Immunohistochemical Localization of Alcohol Dehydrogenase (ADH) in the Stomach Before and After Abstinence of Alcohol in Alcoholics

Using Confocal Laser Scanning Microscopy

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Summary: Previous studies have reported some significant participation by gastric alcohol dehydrogenase (ADH) in alcohol metabolism, similar to that by hepatic ADH. However, the localization of this ADH in the stomach is not yet determined and there has been no study on the localization of ADH in the stomach of alcoholics before and after abstinence from alcohol. The aim of the present study was to reveal any changes between before and after abstinence from alcohol in the immunohistochemical localization of ADH using biopsy specimens from the gastric mucosa. Twenty male alcoholics (aged 47.8±7.4 yrs) gave signed informed consent for this investigation. Esophago-gastro-duodenoscopy (EGD) and gastric corpus biopsy were performed just before abstinence and at one month later. ADH in the biopsy specimens was immunohistochemically examined with an anti-ADH antibody, using confocal laser scanning microscopy. The fluorescence intensity for ADH was compared for each pair of specimens before and after abstinence from alcohol using an image analyzer. Age, total alcohol consumption, degree of gastritis, and the liver function tests of all patients were also analyzed. The strongly immuno-positive cells for ADH in the gastric mucosa were identified as parietal cells. The fluorescence intensity for ADH was significantly higher in those specimens obtained after abstinence than in those before abstinence (p<0.005). The immunoreactivity for ADH in the cells assessed by confocal laser scanning microscopy was greatly improved after abstinence of alcohol, suggesting recovered alcohol metabolism in the gastric mucosa after abstinence from alcohol. The present study, demonstrating the cellular ADH localization in the gastric mucosa before and after abstinence from alcohol, may contribute to clarifying gastric alcohol metabolism in alcoholics.

Key words: gastric ADH, alcoholics, confocal laser scanning microscopy

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Introduction

In human, it is assumed that most alcohol ingested is metabolized by ADH in the liver. A number of studies focusing on ADH in the liver have been conducted (Larsen, 1959; Forsander et al. 1960; von Wartburg, 1971; Winkler et al. 1972; Crow et al. 1977). Recently, localization of ADH in the gastrointestinal tract has been postulated (Pestalozzi et al. 1983), suggesting that orally ingested alcohol could be metabolized in the upper gastrointestinal tract prior to its entry into systemic circulation (Julkunen et al. 1985a, b; Caballeria et al. 1987; Lim et al. 1993). It is now thought that the gastric mucosa is a major site for alcohol metabolism, and the ethanol oxidation system by ADH in the gastric mucosa is now referred to as the first pass metabolism (FPM) of ethanol in the stomach. Gastric ADH has been considered as playing a barrier role in preempting hepatic and central nervous system toxicity. Decreased FPM has been identified in patients after gastrectomy (Caballeria et al. 1989a), in alcoholics (DiPadova et al. 1987), in women (Morgan et al. 1977; DiPadova et al. 1988; Lieber, 1988; Haber et al. 1996), and in patients taking H2-antagonist (Caballeria et al. 1989b; Hernandez-Munoz et al. 1990; Caballeria et al. 1991; DiPadova et al. 1992; Brown et al. 1995). However, there are still many unknown issues related to FPM, including the percentage of ingested alcohol metabolized by gastric mucosa ADH, the effects by alcohol on the gastric mucosa, and the cause for the observed gender difference. Decreased FPM in alcoholics has been suggested to be caused by a reduction in the ADH activity in the gastric mucosa due to alcoholic mucosal injury or by direct disruption of ADH in the gastric mucosa by alcohol (DiPadova et al. 1987). Impairment to the gastric mucosa and decreased ADH activity due to alcohol may underlie depressed FPM. However, no mechanism has yet been identified. To clarify the in vivo origin of depressed alcohol FPM associated with chronic ingestion of alcohol, the presence or absence of macroscopic and histological disorders in the gastric mucosa were investigated using gastric mucosal tissue specimens, collected through biopsy from alcoholics, who were hospitalized to ensure abstinence from alcohol for voluntary rehabilitation. Data were collected on admission and again at after one month of abstinence. The ADH in the gastric mucosa was also studied immunohistologically with an anti-ADH antibody using a confocal laser scanning microscope to investigate the influence of alcohol consumption on gastric mucosal ADH.

