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Forensic significance of concentrations of ethanol in brain tissues following induced acute subdural hemorrhage

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Abstract The concentrations of ethanol in peripheral blood, subdural hematomas and various regions of the brain were determined 1, 2, 4, and 6 h after the induction of a hemorrhage into a subdural space in the right temple of rabbits. The concentrations were compared to cases of intravenous administration of ethanol-free i.v. fluid. Concentrations of ethanol in the subdural hematomas gradually decreased to correspond to those in the peripheral blood. The influence of an intravenous infusion of ethanol-free i.v. fluid was not observed. Concentrations in the brain of the right temporal, parietal and frontal lobes were high and those in the right temporal lobe were maintained during the 6 h of our experiment. Therefore, to determine if a human victim was under the influence of ethanol at the time of injury, we recommend that brain concentrations of ethanol be determined. This is apparently the first study to confirm that the estimation of ethanol in the brain provides a more accurate determination of how much ethanol had been ingested.

Keywords Subdural hematoma · Ethanol distribution · Brain · Cerebral blood flow · Intracranial pressure

Introduction

Acute subdural hemorrhage is the most common lethal injury associated with head trauma [1] whereby the victim may have lost consciousness immediately after sustaining the injury [2] and brain death can occur.

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In forensic toxicology, it is difficult to determine whether a victim who survives for several hours after the injury or who was given medical treatment before death was under the influence of ethanol at the time of injury. Detection of ethanol in subdural hematomas has been reported [3, 4, 5, 6, 7, 8, 9, 10, 11], but we found no documentation of intracranial changes in concentrations of ethanol in a subdural hematoma prior to death.

We induced acute subdural hemorrhages in rabbits by injecting blood into the subdural space and studied the relationship between concentrations of ethanol in the subdural hematoma, various tissues of the brain and in peripheral blood samples. We also tested if concentrations of ethanol in all these regions would be influenced by intravenous infusion (ethanol-free i.v. fluid; Terupack) given during medical treatment.

Materials and methods

Animals

Disease-free male Japanese white rabbits (n = 40) were housed and treated in accordance with accepted principles of laboratory animal care. This study was carried out under regulations determined by the Kyushu University institutional animal investigation committee. The rabbits were 5 months old and weighed 3.54 ± 0.29 kg, all food was withheld for at least 12 h before the start of the experiment and water for drinking was withheld during the experiment.

Pharmacokinetic study of ethanol

Ten rabbits were given a single dose of 10 ml of a 40% ethanol solution per kg body weight by gavage. Blood (4 ml each) collected from the right ear vein into a syringe containing 0.001 g EDTA at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 14 h after ingestion was pharmacokinetically analyzed.

Induction of acute subdural hemorrhages

For preparation of subdural hematomas in rabbits, we used the method reported by Bullock et al. [12], but with some modifications whereby 20 rabbits were divided into 4 groups each containing 5 rabbits, and 40% ethanol solution was given by gavage at a

dose of 10 ml/kg. After 1 h the rabbits were placed in a supine position, and a small incision was made on the right scalp following the injection of 0.1 ml lidocaine hydrochloride (Xylocaine). After exposure of the calvaria, a 5-mm burr hole was drilled in the right parietal bone 0.5 cm from the midline and 2 ml of blood collected from the right ear vein was carefully injected into the subdural space at the rate of 0.1 ml/min. The burr hole was closed with bone wax and wounds on the scalp were closed. The rabbits in each group were kept in a room with a temperature of 20 °C. During the experiment, the animals were unconsciousness and did not react to pain stimulation. Rabbits in each group were killed by exsanguination at 1, 2, 4 and 6 h after the blood had been injected. Peripheral blood, brain tissues (frontal, parietal, right and left temporal and occipital lobes and the cerebellum) and subdural hematoma were immediately taken and analyzed for the presence of ethanol.

Influence of administration of intravenous fluid

Ten other rabbits were divided into 2 groups each containing 5 rabbits and given a single dose of 10 ml/kg of 40% ethanol solution by gavage. Subdural hemorrhages were induced using the same procedure described above. The rabbits were placed in the supine position, and the bladder was catheterized. At 1 h after induction of acute subdural hemorrhage, the right femoral vein was exposed, a catheter was introduced, and infusion of ethanol-free i.v. fluid (Terupack) was started at a rate of 1.0 ml/kg h^{-1} (5 animals) and 2.0 ml/kg h^{-1} (5 animals), respectively. At 3 h after the start of infusion, all rabbits were killed by exsanguination. The peripheral blood, brain tissues (frontal, parietal, right and left temporal and occipital lobes and cerebellum) and the subdural hematoma were analyzed.

