Alcohol & Alcoholism Vol. 37, No. 1, pp. 9–12, 2002

RAPID COMMUNICATION

DIFFERENT BLOOD ACETALDEHYDE CONCENTRATION FOLLOWING ETHANOL ADMINISTRATION IN A NEWLY DEVELOPED HIGH ALCOHOL PREFERENCE AND LOW ALCOHOL PREFERENCE RAT MODEL SYSTEM

MINORI NISHIGUCHI, HIROSHI KINOSHITA*, JAMAL MOSTOFA[1], TADAAKI TANIGUCHI, HARUMI OUCHI, TAKAKO MINAMI, KATSUHIKO HATAKE[2], TAKAO UTSUMI[3], HIROYUKI MOTOMURA[4] and SHIGERU HISHIDA

Department of Legal Medicine and [3]Department of Ophthalmology, Hyogo College of Medicine, 1-1, Mukogawa-cho, Nishinomiya, Hyogo, 663-8501, [1]Department of Forensic Medicine, Kagawa Medical University, 1750-1, Miki, Kita, Kagawa, 761-0793, [2]Department of Legal Medicine, Nara Medical University, 840, Shijo-cho, Kashihara, Nara, 634-8521 and [4]Forensic Science Laboratory, Hyogo Prefectural Police Headquarters, 4-1, Shimoyamate-dori 5-chome, chuo-ku, kobe, 650-8510, Japan

(*Received* 21 *May* 2001; *in revised form* 23 *August* 2001; *accepted* 22 *September* 2001)

Abstract — A significant difference in blood-acetaldehyde concentration was observed between high alcohol-preference (HAP) rats and low alcohol-preference (LAP) rats, newly developed different alcohol preference lines. This difference of acetaldehyde accumulation may be due to cytosolic aldehyde dehydrogenase (ALDH1) polymorphism, which has been reported previously. As the doses of ethanol we employed are higher than that of voluntary drinking, there may be little direct relationship between acetaldehyde accumulation and alcohol preference. We suggest therefore that the ALDH1 polymorphism is associated with alcohol preference in HAP/LAP lines through some other unidentified mechanism.

INTRODUCTION

Acetaldehyde is the initial metabolite of ethanol, which is produced in the liver following ethanol administration. Aldehyde dehydrogenase (ALDH: EC 1.2.1.3) oxidizes a broad class of aldehydes to their carboxylic acids (Lindahl, 1992). ALDH has been found in mitochondria, microsomes and cytosol in rat liver (Tottmar *et al*., 1973; Horton and Barrett, 1975; Lindahl and Evces, 1984) and isozymes are divided into three classes, namely ALDH1 (high K_m, cytosolic form), ALDH2 (low K_m, mitochondrial form) and ALDH3 (inducible cytosolic and microsomal forms) (Lindahl, 1992). Most of the acetaldehyde produced from ethanol is metabolized quickly to acetate by liver ALDH and hence acetaldehyde concentration in blood following ethanol administration is very low (Eriksson, 1973; Eckardt *et al*., 1998). The mitochondrial low K_m ALDH plays a major role in this pathway (Eriksson *et al*., 1975; Svanas and Weiner, 1985; Eckardt *et al*., 1998). In some Oriental populations with a lowered genetic activity of ALDH, high blood concentrations of acetaldehyde are produced following ethanol ingestion (Enomoto *et al*., 1991). As acetaldehyde is a highly toxic metabolite, it can cause adverse symptoms in susceptible individuals, including nausea, headache and palpitations (Enomoto *et al*., 1991). These individuals consume less alcohol than people who have normal activity of ALDH (Higuchi *et al*., 1992). This rapid accumulation of acetaldehyde in blood following ethanol ingestion, due to a lower activity of ALDH, is believed to play a protective role against alcoholism (Harada *et al*., 1982).

High and low alcohol preference rodents, developed by selective breeding, are widely used as animal models in the study of alcoholism (Li *et al*., 1994). There have been many studies performed to assess differences in alcohol preference in these rodents. Previously, there was a report that acetaldehyde concentration following ethanol injection in an alcohol avoidance strain is higher than that of an alcohol preference strain of mice (Sheppard *et al*., 1970). In the AA/ANA rat model developed in Alko by Eriksson (1968), it has been reported that the blood concentration of acetaldehyde following ethanol administration is significantly higher in the ANA rat than in the AA rat (Eriksson, 1973; Koivisto *et al*., 1993). It has been reported that liver ALDH activity in the ANA rat is significantly lower than that of the AA rat (Eriksson, 1973; Koivula *et al*., 1975; Koivisto and Eriksson, 1994). However, the polymorphism of ALDH2 was not associated with alcohol preference in the AA/ANA model (Koivisto *et al*., 1993).

