Clarification of Ethanol-Positive Case Using Urine Serotonin Metabolite Ratio

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Abstract

This paper intends to provide investigators with information useful in determining the presence of postmortem ethanol in fatal accidents and a case history of an accident that involved postmortem alcohol formation is presented. An ethanol-positive fatal case initially reported as being from ingestion was ultimately determined to be from postmortem ethanol production using the ratio of two serotonin metabolites found in urine. This case involved a transportation accident that could have resulted in additional hardships for the victim’s family through loss of compensation and reputation.

Introduction

Postmortem ethanol production in human bodies has been well-documented by many forensic scientists over the past 70 years (1–3). A recent postmortem ethanol article referenced 323 postmortem ethanol papers (4). A study reported in 1993 by the FAA laboratory found that postmortem ethanol occurred in 27% of all ethanol-positive aviation accidents, and that the differentiation of postmortem ethanol production from ingestion could not be determined in 43% of all ethanol-positive aviation accidents (5). Ethanol ingestion could only be confirmed in 30% of all ethanol-positive aviation cases.

Many authors have warned of the dangers in the interpretation of postmortem ethanol positives (1–6). However, problems still arise in fatal accident cases involving severe trauma, thermal injuries, and/or delays in specimen collection. The following case study and discussion provides an overview of the problems that may occur and describes the use of a method (6) for determining the origin of ethanol in postmortem specimens, using a urine serotonin metabolite ratio.

Case Report

Accident description

On a rainy morning in September 2006, at approximately 5:50 Eastern Standard Time, an Ohio State Highway Patrol Trooper (Victim 1, the driver) and Sergeant (Victim 2, the passenger) were involved in a fatal crash after their patrol cruiser traveled across the center line of a two-lane roadway, impacting the vehicle of a driver (Victim 3) traveling in the opposite direction. The cruiser’s fuel cell ruptured and both vehicles were engulfed in flames at impact. This resulted in massive thermal injuries to all parties, as well as extensive trauma. No emergency medical treatment was possible and all three victims were fatalities. It would later be determined that the patrol cruiser was traveling at an estimated speed of 61 to 72 mph at the point of impact and had hydroplaned on the wet pavement.

Non-autopsy laboratory tests

The local county coroner’s office collected non-autopsy specimens from all victims shortly after their deaths. Grey-top tubes containing potassium oxalate and sodium fluoride were used for the collection of blood specimens. Blood specimens taken from the jugular vein of Victim 1 and from the heart cavities of Victim 2 and Victim 3 were analyzed by the Ohio State Highway Patrol Crime Laboratory (OSPCL). The OSPCL used headspace gas chromatography equipped with a Supelco\textsuperscript{TM} 6 in x 1/8 cm stainless steel column packed with 5% carbowax 20M 60/80 carbopack B for the analysis. The limit of detection (LOD) of the procedure was 0.001% with a limit of quantitation (LOQ) of 0.01%. Results below 0.01% are reported as not detected. The OSPCL tests detected no ethanol in the non-autopsy blood specimens.

Autopsy laboratory tests

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It was initially decided by local authorities that autopsies were not needed to determine the cause of death for each victim. Some time later, this decision was reversed and autopsies were scheduled for Victim 2 and Victim 3. The autopsy for Victim 1 was even further delayed to allow for a planned memorial service to take place.

Approximately 34 h after the crash, a contract autopsy facility performed autopsies on Victim 2 and Victim 3. Formalin powder was used to reduce the decomposition of these two victims. Toxicology tests conducted by the coroner’s office on specimens collected at autopsy found no ethanol present in Victim 2, and Victim 3 had a BAC of 0.01%. The procedure,
Whether Victim 1 had consumed ethanol. This novel high-performing liquid chromatography–mass spectrometry (HPLC–MS) technique was developed, described, and reported in the peer-reviewed literature by the Federal Aviation Administration’s (FAA) Bioaeronautical Sciences Research Laboratory in Oklahoma City, OK (6). This procedure involves the simultaneous analysis of two serotonin metabolites whose ratio is significantly altered when ethanol is ingested. Urine samples from both Victim 1 and Victim 2 were analyzed using this procedure. The serotonin metabolite ratio in Victim 1 and Victim 2 was found to have a 5-hydroxytryptophol/5-hydroxyindole-3-acetic acid (5-HTOL pmol/5-HIAA nmol) ratio of 0, well below the 15 cutoff used to determine recent ethanol ingestion, indicating that the ethanol found at autopsy was from postmortem ethanol formation and not from ingestion.

