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Hepatocellular carcinoma in heavy drinkers with negative markers for viral hepatitis

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Abstract

Alcohol has been known to be associated with an increased risk of cancer. We investigated the characteristics of hepatocellular carcinoma (HCC) in heavy drinkers with negative serum markers for viral hepatitis (non-B, non-C) to determine whether ethanol enhances the development of HCC in Japanese patients with or without serum markers for viral hepatitis. Among the 432 HCC cases seen at our hospital between 1995 and 2000, 26 patients had negative serum markers (non-B, non-C) and were heavy drinkers. The mean patient age at the time of HCC diagnosis was 64.2 ± 7.6 years. The mean total ethanol intake was 1617 ± 796 kg. Most of the patients also had liver cirrhosis (LC), although the frequency was significantly higher in non-B, non-C, heavy drinkers HCC cases than in non-B, non-C, non-alcoholic HCC cases. Among the hepatitis C virus (HCV)-positive cases, the mean age at the time of HCC diagnosis was lower in heavy drinkers; this trend was not seen in HBV-positive cases. In HCC cases with heavy drinking, a high frequency of gastrointestinal (oropharynx, esophagus, stomach, colon and anal) cancers was seen. As for the aldehyde dehydrogenase-2 (ALDH2) genotype, the frequency of normal homozygotes was 87.5% in heavy drinkers with HCC and the frequency of heterozygotes was 12.5%; the frequency of heterozygotes was 58.3% in alcoholics with esophageal cancer. More than half of the non-B, non-C, heavy drinkers HCC cases had a normal range of serum alpha-fetoprotein (AFP) levels. These results indicate that heavy drinking enhances HCV-related hepatocarcinogenesis. Whether or not ethanol is directly involved in hepatocarcinogenesis remains controversial, but LC may progress HCC in heavy drinkers even if their serum markers for HBV (including tissue) or HCV are negative. Therefore, close observation, including radiographic examinations, is recommended for non-B, non-C, heavy drinkers with LC. In HCV-positive cases, abstinence or a reduction in daily ethanol intake is recommended.

Keywords: Alcohol; Aldehyde dehydrogenase-2 (ALDH2); Alcoholic liver disease (ALD); Hepatocellular carcinoma (HCC)

1. Introduction

Long-term heavy drinking is known to cause various health problems, such as alcoholic liver dysfunction. The frequency of alcoholic liver disease (ALD) is increasing in Japan, in association with an increase in alcoholic beverage consumption [1,2]. The relationship between alcohol intake and liver dysfunction is well recognized, but the relationship between alcohol intake and the risk of cancer remains unclear. Considerable epidemiological evidence showing that alcoholic beverages were associated with an increased risk of oral cavity, oropharyngolarynx, esophagus and liver cancers and indicating that alcohol was a carcinogen in humans was published by the International Agency for Research of Cancer (IARC) in 1988 [3]. Although a large number of reports on the relationship between alcohol and hepatocellular carcinoma (HCC) have been made, the exact relationship remains controversial.

Previously, HCC was thought to develop as a result of ALD; we now know that many cases are related to hepatitis C virus (HCV) infection. Since widespread serologic surveys of viral hepatitis have been performed, the relationship between alcohol and the hepatitis virus in the development of HCC can be investigated. Some reports have shown that

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HCC and/or liver cirrhosis (LC) developed more rapidly in patients with hepatitis C who were heavy drinkers; consequently, HCC and/or LC was diagnosed at a younger age in heavy drinkers with HCV [4,5]. Furthermore, in patients with HCV cirrhosis, the risk of HCC was larger in heavy drinkers than in either non-drinkers or moderate drinkers [6,7]. On the other hand, the effects of alcohol intake on HBs antigen-positive patients are still controversial. Some reports claim that a habitual high alcohol intake might increase the risk of developing HCC in HBs antigen-positive patients [8,9], while another report showed that alcohol intake was not an independent predictor in HBV cirrhosis [10].

Thus, alcohol may promote carcinogenesis in the liver and may be a risk factor for the progression of chronic viral hepatitis into cirrhosis, which may consequently increase the risk of developing HCC. Since some HCC cases with alcoholic liver fibrosis or cirrhosis and no serologic markers for viral hepatitis have been reported, alcohol seems to be an HCC risk factor that is independent of the hepatitis viral status. Cirrhotic liver caused by long-term heavy drinking may become a precursor to HCC. However, Lieber et al. [11] reported that some alcoholics with HCC did not have cirrhosis, suggesting that ethanol and/or its metabolites may be hepatocarcinogenetic in a manner that is independent of cirrhosis. Carcinogenesis induced developed by chemical agents involves two steps: initiation and promotion. Therefore, ethanol may modulate chemical agent-induced carcinogensis, acting as a tumor promoter. Little is known about the role of ethanol in carcinogenesis, especially in the liver. In the present study, we investigated the characteristics of HCC in heavy drinkers with negative serum markers for viral hepatitis (non-B, non-C) to determine whether ethanol plays a role in the development of HCC independently of the hepatitis virus or enhances hepatocarcinogenesis caused by the hepatitis virus. We also performed aldehyde dehydrogenase-2 (ALDH2) genotyping to determine the relationship between HCC and the ALDH2 genotype in heavy drinkers.

2. Materials and methods

2.1. Subjects with HCC

The subjects consisted of 432 patients with HCC who were consecutively admitted to the Department of Internal Medicine, Keio University Hospital between May 1995 and March 2000. HCC diagnoses were based on histology and/or radiological findings (i.e. abdominal ultrasonography, computed tomography, or angiography). Cirrhosis diagnoses were based on histology and/or laboratory data and the clinical course.

2.2. Definition of negative markers for viral hepatitis

Serum levels HBs Ag, anti-HBs antibodies, anti-HBc antibodies, HBe Ag, anti-HBe antibodies and anti-HCV were determined using commercial EIA kits (Dinabot, Tokyo). Serum HCV-RNA and HBV-DNA levels were measured using Amplicore HCV and the branched DNA probe method (SRL, Tokyo, Japan). All patients were negative for both HBs Ag and anti-HCV antibodies. Although all the markers were not measured in some patients, patients who were positive for even one marker were excluded. Subjects suspected of having autoimmune hepatitis or primary biliary cirrhosis were also excluded.

2.3. Definition of heavy drinkers

The definition of heavy drinkers was based on the Proposed Diagnostic Criteria for Alcoholic Liver Disease [1] which defines a heavy drinker as someone who drinks more than 125 g of alcohol a day continuously for more than 5 years. Information on the drinking profiles of the subjects was obtained by interviewing the patients and/or their families. HBV- and HCV-positive HCC cases were divided into four groups according to their daily ethanol intake: non-drinking group, less than 75 g/day, between 75 and 125 g/day, and more than 125 g/day group.

2.4. Detection of HBx gene in liver tissue

Liver specimens were obtained from six non-B, non-C, heavy drinkers who were HCC patients. HBx gene detection was performed according to method reported by Yotsuyanagi et al. [12]. Briefly, DNA was extracted twice with phenol/chloroform and once with chloroform and then precipitated with ethanol. PCR amplification was performed using a primer set within the X region (5′-CTGGATCCTGCGCGGGACGTCCTT-3′ sense; 5′-GTTCACGGTGGTCTCCAT-3′ antisense). The HBx gene was then detected by Southern blotting using X region-specific probes (5′-GATTCAGCGCCGACGGGGAC-3′).