Recently, high alcohol-preferring rat lines (HAP) and low alcohol-preferring rat lines (LAP) were newly developed from the Wistar rat colony by Hishida (1996), and differences in ALDH activity and in cytosolic ALDH (ALDH1) polymorphism have been observed between these two lines. The activity of liver ALDH in HAP rats was higher than that of LAP rats (Hishida, 1996). Three phenotypes of ALDH1, termed AA, AC and CC type, have been revealed by the band pattern of isoelectric focusing (Negoro *et al*., 1997). The AA type was observed in almost all HAP rats, and almost all LAP rats were CC type (Hishida, 1996). It has also been reported that pharmacological effects of methamphetamine may be influenced by ethanol preference in this line. The HAP rats showed significantly lower dopamine and serotonin release in the striatum and nucleus accumbens than LAP rats following methamphetamine administration (Yamauchi *et al*., 2000). However, there is little information on possible differences in acetaldehyde concentration between these two rat lines following ethanol administration (Hishida, 1996). The present study was designed to investigate differences in ethanol metabolism *in vivo* between the HAP and LAP lines.

*Author to whom correspondence should be addressed.

MATERIALS AND METHODS

HAP and LAP rat lines were developed in the laboratory, Department of Legal Medicine, Hyogo College of Medicine, by selective inbreeding from the Wistar colony of rats. The ethanol preference of the rats was assessed by the one-lever two-liquid chamber method, as previously described (Iso and Sakaki, 1982). Briefly, the voluntary choice of 10% ethanol solution or water was assessed by lever-pressing response following the lever press training by the operant conditioning procedure. The number of responses for 10% ethanol solution or water, and the volume of ethanol consumption were recorded. The ratio of ethanol reinforcement (RER) was defined as the ratio of the number of responses to ethanol solution to the total number of responses to both the ethanol solution and water. The daily ethanol intake was calculated from the total amount of ethanol consumed per kg body wt. The criteria for the classification of alcohol preference was as follows. HAP was defined as the RER >0.50 with a daily ethanol intake >8.0 g/kg, whereas LAP was defined as having an RER <0.39 with a daily ethanol intake <5.49 g/kg (Hishida, 1996).

The alcohol-naive male rats used in this experiment aged from the 22nd–24th generation of the HAP line and the 26th–28th generation of the LAP line, with a weight range of 350–400 g. All animals were housed with a 12-h light/12-h darkness cycle in a temperature- and humidity-controlled environment with free access to food and water. A venous cannula was implanted in the jugular vein in each animal 1 day prior to the experiment, under pentobarbital anaesthesia (50 mg/kg, intraperitoneally). Three experimental groups were employed in each of the rat lines. Rats received an intravenous (i.v.) injection of ethanol at three treatment levels, i.e. 2.0, 3.5 and 5.0 g/kg [in the form of a 20% (v/v) ethanol solution in 0.15 M NaCl]. Ethanol was infused for 10 min at a steady rate. Blood samples (500 µl) were taken immediately prior to i.v. injection of ethanol at t = 0, 30, 60 and 120 min following administration of ethanol. After each blood sampling, an equal volume of heparinized saline was infused. The concentrations of blood ethanol and acetaldehyde were measured by headspace gas chromatography, as previously described (Okada and Mizoi, 1982). Acetaldehyde was purchased from Merck (Munich, Germany). All other reagents were purchased from Wako Pure Chemicals (Osaka, Japan). Following treatment, rats were killed by decapitation and their livers were removed immediately. The preparation of subcellular fractions and isoelectric focusing were performed, as previously described (Negoro *et al*., 1997), to identify the typing of the liver cytosolic ALDH1 polymorphism.

Data are expressed as mean ± SD. Statistical analysis of the data was performed by one-way analysis of variance followed by the Fisher PLSD test. $P < 0.05$ was considered statistically significant. This study was approved by the Animal Investigation Committee, Hyogo College of Medicine.

RESULTS

Table 1 shows blood concentrations of acetaldehyde in each treatment and control group. Acetaldehyde concentration in LAP rats was significantly higher at all time-points of the 5.0 g/kg treatment group and at 30 min with the 3.5 g/kg treatment group ($P < 0.05$), compared with the HAP line. However, there were no significant differences at all other times within the 3.5 g/kg treatment group and at no time within the 2.0 g/kg treatment group.