<table>
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</tr>
</tbody>
</table>

*Approximate time LOD = 0.001 %

LOQ, and LOD for those tests are not available to the authors. Approximately 60 h after the crash, the same contract autopsy facility performed an autopsy on Victim 1. No form of preservation was used for Victim 1, and the body was in an advanced stage of decomposition at the time of autopsy. The specimens collected at autopsy from Victim 1 revealed 0.07% ethanol in urine and 0.08% ethanol in both heart and cavity blood (Table I). Glucose test strips were negative for glucose in the urine, and no vitreous fluid was available for testing. The procedure, LOQ, and LOD for those tests are not available to the authors.

**Interpretation of autopsy test results**

Based on the autopsy toxicology results, the media reported that Victim 1 was intoxicated at the time of the crash. However, an Ohio Highway Patrol investigation found no evidence that the Trooper had consumed ethanol or had acted in an intoxicated manner before or during his tour of duty. The conflicting test results on the non-autopsied and autopsied specimens, and the lack of evidence to support ingestion of ethanol, raised questions about the origin of the ethanol found in the autopsy specimens.

**Urine serotonin metabolite ratio analysis**

The OSPCL was informed of a laboratory test that could, in some cases, differentiate between ingested ethanol and ethanol produced postmortem by microbial action. It was decided that the specimens would be subjected to this test to ascertain whether Victim 1 had consumed ethanol. This novel high-performance liquid chromatography–mass spectrometry technique was developed, described, and reported in the peer-reviewed literature by the Federal Aviation Administration’s (FAA) Bioaeronautical Sciences Research Laboratory in Oklahoma City, OK (6). This procedure involves the simultaneous analysis of two serotonin metabolites whose ratio is significantly altered when ethanol is ingested. Urine samples from both Victim 1 and Victim 2 were analyzed using this procedure. The serotonin metabolite ratio in Victim 1 and Victim 2 was found to have a 5-hydroxytryptophol/5-hydroxyindole-3-acetic acid (5-HTOL pmol/5-HIAA nmol) ratio of 0, well below the 15 cutoff used to determine recent ethanol ingestion, indicating that the ethanol found at autopsy was from postmortem ethanol formation and not from ingestion.

**Discussion**

The distribution of ethanol in the body has been used to differentiate postmortem ethanol from ethanol ingestion. Knowing that vitreous fluid ethanol concentrations are approximately 18% higher than blood ethanol, and that urine ethanol concentrations are approximately 30% or more than the blood ethanol concentrations (assuming the existence of the post-absorptive or elimination phase), it has been suggested that the best approach to differentiate ethanol ingestion from postmortem ethanol is to examine the distribution of ethanol in blood, urine, and vitreous fluid (5,7–9). The first indication that postmortem ethanol formation might have occurred in the autopsied ethanol-positive specimens is the low urine ethanol concentration (0.07%) relative to the blood concentration (0.08%). Based on the normal distribution of ethanol, the urine value should have been around 0.10% and not 0.07%, considering a blood concentration of 0.08%. Vitreous fluid is considered an excellent specimen for determining ingested ethanol, even though it may contain nutrients (glucose) needed for postmortem ethanol formation. Vitreous fluid is encased in the skull and protected from microorganisms found in the intestinal track that are responsible for most of the postmortem ethanol produced in the body (10). No vitreous fluid was available in this case for evaluation.

The possibility of postmortem specimen contamination with ethanol-producing microorganisms increases with a higher degree of trauma to the body. Therefore, postmortem ethanol formation is far more likely to occur in cases involving severe trauma from high-speed crashes, such as aviation accidents or the accident reported in this case. Furthermore, if there is a fire that damages the protective dermal layer of the body, there is an increased opportunity for ethanol-producing microorganisms to invade the body and produce postmortem ethanol.

Time and temperature are factors in the amount of postmortem ethanol produced (11); however, due to the many other variables that affect the production of ethanol, it is impossible to predict postmortem ethanol production solely from the amount of time before specimen collection. Postmortem ethanol production can increase, given the proper conditions, with increased time and temperature. There is, however, a minimum and a maximum temperature under which postmortem alcohol can be produced. Specimens from the driver of the patrol car were not collected until approximately 60 h after death, providing more than enough time for postmortem alcohol to form in the body given the proper conditions. In one aviation fatality resulting from a plane crash at an airport, postmortem specimens were collected 2 h after the accident. An ethanol concentration of 0.055% was found in the pilot’s blood, but no ethanol was found in vitreous fluid, brain, or muscle, strongly indicating that the 0.055% ethanol was from postmortem alcohol formation and not ingestion. The variability of postmortem ethanol formation as a function of time and temperature can also be seen in accidents involving multiple victims, where one body forms postmortem ethanol and the other does not, even though they were in the same accident.

