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Low Km ALDH Isozyme and Alcoholic Liver Injury

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YOSHIHARA, H., N. SATO, T. KAMADA AND H. ABE. *Low Km ALDH isozyme and alcoholic liver injury.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 425–428, 1983.—To assess the relationship between the polymorphism of aldehyde dehydrogenase (ALDH) isozyme and alcoholic liver injury, ALDH isozyme was analyzed by isoelectric focusing electrophoresis in hair roots from normal volunteers and alcoholics with chronic liver disease. Liver biopsy specimens from alcoholics and non-alcoholics with chronic liver disease were also analyzed. It was found that (1) the frequency of low Km ALDH isozyme in hair roots from chronic alcoholics with liver injury was 90%, which was significantly higher than those from normal volunteers (44%) and from non-alcoholics with chronic liver disease (56%); (2) the isozyme pattern of liver specimens analyzed coincided with that of hair roots; (3) the low Km ALDH isozyme-positive subjects including alcoholics showed no facial flushing, and negative subjects showed facial flushing after drinking alcohol. It is concluded that a much higher frequency of low Km ALDH isozyme was found in chronic alcoholics with liver injury. There was no apparent difference in hepatic biochemical and histological findings between chronic alcoholics with and without low Km ALDH isozyme, suggesting that acetaldehyde does not play a primary role in the pathogenesis of alcoholic liver injury.

ALDH isozyme Alcoholic liver injury Acetaldehyde Facial flushing

RECENTLY, it has been suspected (1) that acetaldehyde plays an important role in alcoholic liver injury. It has been reported (2) that the acetaldehyde metabolism in the liver is greatly influenced by the genetic polymorphism of aldehyde dehydrogenase (ALDH) isozyme, which affects the plasma level of acetaldehyde. Genetic polymorphism of ALDH isozymes are also related to facial flushing after drinking alcohol which is mainly due to high plasma level of acetaldehyde [6]. However, it is not known how the polymorphism of ALDH isozyme relates to alcoholic liver injury. The aim of this study is to clarify the relationship between the polymorphism of ALDH isozyme and alcoholic liver injury.

METHOD

Subjects were 16 normal non-alcoholic volunteers (aged 23 to 45, 13 males and 3 females), 16 non-alcoholics with chronic liver disease, diagnosed by histological findings (12 chronic viral hepatitis, 3 liver cirrhosis and 1 primary biliary cirrhosis, aged 16 to 62, 12 males and 4 females) and 31 male chronic alcoholics with alcoholic liver injury. Fourteen out of 31 alcoholic cases were histologically diagnosed as fatty liver (2 cases), liver fibrosis (7 cases) and liver cirrhosis (5 cases). The remaining 17 cases were alcoholic liver cirrhosis (2 cases) and nonspecified alcoholic liver disease (15 cases), as assessed by biochemical studies and total alcohol intake. Hair roots collected from subjects were frozen at −30°C for 1 day to 7 days (average 3 days). Liver biopsy specimens were obtained during laparoscopy. About half of the biopsy sample was used for histological diagnosis and the remaining sample was frozen. ALDH isozymes were analyzed in these frozen samples using the isoelectric focusing electrophoresis method of Harada *et al.* [4]. Facial flushing was assessed by inquiring to normal and alcoholic subjects about facial flushing, hot feeling, and tachycardia after drinking alcohol.

RESULTS

Figures 1 and 2 show aldehyde dehydrogenase (ALDH) isozyme bands of hair roots and liver biopsy specimens in non-alcoholic patients with liver injury and chronic alcoholics with alcoholic liver injury. Two isozyme bands could be detected near the point of pH 5 in the liver specimen sample which were identified as low Km ALDH isozyme (ALDH I) and high Km ALDH isozyme (ALDH II). ALDH III and IV could not be detected with this method. In hair root samples, only low Km ALDH isozyme could be detected and the high Km ALDH isozyme band was too pale to detect. The electrophoretic pattern showed that not only low Km ALDH isozyme positive patients but also negative patients existed.

Frequency of low Km ALDH isozyme in hair roots from normal non-alcoholic volunteers, and non-alcoholics and alcoholics with chronic liver disease is shown in Table 1. The frequency of low Km ALDH isozyme positive cases in 31 chronic alcoholics was 90%, which was significantly higher ($p<0.001$) than in normal non-alcoholic volunteers (44%) or non-alcoholic patients (56%). Three cases among 31 chronic alcoholics were low Km ALDH isozyme negative patients (10%).