Ethanol analysis

Concentrations of ethanol in tissues were determined by gas chromatography, as previously described [13], but with slight modifications. Each sample was examined in triplicate. Each sample of 0.1–0.5 g was weighed and put into a head space vial with 0.1 ml t-butanol solution (1.0 mg/ml, internal standard). The vial was tightly sealed and heated at 60 °C for 10 min, and 1 ml of the head space was injected onto a gas chromatograph, a Shimadzu GC-15 A equipped with a glass column, packed with Porapak Q (80–100 mesh), and a flame ionization detector. The column temperature was maintained at 170 °C and the injection port and detector at 230 °C. Nitrogen was used as the carrier gas at a flow rate of 40 ml/min. The lower detection limit for ethanol was 0.01 mg/g.

Results

Pharmacokinetic study of ethanol in rabbits

Changes in ethanol concentrations in the peripheral blood of the rabbits are shown in Fig. 1. Concentrations in the peripheral blood increased rapidly to reach a maximum at 1 h after oral administration. The average ethanol concentrations at 30 min and 1 h after administration were 2.12 and 3.06 mg/ml, respectively. This high level was maintained for about 1 h. Concentrations in the blood gradually decreased and were undetectable 14 h after ethanol ingestion.

After oral ingestion of 40% ethanol solution (10 ml/kg), the rabbits sometimes staggered around or had difficulty walking after 30 min, a state which reached a peak 1–2 h later. The rabbits gradually recovered after 2 h and had completely recovered 14 h later. Therefore, the intoxicated state of the rabbits reflected the levels of ethanol in the peripheral blood.

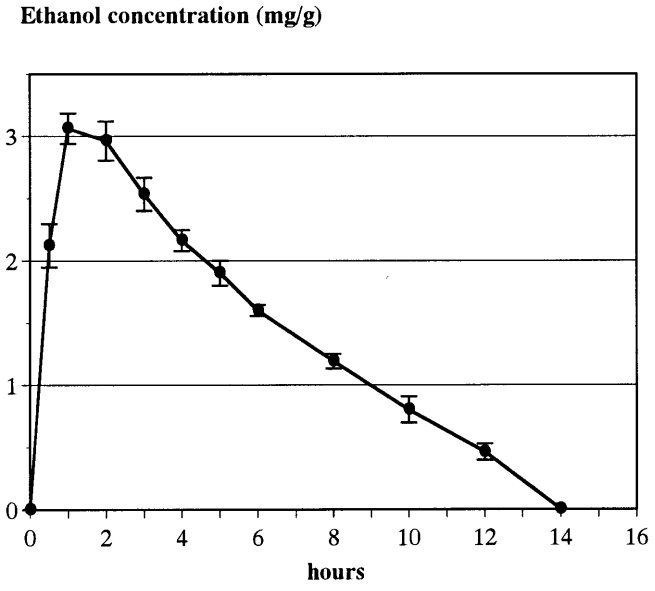

Fig. 1 Changes in ethanol concentrations in the peripheral blood of rabbits (n = 10)

Ethanol levels in subdural hematomas and various brain regions

Concentrations of ethanol in the subdural hematomas and peripheral blood after induction of a subdural hemorrhage are shown in Fig. 2. Concentrations in the subdural hematomas decreased gradually and corresponded to those

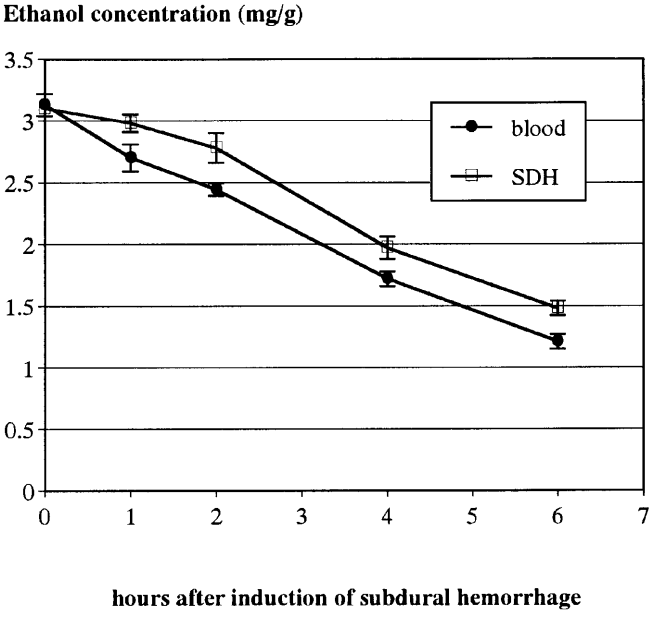

Fig. 2 Ethanol levels in the subdural hematoma and the peripheral blood of rabbits after induction of a subdural hemorrhage (n = 5)