Table 1. Blood-acetaldehyde concentration (µM) after ethanol administration to HAP and LAP rats

Rat line and ethanol dose (g/kg)	Time following ethanol administration (min)		
	30	60	120
HAP (2.0)	12.06 ± 3.40	9.42 ± 1.76	8.36 ± 1.15
LAP (2.0)	12.88 ± 3.70	11.58 ± 2.27	10.53 ± 1.06
HAP (3.5)	14.68 ± 0.76	14.84 ± 0.67	14.58 ± 0.53
LAP (3.5)	20.15 ± 1.83*	16.79 ± 2.24	17.06 ± 1.27
HAP (5.0)	19.03 ± 3.57	18.90 ± 5.86	17.93 ± 2.10
LAP (5.0)	31.58 ± 7.89*	35.23 ± 5.23*	37.60 ± 2.07*

Values are means ± SD (n = 7 or 8). *$P < 0.05$ compared with the same-dosed HAP group.
HAP, high alcohol preference rats; LAP, low alcohol preference rats.

There were no significant differences in blood ethanol concentration between HAP and LAP rats at all three doses (Fig. 1). The typing of liver ALDH1 phenotypes was performed in all rats and showed that all HAP rats demonstrated AA type and that all LAP rats were of the CC type, as shown previously (Negoro *et al*., 1997).

DISCUSSION

The present study demonstrated that LAP rats showed significantly higher concentrations of acetaldehyde in blood following ethanol administration in comparison to HAP rats. The HAP and LAP lines used in the present study were selected by the method of Iso and Sakaki (1982). This method

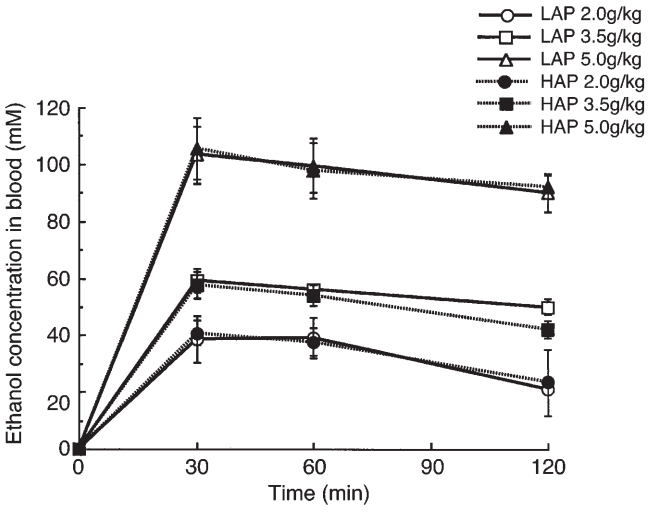

Fig. 1. Time-course of blood-ethanol concentration in high (HAP) and low (LAP) alcohol-preferring rat lines following administration of ethanol at three different doses.
Values are the means ± SD (bars) for n = 7 or 8 rats per group.

has two advantages in comparison to the two bottle method, including: (1) freedom from the problem of positional habit (Myers and Holman, 1966), which is a problem observed in the concurrent choice method; (2) voluntary ethanol ingestion can be observed by this method. Ethanol drinking without food deprivation is a critical factor when assessing any animal model of alcoholism. We believe that this method is more suitable for selection of ethanol preference than the concurrent choice method, and that the HAP and LAP lines are suitable for the study of alcohol preference.

The difference in acetaldehyde levels following ethanol administration has also been observed in other alcohol-preferring lines, such as AA and ANA following 1.5 g/kg ethanol administration (Eriksson, 1973; Koivisto *et al*., 1993). In our experiments, three different doses of ethanol were employed, including 2.0, 3.5 and 5.0 g/kg, and a difference was noted in lineages in the latter two treatment groups. In comparing the HAP and LAP lines, differences in ALDH activity have already been reported (Hishida, 1996), and genetic differences associated with the ALDH1 phenotype have been demonstrated previously (Negoro *et al*., 1997). In humans and rats, a mitochondrial low K_m ALDH (ALDH2) plays a major role in acetaldehyde metabolism *in vivo* (Eriksson *et al*., 1975; Svanas and Weiner, 1985; Eckardt *et al*., 1998). However, the cytosolic ALDH1 in the rat significantly differs from that in the human. The reported K_m value of rat ALDH1 for acetaldehyde is 15 ± 3 μM, which is relatively lower than that in humans (Klyosov *et al*., 1996), suggesting that in the human liver, mitochondrial ALDH2 mainly oxidizes acetaldehyde at physiological concentrations, whereas in the rat liver, both mitochondrial and cytosolic ALDH are functional (Klyosov *et al*., 1996).