2.5. Aldehyde dehydrogenase-2 (ALDH2) genotype

Among the 26 non-B, non-C, heavy drinkers HCC cases in our hospital, 14 ALDH2 genotyping was performed. And at the National Institution on Alcoholism (Kurihama National Hospital), where 10 non-B, non-C HCC cases in 2500 alcoholics from 1993 to 2000, 10 ALDH2 genotyping was performed. Overall 24 ALDH2 genotyping was performed. ALDH2 genotyping was performed on lymphocyte DNA samples using polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) [13]. Briefly, 100–200 ng of genomic DNA was mixed with 5 pmol of each primer (5′-CAAATTACAGGGTCAACTGCT-3′ sense; 5′-CCACACTCACAGTTTTCTCTT-3′ antisense) to produce a total volume of 50 μl containing 50 μM concentration of each dNTP, 1.5 mM of $MgCl_2$, and 1 U of Taq DNA polymerase (Progema, Madison, WI). Thirty-five cycles of PCR (denaturation at 94 °C for 15 s, anneal-

ing at 58 °C for 1.5 min, and polymerization at 72 °C for 30 s) were performed in a Perkin-Elmer Cetus GeneAmp PCR System 9600. After purification, each PCR product was digested with MboII, electrophoresed on a 20% polyacrilamide gel, stained with ethidium bromide, and viewed.

2.6. Statistical analysis

Data were expressed as the mean ± S.D. or as a percentage. And one-way ANOVA and Scheffe's post hoc test or a χ^2-test were used for the statistical evaluations. Statistical significance was set at $P < 0.05$.

3. Results

Among the 432 cases of HCC examined in this study 296 cases (68.5%) were HCV-positive, 70 cases (16.2%) were HBV-positive, 27 cases (6.3%) were both HCV- and HBV-positive, 13 cases (3.0%) did not have serum markers for viral hepatitis and were not heavy drinkers (non-B, non-C, non-alcoholic), and 26 cases (6.0%) were heavy drinkers who did not have serum markers for viral hepatitis (non-B, non-C, heavy drinkers) (Fig. 1).

All of the non-B, non-C, heavy drinkers HCC cases were negative for HBs Ag and anti-HCV antibodies. Twenty-one of 26 were negative for anti-HBc antibodies. We did not examine the anti-HBc antibodies of five non-B, non-C, heavy drinkers HCC cases whose anti-HBs antibodies were negative.

Twenty-five of the 26 non-B, non-C, heavy drinking HCC cases had cirrhosis. Table 1 shows the clinical aspects of these patients compared with the HCV-positive HCC patients. Twenty-five of the 26 cases were male, and the mean age at the time of HCC diagnosis was 64.2 years. The average total ethanol dose was 1617 kg. All of the male cases had drunk more than 1000 kg of alcohol, but one female case had drunk only 900 kg. All cases showed a higher serum gamma-glutamyl transpeptidase (GGTP) activity than the normal range, with a mean value of 215 IU/l. More than half

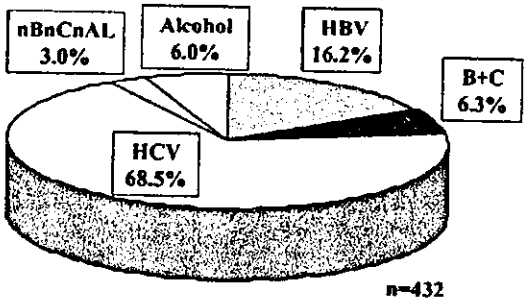

Fig. 1. Etiological trend of HCC. Cases of any HBV markers positive were described as HBV, both HBV and HCV markers positive as B + C, anti-HCV antibody positive as HCV, non-B, non-C, non-drinking as nBnCnAL and heavy drinkers with non-B, non-C as alcohol.

of the cases (64%) had a serum alpha-fetoprotein (AFP) levels that was within the normal range.

Seven cases (26.9%) had other cancers of the gastrointestinal tract. As for their smoking habits, 4% of the subjects smoked between 1 and 10 cigarettes a day, 20% smoked between 11 and 20 cigarettes a day, and 64% smoked more than 21 cigarettes a day. We also investigated the daily intake of alcohol in HCV-positive HCC cases. A higher daily alcohol intake was seen in most of the male population. The mean age at the time of HCC diagnosis was 66.3 years in the non-drinking group, 64.2 years in the less than 75 g/day group, 63.0 years in the 75–125 g/day group and significantly younger (55.7 years) in the more than 125 g/day group. Alcohol consumption did not affect the survival period from the day of HCC diagnosis. The mean total ethanol intake was 1190 kg in the group who drank 75–125 g/day and 1833 kg in the group who drank more than 125 g/day. Sixty-four percent of the non-B, non-C, heavy drinkers group had a normal AFP value, while normal AFP cases in the HCV-positive HCC patients accounted for only 26% in the non-drinking group, 31% in the 75–125 g/day group and 36% in the more than 125 g/day group. The prevalence of normal AFP cases was significantly higher in non-B, non-C, heavy drinkers group than in the HCV-positive, non-drinking group. The prevalence of gastrointestinal cancers was only 6.9% in the non-drinking group and 7.3% in the less than 75 g group, whereas it was 20.0% and 34.8% in the 75–125 g/day and the more than 125 g/day groups, respectively. In the non-drinking group, 72% of the patients did not smoke; however tobacco consumption increased in proportion to the daily alcohol intake. As the daily ethanol intake decreased, the percentage of female cases tended to increase. Although the data for the male cases is shown in parentheses to exclude the effects of gender, the age at the time of diagnosis, the prevalence of gastrointestinal cancer, and smoking habits among the male cases were similar to that observed overall.

Table 2 shows the mean age at the time of HCC diagnosis in the non-B, non-C, heavy drinkers group, the HBV-positive group, the HCV-positive, and the non-B non-C non-alcoholic group. Alcohol consumption did not affect the mean age at the time of HCC diagnosis in the HBV-positive patients. In the non-B, non-C, heavy drinkers group, significantly more HCC cases also had cirrhosis than in the non-B, non-C, non-drinking group. Among the HCV-positive cases, the frequency of cirrhosis showed a tendency to increase with daily alcohol intake in a dose-dependent fashion, but the difference was not significant.

We examined the tissue HBx of six non-B, non-C, heavy drinkers HCC cases (Table 3). They did not have HBx gene in their liver.

