27 ng/mL. The increase in concentration of acetylmorphine in gastric was linear (R^2 = 0.99 and 0.98), while formation in water was non-linear (R^2 = 0.63). The results for morphine sulfate powder were essentially identical to those observed for ER tablets. The initial pH of the two gastric samples were 4.74 and 5.27 respectively; following the addition of the morphine/aspirin tablets and two hours incubation, final pH values were 3.86 and 3.92. The final pH of the water solution was 2.88. This study demonstrates that it is possible to form acetylmorphine *in vitro* by combining morphine and aspirin tablets in both postmortem gastric contents and deionized water. The compound produced in this study was identified as 6-AM by GC/MS. Further investigation must be done to determine whether the compound is actually 6-acetylmorphine, 3-acetylmorphine or a mixture of the two compounds.

Does acetylmorphine form *in vivo*? In addition to the case described above, 10,602 specimens were assayed for opioids by a pain management laboratory using LC/MS/MS. Three cases containing acetylmorphine were found to be inconsistent with heroin usage. A single specimen was listed as having a prescription for morphine and contained codeine, morphine and 6-AM; the other two specimens contained 6-AM but not morphine or codeine. Although *in vitro* formation of acetylmorphine has been demonstrated, these data indicate that *in vivo* formation from the co-administration of aspirin and morphine is unlikely to occur. This may be attributed to inconsistencies in elimination half-lives; half-lives are 13-20 minutes and 1.3-6.7 hours for aspirin and morphine, respectively.

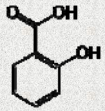
Key Words: Morphine, Acetylsalicylic Acid (Aspirin), Acetylmorphine

Introduction

- Aspirin (acetylsalicylate) is a popular over-the-counter analgesic and antipyretic drug. It is also used as an anti-inflammatory agent for rheumatoid arthritis, and at low doses as an anticoagulant (1).
- Aspirin is a prodrug which is rapidly hydrolyzed to salicylic acid by the liver and enzymes in the blood (2).
- Salicylate is the pharmacologically active drug form (2).
- The half-life of aspirin is only 13-20 minutes; while the half-life of salicylate ranges from 3- 20 hours (1).

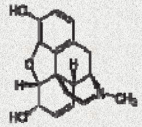


Aspirin

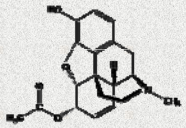


Salicylic Acid

- Morphine is a narcotic analgesic used for moderate to severe pain.
- 6-acetylmorphine (6-AM) and morphine are both metabolites of heroin. These compounds are what account for the narcotic properties of the drug.
- The half-life of 6-AM is 6-25 minutes and the half-life of morphine is 1.3-6.7 hours (3).
- Presence of 6-AM has been considered conclusive evidence of heroin usage.



Morphine



6-acetylmorphine

- At the Cuyahoga County Medical Examiner's Office (CCMEO), 6-AM was detected in the femoral blood of an 85 year old female with a history of a witnessed arrest in bed at a nursing home.
- The decedent was under hospice care for failure to thrive, and had a history of multiple strokes, syncope, hyperkalemia, osteoporosis, anemia and osteomyelitis.
- There was no history of illicit drug use but the decedent was prescribed morphine sulfate elixir (Roxanol®).
- Salicylates were not detected by colorimetry.
- The manner of death was natural and the cause of death was ruled as bronchopneumonia due to hypertensive atherosclerotic cardiovascular disease with remote myocardial and cerebral infarcts, and 6-AM was NOT reported.