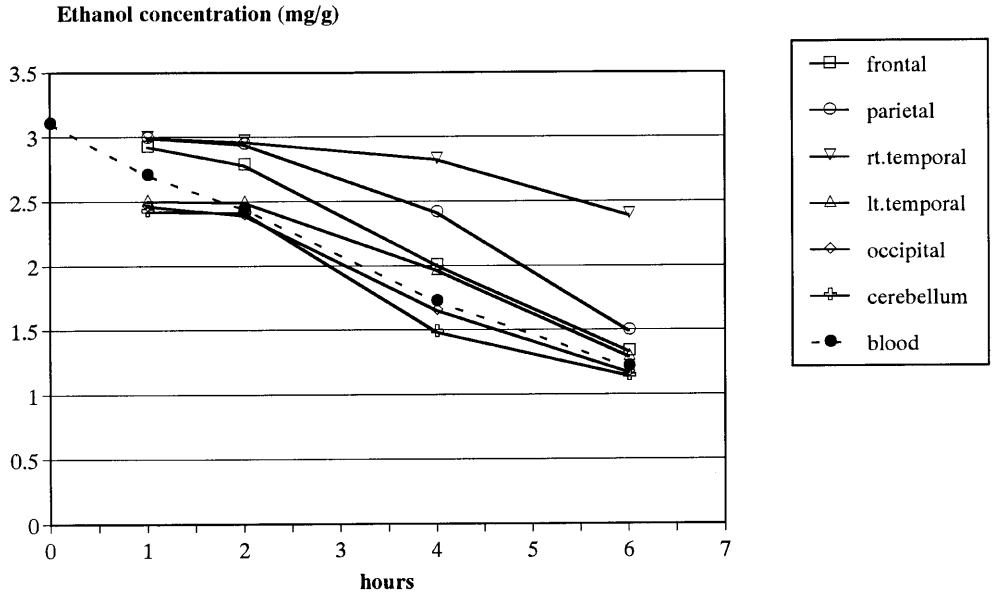

Fig. 3 Ethanol levels in various regions of the brain of rabbits after induction of a subdural hemorrhage ($n = 5$)

in the peripheral blood and in the subdural hematomas they were slightly higher than in the peripheral blood.

Rabbits immediately became unconscious after the induced subdural hemorrhage, and neurological findings such as anisocoria were observed in all rabbits. The brain tissues were slightly softened and many small hemorrhages were found on the surface of the right temporal, parietal and frontal lobes.

The concentrations of ethanol in various regions of the brain after induction of the subdural hemorrhages are shown in Fig. 3. High levels of ethanol in the right temporal, parietal and frontal lobes were evident for 2 h and a high level in the right temporal lobe was maintained for 6 h. Concentrations of ethanol in other regions corresponded fairly well to those in the peripheral blood while those in the cerebellum and occipital lobe were lower than in other brain regions. Differences in concentrations in the various brain regions were markedly increased at 4 h. High concentrations in parietal and frontal lobes at 1 h gradually decreased and corresponded to those in the peripheral blood with the passage of time.

Influence of infusion of ethanol-free i.v. fluid on the ethanol concentrations

When rabbits were infused with 1.0 ml/kg h^{-1} ethanol-free Terupack, the normal state of respiration was maintained. At a rate of 2.0 ml/kg h^{-1}, a dose used for humans, some rabbits hardly breathed due to an increased preload on the heart and lungs at 3 h after this infusion.

Table 1 shows the concentrations of ethanol in peripheral blood, subdural hematomas and brain tissues of 10 rabbits infused with Terupack. Infusion was started 1 h after the induction of the subdural hemorrhage and was continued for 3 h. Concentrations at 4 h after the induction of the subdural hemorrhage without i.v. infusion are also shown in Table 1. Concentrations in peripheral blood and the subdural hematomas were unaltered with both rates of infusion. Ethanol concentrations in the brain of infused rabbits were not significantly different from those in rabbits not infused, except that a higher concentration of ethanol in the cerebellum was evident.

