The effect of green tea on the activity of aldehyde dehydrogenase (ALDH) in the liver of rats during chronic ethanol consumption

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Abstract

Purpose: **Alterations in the redox state during chronic ethanol consumption are associated with the oxidation of ethanol via alcohol and aldehyde dehydrogenase. Among various antioxidants present in food, strong antioxidative effects have been attributed to polyphenols of green tea. The aim of the present study was to investigate the effect of green tea consumption during chronic ethanol intake on the activity of aldehyde dehydrogenase in the liver of rats during maturation and aging.**

Materials and methods: **The activity of ALDH was measured in the livers of rats aged 2 (young), 12 (adult) and 24 months (old). The rats were fed with a control liquid Lieber DeCarli diet, control liquid diet containing green tea (3 g/l), ethanol liquid diet (with increasing ethanol dose from 2.3% to 7%) and ethanol liquid diet containing green tea.**

Results: **Chronic ethanol consumption significantly increased the liver ALDH activity in young and adult rats but decreased this activity in old animals. The drinking of green tea did not alter ALDH activity in ethanol-consuming rats. Drinking green tea alone significantly increased ALDH activity in young and adult rats but did not alter this activity in old rats.**

Conclusions: **These results demonstrate that green tea administered during chronic ethanol consumption does not prevents the changes in the hepatic ALDH activity in the rats at each age.**

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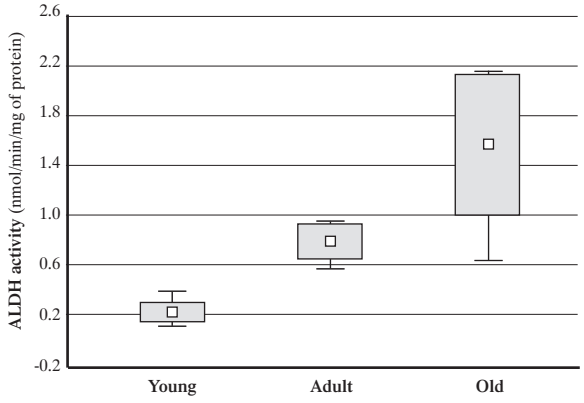
Introduction

Ethanol in mammals is oxidized in the liver to acetaldehyde by alcohol dehydrogenase (ADH), and then to acetate by aldehyde dehydrogenase (ALDH) [1]. In this oxidative metabolic pathway, an excess of reducing equivalents, such as NADH, are generated. Another system capable of carrying out ethanol oxidation is the microsomal ethanol oxidizing system (MEOS) [2]. The altered redox state in the cytosol and mitochondria, and the increase in free-radical production are considered responsible for the different metabolic disturbances following chronic ethanol consumption [1-3].

There are several factors that affect alcohol metabolism. These include food, alcohol abuse, drugs, gender, body weight, body composition and ethnicity. It is generally accepted that age has an effect on alcohol metabolism and that this effect is associated with the activities of alcohol metabolizing enzymes located in the liver and stomach [4-6].

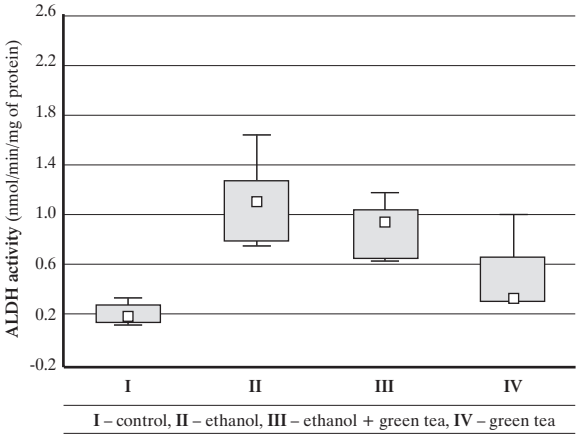
Recently, it has been shown that green tea exhibits antioxidative activity [7,8]. A potential mechanism(s) for such an effect involves radical-scavenging, metal chelating and/or enzyme modulation ability [9,10]. Several compounds of green tea, mainly catechins, have been reported to have a protective action. The molecular mechanisms underlying the effects of catechins in some cases involved inhibition or activation of enzymes. Among other things, it has been observed that green tea inhibits the induction of human cytochrome P450 [11], an enzyme that is induced following chronic ethanol consumption. The objective of the present study was to investigate the effect of green tea drinking together with ethanol on the hepatic activity of ALDH (enzyme involved in redox homeostasis) of rats during maturation and aging. The effect of green tea drinking was also determined separately.

Figure 1. **ALDH activity in the control group according to age of rats**



Squares represent the median values, the boxes represent the second and third interquartile range, and the whiskers represent the overall range

Figure 2. **ALDH activity in the liver of young rats drinking green tea with ethanol**



Squares represent the median values, the boxes represent the second and third interquartile range, and the whiskers represent the overall range

Material and methods

Male Wistar rats aged 2 (200-220 g b.w.), 12 (520-550 g b.w.) and 24 months (750-780 g b.w.) were used in the experiment. The dose of ethanol in this diet was gradually increased from 2.3% (day 1-3), to 4.7% (day 4-6), and to 7% (day 7-28).

