Biochemical Pharmacology, Vol. 52, pp. 1461–1468, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
PII S0006-2952(96)00514-X

Acceleration of the Alcohol Oxidation Rate in Rats with Aloin, a Quinone Derivative of *Aloe*

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ABSTRACT. *Aloe* contains abundant aloin, a C-glycoside derivative of anthraquinone. Based on recent reports indicating that the water extract of *Aloe* enhances the ethanol oxidation rate and also that quinones, in general, have a functional role in elevating the alcohol oxidation rate *in vivo*, we have attempted to identify the quinone derivative contained in *Aloe* that could increase the alcohol oxidation rate. Upon oral administration of aloin (300 mg/kg) given 12 hr prior to the administration of alcohol (3.0 g/kg), the blood alcohol area under the curve (AUC) was found to be decreased significantly (by 40%). This was supported by increases in the rates of blood alcohol elimination and the disappearance of alcohol from the body by 45 and 50%, respectively. Analysis of hepatic triglyceride (TG) levels revealed that both the ethanol and the aloin treatment alone significantly increased the TG levels in a comparable manner; however, the level obtained by the combined treatment of aloin and ethanol was not statistically different from that produced by either treatment alone. The levels of serum L-aspartate:2-oxoglutarate aminotransferase (AST) and L-alanine:2-oxoglutarate aminotransferase (ALT) activities were not increased by acute alcohol intoxication, aloin alone, or by the combined treatment of alcohol and aloin. Pretreatments with aloe-emodin, the anthraquinone aglycone of aloin, resulted in a significantly decreased blood alcohol AUC and an increase in the rate of ethanol disappearance. These results suggested that when the aloin localized primarily in the skin of *Aloe* is ingested, aloe-emodin (the quinone aglycone) may be released, and the released quinone may produce acceleration of the ethanol metabolism rate *in vivo*. BIOCHEM PHARMACOL 52;9:1461–1468, 1996. *Copyright © 1996 Elsevier Science Inc.*

KEY WORDS. *Aloe*; aloin; ethanol metabolism; aloe-emodin; quinone; quinone reductase

In a recent experimental animal study conducted by Sakai *et al.* [1], water extract of *Aloe* was reported to increase the rate of alcohol oxidation. The objective of our study was to determine if this observed increase in alcohol oxidation rate was due to aloin, a component of *Aloe*, which is known to be metabolized to its aglycone, aloe-emodin (a quinone substance) [2].

Aloe has been used as a folk remedy to strengthen the stomach and to relieve constipation for 3000 years [3]. Today, it is widely used as a general health food for some of its acclaimed effects. *Aloe* is known to suppress stomach acid secretion [4], to cure frostbite [5], to have an anti-inflammatory action [6, 7], to cure radiation burns [8–10], to improve blood glucose levels [11], to have an anti-viral action [12], and to modulate the immune response [13–16]. *Aloe* contains many active ingredients, but the best known is aloin (10-glucopyranosyl-1,8-dihydroxy-3-(hydroxymethyl)-9(10H)-anthracenone). Aloin is also called barbaloin, a bitter-tasting yellow crystal, and it s a C-glycoside derivative of an anthraquinone [17]. Although the C-glycoside side chain of aloin is not easily hydrolyzed *in vitro* [18], orally administered aloin is known to be hydrolyzed by esterases secreted by intestinal microflora [19, 20]. Once the C-glycoside is hydrolyzed, it forms aloe-emodin anthrone, which is further auto-oxidized to the quinone aloe-emodin (Fig. 1).

Ethanol is the most widely abused drug in the world; it is well absorbed and distributed, and it produces depression of the central nervous system, among many other toxicities. Ethanol is cleared from the body primarily by hepatic oxidation catalyzed initially by the NAD^+-dependent cytoplasmic ADH,§ (EC 1.1.1.1) to form acetaldehyde [21, 22]. Subsequently, the acetaldehyde is oxidized to acetate by mitochondrial aldehyde dehydrogenase. The resulting acetate is utilized in the mitochondrial Krebs cycle to provide energy. Continued oxidation of ethanol following acute alcohol intoxication results in shifting of the intracellular redox state to a reduced state, and this is illustrated by the decreased NAD^+ to NADH ratio. Under such a condition, due to lack of NAD^+ and the abundance of NADH, the

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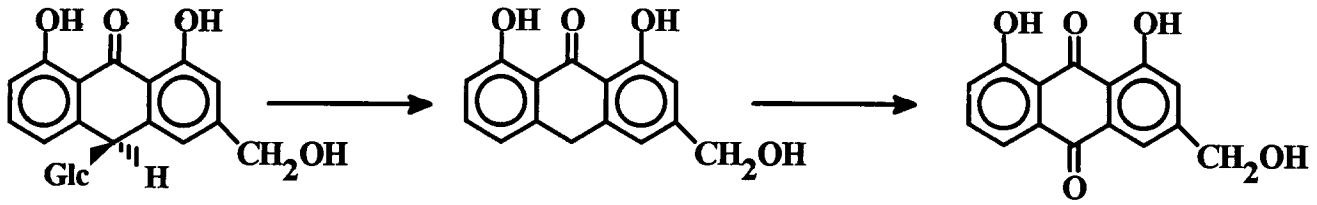
§ *Abbreviations:* ADH, alcohol dehydrogenase; QR, quinone reductase; AST, L-aspartate:2-oxoglutarate aminotransferase; ALT, L-alanine:2-oxoglutarate aminotransferase; AUC, area under the curve; and TG, triglyceride.