Fig. 2 shows the ALDH2 genotypes of HCC patients who were non-B, non-C, heavy drinkers compared to that of healthy cases and alcoholics with esophageal cancer from the Kurihama National Hospital. Twenty-one of the 24 non-B,

Table 1
Characteristics of non-B, non-C, heavy drinkers HCC and HCV-related HCC separated by ethanol intake

	Alcohol ($n = 26$)	HCV > 125 g/day ($n = 23$)	HCV > 75 g/day ($n = 35$)	HCV < 75 g/day ($n = 122$)	HCV only ($n = 116$)
Sex (male/female)	25/1	22/1	33/2	105/17	57/59
Age of diagnosis (years)	64.2 ± 7.7 (63.4 ± 7.1)	55.7 ± 7.0$^{*,**,+}$ (56.4 ± 6.8$^{*,+}$)	63.0 ± 5.9 (62.8 ± 6.0)	64.2 ± 6.7 (63.9 ± 6.7)	66.3 ± 7.9 (63.9 ± 8.5)
Total ethanol dose (kg)	1617 ± 796	1833 ± 387	1190 ± 307	–	0
GGTP (IU/l)	215 ± 198*	129 ± 97*	127 ± 119*	113 ± 116	78 ± 92
Normal AFP (%)	64*	36	31	32	26
Gastrointestinal cancer	26.9% (28.0%)	34.8%* (36.4%*)	20.0%* (25.5%*)	7.3% (7.6%)	6.9% (10.5%)
Cigarette					
0/day	12% (8%)	10% (9%)	31% (30%)	47% (44%)	72% (61%)
1–10/day	4% (4%)	0% (0%)	15% (16%)	12% (14%)	3% (6%)
11–20/day	20% (21%)	35% (32%)	37% (36%)	25% (25%)	9% (6%)
>21/day	64% (67%)	55% (59%)	16% (17%)	16% (17%)	16% (27%)

HCV-positive and drinking more than 125 g/day as HCV > 125 g, 75–125 g/day as HCV > 75 g, less than 75/day as HCV < 75 g, and no drinking as HCV only. Data in parentheses are the data of male eases. ANOVA: age of diagnosis, GGTP; χ^2-test:: % of normal AFP; gastrointestinal cancer.

$^{+}$ $P < 0.01$ vs. Alcohol.

* $P < 0.01$ vs. HCV only.

** $P < 0.01$ vs. HCV > 75 g.

Table 2
Age of HCC diagnosis and % of gastrointestinal cancer and cirrhosis

	Age of diagnosed HCC (years)	Gastrointestinal cancer (%)	Cirrhosis (%)
Non-B non-C heavy drinkers ($n = 26$)	64.2 ± 7.7	26.9	96.5#
HCV non-alcohol ($n = 116$)	66.3 ± 7.9	6.9	87.1
<75 g/day ($n = 122$)	64.2 ± 6.7	7.9	87.7
>75 g/day ($n = 35$)	63.0 ± 5.9	20.0*	88.6
>125 g/day ($n = 23$)	55.7 ± 7.0**,##	34.8*	95.6
HBV non-alcohol ($n = 16$)	56.9 ± 9.2	12.5	68.8
<75 g/day ($n = 28$)	56.0 ± 9.7	10.7	60.7
>75 g/day ($n = 13$)	59.8 ± 7.8	23.1	76.9
>125 g/day ($n = 13$)	58.5 ± 6.3	23.1	69.2
Non-B non-C non-alcoholic ($n = 14$)	60.8 ± 15.4	7.7	35.7

ANOVA: age of diagnosis; χ^2-test: cirrhosis.
* $P < 0.01$ vs. HCV only.
** $P < 0.01$ vs. HCV > 75 g.
# $P < 0.01$ vs. non-B non-C non-alcoholic.

non-C, heavy drinkers HCC cases (87.5%) were ALDH 2 × 1/2 × 1 homozygotes and 3 (12.5%) were ALDH 2 × 1/2 × 2 heterozygotes. Among the entire Japanese population, ALDH 2 × 1/2 × 1 homozygotes account for 58% of the population, ALDH 2 × 1/2 × 2 heterozygotes account for 35%, and 2 × 2/2 × 2 homozygotes account for 7% [14]. The frequency of ALDH 2 × 1/2 × 2 heterozygotes in the non-B, non-C, heavy drinkers group was less than that of overall Japanese population, although the frequency of ALDH 2 × 1/2 × 2 heterozygotes was much higher among alcoholics with esophageal cancer.

4. Discussion

We studied 26 HCC patients, who were heavy drinkers and negative for viral hepatitis markers. The prevalence was 6% of the over all HCC cases. All of them were negative for HBs Ag and anti-HCV antibodies. We excluded cases that had any other serum HBV markers we examined although their HBs Ag was negative. Yotsuyanagi et al. [12] reported that some of the non-B non-C cases had HBV-DNA in cancerous and adjacent non-cancerous liver tissue using RT-PCR and southern blotting method. Thus, HBV-DNA especially the HBx gene may demonstrate hepatocarcinogenesis even if in the cases who were negative for serum HBs Ag. We could not examine all of them. However, all the cases of non-B, non-C, heavy drinkers HCC examined HBx gene in the liver tissue did not have HBx gene. This finding raises a possibility that HCC can develop independently of occult HBV in non-B, non-C, heavy drinkers.

Table 3
HBx gene in the liver tissue of non-B, non-C, heavy drinkers HCC

Number	Sex	Ethanol (kg)	Serum marker			HCC tissue HBx
			HBsAg	HBcAb	HCVAb	
1	M	2000	–	–	–	–
2	M	2000	–	ND	–	–
3	M	1400	–	ND	–	–
4	M	1100	–	–	–	–
5	F	800	–	–	–	–
6	M	1020	–	–	–	–

(–) negative; ND: not done.

Although Lieber et al. [11] reported that cirrhosis was not necessary for the development of HCC in heavy drinkers, 25 of the 26 non-B, non-C, heavy drinkers with HCC also had cirrhosis. Further, the prevalence of cirrhosis was significantly higher in the non-B, non-C, heavy drinkers HCC group than in the non-B, non-C, non-drinking group. These results indicate that alcoholic cirrhosis might progress to HCC in non-B, non-C, heavy drinkers. Non-alcoholic steatohepatitis (NASH) has a histology that closely resembles that of ALD. Recently, the numbers of reports on NASH cases with HCC has been increasing [15,16]. In NASH cases, HCC may develop after a series of clinical events, i.e., steatosis, fibrosis and cirrhosis. The same mechanism may occur in ALD, and cirrhotic liver appears to play an important role in the development of HCC in non-B, non-C cases both in NASH and ALD.

An interesting finding in this study is that more than half of the non-B, non-C, heavy drinkers with HCC had a normal serum AFP level. A few cases had high serum AFP levels, but these cases had huge and/or multiple HCCs. The percentage of HCC patients with a normal AFP value was much higher in the non-B, non-C, heavy drinkers group than in the HCV-positive, non-drinking group. These results suggest that radiographic examinations, such as ultrasonography, computed tomography, etc., may be important in cases with cirrhosis and of heavy drinking, even if the serum tumor marker levels are normal.