The rats were housed in individual cages and pair-fed with either nutritionally control – a liquid Lieber DeCarli diet containing 47% of total energy as carbohydrate, 18% protein, 35% lipid or an identical diet with ethanol substituted isocalorically for carbohydrates (36% of total energy). Liquid diets (control and ethanol) containing 7 g green tea extract/l were also prepared (Camelia sinensis, O. Kuntze, lyophilized extract, TJ Lipton, Englewood Cliffs, NJ). The amount of catechins in this diet, measured by HPLC, was equivalent to the amount of these compounds in 3 g of green tea extract/l water solution, which is frequently the concentration used for human consumption [12]. The content of components in diet with green tea was as follows: epigallocatechin gallate – 337 mg/l, epigallocatechin – 268 mg/l, epicatechin – 90 mg/l, epicatechin gallate – 60 mg/l, and coffeic acid – 35 mg/l. The same age animals were divided into 4 groups (6 animals in each group). The total number of tested rats was 72. All procedures were in accordance with guides for care and use of laboratory animals, and the protocol was approved by the local Animal Care Committee in Białystok.

The control group was fed for 5 weeks a control Lieber DeCarli liquid diet (n=6). The ethanol group was fed for one week the control Lieber DeCarli liquid diet and for the next 4 weeks the ethanol liquid diet (n=6). The green tea group was fed for 5 weeks the control Lieber DeCarli liquid diet containing green tea (n=6). The ethanol and green tea group was fed for one week the control Lieber DeCarli liquid diet containing green tea and for the next 4 weeks the ethanol liquid diet also containing green tea (n=6). Dietary intake was comparable in all groups, with all rats demonstrating consistent weight gain throughout the 5-week feeding period.

The rats were sacrificed with ether anaesthesia, after which the livers were quickly removed and placed in ice-cold 0.15 M NaCl solution, perfused with the same solution to remove blood cells, blotted on filter paper. The organs were weighed and homogenized in 9 ml of 0.25 M sucrose. Homogenates (10%) were centrifuged at 10000 x g for 15 min at 4°C, and the supernatant was kept on ice until assayed.

Aldehyde dehydrogenase (ALDH) activity was measured using the fluorogenic method of Wierzchowski et al. [13] based on oxidation of 6-methoxy-2-naphthaldehyde to the fluorogenic 6-methoxy-2-naphthoate. As described Wierzchowski and co-workers, this naphthaldehyde is very good substrate for liver cytosolic ALDH-1 isoenzyme (class I) and for ALDH-3 isoenzyme (class III), which is not expressed in the liver. The reaction mixture contained 60 µl of 300 µM of 6-methoxy-2-naphthaldehyde, 20 µl of 1 mM of NAD, 2.8 ml of 50 mM Na-pyrophosphate buffer, pH 8.5 and 60 µl of homogenate. The fluorescence was read at an excitation wavelength of 310 nm and an emission wavelength of 360 nm.

Protein concentration was measured according to Lowry using bovine serum albumin as the standard (Sigma kits).

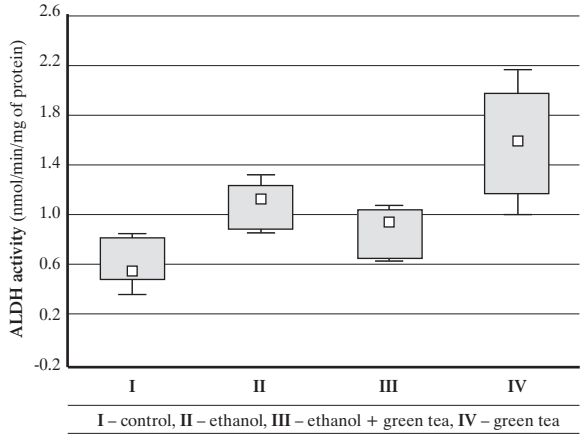
The results are expressed as median and ranges. Statistical analysis was performed using the Mann-Whitney U test. Differences were considered significant at $p<0.05$.

Results

Aldehyde dehydrogenase activity in the liver of rats fed on a control liquid diet increased parallel with the age of the animals, obtaining six-times higher value in old rats than in young (*Fig. 1*). The acitivity in adult rats was 3-times higher than in young.

