EFFECTS OF ETHANOL ON MOUSE LIVER POLYSOMAL DISAGGREGATION BY DIMETHYLNITROSAMINE AND LASIOCARPINE

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1. Introduction

While studying the acute effects of various hepatotoxic agents, an impression was gained that ethanol used as a vehicle may be inhibitory to the action of certain of these agents. One of the situations in which ethanol is protective against the acute effects of hepatotoxic agents may occur when the subject agents require activation by the microsomal mixed function oxidase system. Recent reports [1, 2] by several investigators indicate that ethanol is inhibitory to the microsomal oxidase system *in vivo* as well as *in vitro*. The possible occurrence of such a situation has to be considered in the study of hepatotoxic agents when ethanol is used as the solvent vehicle. Therefore, we investigated a condition in which ethanol inhibits an acute effect, liver polysomal disaggregation due to dimethylnitrosamine, which is activated by the microsomal oxidase system. The simultaneous administration of ethanol prevented the polysomal disaggregation by dimethylnitrosamine. However, ethanol had no effect on polysomal disaggregation by lasiocarpine.

2. Materials and methods

Male Swiss Webster albino mice weighing 14 to 23 g were used. The mice were given Purina laboratory chow and water ad libitum.

The mice were administered a single intraperitoneal injection of 50 mg per kg body weight of the chemical dissolved in either absolute ethanol or in 0.15 M NaCl. The volume of ethanol injected was limited to 0.1 ml per 20 g body weight. This amount of ethanol by itself did not cause polysomal disaggregation. One hour after the test agents were injected the animals were sacrificed. Livers were perfused with ice-cold physiologic saline until they became pale, and were then removed and weighed. Livers were homogenized with 4 volumes of solution containing 10 mM Tris-HCl, (pH 7.6), 5 mM $MgCl_2$, 25 mM KCl, and 0.25 M sucrose in a Potter-Elvehjem type homogenizer with Teflon pestle. The homogenate was then centrifuged at 10,000 *g* for 10 min at 5°. A 10% aqueous solution of sodium deoxycholate was then added to the postmitochondrial fraction to a final concentration of 1%, and 0.2 ml of the fraction was layered onto 4.6 ml of 0.5 M to 1.2 M linear sucrose gradient containing 10 mM Tris-HCl (pH 7.6), 5 mM $MgCl_2$, and 10 mM KCl. The samples were centrifuged at 35,000 rpm in a Spinco Model L centrifuge equipped with a SW 50.1 rotor for 60 min. The gradient was displaced upwards by injecting 1.5 M sucrose to the bottom of the tube through a 20 gauge needle at a flow rate of 0.30 ml per min. The gradient was then analyzed automatically at 260 nm by a Gilford spectrophotometer equipped with flow cell and strip chart recorder.

3. Results

For each treatment 4 to 6 mice were used. The polysomal profiles of animals receiving the same treatment were nearly identical. Fig. 1 illustrates