Among HCV-positive cases HCC was diagnosed at a younger age in the group that drank more than 125 g/day of alcohol. Some previous papers have demonstrated that high ethanol consumption is a risk factor for HCC in HCV-positive patients [5,6]. Our results support this findings

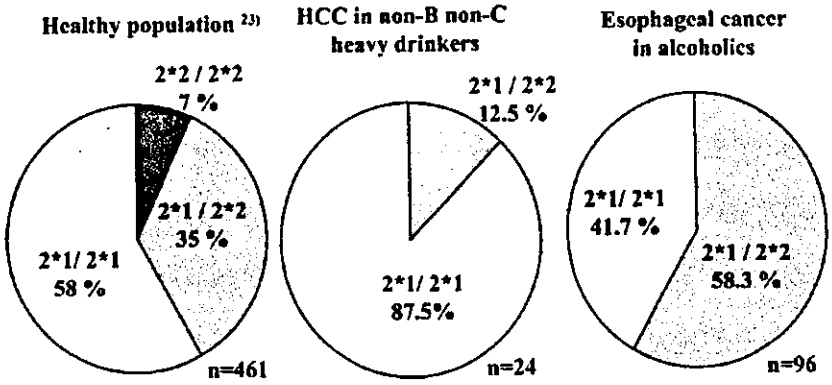

Fig. 2. Aldehyde dehydrogenase-2 genotype of HCC cases in non-B, non-C, heavy drinkers compared to that of healthy population and esophageal cancer in alcoholics.

and suggest that abstinence or a reduced daily ethanol intake should be recommended in HCV-positive cases. Although further studies on HBV-related HCC cases are needed, our results show that large amounts of alcohol consumption did not become an HCC risk factor in HBV-positive cases. Since HBV-related HCC was diagnosed at a younger age than HCV-related HCC, HBV-induced hepatocarcinogenesis may be more severe than HCV-induced hepatocarcinogenesis in heavy drinkers. In non-B, non-C, heavy drinkers with HCC, one of the 26 patients was a woman whose total ethanol dose was less than 1000 kg (900 kg). ALD is more severe in women, even if they have with a lower alcohol intake than men, but little is known regarding gender differences in hepatocarcinogenesis. Although further investigation of female cases is required, our results raise the possibility that women may develop HCC is association with heavy drinking at lower alcohol intakes.

One important characteristic of the non-B, non-C, heavy drinkers with HCC was the high frequency of gastrointestinal (oropharynx, esophagus, stomach, colon, anal) cancers. Seven of the 26 non-B, non-C, heavy drinking HCC cases (26.9%) had gastrointestinal cancers. In the HCV-related HCC cases as well, the frequency of the gastrointestinal cancers increased in accordance with the level of alcohol consumption. These results suggest that large amounts of ethanol consumption may be a risk factor for cancer of the liver and gastrointestinal tract. Since the consumption of cigarettes also increased in parallel with increasing ethanol intake, the effect of smoking may also be involved in the development of gastrointestinal cancers.

The distribution of ALDH2 genotypes differed between heavy drinkers with HCC and esophageal cancer. A higher frequency of ALDH 2 × 1/2 × 1 homozygotes (87.5%) was observed in patients with HCC than in the normal population, while the frequency of ALDH 2 × 1/2 × 2 heterozygotes (58.3%) was higher in patients with esophageal cancers. A mutant allele in the ALDH2 gene, seen in 42% of the Japanese healthy population, markedly diminishes enzyme activity [17]. We have reported that the presence of the ALDH 2 × 2 allele significantly increases the risk of developing these cancers [18]. However, the frequency of ALDH 2 × 1/2 × 2 heterozygotes is lower among HCC patients. These results suggest that the ALDH2 genotype does not affect the development of HCC in heavy drinkers.

Long-term ethanol consumption is known to induce cytochrome P450 2E1 (CYP2E1) [19]. CYP2E1 is an enzyme that metabolizes *N*-nitrosodimethylamine (NMDA), which is present in small quantities in the daily diet, from a procarcinogen to a carcinogen [20]. This metabolic event may enhance hepatocarcinogenesis. Tsutsumi et al. [21] reported that preneoplastic changes in the liver were found only in rats treated with both ethanol and NMDA. These changes were not observed in rats treated with either ethanol or NMDA. Vitamin A levels are known to decrease in the liver after chronic ethanol consumption [22]. This reduction in Vitamin A may enhance hepatic microsomal retinal degradation and contribute to cell differentiation. Despite the above evidence, whether or not ethanol acts as a carcinogen in the liver remains uncertain. Ethanol is unlikely to be an initiator of hepatocarcinogenesis, but it may be a promoter.

Since we did not investigate the presence of hepatitis G virus (HGV) and TT virus (TTV), the possible effects of these viruses on hepatocarcinogenesis cannot be excluded. However, Yotsuyanagi et al. [23] reported that HGV had no association with non-B, non-C HCC. Furthermore, Yamamoto et al. [24] reported that the possibility of TTV-induced hepatocarcinogenesis is low. Although the roles of HGV and TTV in carcinogenesis are unknown, a few reports have demonstrated a relationship between GBV or TTV and hepatocarcinogenesis.

Since ethanol intake in Japan is increasing, the frequency and number of ALD and alcoholic cirrhosis cases are expected to increase. As therapies for viral hepatitis are improved, the frequency of non-B, non-C, alcoholic cases with cirrhosis and HCC may increase. Therefore, in patients with liver cirrhosis whose total ethanol intake is more than 1000 kg, radiographic examination may be necessary to rule-out the possibility of HCC. Moreover, appropriate examinations to detect HCC in heavy drinkers and the

development of specific treatments for HCC will become more important in Japan in the near future.

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Review

Recent understanding of immunological aspects in alcoholic hepatitis

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Abstract

Alcoholic hepatitis is a rate-limiting step in the development of alcoholic liver disease into liver cirrhosis, and approximately half of the heavy drinkers with alcoholic hepatitis develop liver cirrhosis within 5 years. Immunologic mechanisms may be involved in the individual differences in the clinical course of this disease. Endotoxin from the intestine seems to play an important role in neutrophil infiltration of the liver, which induces, and at the same time is induced; by cytokines and chemokines. Kupffer cells and monocytes also have a key role in activating other cell types and producing several cytokines, chemokines, and free radicals. Both cytokines and chemokines up-regulate expression of various adhesion molecules, and adhesion molecules accelerate a cell-to-cell contact that stimulates cytotoxic lymphocytes to cause hepatocyte death. Self-antigens and adducts formed as a result of the degenerative effect of ethanol or aldehyde are targets of antibody-dependent cell-mediated cytotoxicity. Oxygen radicals, NF-kB, and AP-1 are key intracellular factors mediating hepatocyte death in alcoholic hepatitis. Viral infections and alcoholic hepatitis exacerbate each other. Integration of both human investigations and accumulated information from various animal models will gradually clarify the immunological mechanism of alcoholic hepatitis in future.

Keywords: Alcoholic hepatitis; Immunological mechanism; Kupffer cell; Neutrophil; Lymphocyte; Oxygen radicals

Contents

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1. Introduction

Alcoholic liver disease (ALD) includes fatty liver, liver fibrosis, alcoholic (steato)hepatitis, and liver cirrhosis. In the United States, about half of the causes of death in liver cirrhosis is alcohol abuse or alcoholism, a situation which is quite different from Japan, where 80–90% of cause of cirrhosis is chronic viral infection. Although, many ALD patients were found to have chronic hepatitis C after discovery of hepatitis C virus (HCV), alcohol intake is an exacerbating factor of chronic hepatitis C [1], and HCV infection is an exacerbating factor of ALD [2]. Eradication of HCV by interferon therapy may result in an increase in the proportion of ALD among chronic liver diseases in Japan in the future.