In order for postmortem alcohol to form in a specimen, it
must contain nutrients needed by a microorganism to produce alcohol. Under normal conditions, urine lacks the nutrients required for postmortem ethanol production; however, certain medical conditions (diabetes, pancreatitis, low renal threshold, etc.) can cause elevated glucose concentrations in urine and increase the chances of postmortem ethanol production in urine. Tests are routinely performed on urine specimens to check for the presence of glucose. The presence of glucose may suggest the possibility of postmortem ethanol, but the absence of glucose does not exclude postmortem ethanol because ethanol-producing microorganisms can produce ethanol from substrates other than glucose (sucrose, mannose, lactose, etc.). Furthermore, the possibility exists that glucose originally present in the urine was consumed by microorganisms prior to the specimen being collected or analyzed. In individuals who have recently eaten a meal, the nutrients available in the body for postmortem ethanol production are increased. No glucose was found in the urine specimen collected from the driver of the patrol car and no conclusion could be made regarding the origin of the alcohol found in the urine.

Due to the high rate of postmortem ethanol formation in fatal aviation accidents and the frequency at which no determination could be made regarding ethanol origin, it was found to be advantageous to develop a new analytical procedure to determine the origin of ethanol found in pilots who had died in aviation accidents. In 1967, it was reported that the ratio of two serotonin metabolites, 5-HTOL and 5-HIAA, were altered by the ingestion of ethanol and remained altered for up to 16 h after the individual stopped consuming ethanol (12). Researchers discovered that this ratio of serotonin metabolites in urine could be used to determine whether a person had recently consumed ethanol (13). This ratio was initially used in ethanol cessation programs to monitor patients diagnosed with alcoholism. Under normal circumstances, 5-HIAA is found at concentrations up to 100 times as great as 5-HTOL. When ethanol is consumed, the production of 5-HTOL is favored, and the proportion of 5-HIAA:5-HTOL shifts from 100:1 up to 60:40. The determination of this change in serotonin metabolites has been used successfully in aviation accident investigations to differentiate between ingested and postmortem ethanol (14). A ratio of 5-HTOL pmol/5-HIAA nmol below 15 has been proven to be evidence that any ethanol found in the urine specimen is from postmortem ethanol production and not from ingestion. Ratios of 5-HTOL/5-HIAA above 15 usually indicate that the person consumed ethanol within 8–12 h prior to death. An elevated ratio does not exclude the possibility that the ethanol found in the specimen is from a combination of ethanol ingestion and postmortem ethanol production, but a ratio below 15 does exclude ethanol ingestion as a source of ethanol in a specimen. The 5-HTOL/5-HIAA ratio in this case was 0, conclusively proving that the driver of the patrol car had not recently consumed alcohol.

Procedures have been developed at the FAA Civil Aerospace Medical Institute (CAMI) to identify the ethanol-producing microorganisms present in a specimen containing ethanol. This procedure uses a set of DNA probes developed to identify DNA sequences associated with microorganisms capable of ethanol production. The procedure has been used in aviation fatalities to show whether ethanol-producing microorganisms were absent or present in the specimen (15,16). These probes were not needed to show postmortem alcohol formation in this case.

It has been proposed that the presence of volatiles (propanol and butanol) other than ethanol indicates postmortem ethanol formation (17). Research has shown the presence of other volatiles to be an indication of specimen putrefaction, but not necessarily a conclusive indicator of postmortem ethanol production (5,18).

Some investigators believe that concentrations of ethanol above a certain level (0.04%) indicate the ingestion of ethanol (19). However, postmortem ethanol concentrations have been found above 0.300% (5) in postmortem blood specimens taken from aviation accidents. Therefore, no conclusions can be made regarding the absence or presence of postmortem ethanol based solely on the concentration of blood ethanol found.

Summary

Postmortem ethanol production can and does occur in fatal accidents. Therefore, care is necessary in investigating fatal accidents involving severe trauma to the body or in cases where long delays occurred prior to the collection of specimens for toxicological analysis. When abnormal ethanol distribution is found in blood, urine, and vitreous fluid, further testing using serotonin metabolite ratios may be warranted. Finding elevated blood-ethanol concentrations but no ethanol in vitreous fluid and urine should be reported as negative for ingested ethanol and should not require further testing. It is important to realize that an incorrect finding of “intoxication,” in which the person did not consume ethanol, can result in extreme hardship for the family of the deceased through the loss of pension, workers compensation, life insurance, and the reputation of the individual.

Acknowledgments

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