CASE REPORT

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Can Microorganisms Produce Alcohol in Body Cavities of a Living Person?: A Case Report

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ABSTRACT: Unusual endogenous ethanol production in intraabdominal bloody fluid of an individual who was stabbed in the abdomen and who developed peritonitis after a peritoneotomy is discussed. In the intraabdominal bloody fluid, 2.45 mg/g ethanol and 0.079 mg/g n-propanol were detected. The level of ethanol in the heart blood was about 1 mg/g. The level of n-propanol indicates that a large quantity of ethanol was produced endogenously in the intraabdominal bloody fluid. In an animal experiment in which rats were injected with 20 mL of 10% glucose mixed 5:1 with a presumed volume of rat blood into the abdominal cavity after injury of the small intestine to allow enterobacteria to spread into the cavity, a significant quantity of ethanol was produced in the administered fluid while the animals were alive. The antemortem ethanol production in the intraabdominal bloody fluid of the victim might have been caused by the microorganisms responsible for the peritonitis after the operation.

KEYWORDS: toxicology, ethyl alcohol, n-propyl alcohol, ethanol determination in corpses, endogenous production of ethanol

The examination of ethanol in postmortem body fluids and organs of individuals who have suffered from unnatural deaths such as suicide, homicide and accidental death, and sudden natural death, is very important for judging the antemortem alcohol intake. However, the interpretation of postmortem ethanol concentrations in body fluids can be complicated by factors such as postmortem ethanol production in the decomposed body and ethanol diffusion from the stomach containing an extremely high concentration of ethanol [*1–3*]. Nanikawa et al. [*4*] have elucidated that microbial ethanol formation in corpses takes place through a pathway opposite to that of ethanol metabolism in the living body.

However, there has been no report of antemortem production of ethanol in body

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NOTE: In this study, we give concentrations of ethanol and n-propanol as a mass/mass unit, mg/g, because all the specimens were obtained by weight. The concentrations of ethanol and n-propanol shown in this paper can approximately be expressed as a mass/volume unit, g/dL, by dividing each value by 10 although the specific gravity of each specimen is not known.

cavities although that in gastrointestinal tract is well documented [5,6]. Here we describe a case of suspected antemortem ethanol formation in abdominal cavity, along with findings of an animal experiment using rats.

Case History

Around 20:40 h on March 27th, 1991, a 47-year-old man had a quarrel with his friend on the street about 4 h after a drinking session and was stabbed in the abdomen with a knife. The man was immediately hospitalized to undergo peritoneotomy, but he died due to hemorrhagic shock at 23:10 h on the following day. During hospitalization, an estimated blood transfusion volume of 10,385 mL was given to him. Okayama Red Cross Blood Center, a blood supplier, explained that the transfusion blood had totally been free of alcohols and psychotropic drugs.

Autopsy Findings

Autopsy was performed about 11 h after death. The body length and weight were 170 cm and 70 kg, respectively. External examination revealed a surgical incision wound 20 cm long in the median region of the abdomen, and a stab wound 3.5 cm long in the right upper region of the abdomen with a 1 cm I.D. vinyl drain. Internal examination of the body cavities revealed general peritonitis with 350 mL of bloody fluid in the abdominal cavity. The right and left pleural cavities contained 20 mL and 100 mL of bloody fluid, respectively. The heart, weighing 310 g with many posterior epicardial petechiae, contained 50 mL of fluid and uncoagulated blood. The lungs were edematous and congested, the right lung weighing 790 g and the left 650 g. The liver, weighing 1285 g, had a stab wound 4.7 cm long and 6 cm deep in the upper surface of the right lobe. The stomach, with extensive hemorrhage in the mucosa, contained 164 g of dark red viscous blood mixed with a small amount of undigested food material. The bladder contained 2 mL of turbid urine. In other organs, there were no remarkable changes except for congestion.