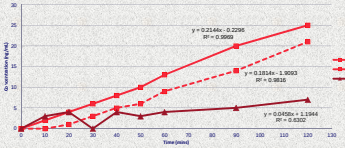
Materials & Methods

- Specimen: Postmortem, opioid-negative gastric samples and deionized (DI) water.
- Gastric samples were obtained from CCMEO cases.
- Pharmaceuticals: 15 mg morphine sulfate extended release (ER) tablets (Watson, Mallinckrodt); 15 mg morphine sulfate powder (Sigma) and 325 mg coated aspirin tablets (Bayer).
- A morphine sulfate ER tablet, an aspirin tablet or both morphine and aspirin tablets were placed into 50 mL gastric or water samples.
- The solutions were placed in a water bath at 37° C (to mimic conditions in the human stomach).
- 1 mL aliquots of the solutions were taken at 10 min intervals from 0 min- 60 min and then at 90 min, 120 min and 26 hours.
- Aliquots were extracted using a previously published UCT solid phase opiate procedure, derivatized with MSTFA and analyzed by GC/MS in SIM mode using matrix matched calibrators and controls.
- The procedure was repeated using morphine sulfate powder in place of ER tablets to determine if the formation of acetylmorphine was limited by the extended release formulation.
- To examine whether the acetylmorphine formation was catalyzed by gastric or microbial enzymes, the experiment was repeated in a gastric specimen containing sodium azide. Because sodium fluoride/potassium oxalate is an additive routinely used in forensic laboratories, incubations including these additives were also performed.
- Further incubations were performed in gastric fluid and DI water to establish how pH influences the formation of acetylmorphine.
- Additionally, cases from both the Cuyahoga County Medical Examiner's Office and Ethos Laboratory were examined to determine whether there were any incidences of unexplained 6-AM results.

Results & Discussion

- Initial studies examined the formation of acetylmorphine in gastric contents and DI water (Figure 1). 6-AM formation in gastric exceeded that in DI water during a 120 min incubation.

Figure 1. Production of 6-AM in gastric and water from a morphine ER tablet/aspirin mix.



Note: Time "0" was sampled at 5 minutes.

At 26 hours 124, 121 and 27 ng/mL 6-AM had been produced respectively.

- Production of acetylmorphine was not inhibited by the addition of sodium azide (R^2 = 0.9798) or sodium fluoride/potassium oxalate (R^2 = 0.9801); indicating formation of acetylmorphine was non-enzymatic.
- Results for the morphine sulfate powder (R^2 = 0.9893) mirrored those of the extended release tablets, indicating the formulation had no impact on the amount of acetylmorphine produced.
- A secondary peak (Figure 2) was detected immediately after the 6-AM peak. This pattern is dissimilar to what is typical of heroin cases seen at CCMEO. The second peak was subsequently identified as 3-acetylmorphine (3-AM) using a certified standard from Lipomed® (Figure 3).
- A full scan analysis was performed on a number of residues from this study; 6-AM and 3-AM were both successfully identified, heroin was NOT detected in full scan.

Results & Discussion

Figure 2. Full scan chromatogram depicting 6-AM and 3-AM elution profile, (obtained from unextracted, derivatized 6-AM (Cerilliant®) and 3-AM (Lipomed®) certified standards).

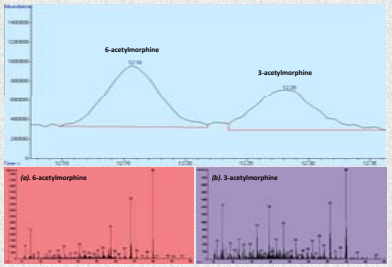


Figure 3. Mass spectra of unextracted, derivatized *(a)*. 6-AM Cerilliant® and *(b)*. 3-AM Lipomed® certified standards.

- Formation of 6-AM and 3-AM were examined at various pHs (1-5), in gastric and DI water (Figure 4). The optimal pH was ≈ 5, under the conditions investigated.
- Figure 5 reveals production of 3-AM exceeded that of 6-AM in both matrices (≈ 8 fold higher in gastric and > 4 fold higher in DI water). Formation of 6-AM in gastric and water was essentially the same (mean gastric:water ratio of 1.19).

Figure 4. The effect of pH on 6-AM and 3-AM formation in gastric and DI water after 26 hours incubation, at a variety of pHs.

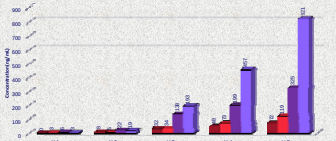


Figure 5. Temporal formation of acetylmorphine in gastric and DI water at pH 5.

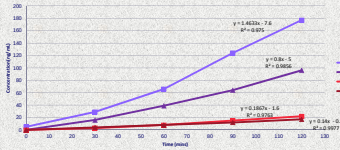
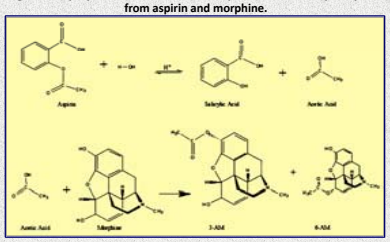


Figure 6. A proposed reaction scheme for production of acetylmorphine from aspirin and morphine.



