ROSIGLITAZONE RELIEVES ACUTE ETHANOL-INDUCED HANGOVER IN SPRAGUE–DAWLEY RATS

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Abstract — Aims: To assess the efficacy of rosiglitazone in blocking ethanol-induced hangover in rats. **Methods:** Rats injected with ethanol (4 g/kg body weight) were subjected to social interaction tests. Levels of aldehyde dehydrogenase 2 (ALD2), involved in an anti-hangover mechanism, were measured by semi-quantitative RT–PCR and western blot analysis. **Results:** Rosiglitazone caused an upregulation of mitochondrial ALD2, thus significantly detoxifying acetaldehyde. **Conclusions:** Rosiglitazone alleviated the symptoms of ethanol-induced hangover by inducing ALD2 expression; this result was reconfirmed by eliminating the effect of rosiglitazone by injecting cyanamide, an ALD2 inhibitor.

INTRODUCTION

Although ethanol has been used to reduce stress, excessive ethanol consumption can have serious negative social consequences. Alcoholism is not a simple problem of heavy drinking. It substantially damages individuals' mental and physical health. Symptoms of ethanol abuse include vomiting, dizziness, thirst, headaches and muscle pain (Swift and Davidson, 1998). Chronic ethanol consumption leads to liver damage, pancreatitis, myocardial infarction and neuropathy and exacerbates the symptoms of tuberculosis and other diseases (Lieber and Leo, 1986, 1992).

Most ethanol consumed is metabolized by aldehyde/alcohol dehydrogenase (ALD), a microsomal ethanol-oxidizing system, and catalase, and by peroxidase in the liver; the remainder is emitted via the kidney and lung. Ethanol administration causes more damage to the liver than to any other organ (Lieber and DeCarli, 1968; Goodman and Gilman, 1975; Lieber, 1985; Lieber and Leo, 1986). Therefore, the efficient detoxification of ethanol is crucial for the preservation of proper liver function.

Acetaldehyde is known to be a primary metabolite of ethanol and is the main cause of hangovers (Kim, 1994). Lieber reported acetaldehyde to be more toxic to the body than ethanol itself (Lieber, 1973). It has been reported that acetaldehyde causes steatohepatitis, hepatic cirrhosis, downregulation of ALD2 expression and impaired vitamin activity via mitochondrial dysfunction (Helander, 1988). Therefore, problems of ethanol toxicity can potentially be solved by effectively decreasing the plasma acetaldehyde concentration.

Rosiglitazone, a class of insulin-sensitizing thiazolidinedione (TZD) drugs, is a peroxisome proliferator-activated receptor (PPAR)-γ agonist. It has been widely used for treating hyperglycaemia and improving insulin resistance (Patel *et al.*, 1999). The metabolic effects of TZDs are mediated by receptor-dependent activation of the PPAR-γ–retinoid X receptor (RXR) complex and subsequent transcriptional activation of target genes (Yki-Jarvinen, 2004). Interestingly, TZDs may also be potent inhibitors of inflammation, in part through receptor-independent mechanisms (Chawla *et al.*, 2001; Takata *et al.*, 2002; Ruan *et al.*, 2003; Mohanty *et al.*, 2004). It is also thought that the anti-inflammatory effects of TZDs may reduce the risk of cardiovascular disease (van Wijk and Rabelink, 2004). There has been one report that the ALD2 promoter contains PPAR response elements (PPREs) (Pinaire *et al.*, 1999). Thus, the expression of ALD2 could potentially be regulated by rosiglitazone.

The purpose of this study was to assess the effects of rosiglitazone on ALD2 expression and associated symptoms of ethanol-induced hangover.

MATERIALS AND METHODS

Animals and study design

Four-week-old male Sprague–Dawley (SD) rats were purchased from Samptako (O San, Korea) and were housed (two per cage) under 12 h light/dark cycles with *ad libitum* access to food and water for 24 weeks. Half of the animals (total $n = 48$) were given 5 mg/kg body weight of rosiglitazone orally for 2 weeks along with standard lab chow *ad libitum*. Because rosiglitazone was originally a metabolic disease drug, it needs time to induce ALD2 expression. This was confirmed in *in vitro* experiments using HepG2 cells (data not shown). After pre-treatment with rosiglitazone, the rats were given one 4 g/kg body weight dose of ethanol orally (AR, $n = 14$) or saline (A, $n = 10$) (Gauvin *et al.*, 1992). Then 10 mg/kg body weight of cyanamide was administered to rosiglitazone plus ethanol-treated rats (ARI, $n = 7$) by intraperitoneal injection as an ALD2 inhibitor. Animals not treated with rosiglitazone (C, $n = 24$) were given standard lab chow *ad libitum*. The animals were given a 10 min social interaction test. Independent rat tissue samples under the same conditions were harvested according to time-dependent plasma alcohol concentration (Fig. 1).