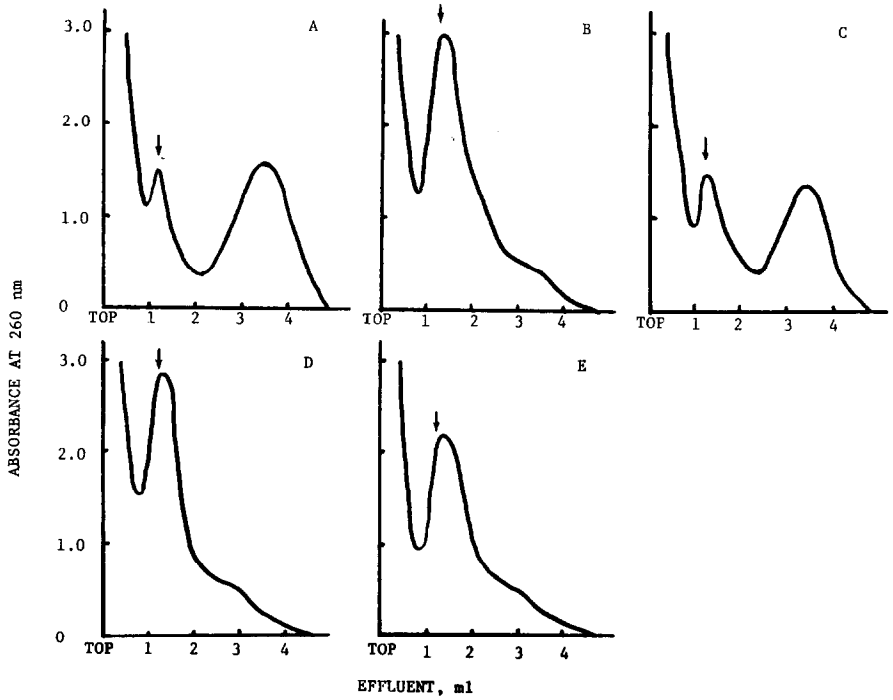

Fig. 1. Polysomal profiles of mouse livers. In each profile the arrow indicates the position of the monosome peak. (A) Normal; (B) 1 hr after injection of dimethylnitrosamine dissolved in saline; (C) 1 hr after injection of dimethylnitrosamine dissolved in ethanol; (D) 1 hr after injection of lasiocarpine dissolved in saline; (E) 1 hr after injection of lasiocarpine dissolved in ethanol.

polysomal profiles of normal and treated livers. Fig. 1A exemplifies a normal mouse liver profile. Fig. 1B indicates complete polysomal disaggregation with only one large monosome peak present 1 hr after administration of 50 mg per kg body weight dimethylnitrosamine in 0.15 M NaCl. Fifty mg per kg body weight dimethylnitrosamine in absolute ethanol, however, did not cause polysomal disaggregation, as evidenced by the normal profile 1 hr after administration (fig. 1C). When ethanol was given as a pretreatment and followed by an aqueous solution of dimethylnitrosamine 45 min later a similar inhibitory effect was obtained. When we extended the study to another hepatotoxic agent, lasiocarpine, a different result was obtained. In a preliminary study a 50 mg per kg body weight injection of lasiocarpine in 0.15 M NaCl was estimated to be an approximate minimal dose to cause almost complete polysomal disaggregation 1 hr after administration (fig. 1D). As can be seen in fig. 1E, ethanol did not prevent polysomal disaggregation due to lasiocarpine when used as a solvent.

4. Discussion

These results indicate that administration of ethanol can prevent an acute effect of dimethylnitrosamine on liver polysomes. Recent investigations suggest that the microsomal-ethanol oxidizing system closely resembles other drug metabolizing systems, and that simultaneous addition of certain drugs and ethanol results in mutual inhibition of microsomal binding [1]. The present results are, therefore, in agreement with the postulated mechanism of activation of dimethylnitrosamine by hepatic microsomal mixed function oxidase system [3] and with the interaction of ethanol with the microsomal oxidase system [1].

The protective effect of ethanol against hepatotoxic agents which require the activation by the microsomal oxidase system is an interesting phenomenon. However, this effect may not occur in chronic ethanol intake which causes the induction of the microsomal oxidase system [1]. This may

result in enhanced microsomal activation and consequent toxicity of certain hepatotoxic agents, including dimethylnitrosamine. This question may merit further investigation.

In view of the postulated activation of pyrrolizidine alkaloids, such as lasiocarpine, by the microsomal system [4, 5] we expected ethanol to inhibit the polysomal disaggregation due to lasiocarpine. However, this expectation did not materialize, even though an approximate minimal dose of lasiocarpine for polysomal disaggregation was used to avoid the possible masking of the ethanol effect by an excess amount of lasiocarpine. The activation of lasiocarpine by the microsomal oxidase system must be very resistant to ethanol.

In any event, care must be taken in the use of ethanol as a solvent in the study of acute drug effects.

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