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Ethanol-induced suppression of interleukin 1-like activity: reversal by a quinone derivative

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Abstract—Chronic ethanol intake impairs several parameters of immune function. Since there is evidence that cytokine production by immune cells may contribute to the immunosuppressive effect of ethanol, we examined interleukin 1 (IL1) production by liver non-parenchymal cells (NPC) in ethanol-fed rats. Male Wistar rats (225–250 g) were fed by continuous intragastric infusion. The source of fat was either saturated fat or polyunsaturated fat. In addition, the effect of a quinone compound on IL1 production was assessed. Animals were fed for various periods: 1 week, 2 weeks, 1 month and 2 months. NPC were isolated and stimulated by lipopolysaccharide. IL1 production by NPC and the ratio of stimulated to unstimulated (S:U) IL1 production were evaluated in the different groups and related to the presence of liver injury. As expected, animals fed corn oil and ethanol (CO + E) developed pathologic liver injury, whereas animals fed saturated fat and ethanol (SF + E) had no liver injury. A progressive decrease in the S:U IL1 ratio was seen in the CO + E group over the 8-week period. The ratio in the SF + E group was higher. The quinone compound reversed the suppressive effect of ethanol on IL1 production. In summary, ethanol-induced suppression of IL1 production was modulated by diet and the presence of liver injury. This suppression of IL1 production was reversed by a quinone compound; the exact mechanism for the reversal of this inhibition is unknown.

Key words: interleukin 1; quinone; liver; Kupffer cells; alcohol

A number of investigators have shown that excessive consumption of ethanol leads to alterations in host defense systems that ultimately result in enhanced susceptibility to infections [1–3]. Chronic ethanol ingestion impairs several parameters of immune function, which include loss of lymphoid cells from the thymus, spleen and peripheral blood [4–6], functional changes in lymphocytes [7], and finally alterations in non-specific immune mechanisms such as natural killer cell activity [8] and granulocyte mobility [9].

The mechanisms underlying the effects described above have not been fully elucidated. Examination of the ethanol-induced immunosuppressive effects suggests that many of the alterations described could be secondary to alterations in cytokine production. There is evidence that ethanol alters cytokine production by various cells, which may contribute to its immunosuppressive effect [10, 11]. Of the many cytokines that control host defense systems, we chose to examine IL1* production by both non-stimulated and lipopolysaccharide-stimulated liver NPC. IL1 participates in the host response to a number of infectious agents to which alcoholics are prone [12]. In addition, IL1 pretreatment also diminishes the lethality of infections in neutropenic animals [13].

To study this issue further, we examined whether the production of IL1-like activity by hepatic NPC was dependent on the presence of pathologic changes characteristic of alcoholic liver injury. This is easily studied in the intragastric feeding model [14] where feeding corn oil and ethanol leads to severe liver injury, whereas animals fed saturated fat and ethanol show no injury [15, 16]. In addition, we also examined the effect of a novel quinone compound, (2E)-3-[5-(2,3-dimethoxy-6-methyl-1,4-benzoquinoyl)]-2-nonyl-2-propenoic acid (E3330) which has been shown to affect cytokine and eicosanoid production by hepatic NPC [17–19].

* Abbreviations: IL1, interleukin 1; NPC, non-parenchymal cells; CO + E, corn oil plus ethanol; SF + E, saturated fat plus ethanol; and CO + E + D, corn oil plus ethanol plus drug.

Materials and Methods

Male Wistar rats, weighing between 225 and 250 g, were fed by continuous infusion of a liquid diet through a permanently implanted gastric tube, as described previously [14–16]. The diet contained corn oil or medium chain triglycerides as the source of fat (25% of calories). The fatty acid composition of the diet is shown in Table 1. Protein, carbohydrate, minerals, and vitamins plus ethanol were administered as previously described [15]. The average caloric distribution for each nutrient was: 25% of total calories as fat, 21% as protein, 12% as carbohydrate, and 42% as ethanol. The amount of ethanol given was modified to maintain high blood alcohol levels for individual rats. For animals receiving E3330, the drug was given at a daily dose of 100 mg/kg body weight. The drug was given via

Table 1. Fatty acid (FA) composition (percentage by weight) of corn oil plus ethanol (CO + E) and saturated fat plus ethanol (SF + E) diets

	% of Total fatty acids	
FA*	CO + E	SF + E
8:0	ND†	52.5
10:0	ND	33.2
12:0	ND	0.52
16:0	11.3	1.4
18:0	1.9	1.0
18:1	25.5	1.0
18:2	59	1.5
Others	2.2	8.1

* The first number signifies the number of carbon atoms in the chain; the second number indicates the number of double bonds in the FA.

† ND = not detectable.