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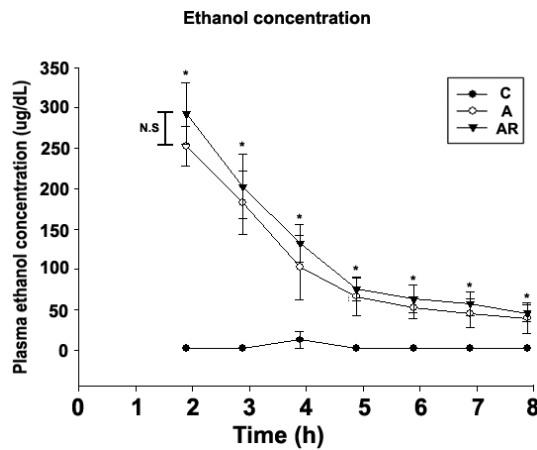
Ethanol concentration

Fig. 1. Plasma ethanol concentration in experimental rats. Data are means ± SD (n = 4). *P < 0.05, ethanol-treated and ethanol plus rosiglitazone-treated group compared with the control group. The difference between the ethanol-treated and ethanol plus rosiglitazone-treated groups was not significant (N.S.). C, control (closed circle); A, ethanol 4 g/kg body weight treatment (open circle); AR, ethanol plus rosiglitazone 5 mg/kg body weight/day treatment (closed triangle).

Determination of plasma ethanol concentration

The plasma ethanol concentration was determined using a rapid analyzer (GL5, Analox Instruments, Lunenburg, MA). The plasma ethanol concentrations in three groups for 8 h are shown in Fig. 1. All experiments using rats were performed in compliance with the European Community Guide for the care and use of Laboratory Animals.

A social interaction test

Overall social activity was scored as the sum of social investigation, contact and play behaviour frequencies. Social investigation was defined as sniffing of any part of the partner's body. Frequency of contact behaviour was scored as the sum of crawling over and under the partner and social grooming. Play fighting was scored by the frequencies of the following behavioural acts and postures demonstrated by test subjects: pouncing or playful nape attack (the experimental subject lunges at the partner with its forepaws extended outward), following and chasing (the experimental animal rapidly pursues the partner) (Meaney and Stewart, 1981; Thor and Holloway, 1984; Vandershuren *et al.*, 1997). The total number of crossovers (movements between compartments) demonstrated by each experimental subject was used as an index of general locomotor activity (Varlinskaya and Spear, 2002). The frequency of social investigation, contact behaviour and play fighting and the total number of crossovers were examined using separate between-group ANOVAs.

Immunoblot analysis

Immunoblot analysis was performed using crude extracts from liver tissue. Samples were resolved by 10% SDS–polyacrylamide gel electrophoresis, transferred to nitrocellulose membrane and blotted with appropriate primary antibodies. The membrane was incubated with peroxidase-conjugated secondary antibodies (1:5000; Santa Cruz Biotechnology, Santa Cruz, CA) and the bound antibody

was visualized using a chemiluminescence reader (Intron, Sungnam, Korea) and X-ray film (Fuji Film, Uetake, Japan). The primary antibody, mouse anti-ALD2, was purchased as synthesized peptides from Peptron (1:1000; Daejeon, Korea).