In our experiments, the line difference in blood acetaldehyde concentration was clearly observed. As this difference was observed at a high dose of ethanol, it may reflect the difference in the high K_m ALDH (ALDH1) activities due to the different phenotypes in HAP/LAP lines. However, the doses of ethanol used were relatively higher than that consumed by voluntary drinking, and the peak concentrations of acetaldehyde following i.v. administration of ethanol were therefore higher than that after voluntary drinking. From the present and previous results, we conclude that ALDH1 polymorphism may be a major factor in acetaldehyde accumulation in HAP/LAP lines, but is unlikely to explain their different alcohol preferences.

Although there are several reports that alcohol preference may correlate with ALDH activity more in the brain than in the liver (Amir, 1978; Socaransky *et al*., 1984), this mechanism is still relatively unknown. There is a report that ALDH is involved in biogenic amine metabolism (Berger and Weiner, 1977). However, oxidation of biogenic aldehydes occurs in mitochondria, and the physiological role of cytosolic ALDH1 is still unknown (Tank *et al*., 1986). Under basal conditions, there was no difference in the extracellular contents of biogenic amines, such as dopamine and serotonin, in striatum and nucleus accumbens between HAP and LAP lines (Yamauchi *et al*., 2000). As acetaldehyde itself has many pharmacological actions (Brien and Loomis, 1983), it may act on the central nervous system (Kinoshita *et al*., 2001), where differences in acetaldehyde elimination may contribute to ethanol preference. However, as we have not yet investigated brain ALDH activity nor metabolites of biogenic amines in both rat lines, further studies are needed to clarify this hypothesis.

REFERENCES

Amir, S. (1978) Brain and liver aldehyde dehydrogenase activity and voluntary ethanol consumption by rats: relations to strain, sex and age. *Psychopharmacology* **57**, 97–102.

Berger, D. and Weiner, H. (1977) Relationship between alcohol preference and biogenic aldehyde metabolizing enzymes in rats. *Biochemical Pharmacology* **26**, 841–846.

Brien, J. F. and Loomis, C. W. (1983) Pharmacology of acetaldehyde. *Canadian Journal of Physiology and Pharmacology* **61**, 1–22.

Eckardt, M. J., File, S. E., Gessa, G. L., Grant, K. A., Guerri, C., Hoffman, P. L., Kalant, H., Koob, G. F., Li, T. K. and Tabakoff, B. (1998) Effects of moderate alcohol consumption on the central nervous system. *Alcoholism: Clinical and Experimental Research* **22**, 998–1040.

Enomoto, N., Takase, S., Yasuhara, M. and Takada, A. (1991) Acetaldehyde metabolism in different aldehyde dehydrogenase-2 genotypes. *Alcoholism: Clinical and Experimental Research* **15**, 141–144.

Eriksson, C. J. P. (1973) Ethanol and acetaldehyde metabolism in rat strains genetically selected for their ethanol preference. *Biochemical Pharmacology* **22**, 2283–2292.

Eriksson, C. J. P., Marselos, M. and Koivula, T. (1975) Role of rat liver aldehyde dehydrogenases in the oxidation of acetaldehyde during ethanol metabolism *in vivo*. *Biochemical Journal* **152**, 709–712.

Eriksson, K. (1968) Genetic selection for voluntary alcohol consumption in the albino rat. *Science* **159**, 739–741.

Harada, S., Agarwal, D. P., Goedde, H. W., Takagi, S. and Ishikawa, B. (1982) Possible protective role against alcoholism for aldehyde dehydrogenase isozyme deficiency in Japan. *Lancet* **ii**, 827.

Higuchi, S., Muramatsu, T., Shigemori, K., Saito, M., Kono, H., Dufour, M. and Harford, T. (1992) The relationship between low Km aldehyde dehydrogenase phenotype and drinking behavior in Japanese. *Journal of Studies on Alcohol* **53**, 170–175.

Hishida, S. (1996) Application of operant conditioning techniques to forensic toxicology: Experimental studies on alcohol and abused drugs. *Japanese Journal of Legal Medicine* **50**, 292–313.