Received 19 May 1995; accepted 22 May 1996.



Aloin (barbaloin) **Aloe-emodin anthrone** **Aloe-emodin**

FIG. 1. Possible degradation pathway of aloin.

ethanol oxidation rate would be slowed and, also, as the consequence of such redox state changes, fatty acid oxidation would be hindered, thus causing fatty liver due to excess accumulation of TGs in liver cells [23].

Quinone compounds are reduced to hydroquinones, and this reaction is catalyzed primarily by QR (EC 1.6.99.2), which is abundant in the cytoplasm of liver cells [24, 25]. This QR reaction proceeds in an opposite direction from that of ADH and as the quinone is reduced to hydroquinone, NAD(P)H is oxidized to $NAD(P)^+$. Thus, when the ADH and QR are operating together in close proximity to each other (both enzymes are localized in the cytoplasm of liver cells in great abundance), quinones such as aloe-emodin may become reduced, thereby regenerating NAD^+ via the QR. This regenerated NAD^+ may then be utilized by the ADH for further oxidation of ethanol. Such a co-operative interaction between ADH and QR, leading to an increased rate of ethanol oxidation, has been demonstrated in a recent series of studies [26, 27], and a mechanistic scheme for the working hypothesis is presented in Fig. 2.

Sakai *et al.* [1] reported that a water extract of *Aloe* could increase significantly the rate of ethanol oxidation by lowering the serum lactate/pyruvate ratio to a non-ethanol (control) level in animal studies. Based on this report and knowing that *Aloe* contains a substantial quantity of aloin, a derivative of anthraquinone [28], the present study was designed to ascertain if aloin could be the substance contained in *Aloe* which has promoted the alcohol oxidation rate. This study was conducted also as a part of a long-term effort to develop a non-toxic amethystic agent from the widely used *Aloe*.

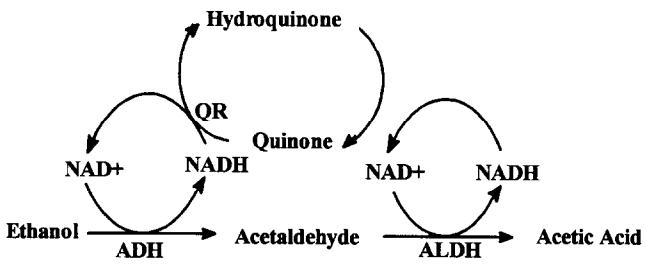


FIG. 2. Cooperative interaction of ethanol oxidation and quinone reduction for the effective shuttling of NAD^+ and NADH for the maintenance of a normal hepatocellular cytoplasmic redox state.

MATERIALS AND METHODS

Chemicals

Aloin and aloe-emodin were purchased from the Sigma Chemical Co. (U.S.A.) and were purified to 99% in our laboratory. Ethanol was purchased from Merck (Germany). The enzyme assay kits used to determine AST and ALT activities were purchased from the Youngdong Pharmaceutical Co. (Korea). Other chemicals used were of reagent grade and were purchased from the Sigma Chemical Co.

Experimental Animals

The three-week-old female Sprague–Dawley rats used in the present study were donated by the Animal Breeding Laboratory of the Yuhan Pharmaceutical Co. (Korea) and were kept at the animal holding facility at Seoul National University with free access to food and water in a 20 ± 1° room maintained under 55 ± 1% humidity and 12-hr light–dark cycles until they grew to over 180 g in weight.

Treatment and Sample Preparations

Aloin (100 and 300 mg/kg) was administered by oral intubation in a corn oil vehicle (100 and 300 mg/mL, respectively; 1 mL/kg) 12 hr prior to ethanol administration. Aloe-emodin (50 mg/kg) was given by i.p. injection in a corn oil vehicle (50 mg/mL; 1 mL/kg) 2 hr before the oral administration of ethanol. A 40% ethanol solution was given to the rats by oral intubation (gavage) at a dose of 3.0 g/kg. At 0.5, 1, 2, 4, 6, 8, and 10 hr after ethanol administration, blood samples (100 μL) were taken from the tail vein to determine the blood alcohol concentrations. Twenty-four hours after ethanol administration, rats were killed under ether anesthesia, blood was obtained by heart puncture, and the liver was removed. The blood was kept at room temperature for a minimum of 30 min to allow clotting. Serum was obtained from the clotted blood by centrifugation at 20,000 *g* for 2 min and then refrigerated. Serum AST and ALT activities were determined within 3

days of collection. The excised liver was washed with 67 mM phosphate buffer (pH 7.0) solution and weighed before being stored frozen at −20° for subsequent determinations of TG levels.

Determination of Blood Ethanol Concentrations

Blood alcohol concentrations were determined by the method of Bonnichsen and Theorell [29]. Briefly, to the 100-μL blood samples collected from the tail vein at various time points, 800 μL of perchloric acid (0.33 N) was added with vortexing and the mixture was centrifuged at 12,000 *g* for 45 sec. A 50-μL aliquot of the supernatant was mixed with 2.4 mL of ethanol assay buffer (pH 8.7 solution containing 75 mM $Na_2P_2O_7 \cdot 10H_2O$, 75 mM semicarbazide HCl, and 20 mM glycine). The blank sample consisted of 50 μL of 0.33 N perchloric acid. To this mixture, 50 μL of 25 mM NAD^+ and 10 μL of 15 kU/mL ADH were added with mixing and incubated for 70 min at 25°. Alcohol levels were determined by calculation from the absorbance of the generated NADH product at 340 nm by the oxidation of alcohol present in the samples.