The rate-limiting step in progression of ALD to liver cirrhosis is the development of alcoholic steatohepatitis, which occurs in 20–30% of heavy drinkers, and steatohepatitis may develop to liver cirrhosis if left untreated. While cytochrome P4502E1 (CYP2E1) is a major microsomal source of oxidative stress and is a candidate for the pathogenesis of alcoholic steatohepatitis [3], approximately 40–50% of the cases of alcoholic steatohepatitis in heavy drinkers have been reported to progress to liver cirrhosis within 5 years. Thus, liver cirrhosis does not occur in all heavy drinkers, and its occurrence is not correlated with the level of alcohol consumption. These observations led to the hypothesis that immunological mechanisms play a role in the development of ALD in addition to individual differences in polymorphisms of CYP2E1 or its expression levels [4], although the precise immunologic mechanisms have not been established.

Animal models are major research tools for understanding the mechanisms of ALD. The initial two reports of animal model were in baboons given a 50% calories alcohol diet [5], and in small animals, rats fed the Lieber–DeCarli diet (36% calories) [6]. Tsukamoto et al. [7] reported continuous intragastric feeding of alcohol to rats and demonstrated that Kupffer cell activation by elevated levels of sinusoidal endotoxin due to increased intestinal permeability to endotoxin caused by alcohol administration was an important event in ALD. Kupffer cells cause oxidative stress following activation of nuclear factor-kappa B (NF-kB), up-regulation of inflammatory cytokines and adhesion molecules, and, finally, inflammatory cell invasion. In rats, these changes are followed by fatty change, patchy necrosis, mild inflammation, and perivenular fibrosis; however, there have been no animal models that are histologically compatible with human ALD, nor have there been any adequate models of viral hepatitis, making it difficult to clarify the pathophysiological mechanisms of ALD.

Immunological mechanisms and immunological abnormalities in ALD have been assessed in terms of both their humoral and cellular aspects. Alcoholic drinking results in two controversial effects on the immunological system. One is that heavy drinking results in a decrease in immunological activity [8], and the other is that an alcohol intake strongly stimulates lymphocytes, leading to inflammation in the liver and a decrease in various immunological markers [9]. There have been many reports about immunological aspects of ALD, but they have not been consistent, because each report has been a reflection of the immunological experimental procedures available when the investigation was performed. For example, various pathogeneses, such as an antibody against Mallory body [10,11], an antibody against lipopolysaccharide [12], an antibody against self-antigen or alcoholic adducts [13–17], cytotoxic T cells [18–21], decrease in cellular immunity [22–24], IgA [25–27], phagocytic activity [28], and cytokines [29–31], have been considered as causes for alcoholic hepatitis in a long research history of alcoholic liver diseases [32]. We review the immunological aspects of alcoholic hepatitis in this article.

2. Role of Kupffer cells in alcoholic hepatitis

Chronic alcohol administration increases intestinal mucosal permeability and the serum lipopolysaccharide (LPS) concentration. LPS binds to LPS-binding protein (LBP), forming an LPS–LBP complex, and this complex binds to the CD14 receptor on the cell membrane of Kupffer cells (KCs). The LSP–CD14 complex reacts with toll-like receptor 4 (Tlr4), which is a membrane-penetration-type receptor, and this stimulates signal transduction and activates nuclear factor-kappa B (NF-kB). Another pathway that activates NF-kB is the oxidative stress caused by LPS itself. NF-kB up-regulates proinflammatory cytokines, tumor necrosis factor (TNF)-α, and cyclooxygenase (Cox)-2, and they induce intrahepatic inflammation. There have been many reports of animal models and human investigations supporting the above scenario as described below.

2.1. Animal models

CD14 and LBP mRNA levels have been demonstrated to correlate well with the extent of liver damage in the Tsukamoto–French model [32]. CD14 has been found to be expressed in KCs and LBP to be expressed in hepatocytes, and alcoholic liver damage has not been induced in CD14-deficient mice or Tlr4-mutant mice [33,34]. NF-kB activation in hepatocytes was demonstrated in the Tsukamoto model [35], but no TNF-α or NF-kB up-regulation or liver damage was observed after ethanol administration to $p47^{Phos}$ knockout (k/o) mice [36]. The $p47^{Phos}$ is a central subunit of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. The findings in this k/o mouse model suggest a scenario, in which the deficiency of NADPH oxidase in KCs cannot induce free-radicals after ethanol administration, and the free-radicals up-regulate NF-kB and TNF-α which then induce liver damage. Intragastric feeding of corn oil and fish oil up-regulated expression of Cox-2 and TNF-α mainly in KCs, that induced necroinflammation in the liver [37]. A significant

role of Cox-2 in hepatic inflammation was also demonstrated in the Cox-2 k/o mice having TNF-α plus galactosamine administration [38].

2.2. Human investigation

Significantly higher levels of TNF-α production has been demonstrated by the monocytes of ALD patients than by the monocytes of healthy controls. The levels of TNF-α, interleukin (IL)-1, and IL-6 in cholangio-endothelial cells and KCs have been found to be significantly higher in ALD patients than in healthy controls [39]. Moreover, monocyte chemoattractant protein-1 and IL-8 are expressed in KCs and may facilitate invasion of the liver by other inflammatory cells.

3. Role of neutrophil invasion

One of the histological characteristics of ALD is neutrophil invasion of the liver. Major neutrophil chemoattractants are CXC chemokines (IL-8, cytokine-induced neutrophil chemoattractant (CINC), macrophage inflammatory protein (MIP)-2, KC, inducible protein (IP)-10, ENA) and RANTES (regulated upon activation, normal T cell expressed and secreted). These chemokines are produced by many different kinds of cells in the liver, including hepatocytes and KCs. On the other hand, several adhesion molecules, such as selectin, which is expressed on vascular endothelium in neutrophil rolling phase, β2 integrin (CD11b, CD18), which is expressed on neutrophils after the rolling phase, and intercellular adhesion molecule (ICAM)-1, which is then expressed on both endothelium and hepatocytes.

TNF-α and chemokine production is increased in both humans with alcoholic hepatitis and animal models [40–43]. Hirano et al. [44] demonstrated that chemokines such as RANTES, are up-regulated by stimulation with TNF-α. Oxidative stress up-regulates redox-reactive transcription factors, such as NF-kB and activator protein (AP)-1, and induces secretion of various cytokines and chemokines [45]. CXC chemokines seem to be produced by KCs, because inactivation of KCs results in attenuation of CXC chemokines and a decrease in liver damage [46]. TNF-α and IL-1 then up-regulate adhesion molecules of neutrophils, hepatocytes, and other cells, and that induces cell-to-cell interaction. The cell-to-cell interactions involve neutrophil-mediated hepatocyte damage [47] or sinusoidal endothelial damage [48,49]. The chronic neutrophil invasion of the liver observed in ALD requires prolonged chemokine production in the liver, because the half-life of neutrophils is short. The cytotoxic effect of neutrophils has been demonstrated in the ischemia–reperfusion model, the endotoxin model, the warm-shock model, the cold-shock model, and a drug toxicity model. Hepatocyte apoptosis also stimulates neutrophil invasion, which then expands inflammation by a positive feedback mechanism [50,51].

Expression of E-cadherin and vascular cell adhesion molecule (VCAM)-1 on the endothelium, in addition to constitutive expression of ICAM-1, is necessary for neutrophil-invasion of the liver parenchyma [52]. Circulating neutrophils are always activated in patients with ALD [53], and neutrophil-activation leads to production of reactive oxygen species (ROS) and Mac-1, followed by increased production of TNF-α and IL-8, leading to hepatocyte toxicity and apoptosis [54]. Serum IL-8 levels have been shown to be correlated with neutrophil-invasion levels [43], which supports the scenario described above.