Isolation of mitochondrial fraction

Tissue lysates were washed in buffer A (100 mM sucrose, 1 mM EGTA and 20 mM MOPS, pH 7.4) and resuspended in buffer B [buffer A plus 5% Percoll, 0.01% digitonin and a cocktail of protease inhibitors: 10 mM aprotinin, 10 mM pepstatin A, 10 mM leupeptin, 25 mM calpain inhibitor I and 1 mM phenylmethylsulfonyl fluoride (PMSF)]. After 15 min of incubation on ice, unbroken cells and nuclei were pelleted by centrifugation at 2500 g for 10 min. The supernatant was centrifuged at 15 000 g for 15 min to pellet mitochondria, which were resuspended in buffer C [300 mM sucrose, 1 mM EGTA, 20 mM MOPS (pH 7.4) and the aforementioned cocktail of protease inhibitors]. The supernatant was centrifuged further at 100 000 g for 1 h. The resulting supernatant and the pellet were designated as the cytosolic and microsomal fractions, respectively. The nuclear pellet was resuspended in 10 mM Tris–HCl (pH 7.5), 2.5 mM KCl and 2.5 mM $MgCl_2$ and isolated after centrifugation at 90 000 g for 30 min through use of 2.1 M sucrose in 50 mM Tris–HCl (pH 7.5) and 5 mM $MgCl_2$.

ALD2 activity assay

The mitochondrial fraction was used to assay the catalytic activity of ALD2. ALD2 catalytic activity was assayed by measuring the increase of absorbance at 340 nm due to the reduction of NAD^+ to NADH following the oxidation of acetaldehyde to acetic acid. The assay method has been described previously (Bostian and Betts, 1978), but was adapted to a 96-well microplate reader using a final volume of 0.2 ml.

RESULTS

Ethanol-induced hangover causes suppressed social investigation in rats, as illustrated in Fig. 2A for our four groups of rats 18 h after ethanol challenge. In contrast to the hangover-associated suppression observed in the ethanol-treated group, rosiglitazone-treated rats showed recovery from hangover-associated social investigation suppression, but not completely. Rosiglitazone plus cyanamide-treated rats did not show a rosiglitazone effect. General locomotor activity (as indexed by the total number of crossovers between compartments) was significantly lower in ethanol-treated rats than in control and in ethanol plus rosiglitazone-treated rats. Decreased locomotor activity during acute ethanol withdrawal (hangover) has also been reported in adult animals tested on the elevated plus maze (Lal *et al.*, 1991; File *et al.*, 1993). Social investigation and contact behaviour tests showed the same patterns as the crossovers tests. But play fighting was not detected in any group (Fig. 2A). As expected, overall social activities were shown to vary among the groups (Fig. 2B).

Because we found behavioural changes in the social interaction test following rosiglitazone treatment, we decided to