Subjects and Methods

Subjects

The subjects included 20 men aged 33 to 62 years (mean 47.8±7.4 years), admitted to hospitals associated with the Second Department of Medicine of Kurume University School of Medicine, for rehabilitation treatment of habitual drinking after being diagnosed as alcohol dependent, between February and April 1996. The objectives and methods of the present study and physical stress caused by tests were explained to the patients. They were
also assured that they would not sustain any disadvantage by nonparticipation in the study. Only patients from whom informed consent was obtained participated in this study and the study protocol was approved by our School Ethics Committee.

Methods

Upper gastrointestinal endoscopy: Upper gastrointestinal endoscopy (GIFQ 200, Olympus, Tokyo) was conducted within one week on admission and again at 4 weeks after admission. Endoscopic findings were evaluated according to the endoscopic division of the Sydney System (Tytgat, 1991). During the endoscopic examination, a biopsy specimen was obtained from the greater curvature of the pylorus, and also from the greater curvature of the body of the stomach, using biopsy forceps (FB25K, Olympus). No medication with antacids or gastric mucosal protective agents was administered between the first and second examinations.

Histopathological assessment and evaluation of the presence of Helicobacter pylori: The biopsy specimen was stained with hematoxylin-eosin (H-E) and graded according to the histological classification of the Sydney System (Price, 1991). When H. pylori was confirmed at even a single site on microscopic examination of Giemsa-stained specimens, then the patient was assessed as positive for H. pylori, while those in whom H. pylori could not be detected at all were assessed as negative for H. pylori (Price, 1991).

Immunostaining using an anti-ADH antibody: The biopsy specimen from the greater curvature of the gastric body was fixed in 10% formalin and embedded in paraffin, and 3 μm-thick tissue sections were prepared. After deparaffinization, the sections were washed sufficiently with cold phosphate-buffered saline (PBS). The sections were allowed to react with whole horse serum (Vector, Burlingame, CA) at room temperature for 30 min and washed sufficiently with cold PBS to prevent any nonspecific reactions. The sections were then treated with mouse anti-human ADH antibody as the primary antibody (diluted with PBS at 1:20) (Advanced Immuno Chemical, Long Beach, CA) at 4°C for 24 hs, then washed sufficiently in cold PBS. Specimens were further treated with a fluorescein isothiocyanate-labeled anti-mouse IgG goat antibody as the secondary antibody (diluted with PBS at 1:40) (Capple Laboratories, Cocharanville, PA) at room temperature for 60 min. After washing sufficiently with cold PBS, the sections were embedded in non-fluorescent glycerin and examined using a confocal laser scanning microscope.

Observation with a confocal laser scanning microscope: Biopsied gastric mucosal specimens stained and treated with anti-ADH antibody were observed with a confocal laser scanning microscope (LSM-GB200, Olympus, Tokyo). To quantify the chromatic features, the fluorescence intensity from the cytoplasm of 10 cells per section was determined, and the fluorescence intensity of the gastric mucosal ADH was expressed as a mean level for the cells.

Statistical analysis: The fluorescence intensity of the gastric mucosal ADH observed by confocal laser scanning microscopy were quantified, and any significant difference in the quantified
fluorescence intensity of the biopsy specimen between that on admission and that at one month after admission was analyzed by the Wilcoxon test. The change in the fluorescence intensity after one month was tested for correlation with age, the cumulative amount of alcohol consumed, the endoscopic severity of gastritis, the degree of inflammatory cell infiltration in the biopsy specimen, and with any abnormality in the liver function tests, using Spearman rank multivariate analysis.

Results

Table 1 shows the ages of the 20 patients with alcohol dependence, the cumulative amounts of alcohol consumed, endoscopic findings from the upper gastrointestinal tract on admission, the degree of inflammatory cell infiltration in the biopsy specimens from the greater curvature of the gastric body, the presence or absence of H. pylori, and the liver function tests. The cumulative amount of alcohol consumed was 1008 g to 7160 g, showing a wide variation. Five patients showed normal endoscopic findings, and 15 showed gastritis. A histological examination revealed that inflammatory cell infiltration was negative in 5, mild in 9, and moderate in 6. There was no patient who showed severe inflammatory cell infiltration.