Biochemical and Enzymatic Assays

The hepatic TG content was determined by the method of Van Handel and Zilversmit [30] as modified by Butler *et al.* [31]. Serum AST and ALT activities were determined by using commercial enzyme assay kits. Briefly, to each 0.5-mL aliquot of pre-warmed (37°) assay mixture kit solutions, 100 μL of serum was added and incubated at 37° for either 60 min (for AST) or 30 min (for ALT), and then 0.5 mL of chromogenic solution containing 2,4-dinitrophenylhydrazine was added. After allowing 20 min at room temperature for color development, 5 mL of 0.4 N NaOH solution was mixed in, and the absorbance at 505 nm was determined. For the blank, 100 μL of distilled water was used.

Calculation of Blood Ethanol Kinetic Parameters

To describe the time course of the blood ethanol curve in an individual animal following a given oral dose of ethanol, we used the Widmark equation [32], based upon the one-compartment open model with zero-order elimination. The slope (β) of the elimination phase was calculated by a linear least squares regression of the descending linear portion of the blood ethanol curve. The derived ethanol concentration at the start of ethanol administration (C_o) was obtained from the y-intercept of the regression line. The volume of distribution (γ) was calculated by dividing the total dose by C_o. The ethanol disappearance rate (β · γ) from the total body was obtained from the product of the slope and the volume of distribution. The AUC was calculated with the trapezoidal method from the beginning of drug administration to the time at which ethanol was not detected.

Statistics

The SPSS computer program was used for statistical analysis. The means and standard errors were calculated for all treatment groups. The data were subjected to ANOVA followed by the Duncan's Multiple Range test to determine which means were significantly different from each other or from controls. Student's *t*-test was used to analyze the results that contained only two groups of data. In all cases *P* values of ≤0.05 were considered to be significant.

RESULTS

Effect of Aloin on Alcohol Metabolism

To determine the effect of administering purified aloin on alcohol metabolism, the aloin was dissolved in corn oil and administered orally to rats at two doses (100 and 300 mg/kg) 12 hr prior to administering the ethanol. The concentrations of blood alcohol were determined at various times following ethanol administration (Fig. 3), and the kinetic parameters were calculated and presented in Table 1. Results (Fig. 3) showed that there were no differences in the blood alcohol level resulting from 100 mg/kg aloin pretreatment. However, there were significant decreases in the blood alcohol concentration in response to pretreatment with 300 mg/kg aloin. The AUC of blood alcohol was decreased markedly, and the rate of ethanol disappearance and the slope during the elimination process were increased markedly in response to the 300 mg/kg aloin pretreatment (Table 1). No differences in these kinetic parameters were observed with the 100 mg/kg aloin pretreatment.

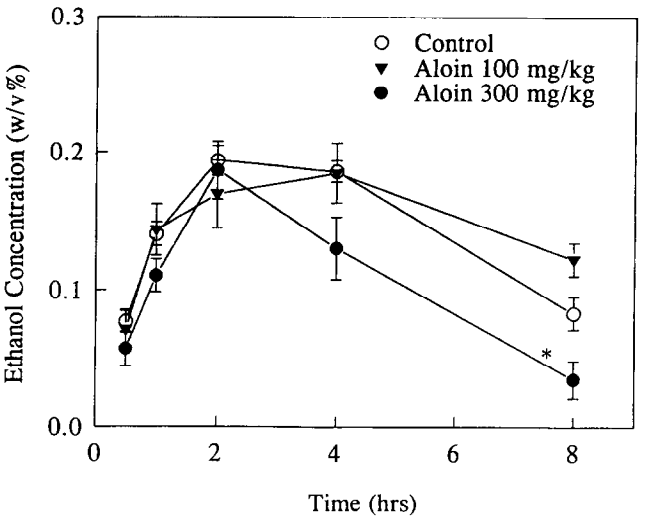


FIG. 3. Effects of aloin on blood alcohol concentrations following oral administration of ethanol to fasted rats. Female Sprague–Dawley rats were fasted overnight prior to oral intubation of ethanol (3.0 g/kg). Aloin dissolved in corn oil was given to rats at doses of 100 and 300 mg/kg by oral intubation at 12 hr prior to ethanol administration. Values are means ± SEM for 8–15 rats. Key: (*) significantly different from the control value (two-way ANOVA followed by Duncan's Multiple Range test, $P < 0.05$).

TABLE 1. Effects of aloin on kinetic parameters of blood ethanol in fasted rats

Group	N	AUC [(mg/mL) · hr]	Slope (β) [mg/(mL · hr)]	Volume of distribution (mL/kg body wt)	Rate of disappearance [mg/(hr · kg body wt)]
Control	15	14.2 ± 1.2	0.24 ± 0.02	1142 ± 48	255 ± 17
Aloin					
100 mg/kg	9	15.0 ± 1.3	0.19 ± 0.02	1303 ± 159	221 ± 21
300 mg/kg	8	8.8 ± 1.4*	0.35 ± 0.05*	1179 ± 156	381 ± 49*

The kinetic parameters were derived separately from each animal and are presented as means ± SEM. The ethanol disappearance rate from the total body was obtained from the product of the slope multiplied by the volume of distribution.