Social interaction test

A

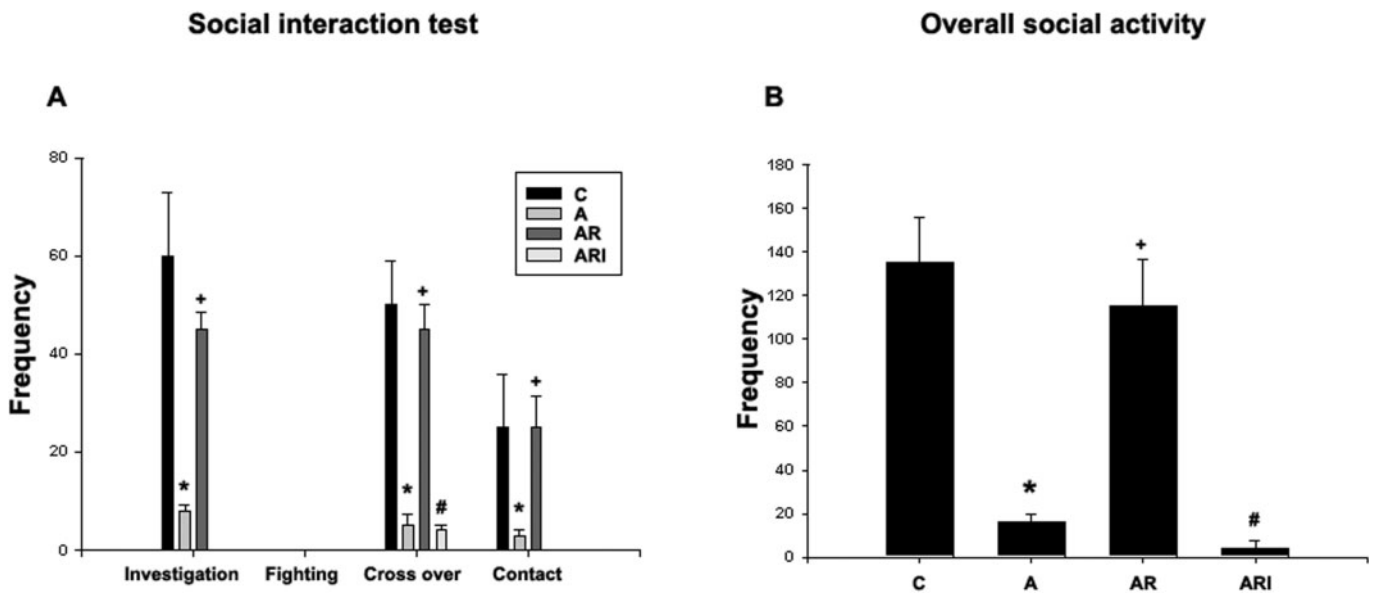## Overall social activity

B

Fig. 2. Social investigation, contact behaviour, play fighting and locomotor activity (overall number of crossovers) during the test session, 18 h after an acute ethanol challenge (**A**). Overall social activity was scored as the sum of social investigation, contact, play fighting and crossover frequencies (**B**). Data for familiar and unfamiliar situations were similar (data not shown). Cyanamide-only treatment was also tested, but that group was not significantly different from the control group. Data are means ± SD. *$P < 0.05$, compared with the control group; +$P < 0.05$, compared with the ethanol treatment group; #$P < 0.05$, compared with the ethanol plus rosiglitazone treatment group. C, control; A, ethanol 4 g/kg body weight treatment; AR, ethanol plus rosiglitazone 5 mg/kg body weight/day treatment; ARI, ethanol plus rosiglitazone plus cyanamide 10 mg/kg body weight treatment.

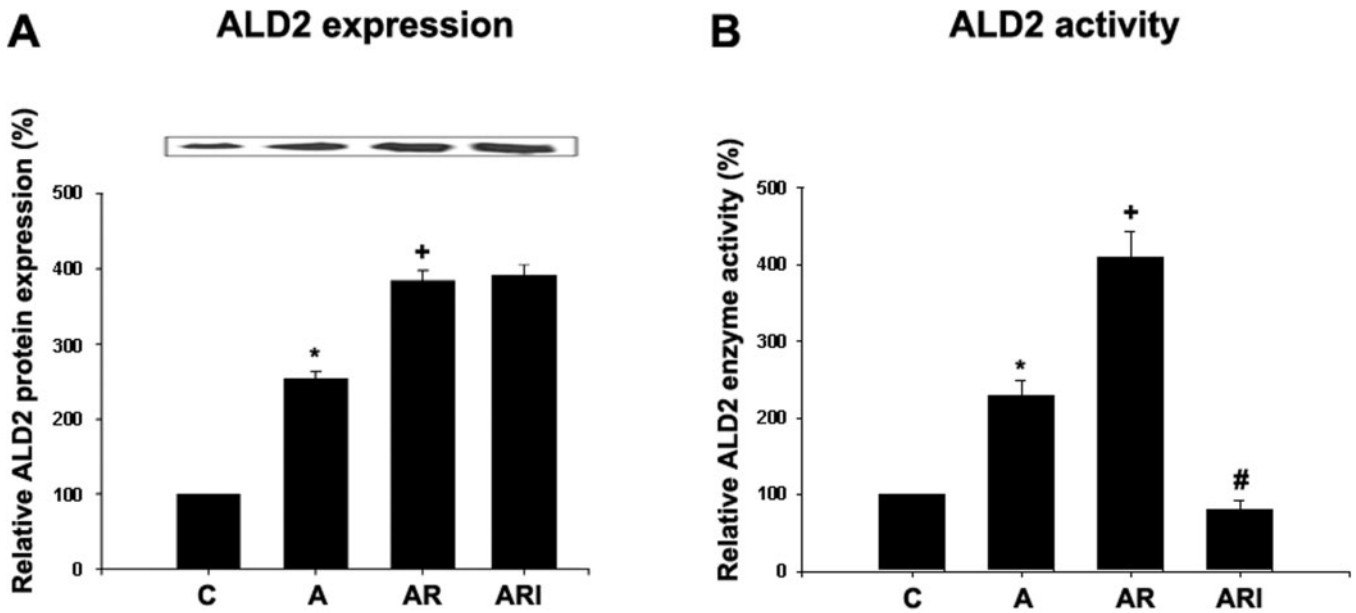**A** ## ALD2 expression

B ## ALD2 activity

Fig. 3. ALD2 expression and activity in experimental rats. ALD2 expression was determined by western blot analysis. The expression of ALD2 was estimated by densitometric analysis of each protein band (**A**). ALD2 activity was measured (**B**). Cyanamide-only treatment was also tested, but that group was not significantly different from the control group. Data are means ± SD ($n = 4$). *$P < 0.05$, compared with the control group; +$P < 0.05$, compared with the ethanol treatment group; #$P < 0.05$, compared with the ethanol plus rosiglitazone treatment group. C, control; A, ethanol 4 g/kg body weight treatment; AR, ethanol plus rosiglitazone 5 mg/kg body weight/day treatment; ARI, ethanol plus rosiglitazone plus cyanamide 10 mg/kg body weight treatment.