Discussion

When death occurs immediately after an injury has been sustained, it is accepted that ethanol concentrations in

Table 1 Concentrations of ethanol in blood, subdural hematomas and brain of rabbits under the influence of an infusion of ethanol-free i.v. fluid (mg/g)

Rate of infusion	Ethanol concentration							
	Blood	Hematoma	Brain region					
			Frontal	Parietal	Right temporal	Left temporal	Occipital	Cerebellum
1.0 ml/kg h^{-1}	1.77 ± 0.07	2.09 ± 0.12	2.06 ± 0.09	2.33 ± 0.34	2.69 ± 0.20	1.99 ± 0.16	1.84 ± 0.16	1.86 ± 0.12
2.0 ml/kg h^{-1}	1.75 ± 0.05	2.00 ± 0.10	2.37 ± 0.41	2.42 ± 0.40	2.76 ± 0.12	2.01 ± 0.18	1.75 ± 0.09	1.83 ± 0.07
No injection	1.72 ± 0.06	1.97 ± 0.09	2.00 ± 0.52	2.41 ± 0.51	2.82 ± 0.09	1.96 ± 0.35	1.65 ± 0.16	1.48 ± 0.13

blood and urine samples obtained at autopsy accurately reflect the situation at the time of the fatal trauma. However, if the victim survives for several hours, ethanol in the blood is gradually metabolized and does not therefore reflect the level of ethanol in the victim's body at the time of injury.

Hirsch [3] suggested that ethanol in a subdural hematoma might be detectable even after ethanol in the blood had been completely metabolized. Other workers also mentioned the usefulness of analyzing ethanol levels in the subdural hematoma [4, 5, 6, 7, 8, 10, 11] and Buchsbaum et al. [9] reported that measurement of ethanol in a subdural hematoma can provide forensically pertinent information, particularly when relatively long or unknown time intervals have passed between injury and death.

In our experiment, the concentrations of ethanol in the subdural hematoma and brain in various regions remote from the subdural hematoma gradually decreased and corresponded to concentrations of ethanol in the peripheral blood. This would suggest that the blood circulation in the subdural hematoma and brain regions continued even after the induced subdural hemorrhage and did not cease up to the time of death. Slightly higher concentrations in the subdural hematoma than in peripheral blood were probably due to a poorer circulation of blood in the subdural hematoma. Therefore, the concentration of ethanol in the subdural hematoma examined at autopsy does not indicate the concentration present at the time of injury.

On the other hand, significantly high concentrations of ethanol were found in the brain just underneath the subdural hematoma, which were considered to stem from the cessation of cerebral blood flow in the brain underneath the subdural hematoma after the induction of a hemorrhage. Cerebral blood flow was probably altered with the occurrence of the severe head injury.

There is some documentation [12, 14] regarding regional cerebral blood flow after severe head injuries. Pfenninger et al. [15] noted a sudden increase in intracranial pressure, cerebral perfusion pressure and a decrease of cerebral blood flow after head injury induced in laboratory animals. They stated that the subdural hematoma pressed directly on brain tissues and regional cerebral blood flow decreased. In our experiments, acute subdural hemorrhages were induced by injecting blood into the subdural space without direct head injury. However, the findings in rabbit brains after induction of a hemorrhage suggest that the subdural hematoma had pressed on the brain as a space-occupying lesion and that regional cerebral blood flow in the brain underneath the subdural hematoma rapidly decreased. Therefore, we considered that concentrations of ethanol underneath the subdural hematoma could provide useful information regarding the concentration of ethanol in the body of a human victim at the time the injury occurred.

An immunohistochemical study was carried out for the age determination of the brain injuries [16, 17]. In the meantime, changes in drug distribution in the brain can be due to cessation of cerebral blood flow, and related data are pertinent to determine the time and progression to brain death [18, 19]. We also reported findings in a victim where concentrations of ethanol in the subdural hematoma did not indicate the blood level at the time of injury and the distribution in the brain was more useful to estimate the physiological state of the victim after severe head injury [13].

