Suppression of Acetaldehyde Accumulation by 4-Methylpyrazole in Alcohol-Hypersensitive Japanese

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Abstract—Alcohol-sensitive Japanese subjects with facial flushing and an increase in heart rate during ethanol intoxication exhibited marked individual variation in accumulation of acetaldehyde. This variation correlated well with the intensity of the above mentioned physiological responses. Oral pretreatment with 10 mg/kg 4-methylpyrazole, which inhibited the ethanol elimination rate by 15–25%, strongly suppressed both acetaldehyde accumulation and the associated responses. Under this condition, the sensitivity to acetaldehyde appeared to be reduced, and the correlation between the acetaldehyde level and the physiological responses disappeared. The effectiveness of even a low dose of 4-methylpyrazole suggests its clinical usefulness for alleviation of acute acetaldehyde toxicity in alcohol-hypersensitive Japanese individuals as well as in disulfiram-treated alcoholics.

A large number of studies have demonstrated the importance of acetaldehyde (AcH), the first metabolite of ethanol, in mediating physiological responses during ethanol intoxication (1, 2). Typical responses in humans to AcH accumulation such as flushing of the face, increase in heart rate and blood pressure changes, are considered to occur through subsequent release of active substances affecting the cardiovascular system (3–5). These unpleasant physiological effects lead to aversion to alcohol drinking in disulfiram (Antabuse)-treated alcoholics and in alcohol-sensitive (flushing) individuals, that is about half of all Orientals (6, 7). In these cases, AcH accumulation is ascribed to an insufficient capacity for AcH oxidation. Administration of disulfiram inhibits (8) aldehyde dehydrogenase (ALDH) which is the responsible enzyme for AcH oxidation, while in flushing Orientals, the absence of one ALDH isozyme with a high affinity for AcH (low-Km ALDH) has been reported (9, 10).

Another possible case for AcH accumulation during ethanol intoxication is the acceleration of ethanol oxidation (11) observed in alcoholics (12). On the contrary, reduction in the ethanol oxidation rate is often highly effective for alleviation of acute AcH toxicity in the subject pretreated with ALDH inhibitor. Partial inhibition of ethanol elimination by infusing a small dose of 4-methylpyrazole (4-MP), a specific inhibitor of alcohol dehydrogenase (13) with low toxicity (14, 15), to cyanamide (another ALDH inhibitor)-treated subjects lead to a rapid fall in the level of AcH and associated responses (16, 17). During the course of our previous experiment investigating AcH accumulation in relation to ethanol and AcH oxidizing capacity, 4-MP was found to be highly effective in reducing AcH levels also in flushing Orientals (18). With further experiments, the present paper describes the effect of 4-MP on blood AcH concentration and cardiac response following ingestion of ethanol.
Materials and Methods

The alcohol drinking experiments were performed with six flushing (age: 22-35, body weight: 52-80 kg) and three non-flushing (age: 22-33, body weight: 62-67 kg) healthy Japanese male subjects. Two or three hr after a light breakfast, each subject drank 20% ethanol solution diluted with unsweetened orange juice. In most cases, 0.5 g/kg ethanol was consumed during a 5 min period and when lower doses were tested, the drinking period was shortened proportionately. In the next 5 hr, the subject sat quietly in a chair, and blood sampling and heart rate counting were done periodically. When 4-MP, purchased from Aldrich Chemical Co., was used on a separate day, it was diluted in 50 ml of orange juice and ingested 1 hr before the ethanol.

Blood samples were taken from an antecubital vein via an indwelling catheter. A blood sample of approximately 1.5 ml was collected by direct dripping into a chilled tube containing 1.5 ml of heparinized semicarbazide solution in isotonic phosphate buffer (pH 7.2) as described by Stowell et al. (17). After immediate mixing followed by centrifugation, an aliquot of the plasma fraction was treated with 0.3 volume of 10% perchloric acid. Ethanol and AcH content in the deproteinized supernatant were estimated by a head-space gas chromatographic procedure (19) as previously described (20). The detection limit of AcH was 0.5 nM in the deproteinized supernatant, which corresponds to about 1.5 M in the whole blood sample.

