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Postmortem Acetaminophen Pharmacokinetics: An Experimental Study of Site and Time-Dependent Concentration Changes

REFERENCE: Gomez, H. F., McKinney, P., Phillips, S., Roberts, D. V., Brent, J., and Watson, W. A., **"Postmortem Acetaminophen Pharmacokinetics: An Experimental Study of Site and Time-Dependent Concentration Changes,"** *Journal of Forensic Sciences,* JFSCA, Vol. 40, No. 6, November 1995, pp. 980–982.

ABSTRACT: Postmortem blood drug concentrations are obtained routinely for assessment of the cause of mortality. However, the relationship of postmortem drug concentration to blood concentrations at the time of death remains poorly characterized. Using Ketamine sedation, 10 New Zealand white rabbits were sacrificed 20 minutes after oral gavage with liquid acetaminophen 160 mg/kg as a model drug. Blood samples were obtained from peripheral (femoral vein) and central sites (heart & inferior cava) over time and compared with heart blood concentrations obtained at the time of sacrifice. The mean ± SE antemortem acetaminophen concentration was 63.1 ± 14.6 mcg/mL. Postmortem central blood concentrations were as follows: T = 3 h: 200.8 ± 129.2 μg/mL, T = 6 h: 100.8 ± 39.6 μg/mL and T = 12 h: 480.8 ± 128.8 μg/mL. Postmortem peripheral site results were: T = 3 h: 50.2 ± 21.4 μg/mL, T = 6 h: 100.8 ± 18.1 and T = 12 h: 117.7 ± 37.2 μg/mL.

Overall, blood acetaminophen concentrations increased significantly over time for central sampling sites. Drug concentration increases seen in the central sampling sites were several times higher than that seen in peripheral blood. Blood samples taken from peripheral sites did not alter significantly. The results of this controlled study were consistent with previous autopsy case series and case reports suggesting that postmortem drug concentrations do not reflect premortem values. Variables affecting postmortem drug concentrations include both postmortem sampling time and anatomic blood collection site.

KEYWORDS: toxicology, postmortem interval, blood drug concentrations, blood collection sites, toxicology, acetaminophen

The investigation of substances found at the scene of a death as well as postmortem blood drug concentrations are often used to implicate one or more potential toxic agents that may have contributed to the cause of a patients demise [*1*], or the behavior of a patient during an accident or homicide [*2*]. However, the assumption that postmortem blood concentrations reflect those at the time of death may be incorrect since the binding and distributive properties of drugs may change significantly when cell death occurs [*3*]. Observations made in autopsy case series have suggested that the interpretation of postmortem drug laboratory results should be made with caution [*4,5*]. Many autopsy case series and postmortem case reports describe up to several-fold differences in drug concentrations between blood samples obtained from different anatomic sites [*2,4–14*].

Although meaningful data has been collected in autopsy case series, there are inherent logistical difficulties in collecting immediate antemortem blood samples and controlling for specific blood sampling time intervals after death. None of the previously published studies, for example, controlled for specific time intervals after death. Furthermore, only one of the prior studies [*14*] compared postmortem drug concentrations to those obtained immediately prior to death. This comparison is necessary to accurately determine the effect of sampling site and time on drug concentration interpretation. Koren [*15*] studied the differences between antemortem and postmortem digoxin concentrations in blood samples obtained from the heart over time using a rat model. The results of the study showed that digoxin redistribution may take place postmortem. However, the Koren study did not evaluate anatomic blood sampling site concentration differences.

In an attempt to characterize the dual variables of time and sampling site, we compared postmortem blood drug concentrations drawn at various sites and times with levels obtained immediately prior to death in a controlled, randomized fashion. This study was designed to test the hypothesis that significant differences in both time and sampling site drug concentrations occur after death.

Materials and Methods

Acetaminophen was used as the model compound in this study since the clinical pharmacokinetics of this drug are well described. The drug has a known small apparent volume of distribution, low lipid affinity, and no known preferential tissue binding.

Ten male New Zealand White rabbits (Starry Pines Ranch, Inc., Denver, Colorado) weighing approximately 2.5 kg each were used for the study. All rabbits were housed in the laboratory at 22 to 24°C and a 12 hour light to dark cycle. They had free access to laboratory chow and deionized water. Food and water were removed from the animals at 12 hours prior to the study. The study had approval of the Animal Use in Research Committee of the Denver General Hospital.

