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Abstract

Sake or bourbon (8g ethanol/kg body weight) was intragastrically administered to rats for 12 days. An equal dose of ethanol in water or an isocaloric glucose solution was administered to control groups. Food was withheld, but water freely provided. Neither mortality nor liver and body weights were different between the alcohol-treated groups. Glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were more elevated in the sake group than in the other groups. Additionally, liver fibrosis was more pronounced, and vacuole formation or steatosis was less in this group. These results suggest that sake is more fibrogenic. Some components other than ethanol, such as long-alkyl chain alcohols, may have been responsible for the differential histopathology.

KEYWORDS: alcoholic liver injury, alcoholic beverages, alcoholic liver fibrosis, longchain alcohols, rats

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EFFECTS OF SAKE AND BOURBON ON LIVER HISTOPATHOLOGY AND FUNCTION IN RATS

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Abstract. Sake or bourbon (8 g ethanol/kg body weight) was intragastrically administered to rats for 12 days. An equal dose of ethanol in water or an isocaloric glucose solution was administered to control groups. Food was withheld, but water freely provided. Neither mortality nor liver and body weights were different between the alcohol-treated groups. Glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were more elevated in the sake group than in the other groups. Additionally, liver fibrosis was more pronounced, and vacuole formation or steatosis was less in this group. These results suggest that sake is more fibrogenic. Some components other than ethanol, such as long-alkyl chain alcohols, may have been responsible for the differential histopathology.

Key words : alcoholic liver injury, alcoholic beverages, alcoholic liver fibrosis, long-chain alcohols, rats.

The incidence of alcoholic liver injury in Japan is increasing parallel to the increase in alcohol consumption (1). Alcoholic liver fibrosis is more frequent in Japan than in Europe or the USA, while alcoholic hepatitis is seen less often (2). This difference in liver pathology may be caused by the various alcoholic beverages consumed. The vehicles of alcoholic beverages are known to contain several long-alkyl chain alcohols such as isoamylalcohol, phenetylalcohol, isobutanol and n-propanol (3). Sake presents the highest level of phenetylalcohol, while bourbon contains the most isoamylalcohol. Numerous studies concerning alcoholic liver injury have been undertaken (4-6), but rarely from the aspect of different alcoholic beverages (7, 8).

MATERIALS AND METHODS

Thirty-six male Sprague-Dawley rats, weighting 250-300 g each, were divided into 4 groups: those given sake, bourbon, ethanol or glucose. Sake contains 17 vol. % ethanol, and bourbon, 45 vol. % ethanol. Bourbon and ethanol were diluted with water to a final concentration of 17 vol. % ethanol. Four g ethanol per kg body weight was administered twice daily for 12 days. An isocaloric amount of glucose, dissolved in a similar volume of water, was given to the control group. The beverages were administered to rats through a stomach tube while

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under ether anesthesia. Rats were deprived of all food, but allowed free access to water. From the 9th to 12th day, blood samples were obtained from the abdominal aorta for liver function tests: total bilirubin, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), GOT/GPT ratio, alkaline phosphatase (ALP), luecine aminopeptidase (LAP) and γ-glutamyl-transpeptidase (γ-GTP). Transaminase was evaluated after logarithm conversion to achieve a normal distribution.

Immediately after sacrificing, the liver was irrigated via the thoracic aorta with 500 ml of Ringer solution and fixed with 100 ml of 1 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The liver was then removed and prepared for light microscopy and scanning electron microscopy (SEM) by standard procedures (9). The histological specimens were examined independently by 5 doctors, under double blind random conditions.

RESULTS

Mortality and morbidity. In the 3 alcoholic groups, 7 rats (24 %) died within 8 days, 3 in the sake group, and 2 each in the ethanol and bourbon groups. Thereafter, rats were sacrificed when they became weak. However, 5 rats were not sacrificed as they died during the night. Thirteen (33 %) additional rats in the alcoholic groups died or were sacrificed before the end of the experiment. Only seven rats survived the entire treatment. The mortality rate did not differ among the alcoholic groups (Table 1). In the glucose-fed group, two rats were sacrificed on day 10. This situation is in contrast to that observed in the ethanol-administered animals. Body weight loss at the time of death in the sake (37 %) and bourbon (37 %) groups was greater than in the ethanol (31 %) and glucose (30 %) groups. The liver weight to body weight ratio of the sake (4.4 %) and ethanol (4.4 %) groups was significantly greater than that of the bourbon (3.8 %) and glucose (3.6 %) groups (Table 1).

Liver function. Total bilirubin of rats in the sake group was significantly higher than in the bourbon, ethanol and glucose groups. GOT and GPT activities varied between the 4 groups (Table 2); in descending order were the sake, bourbon, ethanol and glucose groups. The GOT/GPT ratio was the opposite, with the glucose group presenting a higher ratio than the other groups. LAP values of the glucose group were higher than those of the sake and bourbon groups. ALP and γ-GTP did not differ between the 4 groups (Table 2).