The relationship between low Km ALDH isozymes and facial flushing after drinking alcohol was analyzed in normal

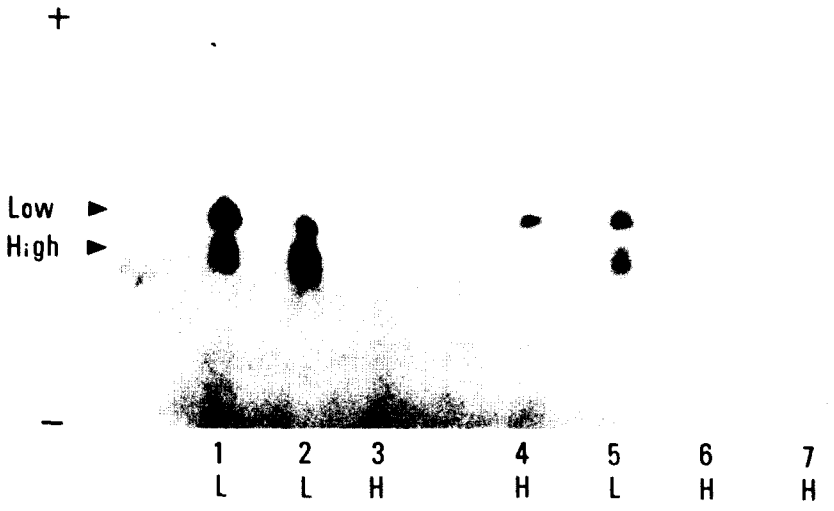

FIG. 1. Aldehyde dehydrogenase (ALDH) isozyme bands of hair roots and liver specimens in 7 chronic alcoholics with liver injury using isoelectric focusing electrophoresis. Numbers 1, 2 and 5 are of liver specimens (indicated as "L") and numbers 3, 4, 6 and 7 are of hair roots ("H"). Patient Nos. 3, 6 and 7 lack low Km ALDH isozyme band in their hair roots. Low Km and high Km ALDH isozymes are shown as "Low" and "High" in the figure, respectively.

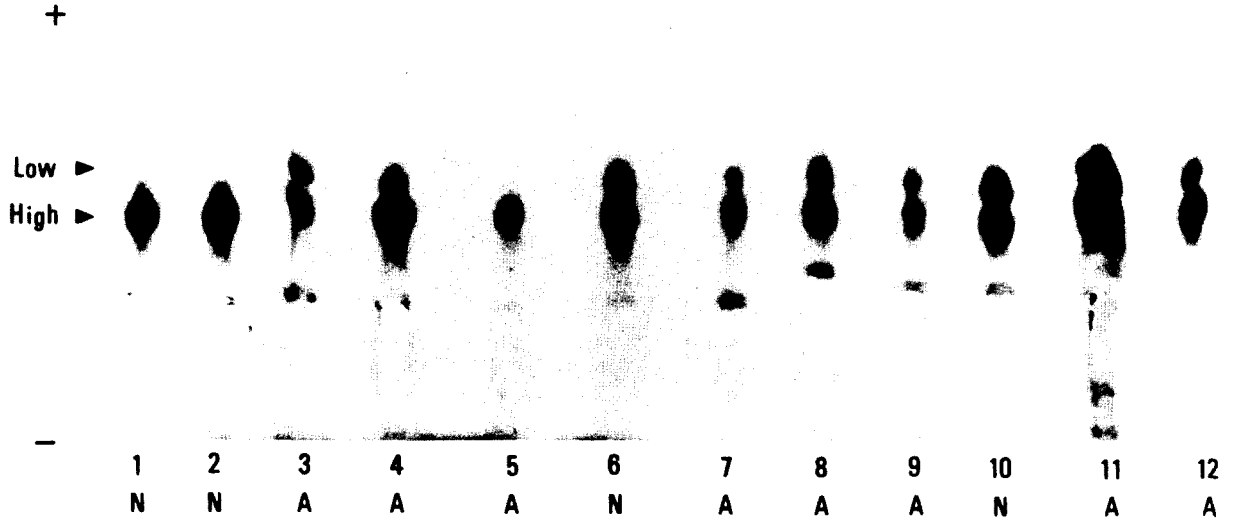

FIG. 2. Typical representative of ALDH isozymes of liver specimens in 4 non-alcoholic and 8 alcoholic patients with chronic liver disease. NA, non-alcoholic; A, alcoholic. Patient Nos. 1, 2 and 5 lack low Km ALDH isozyme band.

non-alcoholic volunteers. It was confirmed that cases with low Km ALDH isozyme showed no facial flushing after drinking alcohol, and cases lacking low Km ALDH isozyme showed facial flushing.