Toxicologial Findings

Analytical Methods for Ethanol and n-Propanol

Head space gas chromatography using t-butanol as an internal standard was employed for quantitation of ethanol and n-propanol [7]. The apparatus used was a Shimadzu GC 4CM-PF equipped with a 200× 0.3 cm I.D. glass column, packed with 25% PEG 1000 on 80/100 Shimalite. The temperatures of the injection port and column were 110°C and 90°C, respectively. The carrier gas was nitrogen with a flow rate of 40 mL/min. The lower quantitation limits for ethanol and n-propanol were 0.01 mg/g and 0.001 mg/g, respectively.

As shown in Table 1, 2.45 mg/g ethanol and 0.079 mg/g n-propanol were detected in the intraabdominal bloody fluid. Ethanol and n-propanol concentrations in the urine and gastric contents were 2.03 mg/g and 0.021 mg/g, and 2.19 mg/g and 0.060 mg/g, respectively. Meanwhile, ethanol levels in the blood in the left and right sides of the heart, pericardial sac fluid, aortic blood, right intrathoracic bloody fluid, cerebrospinal fluid and vitreous humor were 0.89 mg/g, 0.97 mg/g, 0.96 mg/g, 0.98 mg/g, 0.89 mg/g, 0.90 mg/g and 0.85 mg/g, respectively. No n-propanol was detected in any of the specimens.

TABLE 1—*Ethanol and n-propanol concentrations in various body fluids of the victim.*

Samples	Concentrations (mg/g)	
	Ethanol	n-Propanol
Right intrathoracic bloody fluid	0.89	N.D.
Pericardial sac fluid	0.96	N.D.
Heart blood (left side)	0.89	N.D.
Heart blood (right side)	0.97	N.D.
Aortic blood	0.98	N.D.
Cerebrospinal fluid	0.90	N.D.
Vitreous humor	0.85	N.D.
Intraabdominal bloody fluid	2.45	0.079
Urine	2.03	0.021
Gastric contents	2.19	0.060

Experimental

Under anesthesia with diethyl ether vapor, nine male Wistar rats weighing about 300 g were given injuries in the small intestine, about 3 cm long, with scissors to allow enterobacteria to spread into the abdominal cavity. The operation wounds in the abdomen were then closed with binder clips, and the treated rats were divided into three groups.

Group A—Twenty milliliters of 10% glucose mixed 5:1 with a presumed volume of rat blood (glucose/blood) was injected into the abdominal cavity.

Group B—Twenty milliliters of saline mixed 5:1 with a presumed volume of rat blood (saline/blood) was injected instead of the glucose/blood.

Group C—The rats were sacrificed by injecting air into the heart. Twenty milliliters of the glucose/blood was injected into the abdominal cavity of the carcasses.

Rats in each group were placed in a room at a temperature of about 20°C.

About 0.2 mL of intraabdominal fluid from each animal was collected via a 1 mm I.D. vinyl tube connected to a 2 mL plastic syringe at irregular time intervals. In groups A and B, heart blood was also collected at the time of death by cardiac puncture using the plastic syringe.

Quantitation of ethanol and n-propanol in the body fluids was performed by headspace gas chromatography.

Results

In group A, the rats died 15 to 19 h after the glucose/blood had been injected into the abdominal cavity. Twelve hours after the treatment, 0.13 ~ 0.33 mg/g ethanol and N.D. ~ 0.004 mg/g n-propanol were detected in the intraabdominal fluid. At the time of death, the concentrations of ethanol and n-propanol reached 0.38 ~ 0.63 mg/g and 0.002 ~ 0.006 mg/g, respectively (Fig. 1 and Table 2). However, ethanol concentrations in heart blood at the time of death were as low as 0.05 ~ 0.07 mg/g. n-Propanol was not detected in any heart blood (Table 2). The intraabdominal fluid contained 0.39 ~ 0.53 mg/g ethanol and 0.004 ~ 0.016 mg/g n-propanol 24 h after the treatment (Fig. 1).

Group B treated with the saline/blood showed little production of ethanol in the intraabdominal fluid by 24 h after the treatment (Fig. 1). The rats in this group died 18 to 22 hours after the treatment. No ethanol or n-propanol was detected in the heart blood at the time of death (Table 2).