* Significantly different from control (one-way ANOVA followed by Duncan's Multiple Range test, $P < 0.05$).

Effect of Aloin on the Ethanol-Derived Acute Hepatic Toxicity

Accumulation of TGs in the liver is a sensitive indicator of hepatotoxicity caused by acute alcohol intoxication [33]. To determine if aloin is able to protect against alcohol-induced hepatic TG accumulation, hepatic TG levels were determined in rats treated with no ethanol (control), with ethanol alone (24 hr after 3 g ethanol/kg), with aloin alone (36 hr after 300 mg aloin/kg), and with ethanol and aloin together (24 hr after ethanol but 36 hr after aloin). Results shown in Fig. 4 indicate that hepatic TG contents were increased significantly by ethanol exposure alone, by aloin treatment alone, and by combined ethanol and aloin administration. Although the data suggest an almost additive increase in TG levels, statistical analysis by one-way ANOVA indicates that the increases seen with alcohol alone, aloin alone, and with combined treatment were not significantly different, thus suggesting an effect that is much less than additive.

Having observed that aloin alone could induce the elevation of hepatic TG levels, we sought to determine if additional manifestations of hepatotoxicity such as hepatocellular necrosis were induced by aloin pretreatment alone or by the combined pretreatment of aloin and ethanol. Hepatocellular necrosis caused by one-electron reduction metabolism of quinones can result in the release of hepatocellular cytoplasmic enzymes such as AST and ALT into the systemic circulation. Thus, we determined AST and ALT activities at 24 hr after ethanol or water (control) administration with or without aloin pretreatment. Results shown in Fig. 5 indicate that none of these pretreatments caused hepatocellular necrosis. These results are different from those observed for TG in that aloin did not affect this parameter of hepatotoxicity.

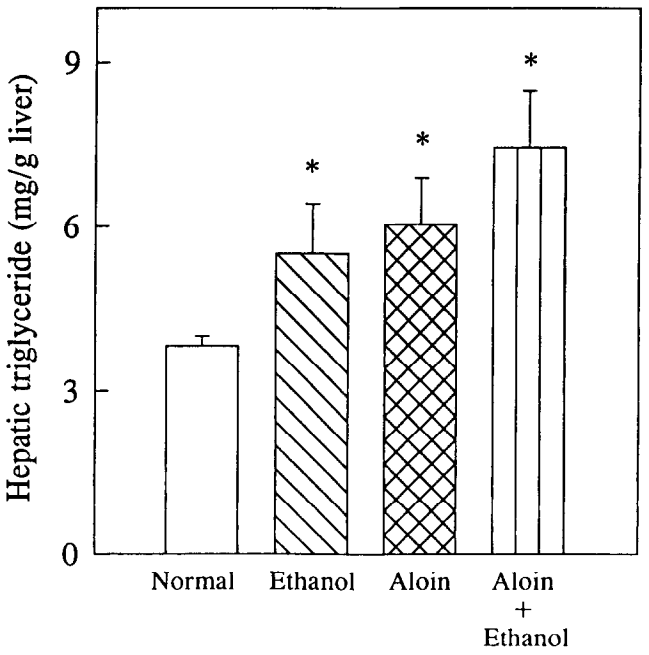


FIG. 4. Effect of aloin on hepatic TG accumulation induced by ethanol. All treatments were the same as described in Fig. 3. Aloin (300 mg/kg) was given to rats 12 hr prior to administering the ethanol; 24 hr after the ethanol administration, the rats were killed and their livers were excised for TG determinations. Values are means ± SEM for 4 rats. Key: (*) significantly different from the normal value (one-way ANOVA followed by Duncan's Multiple Range test, $P < 0.05$).

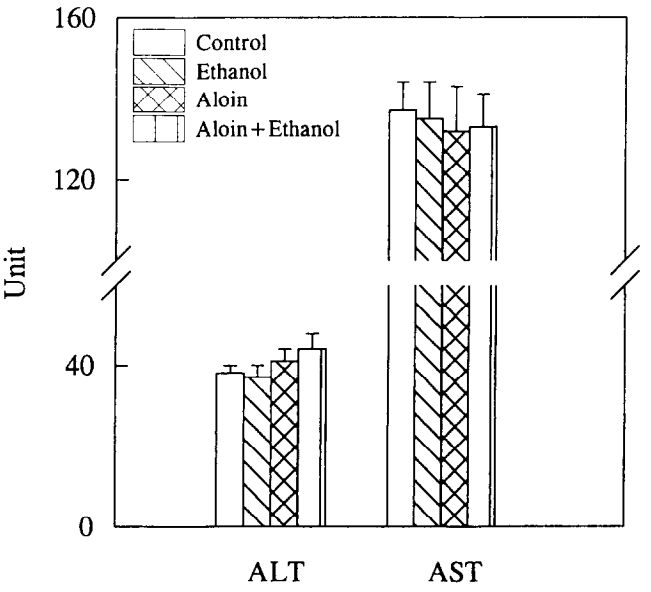


FIG. 5. Effect of aloin on serum AST and ALT levels in rats 24 hr after ethanol administration. All treatments were the same as described for Fig. 4. Values are means ± SEM for 4 rats.