confirm the relationship of rosiglitazone treatment to ALD2 expression. To analyze aldehyde metabolism, expression of ALD2, which is known to catalyze aldehyde, was assessed by western blot analysis in the four groups. As shown in Fig. 3A, ALD2 expression was higher in rosiglitazone-treated rat livers than in those of control and ethanol-treated rats. But cyanamide influenced only ALD2 activity, not its expression (Fig. 3B).

DISCUSSION

Hangover-related anxiety can be indexed in laboratory animals by assessing the suppression of social interactions normally seen when two animals are placed together (social inhibition). Social interaction tests have been effectively applied to the investigation of anxiety-related behaviour in rats (File and Seth, 2003) including behaviours related to ethanol withdrawal (File *et al*., 1989, 1991, 1992). The social interaction test is a model of generalized anxiety (File, 1995) and has several advantages over other animal models of anxiety. It does not involve ecologically unusual conditions such as food and water deprivation or the use of electric shock. The social interaction test does not require extensive pre-training procedures and, hence, can be easily applied to the assessment of hangover-related anxiety in rats. In the conventional social interaction test, a pair of rats is placed in an experimental cage and the time that they spend in active social interaction is recorded. A summed measure of a variety of different elementary behavioural acts, including sniffing, grooming, chasing and wrestling, crawling under or over, nape attacks and biting, has traditionally been used for assessment of anxiogenic drug effects in this test situation. However, rats engage in distinct forms of interactive social behaviours that include social investigation, contact behaviour and play fighting. Each of these forms of social activity is influenced differently by drug treatment (Beatty *et al*., 1982; Soffie and Bronchart, 1988; Vandershuren *et al*., 1997), has a distinct ethological pattern (Meaney and Stewart, 1981; Panksepp *et al*., 1984; Thor and Holloway, 1984; Vandershuren *et al*., 1997) and hence may be mediated by different neural systems.

This study assessed the effects of acute ethanol hangover on different forms of social behaviour (social investigation, contact behaviour and play fighting) in control, ethanol-treated, ethanol plus rosiglitazone-treated and rosiglitazone-only-treated rats. The results of this study indicate that rosiglitazone reversed ethanol's depression of social activities and that it is able to eliminate acetaldehyde by regulating the expression of ALD2.

As expected, we confirmed more induced ALD2 expression in ethanol plus rosiglitazone-treated rats than in rats treated with ethanol only. Thus, we supposed that rosiglitazone enabled recovery from hangover in rats by effectively decreasing the plasma acetaldehyde concentration. The social activities of rosiglitazone-only-treated rats were similar to those of the controls (data not shown)

Unfortunately, we could not directly measure plasma acetaldehyde concentrations because of acetaldehyde's highly evaporative nature and the potential for measurement inaccuracy in our laboratory setting. But we used the second best option: we solved the problem by treating rats with cyanamide, an ALD2 inhibitor. Cyanamide eliminated the effects of rosiglitazone in the social interaction test (Fig. 2) as well as ALD2 activity (Fig. 3B) without changing the ALD2 expression level (Fig. 3).

Interestingly, the present study shows that rosiglitazone may be able to decrease plasma acetaldehyde concentration in a time-dependent manner without changing plasma ethanol concentrations (Fig. 1). This result shows that rosiglitazone can selectively eliminate the very toxic effects of acetaldehyde by inducing ALD2 expression.

In summary, our results show that rosiglitazone combats ethanol-induced hangover in rats. These results may open up a new clinical avenue for treating acute and severe ethanol-induced hangover. Further studies of the anti-hangover mechanism and ALD2 mechanism of rosiglitazone are necessary, and the cost of rosiglitazone needs to be reduced if it is to be used as a simple anti-hangover drug.

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