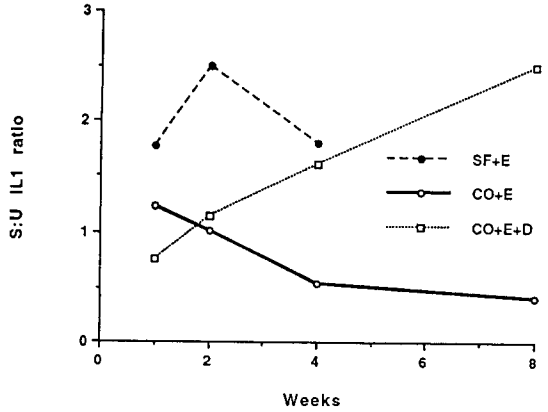

Fig. 1. Ratio of lipopolysaccharide-stimulated (S) to spontaneous (unstimulated, U) levels of IL1-like activity in non-NPC supernatants from various experimental groups. The supernatants were collected after pooling of NPC from 4–5 rats/group. This was done in order to collect an adequate amount of NPC. The IL1-like activity was assayed using the conventional thymocyte proliferation assay referred to in Materials and Methods.

the intragastric cannula as a bolus dose at the same time every day. Some of the animals in the present study have been included previously in a separate study comparing the effect of E3330 on alcoholic liver injury. Eleven sets of experiments (4–5 rats/group) were performed in which three groups of animals were fed the corn oil and ethanol (CO + E) diet, three groups of animals were fed saturated fat and ethanol (SF + E), and the other three groups were fed the CO + E diet plus E3330 (CO + E + D). The different groups of animals were killed at intervals of 1 week, 2 weeks and 1 month after the start of feeding. In addition, two groups (CO + E and CO + E + D) (5 rats/group) were killed at 2 months.

NPC were harvested from the livers of anesthesthetized rats, using isolation procedures and buffers described previously [20]. In all experimental groups, NPC from 4 to 5 rats were combined. This allowed us to collect an adequate number of NPC for stimulation by lipopolysaccharide. Two suspensions of NPC were prepared and left overnight; to one suspension 200 ng/mL of lipopolysaccharide was added. IL1-like activity was measured in both unstimulated and stimulated NPC supernatants using a modification [21] of an assay of thymocyte comitogenic response [22]. This was done in order to determine spontaneous IL1 production by NPC as well as the maximal response of these cells to lipopolysaccharide. For each group of animals, the ratio of lipopolysaccharide stimulated to non-stimulated IL1 was calculated. In addition, the stimulated IL1 levels were also compared in the different groups. All experiments using NPC supernatant were performed in duplicate, and the mean level for IL1-like activity was used.

Results and Discussion

The weight gain and blood alcohol levels (range 150–300 mg/dL) were not significantly different between the groups. As expected, the animals fed corn oil and ethanol developed pathologic liver injury, whereas the animals fed saturated fat and ethanol did not.

Figure 1 shows sequential changes in the ratio of stimulated to unstimulated (S:U) IL1 levels of the NPC supernatant in the various groups. A progressive decrease in this IL1 ratio was seen in the CO + E group. In contrast, in the animals fed SF + E, the ratio of stimulated to unstimulated IL1 levels remained higher than unstimulated levels throughout the test period. Animals given E3330 had a progressive increase in the ratio of S:U IL1 levels.

Figure 2 shows that stimulated IL1 levels declined between weeks 2 and 4 in both CO + E and SF + E groups. However, the levels in the SF + E group at 2 weeks were much higher. The decline in IL1 levels occurred when alcohol consumption was close to maximal levels (up to 17 g/kg/day), which was approximately twice the intake at 1 week (8 g/kg/day). Furthermore, between 2 and 4 weeks, maximal blood alcohol levels were also achieved. In the animals given E3330, the stimulated IL1 levels increased from 1 week onwards. Thus, it would appear that ethanol at high concentrations, regardless of the source of the dietary fat, suppresses stimulated IL1 production by liver NPC. This suppressive effect was reversed by E3330. The reversal by E3330 of this suppressive effect of ethanol on stimulated IL1 production is shown clearly in Fig. 2C, which demonstrates that the ratio of stimulated IL1 levels in the E3330-treated group compared with non-drug-treated animals remained high at both 4 and 8 weeks.

It is generally believed that IL1 is not constitutively produced but that its synthesis requires appropriate activation followed by synthesis of new mRNA and protein [23]. Our studies show that "unstimulated" NPC secrete detectable levels of IL1 which probably is in response to low levels of endotoxin present in the blood of alcohol-fed animals [24]. In contrast, Martinez *et al.* [25], using a liquid diet model (Lieber–DeCarli), showed an increase in IL1 secretion by Kupffer cells in the absence of detectable endotoxin. One possible interpretation of this difference is the blood alcohol levels seen in the different models. With lower levels of blood alcohol as seen with the Lieber–DeCarli diet, IL1 production by liver NPC is probably stimulated, whereas at higher blood alcohol levels, seen in the intragastric feeding model, IL1 production is probably suppressed.