Histological evaluation. The grade of fibrosis was classified in the specimens stained with azan and Pap's silver solution by the following criteria: (−), no fibrosis; (±), slight fibrosis, and (+), marked fibrosis. In the sake group, distinct fibrosis was observed in 5 rats (Table 3, Fig. 1), whereas only slight, if any, fibrosis was noted in the other groups ($p < 0.05$). Collagen fibers were increased (Fig. 2), and microfibrils were observed surrounding fat storing cells (Fig. 3). Focal necrosis was observed in the bourbon, ethanol and glucose groups, but not in the sake group. There was, however, no significant difference. Cellular infiltration was confined to the necrotic areas. Massive necrosis was not noted in any group. Vacuole formation and steatosis were considered together as they appeared similar

TABLE 1. MORTALITY, BODY WEIGHT LOSS AND LIVER WEIGHT/BODY WEIGHT RATIO

	No. of rat	day of sacrifice (death)	body weight loss (%)	L•W/B•W ratio (%)
Sake group n = 9	1	(2)		
	2	(6)	21	6.3
	3	(8)	27	
	4	9	31	5.0
	5	9	34	4.0
	6	10	38	4.4
	7	11	33	4.2
	8	12	44	3.7
	9	12	41	5.0
			37 ± 5.0[a]	4.4 ± 0.5
Bourbon group n = 10	1	(4)	13	
	2	(4)	9	
	3	9	22	4.1
	4	10	31	4.3
	5	10	36	3.3 ※
	6	(12)	39	
	7	(12)	36	
	8	12	38	4.1
	9	12	38	3.7
	10	12	41	3.8
			37 ± 3.2	3.8 ± 0.4
Ethanol group n = 10	1	(3)	8	
	2	(5)	15	
	3	(9)	27	
	4	(9)	30 ※※ ※	
	5	(9)	28	
	6	9	30	3.8
	7	9	30	3.9
	8	10	31	5.3
	9	12	36	4.8
	10	12	37	4.0
			31 ± 3.6	4.4 ± 0.7
Glucose group n = 7	1	10	25	3.8
	2	10	28	3.4
	3	12	※※※ 30 ※※	3.4 ※※※
	4	12	29	3.8
	5	12	32	3.2 ※
	6	12	32	3.8
	7	12	31	
			30 ± 2.5	3.6 ± 0.3

a : Values are expressed as mean ± SD

Significant difference : ※ $P < 0.05$, ※※ $P < 0.01$, ※※※ $P < 0.001$

L.W : liver weight, B.W : body weight

TABLE 2. LIVER FUNCTION TESTS

	No. of rat	T-Bil[a] (mg/dl)	GOT[b] (IU/L)	GPT[b] (IU/L)	GOT/GPT[a]	ALP[a] (IU/L)	LAP[a] (IU/L)	γ-GTP[a] (IU/L)
Sake group n = 6	4	0.33	194	134	1.45	76	64	1
	5	0.54	412	192	2.15	87	60	2
	6	0.45	979	555	1.76	66	56	1
	7	0.54	335	199	1.68	122	59	2
	8	0.65	1574	1020	1.54	76	69	2
	9	0.29	1140	685	1.66	221	46	3
		0.47±0.14	601 (265,1360)	355 (155,809)	1.71±0.24	108±59	59±8	1.8±0.8
Bourbon group n = 6	3	0.37	191	148	1.29	121	58	4
	4	0.33	138	86	1.60	62	52	1
	5	0.34	405 ※	149 ※	2.72	134	64	1
	8	0.39	216	70	3.09	61	56	0
	9 ※	0.22	253	138	1.83	48	60	1
	10	0.42	692	454	1.52	109	64	2
		0.35±0.07	272 (152,485)	142 (74,272)	2.01±0.73	89±36	61±7	1.5±1.4
Ethanol group n = 5	6	0.30	148	65	2.28	102	96	2
	7	0.34	133 ※	78 ※	1.71	71	67	1
	8	0.26	119 ※※	105 ※※	1.13	66	49	1
	9 ※	0.33	158	65	2.43	69	86	1
	10	0.30	148	81	1.83	59	67	1
		0.31±0.03	141 (126,157)	78 (64,94)	1.98±0.64	73±17	73±18	1.2±0.5
Glucose group n = 7	1	0.38	108	29	3.72	92	66	2
	2	0.58 ※※	134	21	6.38	65	81	2
	3	0.32	161	27	5.96 ※※※※※※	53	78 ※※※※	1
	4	0.35	72 ※ ※※※ ※※※	27 ※※※ ※※※	2.67	74	75	3
	5	0.35	66	13	5.08	79	67	1
	6	0.41	84	17	4.94	59	74	1
	7	0.13	43	20	2.15	95	67	1
		0.35±0.11	87 (56,138)	21 (16,28)	4.41±1.61	74±16	73±6	1.6±0.8

a: Values are expressed as mean ± SD

b: Values are expressed as mean (mean − SD, mean + SD) after logarithm conversion

Significant differance: ※P < 0.05, ※※P < 0.01, ※※※P < 0.001

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TABLE 3. LIVER HISTOPATHOLOGY