There is no neutrophil invasion in either the Lieber–DeCarli model; or the Tsukamoto–French model, however, LPS administration in these models was followed by increased expression of CXC chemokines and adhesion molecules and neutrophil invasion of the liver [55–57]. This phenomenon suggests that the most important factor in the establishment of the neutrophil-invasion in the liver seen in ALD is the supply of endotoxin from the intestine.

4. Role of lymphocyte invasion

Circulating lymphocytes in ALD patients may be trapped by the sinusoidal endothelium by adhesion with VCAM-1, ICAM-1, 2, or vascular adhesion protein (VAP)-1 [58], and then they invade into hepatic parenchyma by several CXC chemokines, such as monokine induced by gamma-interferon (KIG), interferon-inducinble T cell alpha chemoattranctant (ITAC) and IL-10, which are up-regulated in hepatitis [40,42,59,60]. The CD31 molecule is thought to be responsible for invasion through inter-endothelial tight junctions [61].

Necroapoptosis in alcoholic hepatitis is thought to be caused by cytokines, such as TNF-α, and free-radicals, such as nitric oxide (NO). The precise role of lymphocytes in ALD, however, is still a matter of controversy. Chedid et al. [62] and Sakai et al. [21] showed increased numbers of intrahepatic CD8+ cells and CD44+ cells, and decreased numbers of B cells and natural killer (NK) cells. NK activity is decreased in animal models [63], but the numbers and activity of CD3–CD56+ cells (compatible with NK cells) in the peripheral blood of ALD patients are increased, while their intrahepatic numbers are decreased [62], in contrast to viral hepatitis. Summarizing the findings in many reports, TNF-α and IL-6 are up-regulated in alcoholic hepatitis patients but IFN-γ is downregulated. T cells that produce TNF-α and IFN-γ are classified as CD57+ cells and are reactive to T helper (Th)1-type cytokines [9], but there are individual differences in the production of these cytokines [8], probably because of promoter polymorphisms [64,65]. It has also been reported that T cells are activated in chronic drinkers who drink more than 80 g/day ethanol, and that the activation persists after they stop drinking [8]. Production of these cytokines has also been demonstrated in animal models.

The CD8/CD4 ratio is said to be higher in the liver than in peripheral blood, and French and co-workers [66] demonstrated that CD4+ cells are predominant in zone 3 and CD8+ cells are predominant in zone 1, although both cells are seen in the portal area. MHC class I expression correlates with levels of portal inflammation and interface hepatitis, and MHC class II expression correlates with hepatocyte necrosis and appearance of Mallory bodies. CD29, CD45 RA, and CD45 RO, which are important for recognition of allo antigens or for adhesion, are highly expressed in the necrotic area and/or in Mallory-positive liver. These findings indicate that cytotoxic T cells (CTLs) are important to the progression of alcoholic hepatitis as well as to the progression of viral hepatitis.

Fas-Fas-ligand (FasL)-mediated CTL and TNF-α play important roles in chronic hepatitis C virus (HCV) infection [67], and alcohol abuse may exaggerate the cytotoxic process [68] because alcohol increases Fas/Fas-L expression [69,70]. Expression of another important cytotoxic mediator, perforin-granzyme, requires MHC class I expression, and alcohol intake increases its expression, suggesting that alcohol drinking increases CD8+ T cell-mediated cytotoxicity in the liver [71].

5. Role of antibody-dependent cell-mediated cytotoxicity

Antibody-dependent cell-mediated cytotoxicity (ADCC) is thought to be involved in the liver damage in alcoholic hepatitis. Autoantibodies against CYP3A4 and CYP2E1 are found in 20–30% and 10–20%, respectively, of healthy individuals [72], and the titers of these autoantibodies in the Tsukamoto–French model rise to two- to three-fold above the control level 1 month after the start of feeding and they correlate with levels of liver damage. Administration of chlormethiazole, an inhibitor of CYP2E1, reduced CYP2E1 activity as well as the autoantibody titers [73]. A similar phenomenon has also been observed in regard to other autoantibodies, such as anti-hydroxyethyl adduct and anti-malondialdehyde adduct, in both animal models and humans [74]. These adducts are produced during oxidation of ethanol by CYP2E1. Accumulation of the autoantibodies to these adducts is observed on hepatocyte membranes by confocal microscopy [75], suggesting that the ADCC mechanism may operate in alcoholic hepatitis [66,76]. Vidali et al. [77] recently reported that polymorphism in the exon 1 of CTL antigen (CTLA)-4 gene induces disregulation of T-cell proliferation, leading to production of autoantibody against CYP2E1.

6. Role of monocytes

The peripheral blood monocytes of alcoholic hepatitis patients produce more TNF-α, both in the presence and absence of stimulation by LPS, than those of healthy controls [31], and TNF receptor expression is increased [30]. The expression levels are always correlated with progression to liver cirrhosis, and the same phenomenon is observed in regard IL-6 production. The production levels and serum levels of MCP-1 and MIP-1 are significantly higher in alcoholic hepatitis patients than in healthy controls [42,78]. A recent study demonstrated that acute alcoholic stimulation inhibits cytokine production from monocytes, but chronic stimulation up-regulates production of cytokines and reactive oxygen from monocytes [79], suggesting that pathophysiological mechanisms are different between acute injury and chronic injury. Thus, inhibition of monocyte activation is now going to be the target of the treatment of alcoholic hepatitis in the future.

Chronic ethanol administration to rats for 6–8 weeks makes hepatocytes more susceptible to injury by TNF-α [80], and the mechanism of the change in susceptibility is attributable to a change in the membrane permeability of mitochondria [81]. Recent studies have shown that liver injury always correlates with genomic polymorphisms of manganese superoxide dismutase [82], while a negative report appeared after then. Further studies are needed in regard to this matter [83].

7. Conclusion

We have reviewed the immunological aspects of alcoholic hepatitis. Although many factors such as hypoxia have been implicated to have a significant role in the pathogenesis of alcoholic liver disease [84], immunologic factors seem to be still important for making its individual difference. The major reason why the precise immunological mechanism has not been understood in the pathogenesis of alcoholic hepatitis is a lack of adequate animal models, the same as in viral hepatitis. Integration of human investigations and accumulated information from various animal models will gradually clarify the immunological mechanisms of alcoholic hepatitis in the future.

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Lifestyle guidance for patients with chronic liver diseases; information provision via educational classes on liver diseases

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Abstract

Patients with chronic liver disease need information on the disease to keep their good quality of life. Educational class on liver disease is one of solutions for that. We have started the class in 1992, and continued to run it once per month. Exchange of information among patients at group work is also helpful to relief their anxiety related to their disease. Many hospitals are now preparing to establish such classes in Japan. In this article, we present tips on providing information to patients with chronic liver diseases and advice on class management.