In group C, a small amount of ethanol, 0.09 ~ 0.17 mg/g, was detected in the intraab-

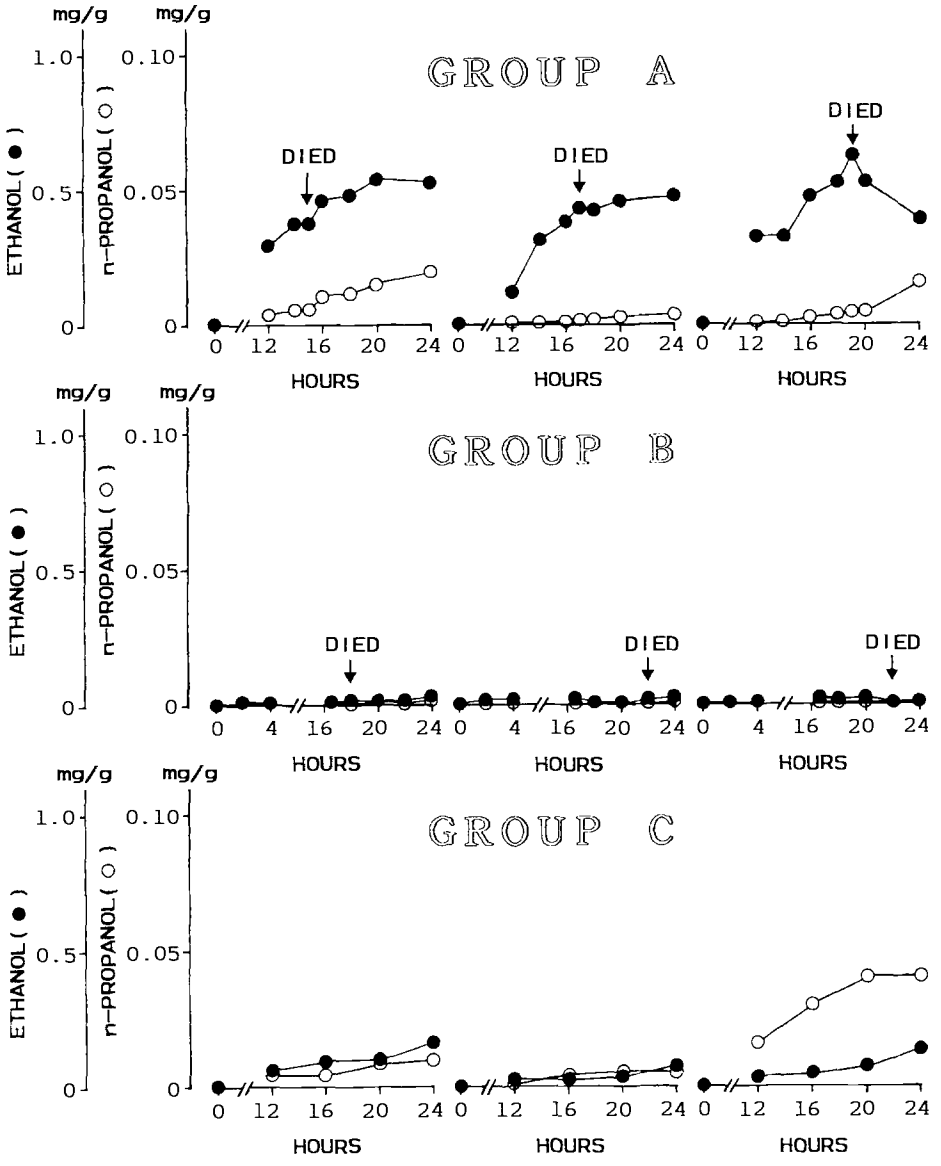

FIG. 1—*Alcohol production in intraabdominal fluid of rats with intestinal injury and injected intraabdominally with 20 mL of glucose/blood (group A) or saline/blood (group B), and that of rat carcasses with intestinal injury and injected intraabdominally with 20 mL of glucose/blood (group C).*