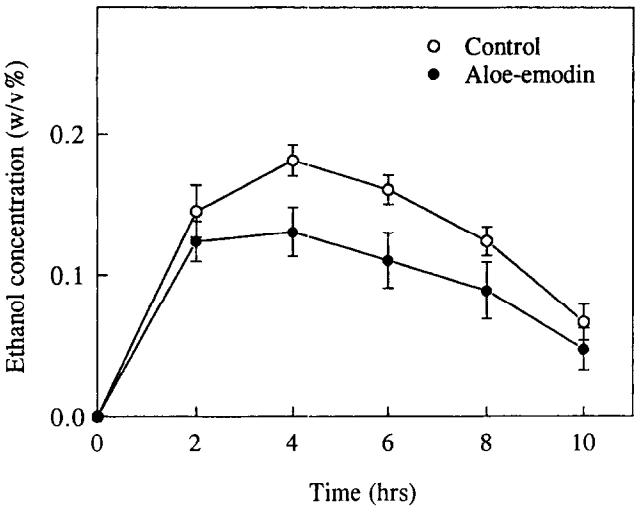


FIG. 6. Effects of aloe-emodin on blood alcohol concentrations following oral administration of ethanol to fasted rats. Female Sprague–Dawley rats were fasted overnight prior to oral intubation of ethanol (3.0 g/kg). Aloe-emodin dissolved in corn oil was given to rats at a dose of 50 mg/kg i.p. 12 hr prior to ethanol administration. Values are means ± SEM for 7 or 8 rats.

Effect of Aloe-Emodin Quinone, the Aglycone Derived from Aloin, on Ethanol Metabolism

We hypothesized that the aloin-induced increase of the ethanol oxidation rate was due to its quinone metabolite, aloe-emodin. To test this hypothesis, 2 hr before the oral administration of ethanol rats were given 50 mg/kg aloe-emodin in corn oil by the i.p. route to enhance delivery of the quinone substance to the liver. As shown in Fig. 6, aloe-emodin pretreatment produced marked decreases in blood alcohol levels. In Table 2, it is shown that aloe-emodin pretreatment significantly reduced the AUC and increased the rate of ethanol disappearance. Attempts were made to increase the ethanol metabolism rate by increasing the administered dose of aloe-emodin up to 300 mg/kg, but it proved lethal (data not shown).

DISCUSSION

In the overall pathway of ethanol oxidation, the first step, namely the oxidation of ethanol to acetaldehyde, is considered to be the rate-limiting step. The reaction occurs primarily in the liver and is catalyzed by ADH, an NAD^+-dependent enzyme localized principally in hepatocellular cytoplasm [34]. However, it is still a matter of debate as to whether this ADH reaction is rate-limited *in vivo* by the slow turnover rate of ADH or by the slow capacity of liver to regenerate cytosolic NAD^+ from NADH produced during alcohol oxidation [35]. In connection with this debate, several *in vivo* attempts have been made by Higgins [36] to demonstrate the importance of rapid NAD^+ availability in influencing the rate of ethanol oxidation. For example, when an excellent electron acceptor such as methylene blue was administered to experimental animals to enhance oxidation of NADH to NAD^+ non-enzymatically, the elevated level of NADH and the decreased level of NAD^+ (decreased hepatic NAD^+ to NADH ratio) induced by ethanol administration was reversed and the *in vivo* rate of ethanol oxidation was observed to be accelerated [37, 38]. Thus, it appeared that ready availability of NAD^+ increases the turnover rate of ADH and enhances the ethanol oxidation rate.

The ADH reaction catalyzing the oxidation of ethanol is known to proceed as follows: NAD^+ binds to ADH, first, by forming an ADH–NAD^+ complex; then, ethanol binds to this binary complex and forms a ternary complex. On the ternary complex, ethanol is oxidized to acetaldehyde and the NAD^+ is reduced to NADH. Acetaldehyde then leaves the ternary complex quickly but the NADH evacuates slowly, thus hindering the binding of NAD^+ to ADH for continued rapid oxidation of ethanol [22]. Hence, the slow speed of NADH departure limits the turnover rate of ADH, and this constitutes the rate-limiting step in the ethanol oxidation process. Based on this mechanism, it was suggested by Cha and Heine [39] that if there is an enzymatic reaction, such as lactate dehydrogenase converting pyruvate to lactate, which utilizes NADH and regenerates NAD^+ in the vicinity of ADH within the cytoplasm, it may strip the NADH off the residual ADH–NADH complex and accelerate the ADH turnover rate, thus, enhancing the overall rate of alcohol oxidation.

Indeed, in the hepatic cytosol, there is an enzyme system known as QR, formerly called DT-diaphorase, which catalyzes the two-electron reduction of quinones to hydroquinones. The enzyme is present at relatively high levels in the liver and is known to be induced by the administration of some food-additive hydroquinones such as butylated hydroxyanisole (BHA) and some xenobiotics [39–41]. The QR reaction proceeds in a directly opposite sequence from

TABLE 2. Effects of aloe-emodin on kinetic parameters of blood ethanol in fasted rats

Group	N	AUC [(mg/mL) · hr]	Slope (β) [mg/(mL · hr)]	Volume of distribution (mL/kg body wt)	Rate of disappearance [mg/(hr · kg body wt)]
Control	8	14.4 ± 1.4	0.23 ± 0.03	1044 ± 66	269 ± 13
Aloe-emodin	7	9.9 ± 1.6*	0.21 ± 0.02	1448 ± 276	323 ± 16*

The kinetic parameters were derived separately from each animal and are presented as means ± SEM. The ethanol disappearance rate from the total body was obtained from the product of the slope multiplied by the volume of distribution.