1. Introduction

Patients with chronic liver diseases require detailed information on the various aspects of their disease [1]. For example, the recent media focus on hepatitis C virus associated with contaminated blood products has led to patients with hepatitis C virus infection becoming anxious about their future. Because they are also worried that the viruses will infect their family and other people, they should be provided with education about prophylaxis [2]. Various new pharmaceuticals, such as interferon and other antiviral agents, have been developed and placed on the market, but their severe side effects are also reported in the media, leading to confusion about the new pharmaceuticals [3]. Because hepatic cancer may develop during their clinical course, patients also suffer from cancer-related anxiety. Once cancer develops, the importance of information provision increases when patients are informed the name of their diseases and when the therapeutic regimen is selected. Various techniques have been developed for treating esophageal varices and liver cancer, which are common complications of liver cirrhosis, and patients need to be involved in the selection of therapeutic regimens. When the cancer advances, palliative treatment is required, and care for patients' families also becomes important [1].

The importance of rest and a high protein diet for patients with chronic liver diseases was overemphasized for many years in Japan, which led some patients to pay too much attention to resting, and others to suffer from hepatic encephalopathy due to an excessive intake of high protein foods. Furthermore, obesity is recently reported to be a risk factor for the development of liver diseases, such as fibrosis and cancer [4–14]. In order to educate about a truly balanced lifestyle, we should work to overturn common misperceptions held by the patients.

Although information provision to patients is difficult, patients require appropriate and comprehensive information on chronic liver diseases. Falvo [15] stated that 'patient education is a right of patients and a duty of medical staff.' However, in Japan, many patients are at a loss what to do due to insufficient information from medical staff.

In order to respond to these patient demands, we started an educational class on liver diseases in 1992, and continue to run this class once per month. This class has been improved so that, information is not only unilaterally provided from doctors and nutritionists, but is also exchanged and shared among the patients. In recent years in Japan, hepatologists

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Table 1
Facilities with laboratories for liver disease

	In operation	Preparation underway	Interested	Total	Facilities certified by the Association of Gastroenterology	Educational facilities certified by the Association of Internal Medicine
Hokkaido	3	1	26	30	39	42
Tohoku	3	2	25	30	43	61
Kanto/Shin-etsu	7	1	11	19	83	126
Tokyo	5	2	19	26	67	67
Yokohama	3	2	10	15	34	38
Tokai	3	1	2	6	84	110
Kyoto/Hokuriku	5	1	8	14	42	62
Osaka	9	5	37	51	91	115
Chugoku	6	2	32	40	52	56
Shikoku	6	4	6	16	24	39
Kyushu	14	4	11	29	54	75
Total	64	25	188	277	613	791

Based on research by the Ajinomoto Pharmaceutical Co. Ltd. June 2003.

have belatedly realized the necessity of providing education and information to patients with chronic liver diseases, and about 10% of postgraduate education hospitals in Japan now run educational classes on liver diseases (Table 1). Many hospitals are now preparing to establish such classes. Therefore, we expect that educational classes on liver diseases will quickly become common. In this article, we present tips on providing information to patients with chronic liver diseases and advice on class management.

2. Goals of educational classes on liver diseases

The primary goal of educational classes on liver diseases is to improve the quality of life (QOL) of patients with liver diseases. For patients with HIV infection, the necessity of mental health support from counselors has been discussed extensively, but medical staffs rarely recognize that this is also a necessity for patients with HCV or HBV infection [16–19]. It has been reported that patients with HCV-related chronic hepatitis who were informed of their viral infections had a lower QOL than patients who were not informed [20]. However, considering that information disclosure and transparency are presently required, it is unacceptable not to inform patients of their HCV infections. Therefore, the issue is how to provide information without decreasing QOL. Providing information in an appropriate way may help the patients to lead happier, more active lives.

3. Preparations for opening classes

Firstly, a room is booked for class, and posters are displayed on the wall of the out-patient clinic in order to inform patients of the class opening. We display posters on the wall of our out-patient clinic to inform patients about our monthly class, which is open-access for patients. We have 30–50 patients a month in our class. The number of patients who are starved of information is larger than we think. We also request that doctors in other departments, such as general surgery and radiology, participate in lectures on liver diseases, which are held once or twice per year. When we broadly announce these lectures using large posters, more than 200 people want to attend, which is over the capacity of the hall. Therefore, we use a pre-registration system for these lectures. When an educational class is opened for the first time, one option is to adopt a pre-registration system, taking into consideration the preparation of documents and the size of the classroom.

The speech content is summarized using presentation software (PowerPoint) [1], and slide content is printed out and given to patients as handouts. Recently, many patients are disappointed or complain if handouts are not prepared. Therefore, it is advisable to prepare handouts, even though the actual information content may only be an overview.

4. Frequency and duration of classes and detailed content

We run classes once per month and repeat four themes. Classes are held on Saturday mornings, because it is easy for patients to attend. Each class lasts approximately 2 h, and we give a lecture of 45 min–1 h, including the question period. After the lecture, there is a question-and-answer session (30 min) and a group work session (30 min).

The four themes picked up in classes are (1) lifestyle precautions for patients with liver diseases, (2) what liver examinations are checking for, (3) chronic hepatitis; interferon therapy and antivirus therapy, and (4) complications of liver cirrhosis and their treatments. If there are too many themes, patients will hesitate in attending, and medical staff will also acquire the added burden of extra preparation time. Therefore, we limit the themes to four and repeat the same themes, and gradually add new content in each class. When the same theme is repeated every four classes, even if patients have not been able to attend for 1 month, they are able to attend a class which deals with the same theme after just three more classes.

Some consider that classifying patients by clinical stage or by cause of liver diseases is efficient for class management. However, we aim to operate a class management system that allows patients at all clinical stages of liver diseases to participate and encourage each other. Therefore, we do not specifically target the patients for classes. In fact, at our classes, we sometimes observe patients with liver cirrhosis encouraging patients with chronic hepatitis. It is better to request nutritionists and nurses to join classes, because many of them are interested in patient education. In the Japanese medical system, we can claim health care costs as group nutritional guidance when nutritionists join. We consider that this kind of patient education should eventually be provided by nutritionists and nurses. However, to continue these patient classes in Japan, physicians should first understand their significance.

In Japan, many doctors still emphasize the importance for patients to rest. However, patients with chronic diseases require information about how much exercise they can do without problems, instead of how much rest they should take [21–23]. We attempted to give exercise guidance to patients with chronic hepatitis or liver cirrhosis, and found that they were able to do aerobic exercise without causing deterioration of their liver and nervous functions. When exercise intensity is within 60% of its maximum, exercise does not increase blood ammonia levels. In terms of ammonia metabolism and sugar metabolism, muscles work similarly in the liver and may compensate for hepatic dysfunction. Therefore, in order to maintain muscle mass, it is important to actively instruct patients to do some exercise, rather than to rest for long-periods of time or throughout their life.

5. Provision of information among patients: group work

When there are not too many participants (around 20 patients), active participation may be encouraged by the question-and-answer session alone. However, if the number of participants is larger than this, many patients are discouraged from active participation, such as asking questions and presenting their opinions. Therefore, we make groups of approximately five patients and let them actively exchange their opinions in these smaller groups. Group work allows patients to talk about their clinical conditions, examinations, therapies, problems that they have and their strategies for dealing with such problems with other patients (Table 2). The perspective on information that is exchanged among patients is different from that provided by doctors, the former information being more familiar and authentic for patients. In fact, during group work, patients become more active than we had anticipated and confer about a wide range of issues in a positive manner. In these groups, patients discuss issues that they do not usually talk about with doctors. Many people are concerned that group work does not work well in small communities or in rural areas, because there may be many acquaintances in a group. However, the medical staff from an institution in a rural area, who had visited our institution, attempted group work and also reported that it worked well.