TABLE 2—*Ethanol and n-propanol concentrations in the intraabdominal fluid and heart blood collected at the time of death from rats given an intraabdominal injection of 20 mL of glucose/blood (group A) or saline/blood (group B).*

	Survival time (h)	Concentrations (mg/g)			
		Intraabdominal fluid		Heart blood	
		Ethanol	n-Propanol	Ethanol	n-Propanol
Group A	15	0.38	0.006	0.05	N.D.
	17	0.44	0.002	0.06	N.D.
	19	0.63	0.005	0.07	N.D.
Group B	18	N.D.	N.D.	N.D.	N.D.
	22	0.02	0.001	N.D.	N.D.
	22	0.03	N.D.	N.D.	N.D.

dominal fluid of carcasses 24 h after the treatment. The n-propanol concentration was 0.005 ~ 0.041 mg/g (Fig. 1).

Discussion

In postmortem body fluids and organs, a significant quantity of ethanol can be produced through a pathway opposite to that of ethanol metabolism in the living body in the presence of alcohol dehydrogenase and aldehyde dehydrogenase group of multiplying bacteria, using carbohydrates as substrates [*4*]. Although there is currently no proven method for determining accurately how much ethanol might have been produced postmortem and how much was present antemortem in corpses showing moderate to advanced putrefaction, n-propanol, which is produced with ethanol postmortem, can be an indicator of postmortem ethanol production because normally it does not exist in the living body [*8,9*]. The postmortem production of ethanol is significantly higher in blood than in muscle, whereas the production of n-propanol is essentially equal in both tissues [*9*]. It has been clarified that the ratio of the concentration of endogenous ethanol to that of n-propanol is less than 10:1 in muscle and less than 20:1 in blood [*9*].

In the present case, the ethanol concentration in the intraabdominal bloody fluid was much higher than that in other blood specimens. As for the possible origins of the ethanol, a) diffusion from the stomach, b) bleeding from the injured liver containing a high concentration of ethanol, and c) endogenous production can be considered. However, the ethanol that had been ingested about 4 h before the victim sustained the liver injury would mostly have absorbed from the gastrointestinal tract. Thus, the ethanol concentration in the stomach contents and blood in the liver would have had little effect on the ethanol concentration in the intraabdominal bloody fluid during hospitalization. Despite the short postmortem time interval of about 11 h, the level of n-propanol in the intraabdominal bloody fluid, 0.079 mg/g, was equal to that in a severely decomposed body [*10*]. It is evident that a significant quantity of endogenous ethanol was present in the bloody fluid. However, such a large amount of n-propanol cannot be produced in a corpse that has been left at room temperature for only 11 h after death [*2*].

Therefore, we performed an animal experiment using rats to elucidate this phenomenon. Rats whose small intestine had been injured to allow enterobacteria to spread into the abdominal cavity to induce peritonitis artificially, were injected intraabdominally with 20 mL of glucose/blood or saline/blood. In the rats treated with the glucose/blood, a significant quantity of ethanol was produced in the intraabdominal fluid by the time of death, although the n-propanol concentration was very low. In contrast, a negligible

quantity of ethanol was formed in the saline/blood-treated rats. In the rat carcasses treated with the glucose/blood, only a small amount of ethanol was detected in the intraabdominal fluid. The results indicate that, even in the living body, a significant quantity of ethanol may be produced in intraperitoneal fluid of patients suffering from peritonitis as a result of microorganisms contamination. Thus, from the animal experiment, we could concluded that, in the present case where the deceased had suffered from severe peritonitis, a significant amount of endogenous ethanol might have been produced in the intraabdominal bloody fluid containing a large amount of substrates such as glucose and amino acids resulting from the transfusion, although the microorganisms which induced the peritonitis were not identified. To our knowledge, this is the first report of antemortem ethanol production in a body cavity although that in gastrointestinal tract is well documented [*5,6*].

Also, the levels of n-propanol in the urine, 0.021 mg/g, and gastric contents, 0.060 mg/g, indicate that a large quantity of endogenous ethanol might have been produced in both fluids due to spread of the microorganisms that induced peritonitis.

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