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Age (y)</th>
<th>Amounts of Alcohol Consumed (g)</th>
<th>Endoscopic Findings</th>
<th>Degree of Inflammatory Cell Infiltration</th>
<th>H. pylori</th>
<th>ALT (IU/L)</th>
<th>GGT (IU/L)</th>
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<td>6048</td>
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<td>250</td>
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GASTRIC ADH IN ALCOHOLICS

Fig. 1. A: Hematoxylin-eosin staining of a biopsy specimen from the greater curvature of the gastric body (×400). B: Confocal laser scanning micrograph with immunostaining using an anti-alcohol dehydrogenase antibody shows bright fluorescence in the cytoplasm of parietal cells.

Fig. 2. Confocal laser scanning micrograph (A) on admission, and (B) at one month after admission, in the same case. The fluorescence intensity in the cytoplasm after one month of abstinence (B) was distinctly greater than that shown in (A) on admission.
Fig. 3. The fluorescence intensity for alcohol dehydrogenase in the cytoplasm was determined, and the mean fluorescence intensity for 10 cells was obtained.

Fig. 4. The fluorescence intensity for alcohol dehydrogenase in the gastric mucosa on admission, and at one month of abstinence from alcohol in 20 patients with alcohol dependence. The fluorescence intensity for alcohol dehydrogenase after one month of abstinence was significantly higher than that on admission ($p<0.005$).
infiltration. The overall rate of positivity for H. pylori was 25%. There was no correlation between either age or the amount of alcohol consumed and the severity of gastric mucosal disorders. Fig. 1 shows an H-E stained biopsy gastric mucosal specimen and the confocal laser scanning microscopic findings on immunostaining with an anti-ADH antibody. Bright fluorescence was demonstrated in the cytoplasm of parietal cells. Fig. 2 shows the confocal laser scanning microscopic findings observed on immunostaining with anti-ADH antibody in the biopsy gastric mucosal specimens from the same patient with alcohol dependence, on admission and at one month after admission. The fluorescence intensity of ADH was distinctly increased in the gastric mucosa after alcohol abstinence for one month. The fluorescence intensity of ADH was quantified by the methods shown in Fig. 3. As shown in Fig. 4, the fluorescence intensity from the gastric mucosal ADH at one month after admission was significantly higher than that on admission (p<0.005), showing a significant recovery in the fluorescence intensity of gastric mucosal ADH due to abstinence. The rate of recovery in the fluorescence intensity of the gastric mucosal ADH on admission compared to the fluorescence intensity at one month after admission did not correlate with patient age, cumulative amount of alcohol consumed, presence or absence of endoscopic mucosal disorders, degree of histological inflammatory cell infiltration, presence or absence of H. pylori, or with the abnormality of the liver function tests.