Conclusions

- *In vitro* production of acetylmorphine occurred when a mixture of morphine and aspirin were incubated at 37° C in both postmortem gastric contents, as well as deionized water.
- 6-AM and 3-AM were both produced in this study, heroin was not. A morphine only control did not form acetylmorphine, therefore the acetylmorphine produced in this study was not generated from the instrument or procedure.
- In the original formation study, 6-AM concentrations increased in both gastric samples at a linear rate over time (Gastric 1: R^2= 0.9816, Gastric 2: 0.9969). 6-AM concentrations in the DI water also increased over time, but not in a linear fashion (R^2= 0.6302). The pH was not controlled during early experimentation (Figure 1) (DI water = pH 7) which led to the conflicting results seen in Figure 5.
- Linearity was not due to the time release properties of the tablets; results using morphine powder were essentially the same to those of the ER tablets.
- The formation of acetylmorphine was non-enzymatic, in that acetylmorphine production was linear and equivalent in the gastric with (R^2= 0.9798) and without (R^2= 0.9826) sodium azide.
- Under the conditions used, 3-AM formation was optimal at pH 5 and in the gastric medium. 6-AM formation was also optimal at pH 5, but was not affected by the matrix.
- 3-AM is preferentially produced when aspirin and morphine interact. Based on steric factors, the alcohol at the "3" position may be easier to acetylate, which would lead to formation of 3-AM before 6-AM (personal communication with Dr. Randall Clark, Professor of Medicinal Chemistry at Auburn University) (4).
- It is expected that acetylmorphine does not form *in vivo* to any forensically significant extent. In cases where there is concern of acetylmorphine production, identification of 3-AM would be crucial in making that determination. 3-AM is an analyte that is **not** detected in heroin case analysis at CCMEO.
- Over 6,000 CCMEO postmortem cases and 10,602 specimens from Ethos Labs were investigated for the possibility of *in vivo* formation of 6-AM. There was only one suspicious 6-AM result at CCMEO, and three 6-AM positives found to be inconsistent with heroin usage from Ethos (one case contained codeine, morphine and 6-AM and was most likely a heroin abuser, while the other two contained only 6-AM).
- The Ethos cases identifying only 6-AM may have contained conjugated morphine. Perhaps further hydrolysis of the specimens would produce the necessary morphine evidence to support heroin use (5). Other explanations may include administration of heroin too closely to sampling, morphine concentrations below cutoff or polymorphism of heroin metabolism (6).
- A possible explanation for the 6-AM detected in the elderly female is contamination of the pharmaceutical morphine being administered. The morphine powder examined in this study from CCMEO exhibited presence of acetylmorphine in the bottle, supporting this hypothesis.

References

1) Baselt RC. Disposition of Toxic Drugs and Chemicals in Man. 8th Edition. Foster City (CA) : Biomedical Publications, 2008; 22-24.
2) Levine B, editor. Principles of Forensic Toxicology. 3rd Edition. Washington, DC: AACC Press, 2009.
3) Baselt RC. Disposition of Toxic Drugs and Chemicals in Man. 8th Edition. Foster City (CA): Biomedical Publications, 2008; 730-733; 1057-1060.
4) Bedford KR, Nolan SL, Onrust R, Siegers JD. The Illicit Preparation of Morphine and Heroin From Pharmaceutical Products Containing Codeine: "Homebake" Laboratories in New Zealand. Forensic Science International 1987 34(3):197-204.
5) Stout PR, Lewallen C, Bynum N, Freed T, Rehder-Silinski M, Ropero-Miller JD, et al. Anomalous Results of Morphine and 6-Acetylmorphine in Urine Specimens. SOFT-TIAFT; 2011 SEPT 25-30; San Francisco.
6) Shulte TF. Interpretation of the Presence of 6-Monoacetylmorphine in Federally Regulated Urine Testing. Research Triangle Park (NC): American Association of Medical Review Officers, MRO Alert; 2010 DEC Volume XXI, No. 9.

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