Results

Administration of 0.5 g/kg ethanol resulted in accumulation of AcH in all the tested flushing subjects, but none of the non-flushing subjects had levels of more than 5 nM (Table 1). Though there were rather small individual variations in both peak blood ethanol levels and ethanol elimination rates (18), marked variation was noted with blood AcH levels in the flushing subjects, particularly at their peaks after 30 min (40-270 nM) and after 1 hr (20-200 nM). In parallel with blood AcH levels, there were also individual variations in physiological responses. Those who accumulated higher amounts of AcH revealed a higher increase in heart rate and degree of flushing, while in the subjects with weaker response, lower levels of blood AcH were obtained. Good correlation between blood AcH level and cardiac response is further demonstrated in Fig. 1. The plot of percent change in heart rate as a function of logarithmic concentration of AcH demon-

Table 1. Peak blood acetaldehyde levels after 30 min of ethanol intake

| Treatment   | Flushing subjects | Non-flushing subjects |
|-------------|-------------------|----------------------|
| Control     | 136.6±92.4 (6)    | 2.6±2.4 (3)          |
| 4-MP        | 33.0±16.7 (6)     |                      |

Ethanol (0.5 g/kg) was ingested with or without oral treatment of 10 mg/kg 4-methylpyrazole (4-MP) 1 hr before ethanol. Each value represents the mean±S.D. (N).
strates a positive linear relation above 30 \text{nM} \text{AcH}, and the correlation coefficient was calculated to be 0.876 (P<0.01). On the other hand, the cardiac response is evidently independent of blood ethanol level (Fig. 2).

When the same flushing subjects were tested with the same ethanol dose 1 hr after oral administration of 10 mg/kg 4-MP, both \text{AcH} accumulation and associated physiological responses were effectively suppressed through a 20.4±4.2% reduction of ethanol elimination rate (18). Though peak \text{AcH} levels observed after 30 min again showed large individual variation in a range between 15–65 \text{nM} (Table 1), the physiological sensitivity to \text{AcH} was apparently lowered. With 4-MP, the plot of heart rate change versus \text{AcH} concentration was limited to the lower range of heart rate change between 90–120% of the control, and a positive relation was not apparent (Fig. 1). As to facial flushing, there was no evident change in any of the 4-MP-treated subjects even in the one with 50 \text{\mu M} \text{AcH}.

To one subject who showed pronounced tachycardia and facial flushing with over 200 \text{\mu M} \text{AcH} level in the control experiment (Fig. 3a), different doses of 4-MP and ethanol were given. As shown in Fig. 3b, with partial inhibition of \text{AcH} accumulation through 8% reduction of ethanol elimination by 7 mg/kg 4-MP, the heart rate remained fairly normal. Accumulation of \text{AcH} was suppressed more efficiently by 10 mg/kg 4-MP, both \text{AcH} accumulation and associated physiological responses were effectively suppressed through a 20.4±4.2% reduction of ethanol elimination rate (18). Though peak \text{AcH} levels observed after 30 min again showed large individual variation in a range between 15–65 \text{nM} (Table 1), the physiological sensitivity to \text{AcH} was apparently lowered. With 4-MP, the plot of heart rate change versus \text{AcH} concentration was limited to the lower range of heart rate change between 90–120% of the control, and a positive relation was not apparent (Fig. 1). As to facial flushing, there was no evident change in any of the 4-MP-treated subjects even in the one with 50 \text{\mu M} \text{AcH}.

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![Fig. 2. Correlation between blood ethanol (EtOH) concentration and change in heart rate. O, without 4-MP; •, with 4-MP pretreatment.](image)

![Fig. 3. Effect of different doses of ethanol and 4-MP on acetaldehyde accumulation and heart rate.](image)
4-MP which brought about a 15% reduction in ethanol elimination (Fig. 3c). Under this condition, though the peak AcH was still over 50 μM, the heart rate remained unchanged, and there was no visible alteration in facial color. On the other hand, the peak AcH level of 60 μM attained by ingestion of a lower (0.2 g/kg) dose of ethanol without 4-MP increased the heart rate (Fig. 3d), and there was a mild facial flushing. Even with 0.1 g/kg ethanol producing a lower amount of AcH (Fig. 3e), a slight change was detected for the two responses.