* Significantly different from control ($P < 0.05$, t-test).

that of ADH. Thus, QR binds NADH first and then the quinone, forming a ternary complex. On the ternary complex, quinone is reduced to hydroquinone and NADH is oxidized to NAD^+. While the hydroquinone is thought to leave QR quickly, the NAD^+ departs the QR slowly, and this slow departure step is known to constitute the rate-limiting step in the overall QR reaction. Thus, when the QR and ADH reactions are operating together in close proximity to each other (both enzymes are localized in the cytoplasm of the liver cell and are present in great abundance), quinones like menadione (vitamin K_3) or *tert*-butylquinone (produced from the administered BHA) are reduced to their respective hydroquinones more quickly in the presence of ethanol and, in turn, ethanol is oxidized more quickly in the presence of quinones. Such a cooperative interaction between QR and ADH *in vivo* enhancing the ethanol oxidation rate has been demonstrated in a recent series of studies reported by this laboratory [26, 27] and the working mechanistic hypothesis is presented as a scheme in Fig. 2.

As part of the continuing effort to develop non-toxic amethystic agents (i.e. substances that enhance the alcohol oxidation rate and that allow quick recovery from alcohol intoxication), we have unsuccessfully tested the amethystic effects of some polyphenols, known to be present in tea, that could be oxidized easily to quinones (unpublished results). Thus, we have turned our attention to the report of Sakai *et al.* [1] which indicated that water extracts of a widely used health food, *Aloe*, could enhance the oxidation rate of alcohol. The skin of *Aloe* plants contains a substantial quantity of aloin, a C-glycoside of anthraquinone [18, 28], which when ingested, is readily hydrolyzed to aloe-emodin anthrone by the intestinal bacterial esterases [19, 20]. The released aloe-emodin anthrone can be readily auto-oxidized to aloe-emodin quinone within the intestine [19]. We therefore postulated that the orally administered *Aloe* extracts could release aloe-emodin quinone which, when absorbed in the liver, could bring about an enhanced rate of alcohol oxidation via cooperative interaction between QR and ADH (Fig. 6 and Table 2).

The present results appear to support this hypothesis: aloin and aloe-emodin pretreatments were capable of reducing the blood ethanol AUC and increasing the ethanol disappearance rate from the body. According to a report published by Ishii *et al.* [42], at least 6 hr was required to release sufficient aloe-emodin from orally administered aloin. However, Sakai *et al.* [1] observed an enhanced alcohol oxidation rate with a 30-min pretreatment of *Aloe* extracts, suggesting that sufficient aloe-emodin had been released by this early time point. This could be explained by the fact that, while the yellow-colored extract of *Aloe* skin is known to contain the quinone derivatives, it may also contain the already hydrolyzed aloe-emodin anthrone or other hydrolyzed quinones. Our experiments with purified aloin showed that at least 6 hr of pretreatment was required to produce a significant reduction in the AUC of blood alcohol and that maximal effects were observed by a 12-hr pretreatment (data not shown).

Although the calculated slope and volume of distribution produced by the two drug treatments (Tables 1 and 2) appear to be different or even opposite (in the case of slope), apparent contradictions can be basically eliminated by a statistical review. In comparing the blood ethanol concentration results shown in Fig. 3 (aloin) and Fig. 6 (aloe-emodin), it can be seen that while aloe-emodin altered the absorption kinetics (0–4 hr), aloin did not. Furthermore, while aloe-emodin did not appear to affect the elimination slope markedly (4–10 hr), aloin treatment had increased the elimination slope markedly. Thus, when these differences are entered into the calculation of apparent volume of distribution, the aloe-emodin appeared to increase the volume of distribution. This would explain the apparent discrepancy in the results shown in Tables 1 and 2. The mechanisms involved in this discrepancy may have been due to the fact that aloin is a glycoside and aloe-emodin is an aglycon. Aloin is a glycoside of the aloe-emodin quinone and must be hydrolyzed by the intestinal microflora before affecting ethanol metabolism. Thus, aloin was given by the oral route and aloe-emodin was given the i.p. route. Aloe-emodin was administered i.p. because we wished a rapid or efficient absorption of the quinone substance directly into the liver. Because the two routes of administration were different, it was not surprising to see that the treatments became effective at different phases.

Many previous attempts have been made to increase the rate of ethanol metabolism, but as of yet none has changed the rate by more than 50% [36]. The failure of any single agent to produce a more extensive increase in the metabolism rate of ethanol appears to have possible explanations; when one process is accelerated, another becomes rate limiting. Applying this concept to our model, even though the reoxidation of NADH was stimulated by treatments of aloin or aloe-emodin so that the cytosolic redox state would remain at the non-ethanol-treated level, the level of ADH (V_{max}) may have assumed the role of primary control for the ethanol metabolism rate. Therefore, combinations of agents affecting several processes concomitantly may be required for further acceleration of the ethanol metabolism rate. In our experiments, treatment with aloin resulted in about a 50% increase in the ethanol disappearance rate (Table 1). This indicates that the ethanol metabolism rate may not be further enhanced unless total ADH activity is also increased, which has yet to be demonstrated successfully.