After ketamine sedation, the rabbits were randomized and

Received for publication 25 Jan. 1994; revised manuscript received 37 March and 1 May 1995; accepted for publication 1 May 1995.

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Presented at the AAPCC/AACT/ABMT/CAPCC Annual Scientific Meeting in Tampa, Florida, October 1992.

administered a solution of acetaminophen 160 mg/kg by oral gavage. Twenty minutes later the animals were sacrificed using pentobarbital anesthesia. Under the assumption that there would be no significant antemortem differences between venous and cardiac chamber antemortem blood in the living animal, blood samples were drawn from the heart immediately prior to sacrifice. Postmortem peripheral blood levels were collected from the left and right femoral sites. According to prior randomization, half of the rabbits had femoral blood drawn 3 and 12 hours postmortem, and half had femoral blood drawn at 6 and 12 hours postmortem. Central site specimens were collected from the heart and inferior vena cava. Blood specimens were centrifuged; serum was removed and placed in commercial tubes (vacutainer, Becton-Dickinson, Rutherford, NJ) containing sodium fluoride. The acetaminophen concentrations were determined by Enzyme Immunoassay (SYVA Corporation, San Jose, California). The sensitivity of this Immunoassay for acetaminophen levels above 10 mcg/mL is 100%. The assay has a specificity of 100% according to the manufacturer. The collected data are reported as mean ± standard error (SE). Statistical analysis of the results utilized analysis of variance (ANOVA) and Tukey's multiple comparison of means. A P value of less than 0.05 was defined as statistically significant.

Results

One of the rabbits died prior to the 20 minute post-ingestion sacrifice time, thus leaving a total of nine animals for site collection and analysis. Of the remaining rabbits, there were significant blood acetaminophen concentration differences at time and site intervals tested with ANOVA P = .001 (see Fig. 1). The mean ± Se antemortem acetaminophen concentration was 63.1 ± 14.6 μg/mL. Postmortem central blood concentrations were as follows: T = 3 h: 200.8 ± 129.2 μg/mL, T = 6 h: 100.8 ± 39.6 μg/mL and T = 12 h: 480.8 ± 128.8 μg/mL. Postmortem peripheral site results were: T = 3 h: 50.2 ± 21.4 μg/mL, T = 6 h: 100.8 ± 18.1 and T = 12 h: 117.7 ± 37.2 μg/mL. Tukey's multiple comparison of means showed no significant difference in the peripheral blood acetaminophen concentrations at all time periods studied in comparison to antemortem concentrations. In contrast, increases in central acetaminophen concentrations over time were significant with the mean central 12 hour levels increasing over mean antemortem levels several fold.

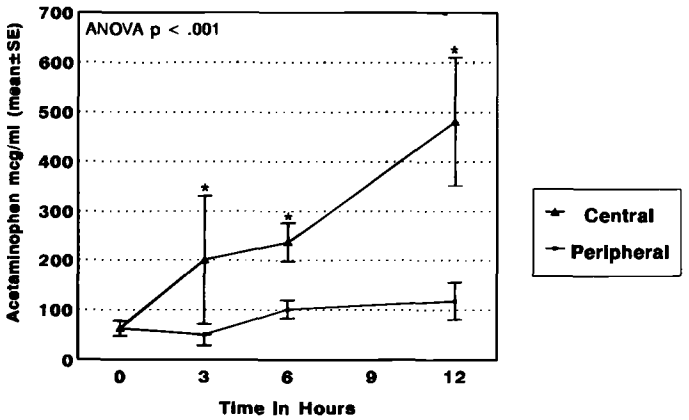

FIG. 1—*Antemortem and postmortem levels (central and peripheral sites) over time.* *Tukey's significance.

Discussion

Our study demonstrated that postmortem blood samples may not accurately reflect the concentration of this model drug at the time of death and that both the elapsed time interval and the sampling site are important variables. We demonstrated significant increases over time in postmortem central sampling sites when compared to antemortem concentrations. Furthermore, higher postmortem central site acetaminophen concentrations were noted at each tested time point, compared not only to antemortem levels, but also to peripheral concentrations obtained at each respective time point. Overall, postmortem peripheral acetaminophen blood concentrations did not change significantly. However, since all antemortem drug concentrations were obtained from the heart under the assumption that heart and peripheral site drug concentrations would be the same in the living animal, it remains possible that significant postmortem concentration changes may have been missed if there had been incomplete drug distribution to more peripheral anatomical sites prior to sacrifice.