The effect of the quinone compound in reversing the suppressive effect of ethanol on IL1 is a novel finding. E3330 has a number of diverse actions that may be relevant to this observation. E3330 has been shown to inhibit tumor necrosis factor production by several types of macrophages [17]. In addition, E3330 inhibits the generation of leukotriene B_4 and thromboxane B_2 [18, 19]. Previous studies evaluating eicosanoid production by rat Kupffer cells in the presence of lipopolysaccharide show that indomethacin (a cyclooxygenase inhibitor) up-regulates IL1 production by Kupffer cells [26]. Since one of the actions of E3330 is inhibition of thromboxane generation, which is also seen with cyclooxygenase inhibition, this eicosanoid–cytokine interaction may be one mechanism which explains the stimulation of IL1 by E3330. Prostaglandin E_2 (PGE_2) has been shown to down-regulate IL1 production by Kupffer cells in partially hepatectomized animals [23]. Whether the decline of PGE_2 production seen in ethanol-fed rats given E3330 [19] leads to enhanced IL1 production is unknown but is suggested by the present study.

The other additional possibility that should be considered in the interpretation of decreased IL1 production by ethanol is that the presence of liver injury at 1 and 2 months could decrease the yield of NPC. We consider this an unlikely possibility in view of the fact that no significant differences in pathology were seen between the drug and the non-drug treated group at 1 month [19], and the differences in IL1 production are already obvious. It should be recognized that the data from the IL1 assay must be interpreted with the understanding that the NPC supernatants may contain additional growth-stimulating or growth-inhibiting activity that could lead to potential false-positive or false-negative

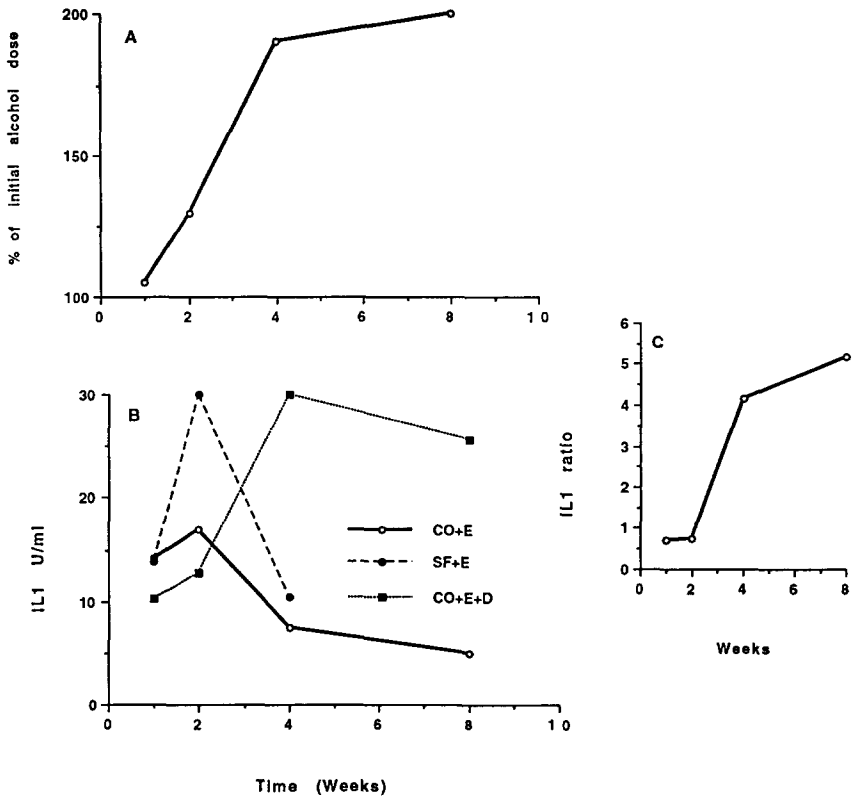

Fig. 2. IL1-like activity in lipopolysaccharide-stimulated NPC supernatant in the various experimental groups. (A) Ethanol intake over time. (B) Effect of diet on inhibition of IL1 production. (C) Reversal of IL-1 inhibition by E3330.

results [27]. For this reason, IL1 activity in our experiments is referred to as IL1-like activity.

In summary, we have shown that the ethanol-induced suppression of IL1 production was modulated by diet and that a dietary regimen that eventually causes liver injury led to a marked suppression of lipopolysaccharide-stimulated IL1 production. This ethanol-induced suppression of IL1 production was reversed by a quinone compound. The exact mechanism for the reversal of this inhibition is unknown.

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