At the start of group work, we always explain the rules and present examples of topics (Fig. 1). For this, we present

Table 2
What is group work?

Its objective is to utilize information exchange between patients, and not to provide information solely from doctors to the patients
In most cases, the information provided to patients from other patients is more beneficial than the information provided by doctors
Discussion of experiences, including those regarding liver biopsies, TAE, PEIT and RF
How to deal with such problems as leg cramps, lethargy, fatigue, or depression
Patients can see the prospects for the future course of their illness by observing other patients
Patients with chronic hepatitis will understand the circumstances of the daily life of patients with cirrhosis or HCC
Doctors will give advice to the patients when needed

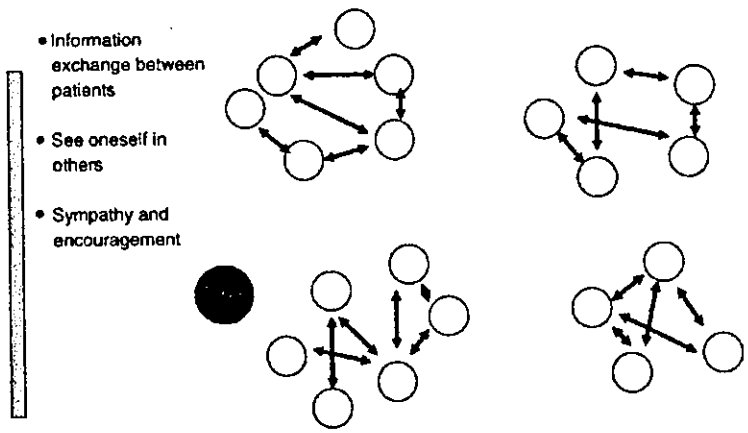

Fig. 1. Group work format. Information exchange between patients. See oneself in others. Sympathy and encouragement.

Table 3
Group work rules

1. Discuss your problem with, and how you are bothered by, having liver disease
2. Give priority to patients with the greatest need for discussion, or to new patients
3. The discussion with each patient will last from 3–5 min
4. Start with a short introduction. You do not have to give your real name; an assumed name or nickname will be fine
5. Your opinion is for reference purposes only. Do not try to push your opinion on others
6. Raise your hand and ask to be recognized when you want to ask for the opinion of a doctor or medical personnel

the slide shown in Table 3. Attention should be paid not to let discussion place disproportionate weight on one topic, such as folk remedies or the soliciting of patients to join particular religious groups. It is better to mix patients, who have experienced group work several times with patients who have never experienced it. When group discussion is not so active, medical staff needs to help (e.g. by providing a topic that works as a trigger for discussion).

6. Collecting fees and countermeasures against costs, including the cost of lectures

At present, our classes are free. We expect that the day will soon come when the necessity of providing education to patients with chronic liver diseases is understood, and when we can claim the medical expense for this education as a health care cost. At present, as previously mentioned, when nutritionists join, costs can be claimed as group nutritional guidance. Additionally, another option is that participation in classes is defined as self-motivating instead of compulsory, and that patients pay for the cost of the room and documents.

7. Problems that are frequently faced in class management, and tips for continuing classes long-term

The ultimate goal of these educational classes is to improve the QOL of patients. When medical staff speak to patients in an authoritative manner or preach to them, fewer patients want to continue attending classes, so improvement of their QOL cannot be achieved. In order for classes to continue for a long-time, the term 'empowerment' should be deeply understood by med staffs [14], and in class management, we should always consider how to make the lives of patients with chronic diseases meaningful and satisfying. When too many medical staffs are involved in classes, it is difficult to achieve improved efficiency and simplified health care (which is also a goal of opening classes), and it also becomes difficult to continue classes from a financial perspective.

8. Conclusion

Starting educational classes on liver diseases is not as difficult as it seems. When the opportunity arises, it would be a good idea to open classes because of the multiple benefits they bring. Once you open a class, you will encounter problems and will be able to use your experiences to improve future classes. These educational classes will be beneficial and able to continue successfully if patients can become less anxious about their diseases and feel even a slight sense of relief by the end of each class.

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HEPATOLOGY

Kupffer cell depletion attenuates superoxide anion release into the hepatic sinusoids after lipopolysaccharide treatment

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Abstract

Background and Aim: The mechanisms involved in the beneficial effect of gadolinium chloride against endotoxin-induced liver damage were studied.

Methods: Superoxide anions released into the hepatic sinusoids were examined in a liver perfusion model using the cytochrome C method.

Results: Gadolinium chloride treatment fully depleted ED2-positive cells from the liver and significantly attenuated superoxide anion release after a lipopolysaccharide or tumor necrosis factor-α (TNF-α) challenge. Moreover, gadolinium chloride treatment resulted in a significant decline in endothelial cell damage in the hepatic sinusoids as assessed by the purine nucleoside phosphorylase/glutamic–pyruvic transaminase ratio in the liver perfusate. Although gadolinium chloride treatment did not affect the level of serum TNF-α, it significantly reduced that of interleukin (IL)-8 and neutrophil migration in the hepatic sinusoids after the lipopolysaccharide challenge.

Conclusion: These data suggest that a reduction of the superoxide anion level in the hepatic sinusoids in acute endotoxemia and subsequent reduction of neutrophil migration into the liver may indicate that gadolinium chloride treatment suppresses the progression of liver damage in acute endotoxemia.

Key words: free radical, $GdCl_3$, Kupffer cell, macrophages, neutrophils.

INTRODUCTION

Endotoxin from intestinal bacteria reaches the liver via the portal vein.[1] The endotoxin is mainly eliminated by Kupffer cells, the resident macrophages of the liver. Excessive endotoxin, however, can cause liver injury,[2,3] and there are numerous lines of evidence indicating that Kupffer cells play a central role in the progression of endotoxin-dependent liver injury.[4] The recently prevailing view is that treatment with gadolinium chloride attenuates the progression of endotoxin-related liver damage via inactivation of Kupffer cells.[5,6] However, the exact mechanisms behind this phenomenon are, so far, unclear.

Bautista and Spitzer demonstrated that treatment with lipopolysaccharide (LPS), the main component of endotoxin, results in superoxide anion release into the hepatic sinusoids.[7] They also showed that tumor necrosis factor-α (TNF-α) plays a crucial role in the superoxide anion formation.[8] We were able to confirm these results, and our preliminary data indicated that superoxide anion release into the hepatic sinusoids might cause damage to hepatic sinusoidal endothelial cells.[9] Moreover, Bautista and Spitzer used cultured cells to show that superoxide anions are mainly released from Kupffer cells, whereas the participation of endothelial cells and hepatocytes was minimal, at least after phorbolmyristate ether treatment.[10] Thus, it is conceivable that Kupffer cell depletion diminishes superoxide anion level in the hepatic sinusoids in acute endotoxemia and subsequent neutrophil migration into the liver. This supports the reported finding that gadolinium chloride treatment suppresses the progression of liver damage caused by acute endotoxemia.

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