Analysis of ALDH isozyme in both hair roots and liver specimens was carried out for 15 chronic alcoholics with alcoholic liver injury. The isozyme pattern of liver specimens coincided with that of hair roots (Table 2): thirteen among 15 cases were low Km ALDH isozyme positive, while the remaining 2 cases were low Km ALDH isozyme negative patients. The low Km ALDH isozyme positive alcoholics showed no facial flushing, while the low Km ALDH isozyme negative alcoholics showed facial flushing after drinking alcohol. Among 3 cases lacking low Km ALDH isozyme, one was diagnosed histologically as having liver cirrhosis. The remaining 2 cases were diagnosed as nonspecified alcoholic liver disease, as assessed by biochemical studies.

The clinical data of hepatic function tests in 31 chronic alcoholics on admission and one week after are shown in Fig. 3. The levels of serum total bilirubin, r-GTP and GOT reduced or were normalized after one week abstinence in not

TABLE 1

FREQUENCY OF LOW Km ALDH ISOZYME POSITIVITY IN HAIR ROOTS FROM NORMAL VOLUNTEERS, AND ALCOHOLIC AND NON-ALCOHOLIC PATIENTS WITH CHRONIC LIVER DISEASE

Subjects (Number)	Low Km ALDH isozyme positive	negative
Normal Volunteers (n=16)	7 (44%)	9 (56%)
Patients with chronic liver disease (n=47)		
Non-alcoholics (n=16)	9 (56%)	7 (44%)
Alcoholics (n=31)	28 (90%)	3 (10%)

ALDH, aldehyde dehydrogenase.

TABLE 2

LOW Km ALDH ISOZYME OF HAIR ROOTS AND LIVER BIOPSY SPECIMENS IN CHRONIC ALCOHOLICS WITH LIVER INJURY

Case	Sex	Age	Liver histology	Flushing	Low Km ALDH Hair roots	Liver
(1) J.K.	m	53	Fatty liver	−	+	+
(2) K.T.	m	40	*	+	−	−
(3) C.M.	m	41	Liver fibrosis	−	+	+
(4) T.O.	m	41	Liver fibrosis	−	+	+
(5) M.T.	m	41	Liver fibrosis	−	+	+
(6) Y.Y.	m	54	Liver fibrosis	−	+	+
(7) N.H.	m	43	Fatty liver	−	+	+
(8) M.T.	m	47	Liver fibrosis	−	+	+
(9) M.M.	m	55	Liver fibrosis	−	+	+
(10) T.S.	m	39	Liver cirrhosis	−	+	+
(11) Y.M.	m	50	Liver cirrhosis	−	+	+
(12) H.Y.	m	56	Liver cirrhosis	+	−	−
(13) Y.Y.	m	48	Liver fibrosis	−	+	+
(14) W.H.	m	57	Liver cirrhosis	−	+	+
(15) K.Y.	m	48	Liver cirrhosis	−	+	+

ALDH, aldehyde dehydrogenase.
+, positive; −, negative.
*, not studied.
m, male.

only low Km ALDH positive patients, but also low Km ALDH negative patients.

DISCUSSION

Human liver possesses 4 ALDH isozymes which are named ALDH I (fast migrating, low Km for acetaldehyde), ALDH II (slow migrating, high Km for acetaldehyde), III and IV (very slow migrating, very high Km for acetaldehyde). The former two isozymes are supposed to play an important role in metabolism of acetaldehyde, while the latter two isozymes are insignificant [3]. The present study showed that 44% of Japanese normal non-alcoholic volunteers had low Km ALDH isozyme (ALDH I) in their hair roots, which is agreeable with the previous reports of Harada *et al.* [4]. On the other hand, in chronic alcoholics, a much higher frequency (90%) of low Km ALDH isozyme-positive patients was found compared not only to normal volunteers but also to non-alcoholic patients with chronic liver disease. The results suggest that the existence of this enzyme is closely connected with alcoholic liver injury. This close connection of high frequency of low Km ALDH isozyme positivity in chronic alcoholics with liver disease may partly be explained as follows: low Km isozyme positive men can drink much more alcohol because they do not show the acute toxic symptoms due to acetaldehyde, while low Km isozyme deficients drink much less because of acute toxic symptoms.