Discussion

In the present study, we have demonstrated significant immunolocalization of ADH in the human gastric mucosa using an anti-ADH antibody and a confocal laser scanning microscope. In the alcoholics, the immunolocalization of ADH, predominantly demonstrated in parietal cells of the gastric mucosa, was reduced on admission (during alcohol loading) and this significantly recovered after alcohol abstinence for one month. Pestalozzi et al. (1983) postulated the localization of ADH in the gastrointestinal tract, and since then many studies have been made on gastric mucosal ADH. Julkunen et al. (1985a) determined the blood levels of ethanol and acetate after ethanol was administered intravenously, and perorally. They demonstrated that the blood level of ethanol administered intravenously was higher than that of ethanol administered perorally, and that the blood level of acetate, which is a metabolite of ethanol, was higher after the peroral administration of ethanol than after intravenous administration. Thus, Julkunen et al. concluded that ethanol was metabolized in the gastrointestinal tract. When comparing blood levels of alcohol administered intravenously or perorally in the same volumes, the blood alcohol level was higher after intravenously-administered than after perorally administered. The difference between the two is thought to reflect a volume of alcohol that did not enter systemic circulation due to FPM. The study of the FPM of ethanol has attracted considerable attention. FPM has been reported to be greater after
meals than during fasting, to be less in gastrectomized patients than in healthy persons, and to be lower in women than in men (DiPadova et al. 1987; DiPadova et al. 1988; Caballeria et al. 1989a). Persons who consume alcohol excessively, show a significantly greater depression in FPM than do healthy persons. Some reports have shown that FPM is significantly elevated after 2 weeks of abstinence and to approximately the same degree as in healthy persons after 3 weeks of abstinence (DiPadova et al. 1987). Disorder in the gastric mucosa itself due to a decrease in defense activity could contribute to a depressed FPM. One study has shown that FPM was depressed with gastric mucosal ADH hypoactivity due to chronic administration of ethanol, in rat (Julkunen et al. 1985a). An experimental report has also shown that the gastric mucosa damaged by pure ethanol was quickly regenerated (Lacy et al. 1984; Ito et al. 1985). Mario et al. found ADH activity in a homogenate of the gastric mucosa (using human biopsy gastric mucosal specimens), and demonstrated that this ADH activity was significantly lower in patients with alcohol dependence than in healthy persons (Frezza et al. 1990). Although there are many reports on heavy drinking in combination with several medications, heavy drinking affects tissues in various ways (Hasumura et al. 1974; Sato et al. 1981; Seeff et al. 1986; Swerdlow, 1990). In this study, we examined specimens from alcoholics to discover whether the influence of chronic alcohol intake on ADH in the gastric mucosa was a disorder secondary to gastric mucosal injury or was instead induction of a direct decrease in ADH activity. Changes in the fluorescence intensity of gastric mucosa ADH over one month of abstinence were observed, as well as changes in the ADH localization in the gastric mucosa. The fluorescence intensity was marked in the parietal cells, indicating abundant localization of ADH in these cells, while the epithelial cells and other cells were hardly stained. These findings are slightly different from those of a previous report (Pestalozzi et al. 1983). When the macroscopic findings and the histological findings at biopsy were compared, inflammatory cell infiltration, involving both plasmacytes and monocytes, was observed in gastric mucosal specimens from 15 of the 20 patients with alcohol dependence on admission, suggesting a chronic disorder in the gastric mucosa. However, there was no significant improvement in this finding after abstinence from alcohol. Therefore, the inflammatory lesion in the gastric mucosa in the present patients was not attributed to alcohol ingestion but to some other causes. There was no correlation between the presence or absence of H. pylori and the fluorescence intensity of ADH. The rate of positivity for H. pylori in patients with alcohol dependence was 25%, which was lower than that in non alcohol dependent men of the same age group (Asaka et al. 1992). Atrophied mucosa shown as a changed pH in the stomach due to alcohol ingestion has been reported to influence the rate of positivity for H. pylori. Our study on gastric mucosal specimens collected through biopsy from 20 patients has revealed the absence of any atrophy. Further studies are needed on the
relationship between alcohol and H. pylori. The patients were not treated with antiulcerative medication. Some H2-antagonists have been shown to reduce the ADH activity in the stomach (Caballeria et al. 1989b, 1991; Hernandez-Munoz et al. 1990; DiPadova et al. 1992; Brown et al. 1995). All of our 20 patients included in the present study were not treated by H2-antagonists. Our findings were consistent with those of a previous report that ADH activity in homogenate gastric mucosa was significantly reduced in alcoholics (Frezza et al. 1990). These results suggested that depression in the fluorescence intensity of the gastric mucosal ADH can be attributed to hypoactivity of ADH due to excessive ethanol metabolism regardless of any disorders in the gastric mucosa. In the future, it will be possible to semiquantitate the amount of ADH in the gastric mucosa using a confocal laser scanning microscopy on a formalin-embedded biopsy specimen from the gastric mucosa without measuring the activity of ADH in the homogenate of the gastric mucosa. This method may be easy to apply in clinical practice. It is assumed that disorders in ADH in the gastric mucosa enhance the alcohol load on the liver and exacerbate liver damage and induces alcoholic liver diseases. Therefore, understanding ADH in the gastric mucosa may facilitate the development of some therapy for these disorders, and further studies on ADH in the gastric mucosa are important.

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References