The primary cause of alcohol-induced fatty infiltration of liver is thought to be due to an increased synthesis of lipids within the liver and also to an increased mobilization from adipose tissue into the liver in the face of the blocked free fatty acid oxidation caused by excess NADH [43]. Thus, upon improved oxidation of NADH and regeneration of NAD^+ by utilizing the QR with provision of quinone substrates (aloe-emodin quinone), the rate of alcohol metabo-

lism was demonstrated to increase (or the blood alcohol AUC was shown to decrease). This situation would also allow the fatty acid oxidation to proceed at a faster rate and perhaps push towards lowering the TG levels in the liver. Thus, based on the results presented in Table 1 that show reductions of blood alcohol AUC with prior intubation of aloin, it was expected that the increased hepatic TG level caused by alcohol intoxication would be decreased by aloin pretreatment. However, for unknown reasons, the aloin treatment alone elevated the hepatic TG content (Fig. 4). Since the elevation in TGs from the combined treatment of aloin and ethanol was not significantly different from that produced by either treatment alone, it is possible that the aloin pretreatment may have mediated some of the alcohol-dependent accumulation of TGs in the liver. It was interesting to note that, while the single treatment of aloin alone or the combined treatment of alcohol and aloin had caused accumulation of hepatic TGs, there were no increases in serum AST or ALT activities. This result indicated that the accumulation of hepatic TG itself does not lead to hepatocellular necrosis.

In conclusion, aloin, a C-glycoside derivative of anthraquinone localized in the skin of *Aloe*, may release the quinone when ingested, and the released quinone (aloe-emodin), in turn, may accelerate the rate of ethanol metabolism *in vivo*. Further research is required to ascertain whether aloin-emodin or other aloin derivatives can effectively increase the ethanol oxidation rate without inducing any hepatotoxicity, such as hepatic TG accumulation.

This work was supported by a grant from the Ministry of Health and Social Affairs of Korea.

References

1. Sakai K, Sattoh Y, Ikawa C and Nishhata T, Effect of water extracts of Aloe and some herbs in decreasing blood ethanol concentration in rats. *Chem Pharm Bull (Tokyo)* **37:** 155–159, 1989.
2. Grindlay D and Reynolds T, The *Aloe vera* phenomenon: A review of the properties and modern uses of the leaf parenchyma gel. *J Ethnopharmacol* **16:** 117–151, 1986.
3. John SH, A drug for all seasons: Medical and pharmacological history of Aloe. *Bull NY Acad Med* **66:** 647–659, 1990.
4. Hirata T and Suga T, Biologically active constituents of leaves and roots of *Aloe arborescens var. natalensis*. *Z Naturforch* **32c:** 731–734, 1977.
5. McCauley RL, Heggers JP and Robson MC, Frostbite. Methods to minimize tissue loss. *Postgrad Med* **88:** 67–77, 1990.
6. Davis RH, Rosenthan KY, Cesario LR and Rauw GA, Processed *Aloe vera* administered topically inhibits inflammation. *J Am Podiatr Med Assoc* **79:** 395–397, 1989.
7. Saito H, Ishiguro T, Imanishi K and Suzuki I, Pharmacological studies on a plant lectin aloctin A. II. Inhibitory effect of aloctin A on experimental models of inflammation in rats. *Jpn J Pharmacol* **32:** 139–142, 1982.
8. Lushbaugh CC and Hale DB, Experimental acute radiodermatitis following beta irradiation. V. Histopathological study of the mode of action of therapy with *Aloe vera*. *Cancer* **6:** 690–698, 1953.
9. Davis RH, Parker WL, Samson RT and Murdoch DP, Isolation of stimulatory system in Aloe extract. *J Am Podiatr Med Assoc* **81:** 473–478, 1991.
10. Rodriguez-Bigas M, Cruz NI and Suarez A, Comparative evaluation of *Aloe vera* in the management of burn wounds in guinea pigs. *Plast Reconstr Surg* **81:** 386–389, 1988.
11. Ajabnoor MA, Effects of aloes on blood glucose levels in normal and alloxan diabetic mice. *J Ethnopharmacol* **28:** 215–220, 1990.
12. Sydiskis RJ, Owen DG, Lohr JL, Rosler KA and Blomster RN, Inactivation of enveloped viruses by anthraquinones extracted from plants. *Antimicrob Agents Chemother* **35:** 2463–2466, 1991.
13. Hart LA, van den Berg AJ, Kuis L, van Dijk H and Labadie RP, An anti-complementary polysaccharide with immunological adjuvant activity from the leaf of parenchyma gel of *Aloe vera*. *Planta Med* **55:** 509–512, 1989.
14. Hart LA, Nibbering PH, van den Barselaar MT, van Dijk H, van den Berg AJJ and Labadie RP, Effects of low molecular constituents from *Aloe vera* gel on oxidative metabolism and cytotoxic and bactericidal activities of human neutrophils. *Int J Immunopharmacol* **12:** 427–434, 1990.
15. Womble D and Helderman JH, Enhancement of alloresponsiveness of human lymphocytes by acemannan (Carrisyn™). *Int J Immunopharmacol* **10:** 967–974, 1988.
16. Shida T, Yagi A, Nishimura H and Nishioka I, Effect of Aloe extract on peripheral phagocytosis in adult bronchial asthma. *Planta Med* **51:** 273–275, 1985.
17. Wyk BE, Oudtshoorn MCB and Smith GF, Geographical variation in the major compounds of *Aloe ferox* leaf exudate. *Planta Med* **61:** 250–253, 1995.
18. Franz G and Grun M, Chemistry, occurrence and biosynthesis of C-glycosyl compounds in plants. *Planta Med* **47:** 131–140, 1983.
19. Hattori M, Kanda T, Shu YZ, Akao T, Kobashi K and Namba T, Metabolism of barbaloin by intestinal bacteria. *Chem Pharm Bull (Tokyo)* **36:** 4462–4466, 1988.
20. Che QM, Akao T, Hattori M, Kobashi K and Namba T, Isolation of human intestinal bacterium capable of transforming barbaloin to aloe-emodin anthrone. *Planta Med* **57:** 15–19, 1991.
21. Theorell HS and Bonnichsen R, Studies on liver alcohol dehydrogenase. I. Equilibria and initial reaction velocities. *Acta Chem Scand* **5:** 1105–1126, 1951.
22. Theorell HS and Chance B, Studies on liver alcohol dehydrogenase. II. The kinetics of the compound of horse liver alcohol dehydrogenase and reduced diphosphopyridine nucleotide. *Acta Chem Scand* **5:** 1127–1144, 1951.
23. Lieber CS and Savolainen M, Ethanol and lipids. *Alcohol Clin Exp Res* **8:** 409–423, 1984.
24. Lind C, Hochstein P and Ernster L, DT-Diaphorase as a quinone reductase: A cellular device against semiquinone and superoxide radical formation. *Arch Biochem Biophys* **216:** 178–185, 1982.
25. Prochaska JJ and Talalay P, The role of NAD(P)H:quinone reductase in protection against the toxicity of quinones and related agents. In: *Oxidative Stress: Oxidants and Antioxidants* (Ed. Sies H), pp. 195–211. Academic Press, London, 1991.
26. Chung JH, Rubin RJ and Cha YN, Effects of vitamin K_1 and menadione on ethanol metabolism and toxicity. *Drug Chem Toxicol* **164:** 383–394, 1993.
27. Chung JH, Cha YN and Rubin RJ, Role of quinone reductase in *in vivo* ethanol metabolism and toxicity. *Toxicol Appl Pharmacol* **124:** 123–130, 1994.
28. Groom QJ and Reynolds T, Barbaloin in Aloe species. *Planta Med* **53:** 345–348, 1987.
29. Bonnichsen RK and Theorell H, An enzymatic method for the micro-determination of ethanol. *Scand J Clin Lab Invest* **3:** 58–62, 1951.