Surprisingly, there existed 3 cases lacking low Km ALDH isozyme among 31 chronic alcoholics. Inquiring to patients or their family about alcohol intake confirmed their high alcohol intake. They drank alcohol usually from morning till

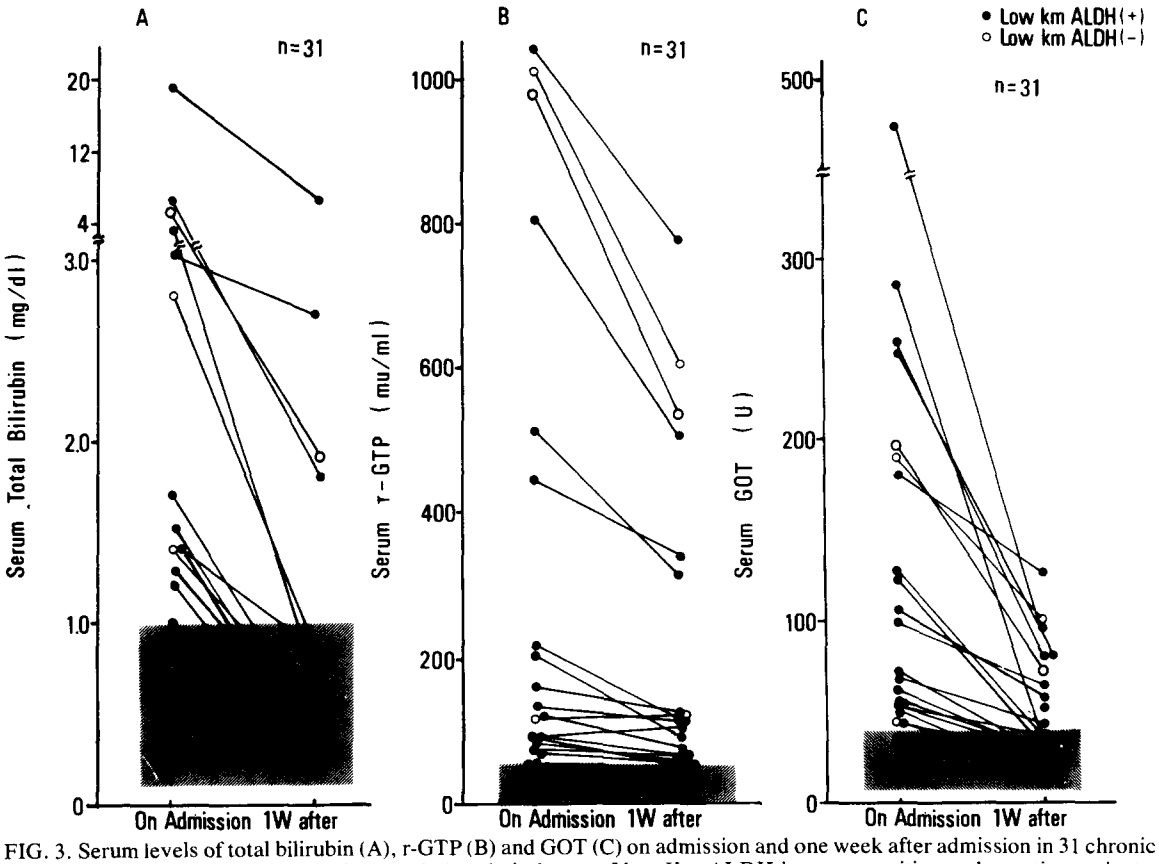

FIG. 3. Serum levels of total bilirubin (A), r-GTP (B) and GOT (C) on admission and one week after admission in 31 chronic alcoholics with liver injury. Open circle and closed circle are of low Km ALDH isozyme positive and negative patients, respectively.

night for more than ten years. They showed facial flushing as an initial symptom after drinking alcohol. Mizoi *et al.* [6] reported that facial flushing was closely related to the blood level of acetaldehyde. They suggested that low Km isozyme deficients would have elevation of blood acetaldehyde level resulting in facial flushing when they drink alcohol. Therefore, it is suspected that these low Km isozyme deficients would show significantly higher levels of acetaldehyde in blood as well as liver after drinking alcohol than the low Km isozyme positive cases. Considering the cytotoxicity of acetaldehyde [1], it may be supposed that patients lacking low Km isozymes are more susceptible to alcoholic liver injury and may show higher progression than those with low Km ALDH isozymes. Nevertheless, there was no apparent difference in hepatic function tests and histological findings between patients with and without low Km ALDH isozyme. This suggests that acetaldehyde does not play a primary or progressive role in alcoholic liver injury. It is well known that morphological and functional damage of mitochondria is the early manifestation of alcoholic liver injury [7,8]. Low Km ALDH isozyme is supposed to be localized in mitochondria [5]. Therefore, it may be suggested that the presence of low Km ALDH isozyme in mitochondria may induce mitochondrial overload due to greater acetaldehyde oxidation in this organelle. Further study is necessary to solve the mechanism of alcoholic liver injury.

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