Discussion

Oral pretreatment of flushing subjects with 4-MP was highly effective in suppressing AcH accumulation during ethanol intoxication. A similar effect was found with 4-MP infusion into ALDH inhibitor-pretreated subjects followed by ethanol intake (16, 17). In both above-mentioned subjects, about 20% inhibition of ethanol oxidation (AcH production) reflected by the reduced rate of ethanol elimination was sufficient for evidence of the effectiveness. From these results plus previous findings of no significant differences in ethanol elimination rates among flushing Japanese, nonflushing Japanese and Caucasian subjects (6, 18, 21), it may be suggested that flushing Orientals lacking low-Km ALDH isozyme (9, 10) have a capacity to oxidize approximately 80% of the AcH being produced from ethanol. Since both ethanol and AcH are oxidized mostly in the liver, hepatic output of the resting unoxidizable AcH would indicate the level of peripheral AcH.

As to non-flushing Orientals and Caucasians possessing both the low-Km ALDH and another major ALDH isozyme found in every human with a higher Km value for AcH (22, 23), they appear to have a sufficient capacity for AcH oxidation to prevent hepatic AcH output under normal conditions, but there is not such a high capacity to cover the 50% increase in ethanol oxidation observed in Caucasian alcoholics (11, 12). Therefore, in alcohol aversion therapy, partial inhibition of ALDH activity probably leads to a condition similar to that of flushing Orientals who show significant accumulation of AcH during ethanol intoxication. Indeed, such was found in rats by Tottmar and Hellström (24).

Acute physiological responses to ethanol-derived AcH seem to occur with the subsequent release of active substances affecting the cardiovascular system (3-5). Using electrically driven left atria isolated from guinea pigs, Walsh et al. (25) observed a positive inotropic response to exogeneous AcH which could be inhibited by propranolol or reserpine pretreatment. This response increased linearly as a function of logarithmic concentrations of AcH in a range between 0.1-3 mM. In the present experiment without 4-MP, the positive chronotropic response was similarly related to ethanol-derived AcH concentration above 30 μM (Fig. 1). Because both positive inotropic and chronotropic responses occur with β-adrenergic stimulation, and blood levels of norepinephrine and epinephrine are elevated in flushing Japanese subjects during ethanol intoxication (3, 4), it seems highly probable that release of these catecholamines and the resulting effects on cardiac function depend on the accumulated levels of AcH.

The higher threshold level of AcH (100 μM) which exerts a significant effect on the isolated atria (25) compared with human cardiac function (30 μM) in the present study may in part be explained by the stimulating effect of AcH on the adrenal gland to release these two catecholamines (26) as well as on adrenergic nerve terminals in myocardial tissue containing mostly norepinephrine (27). Thus, in combination with the result showing a similar relation between AcH level and the responses obtained by giving different doses of ethanol to the same subject (Fig. 3), it is strongly suggested that individual differences in sensitivity to alcohol among flushing Orientals are determined mainly by the AcH level itself. Differences in releasing reactivity of catecholamine storing sites to AcH seem to contribute to a much lesser extent.

Compared with pyrazole, 4-MP is a more specific alcohol dehydrogenase inhibitor (13) with less toxicity (14, 15). The dose of 4-MP used in the present study on humans was much lower than the daily dose given in the
chronic rat experiment in which no toxic effect was observed (14). However, the apparently lowered sensitivity to AcH seen with 4-MP pretreatment may be related to pharmacological effects of 4-alkylated pyrazoles which do lower muscle tone, motor activity and body temperature (28), and such effects may antagonize the sympathomimetic action of AcH. Furthermore, taking account of the altered functions of the central nervous systems and lipid metabolism which can lead to hepatic damage by various pyrazole derivatives (29), the possible toxic side effects of 4-MP at even lower dose must be carefully taken into consideration. Though further investigation on such points would be required, 4-MP may be clinically useful in acute treatment of strong alcohol flushing and tachycardia of alcohol-hypersensitive Orientals as well as of disulfiram-treated alcoholics (15, 16).

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