30. Van Handel E and Zilversmit DB, Micromethod for direct determination of serum triglycerides. *J Lab Clin Med* **50:** 152–157, 1957.
31. Butler WM, Maling HM, Horning MG and Brodie BB, The direct determination of liver triglycerides. *J Lipid Res* **2:** 95–96, 1961.
32. Widmark EMP, Verteilung und unwandltung des athyl alkohols in organismus des hundes. *Biochem Z* **267:** 128–134, 1933.
33. Ryle PR, Chakraborty J and Thomson AD, The role of the hepatocellular redox state in the hepatic triglyceride accumulation following acute ethanol administration. *Biochem Pharmacol* **35:** 3159–3164, 1986.
34. Lieber CS, Alcohol and the liver: 1984 update. *Hepatology* **4:** 1243–1260, 1984.
35. Dawson AG, What governs ethanol metabolism? In: *Metabolic Regulation* (Ed. Ochs ES), pp. 87–91. Elsevier, New York, 1985.
36. Higgins JJ, Control of ethanol oxidation and its interaction with other metabolic systems. In: *Biochemistry and Pharmacology of Ethanol* (Eds. Majchrowicz E and Noble EP), pp. 249–351. Plenum Press, New York, 1979.
37. Berry MN, Fanning DC and Wallace PG, Ethanol oxidation in isolated hepatocytes. *Adv Exp Med Biol* **132:** 403–411, 1979.
38. Ryle PR, Chakraborty J and Thomson AD, The effect of methylene blue on the hepatocellular redox state and liver lipid content during chronic ethanol feeding in the rat. *Biochem J* **232:** 877–882, 1985.
39. Cha Y-N and Heine HS, Comparative effects of dietary administration of 2(3)-*tert*-butyl-4-hydroxyanisole and 3,5-di-*tert*-butyl-4-hydroxytoluene on several hepatic enzyme activities in mice and rats. *Cancer Res* **42:** 2609–2615, 1982.
40. De Long MJ, Prochaska HJ and Talalay P, Induction of NAD(P)H:quinone reductase in murine hepatoma cells by phenolic antioxidants, azo dyes, and other chemoprotectors: A model system for the study of anticarcinogens. *Proc Natl Acad Sci USA* **83:** 787–791, 1986.
41. Riley RJ and Workman P, DT-Diaphorase and cancer chemotherapy. *Biochem Pharmacol* **43:** 1657–1669, 1992.
42. Ishii Y, Tanizawa H, Ikemoto C and Takino Y, Studies of Aloe. I. Cathartic effects. *Yakugaku Zasshi* **101:** 254–258, 1981.
43. Day CP and Yeaman SJ, The biochemistry of alcohol-induced fatty liver. *Biochim Biophys Acta* **1215:** 33–48, 1994.