The site dependent acetaminophen concentration changes seen in our study are noted to be consistent with a previous autopsy case report where the intracardiac blood concentration of acetaminophen was noted to be 630 μg/mL compared to a femoral blood concentration of 300 μg/mL [*16*]. In contrast, in a second case report involving multiple drug ingestion, significant sampling site dependent blood concentration differences were seen with imipramine, but not with acetaminophen [*8*]. Since the second case report cited above involves a multiple drug ingestion, the possibility of a drug-drug interaction inhibiting increases in postmortem acetaminophen blood concentrations cannot be ruled out.

Acetaminophen was used as a model compound in this study since premortem pharmacokinetics in this drug are well understood with the drug having a known small apparent volume of distribution, low lipid affinity, and no known preferential uptake of drug by tissues. We therefore might have predicted that intravascular postmortem acetaminophen concentrations would decrease over time as drug distributes from the intravascular compartment to surrounding tissues. Although this study was not designed to investigate the cause of postmortem drug concentration changes, there are several possible explanations for the postmortem redistribution to the central vascular compartment seen in this acetaminophen drug model. These include: 1) diffusion from specific tissue sites of higher concentration post ingestion (such as the liver or mesenteric/portal vessels) to central vessels in close proximity, or 2) diffusion of unabsorbed drug in the stomach to the heart and inferior cava. Another factor that may contribute to drug concentrations over time is that the collection of blood specimens over time might tend to "circulate" blood from regions of higher concentration. Thus, what might be construed as concentration changes at a specific anatomic site, might simply reflect levels from "circulated" blood from a different site, such as the heart. Peripheral vessels may be protected anatomically by distance from drug diffusion from the GI tract or major organs [*8*]. The data in this study are in agreement with previous observations that a better estimate of antemortem blood drug concentrations may be made by collecting blood from a femoral vein or other peripheral blood vessel [*7*], provided that the drug is not preferentially concentrated in skeletal muscle [*8*].

Postmortem drug redistribution reported in autopsy case series with other medications suggest numerous other contributing factors. These include depletion of energy dependent processes [*17*] that may concentrate drug in specific tissues, changes in the perme-

ability of intertissue barriers after death, as well as postmortem changes in pH and ionic strength of intra- and extracellular fluids [*18*], which would allow drugs to redistribute down a concentration gradient.

Other potential factors that may influence postmortem blood drug concentrations are route of administration, and acute versus chronic dosage prior to death [*1*]. A theory on postmortem drug redistribution based on work by Fallani demonstrating postmortem movement of blood through vasculature (through intravascular pressure caused by gas formation) [*19*] is that drug from different sites may mix and equilibrate over time in severely decomposed bodies [*7*]. Presumably this would have the effect of minimizing anatomical site blood drug concentration differences over time in putrefied bodies. Since our sampling stopped at twelve hours, such an effect would not have been expected to occur in this experiment.

Although our model demonstrated postmortem increases in central blood acetaminophen levels, drug concentrations may theoretically *decrease* over time if intravascular concentrations were higher than surrounding tissue concentrations at the time of death. This might occur, for example if death occurred prior to completion of the distribution phase following a rapid intravenous infusion of drug. Another situation where lower postmortem drug concentrations over time might occur would be in postmortem metabolism of cocaine (by blood cholinesterases). Cocaine has been shown to hydrolyze rapidly in blood in in vitro experiments [*2,20*].

Thus, our study, and the data reviewed above indicate that multiple factors appear to influence blood drug concentrations determined postmortem. The implications of this are significant since postmortem blood concentrations are frequently used as an indication of toxin concentrations at the time of death. It appears that unless the postmortem pharmacokinetics of a particular substance have been studied and characterized, any inferences based on drug concentrations obtained postmortem must be done cautiously.

Summary

We evaluated postmortem drug concentration differences at central and peripheral blood sites over time in a controlled, randomized fashion using acetaminophen as a model drug. In this animal model, central acetaminophen concentrations did not reflect antemortem concentrations and increased with time. Concentrations of the drug taken from peripheral sites did not change significantly. Both time and sampling sites should be considered when interpreting postmortem blood drug concentrations. This study is consistent with and supports previous observations that peripheral blood drug concentrations may reflect antemortem drug levels more accurately than central sites.

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