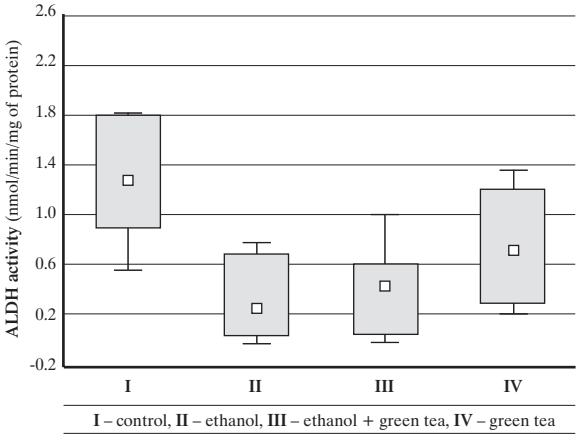
Chronic ethanol consumption caused a significant increase in ALDH activity in the liver of young and adult rats but lead to

Figure 3. **ALDH activity in the liver of adult rats drinking green tea with ethanol**



Squares represent the median values, the boxes represent the second and third interquartile range, and the whiskers represent the overall range

Figure 4. **ALDH activity in the liver of old rats drinking green tea with ethanol**



Squares represent the median values, the boxes represent the second and third interquartile range, and the whiskers represent the overall range

decrease in ALDH activity in old animals when compared to the control group ($p<0.05$) (*Fig. 2*, *3* and *4*). The drinking of ethanol with green tea by young, adult and old rats did not change the activity of ALDH in comparison with the ethanol alone group ($p>0.05$). Administration green tea alone to young and adult rats significantly increased ALDH activity when compared to the control group ($p<0.05$). Drinking green tea alone by old rats did not influence ALDH activity in comparison with the control group ($p>0.05$).

Discussion

The main findings of our study are that chronic ethanol consumption significantly increased the liver ALDH activity in young and adult rats and that drinking ethanol with green tea by rats at each age did not change this activity when compared to the ethanol alone group. These data concerning the effect of ethanol consumption on the activity of enzymes involved in ethanol metabolism are in conflict with some of the literature. Some previous reports have found an increase of ALDH activity in the rodent liver cytosol, mitochondria and microsomes following chronic administration of alcohol [14,15]. Previous works by Guerri et al. also reported a 2-fold increase in the liver ALDH of ethanol fed rats (for 7 week) [16]. As Vaananen et al. noted, even 12 weeks consumption of ethanol did not cause histological changes in the rat liver parenchyma [15]. However, despite the lack of parenchymal lesions they observed an increase in low K_m aldehyde dehydrogenase. In our study, the rats were fed with an ethanol liquid diet for 4 weeks and we did not observe changes in liver histology. In a more recent study, Vidal et al. reported that chronic alcohol abuse reduced ALDH activity (mainly low K_m form) in patients with alcoholic cirrhosis but did not depress ALDH activity in patients without alcoholic liver diseases [17]. It has been established that increases in the rate of ethanol metabolism require the contribution of ethanol metabolizing enzymes. However, on the basis of the results obtained by Lumeng et al., it could be suggested that a reduction in the rate of ethanol elimination in fasted rats may be caused by decreasing ADH activity [18]. The increase in ALDH activity after chronic ethanol drinking in the present study may be caused by the presence of a substrate for this enzyme: acetaldehyde. Recently, Badger et al., for the first time, demonstrated the existence of an ethanol-dependent induction of rat class I hepatic ADH [19]. Enhancing activity of liver class I ADH promotes the generation of acetaldehyde, which may result in an induction of ALDH activity.

Our data show that drinking green tea alone significantly increased ALDH activity in young and adult rats. The flavonoids of green tea have been found to affect (inhibition or activiation) activities of several enzymes, e.g. monooxygenase, lipoxygenases, cyclooxygenase, histydyne decarboxylase, cyclic AMP phosphodiesterase [20]. The increase of ALDH activity observed after green tea consumption may have been caused by the activation of one of these enzymes by the catechins in green tea. The other isoflvonoids isolated from plants that affect the alcohol pharmacokinetics and alcohol-drinking behaviour in rats are daidzin, daidzein and puerarin [21]. Daidzein is a reversible inhibitor of alcohol dehydrogenase [22], and daidzin of mitochondrial aldehyde dehydrogenase (class II ALDH) [23]. In our study we have estimated the activity of ALDH with the substrate for class I ALDH (cytosolic). However, none of the 3 isoflavonoids administrated orally affected liver alcohol dehydrogenase or aldehyde dehydrogenase activities, as it was reported for intraperitoneal administration [21].

When we demonstrated the stimulation of ALDH activity by the consumption of green tea, other authors showed the inhibition of cytochrome P450 2E1 activity under similar conditions [11]. Among the compounds in green tea, the most effective cytochrome P450 2E1 inhibitor was epigallocatechin gallate.

The activation of the MEOS system following chronic ethanol consumption generates free radicals. The green tea antioxidant potential has been attributed to a free radical scavenging effect. Thus, drinking green tea may cause the inhibition of one pathway (MEOS) and the activation of another pathway (ALDH) for hepatic ethanol elimination (our study). The induction of ALDH activity in the liver of rats drinking tea was observed in young and adult rats but not in the old animals. This discrepancy might been caused by differences in ALDH activity at various ages. In partial support of this hypothesis, the activity of ALDH in the old control rats was much higher than the activity in the young and adult animals. Taking into account that the acetaldehyde is the most toxic product generated during ethanol metabolism, these findings suggest that the older subjects have the better defence against the negative consequences of alcohol abuse than the younger subjects.

Conclusions

We conclude that the consumption of green tea following chronic ethanol administration did not prevents the changes in the hepatic activity of ALDH in the rats at each age.

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