Horton, A. A. and Barrett, M. C. (1975) The subcellular localization of aldehyde dehydrogenases in rat liver. *Archives of Biochemistry and Biophysics* **167**, 426–436.

Iso, H. and Sakaki, N. (1982) Lever press responding as a measure of ethanol preference in rats. *Psychopharmacology* **76**, 36–39.

Kinoshita, H., Jessop, D. S., Finn, D. P., Coventry, T. L., Roberts, D. J., Ameno, K., Ijiri, I. and Harbuz, M. S. (2001) Acetaldehyde, a metabolite of ethanol, activates the hypothalamic–pituitary–adrenal axis in the rat. *Alcohol and Alcoholism* **36**, 59–64.

Klyosov, A. A., Rashkovetsky, L. G., Tahir, M. K. and Keung, W.-M. (1996) Possible role of liver cytosolic and mitochondrial aldehyde dehydrogenases in acetaldehyde metabolism. *Biochemistry* **35**, 4445–4456.

Koivisto, T. and Eriksson, C. J. P. (1994) Hepatic aldehyde and alcohol dehydrogenases in alcohol-preferring and alcohol-avoiding rat lines. *Biochemical Pharmacology* **48**, 1551–1558.

Koivisto, T., Carr, L. G., Li, T.-K. and Eriksson, C. J. P. (1993) Mitochondrial aldehyde dehydrogenase (ALDH2) polymorphism in AA and ANA rats: lack of genotype and phenotype line differences. *Pharmacology, Biochemistry and Behavior* **45**, 215–220.

Koivula, T., Koivusalo, M. and Lindros, K. O. (1975) Liver aldehyde and alcohol dehydrogenase activities in rat strains genetically selected for their ethanol preference. *Biochemical Pharmacology* **24**,1807–1811.

Li, T.-K., Lumeng, L., McBride, W. J. and Murphy, J. M. (1994) Genetic and neurobiological basis of alcohol-seeking behaviour. *Alcohol and Alcoholism* **29**, 697–700.

Lindahl, R. (1992) Aldehyde dehydrogenases and their role in carcinogenesis. *Critical Reviews in Biochemistry and Molecular Biochemistry* **27**, 283–335.

Lindahl, R. and Evces, S. (1984) Rat liver aldehyde dehydrogenase. *Journal of Biological Chemistry* **259**, 11986–11990.

Myers, R. D. and Holman, R. B. (1966) A procedure for eliminating position habit in performance-aversion test for ethanol and other fluids. *Psychonomic Science* **6**, 235–236.

Negoro, M., Hatake, K., Taniguchi, T., Ouchi, H., Minami, T. and Hishida, S. (1997) Liver cytosolic aldehyde dehydrogenase (ALDH1) polymorphism and its inheritance in Wistar rats. *Japanese Journal of Alcohol and Drug Dependence* **32**, 182–188.

Okada, T. and Mizoi, Y. (1982) Studies on the problem of blood acetaldehyde determination in man and its level after alcohol intake. *Japanese Journal of Alcohol Studies and Drug Dependence* **17**, 141–159.

Sheppard, J. R., Albersheim, P. and McClearn, G. (1970) Aldehyde dehydrogenase and ethanol preference in mice. *Journal of Biological Chemistry* **245**, 2876–2882.

Socaransky, S. M., Aragon, C. M. G., Amit, Z. and Blander, A. (1984) Higher correlation of ethanol consumption with brain than liver aldehyde dehydrogenase in three strains of rats. *Psychopharmacology* **84**, 250–253.

Svanas, G. W. and Weiner, H. (1985) Aldehyde dehydrogenase activity as the rate-limiting factor for acetaldehyde metabolism in rat liver. *Archives of Biochemistry and Biophysics* **236**, 36–46.

Tank, A. W., Deitrich, R. A. and Weiner, H. (1986) Effects of induction of rat liver cytosolic aldehyde dehydrogenase on the oxidation of biogenic aldehydes. *Biochemical Pharmacology* **35**, 4563–4569.

Tottmar, S. O. C., Pettersson, H. and Kiessling, K.-H. (1973) The subcellular distribution and properties of aldehyde dehydrogenases in rat liver. *Biochemical Journal* **135**, 577–586.

Yamauchi, J., Marukawa, S. and Hishida, S. (2000) Simultaneous administration of ethanol emphasizes the effect of methamphetamine on central nervous system in rat with high alcohol preference. *Japanese Journal of Alcohol and Drug Dependence* **35**, 28–47.