With respect to the influence of intravenous infusion, concentrations of ethanol in peripheral blood and subdural hematoma remained unchanged with the two different rates of infusion tested. Ameno and Nanikawa reported that ethanol concentrations in the blood of rabbits did not decrease with i.v. administration of physiological saline [20]. As for concentrations in the brain, high levels of ethanol in the cerebellum were observed in our i.v.-infused rabbits probably due to a high infusion pressure and early cessation of cerebral blood flow in the cerebellum. Therefore, concentrations of ethanol in the subdural hematoma correspond to those in the peripheral blood and do not indicate concentrations present at the time of injury. The concentrations of ethanol in the brain of various regions depend on the cerebral blood flow of the victim, and determination of levels in the brain underneath the subdural hematoma will be pertinent to determine if the victim was under the influence of ethanol at the time of injury. This is apparently the first study to confirm the usefulness of measurements of ethanol in brain tissues.

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References

1. DiMaio DJ, DiMaio VJM (1989) Forensic pathology. Elsevier, Amsterdam, p 156
2. Wilberger JE Jr, Harris M, Diamond DL (1991) Acute subdural hematoma: morbidity, mortality, and operative timing. J Neurosurg 74:212–218
3. Hirsch CS (1973) Ethanol in sequestered hematomas. Am J Clin Pathol 59:429–433
4. Freireich AW, Bidanset JH, Lukash L (1975) Alcohol levels in intracranial blood clots. J Forensic Sci 20:83–85
5. Nanikawa R, Ameno K, Hashimoto Y (1977) Medicolegal aspects on alcohol detected in autopsy cases. Alcohol levels in hematomas (in Japanese with English abstract). Nippon Hoigaku Zasshi 31:241–247
6. Smialek JE, Spitz WU, Wolfe JA (1980) Ethanol in intracranial clot. Report of two homicidal cases with prolonged survival after injury. Am J Forensic Med Pathol 1:149–150
7. Cassin BJ, Spitz WU (1983) Concentration of alcohol in delayed subdural hematoma. J Forensic Sci 28:1013–1015
8. Eisele JW, Reay DT, Bonnell HJ (1984) Ethanol in sequestered hematomas: quantitative evaluation. Am J Clin Pathol 81:352–355
9. Buchsbaum RM, Adelsen L, Sunshine I (1989) A comparison of post-mortem ethanol levels obtained from blood and subdural specimens. Forensic Sci Int 41:237–243
10. Riggs JE, Schochet SS Jr, Frost JL (1998) Ethanol level differential between postmortem blood and subdural hematoma. Mil Med 163:722–724
11. Moriya F, Hashimoto Y (1998) Medicolegal implications of drugs and chemicals detected in intracranial hematomas. J Forensic Sci 43:980–984
12. Bullock R, Butcher SP, Chen MH, Kendall L, MacCulloch J (1991) Correlation of the extracellular glutamate concentration with extent of blood flow reduction after subdural hematoma in the rat. J Neurosurg 74:794–802

13. Takahashi K, Ikeda N, Kudo K, Ohtsuka Y (1999) Ethanol distribution in the brain of a victim autopsied after acute subdural hemorrhage. Legal Med 1:111–113
14. Marion DW, Darby J, Yonas H (1991) Acute regional cerebral blood flow changes caused by severe head injuries. J Neurosurg 74:407–414
15. Pfenninger EG, Reith A, Breitig D, Grünert A, Ahnefeld FW (1989) Early changes of intracranial pressure, perfusion pressure, and blood flow after acute head injury. 1. An experimental study of the underlying pathophysiology. J Neurosurg 70:774–779
16. Hausmann R, Kaiser A, Lang C, Bohnert M, Betz P (1999) A quantitative immunohistochemical study on the time-dependent course of acute inflammatory cellular response to human brain injury. Int J Legal Med 112:227–232
17. Hausmann R, Betz P (2000) The time course of the vascular response to human brain injury – an immunohistochemical study. Int J Legal Med 113:288–292
18. Jitsufuchi N, Kudo K, Imamura T, Nagata T (1996) Brain death diagnosed by forensic analysis of drug distribution in human tissues. II. Distribution of caffeine in brain death. Nippon Hoigaku Zasshi 50:72–77
19. Jitsufuchi N, Kudo K, Imamura T, Kimura K, Ikeda N (1997) Distribution of drugs in various tissues in a brain dead man. Forensic Sci Int 90:103–109
20. Ameno K, Nanikawa R (1976) Studies on the alcohol examination in autopsy cases. Blood alcohol levels in cases of profuse bleeding (in Japanese with English abstract). Nippon Hoigaku Zasshi 30:36–41