	No. of rat	Fibrosis	Focal necrosis	Vacuole or steatosis
Sake group n = 6	4	+	−	++
	5	+	−	+
	6	+	−	+
	7	+	−	+
	8	+	−	+
	9	±	(+)#	+
Bourbon group n = 6	3	−	+	++
	4	−	−	++
	5	−	−	++
	8	±	+	+
	9	±	−	+
	10	±	+	++
Ethanol group n = 5	6	±	+	++
	7	−	+	++
	8	−	+	++
	9	±	+	++
	10	±	−	++
Glucose group n = 6	1	±	+	++
	2	−	−	++
	3	−	−	+++
	4	±	−	+
	5	±	+	++
	6	±	−	+++

Omitted because of artificial bleeding of upper G-1 tract

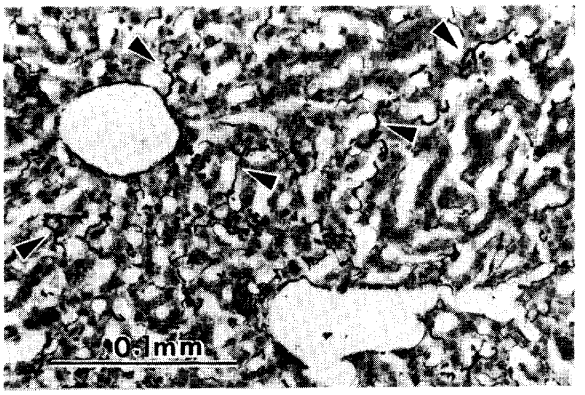

Fig. 1. Liver fibrosis (arrow heads) in a sake-treated rat (Pap's silver staining).

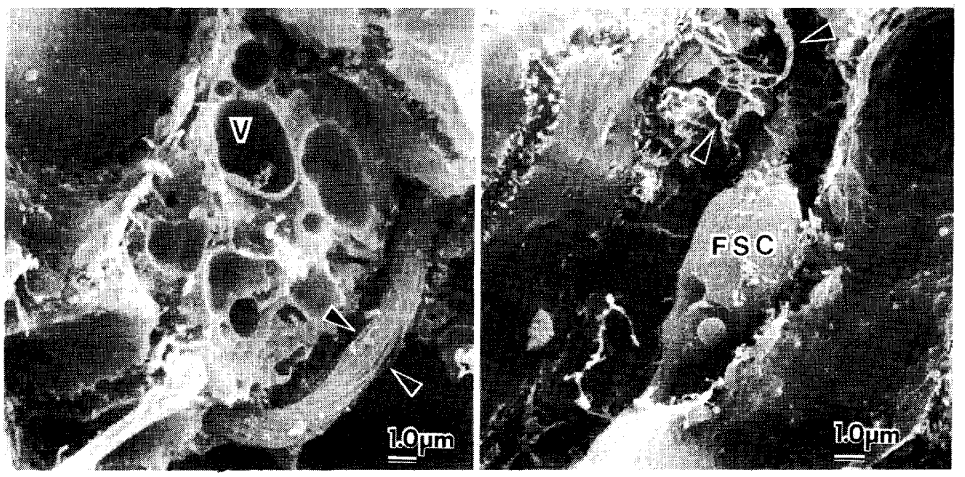

Fig. 2. (*left*) Scanning electron micrograph showing a collagen fiber (arrow heads) and vacuoles (v) in a sake-treated rat.

Fig. 3. (*right*) Scanning electron micrograph showing microfibrils (arrow heads) around a fat storing cell (FSC) in a sake-treated rat.

under the light microscope. The grading was as follows: (−), no vacuole formation or steatosis; (+), mild vacuole formation or steatosis observed only in hepatocytes of the central zone; (++), moderate vacuole formation or steatosis observed in the central and middle zone, and (+++), severe vacuole formation or steatosis observed diffusely throughout the whole lobule. All the rats presented some degree of vacuolization. The sake group presented slighter changes than the ethanol or glucose group ($p < 0.05$), while the buorbon group was intermediate.

DISCUSSION

Liver fibrosis developed most markedly in the sake-administered group. Microfibrils were often observed by SEM around the Ito cells, supporting the relation between fibrosis and these cells (10-13). Focal necrosis and significant steatosis were absent from this group, though present in the control and other test groups. As other variables, such as ethanol and caloric intake, were controlled, the differential histopathology was probably due to other components, the prime suspects being long-chain alcohols (3). Vacuole formation or steatosis appears during starvation (14) and after ethanol administration (6). This reaction was mild in the sake-treated rats, possibly due to sake possessing a higher caloric content as glucose than the other alcoholic beverages (3).

The sake, bourbon and ethanol groups presented elevated GOT and GPT activities. The glucose group showed normal GPT and slight elevation of GOT. Elevation of the transaminases could be explained by vacuolar degeneration of hepatocytes. It has been reported that starvation causes GOT elevation, with small amounts of glucose preventing this (15). Therefore, a small portion of the

GOT increase seen in our study was possibly due to starvation.

In conclusion, sake was more fibrogenic than the other alcoholic beverages. Components of the beverages other than ethanol are thought to be responsible for these differences in liver histopathology.

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