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Effect of Polyethylene Glycol 400 on Adriamycin Toxicity in Mice

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Abstract—*The effect of a widely used organic solvent, polyethylene glycol 400 (PEG 400), on the toxic action of an acute or chronic treatment with adriamycin (ADR) was evaluated in mice. PEG 400 impressively decreased both acute high-dose and chronic low-dose-ADR-associated lethality. Light microscopic analysis showed a significant protection against ADR-induced cardiac morphological alterations. Such treatment did not diminish the ADR antitumor activity in L1210 leukemia and in Ehrlich ascites tumor.*

INTRODUCTION

ADR-INDUCED cardiomyopathy (CMP) is a severe total dose-limiting side-effect in patients treated with this powerful antineoplastic agent [1]. Several studies on animals and man have therefore been performed to clarify the mechanism of the toxic activity and also to find possible protective substances for this untoward cardiac effect [1].

In recent investigations conducted in our laboratory into the possible usefulness of various chemicals in preventing ADR CMP, we obtained promising results with a very common pharmacological vehicle, PEG 400.

PEG 400 is a condensation polymer of ethylene oxide and water, represented by the formula: HO $(C_2H_4O)_nH$, in which n varies from 8 to 10. This substance has been assumed to be a relatively inert and safe solvent for pharmaceuticals, although in recent years some limits to its administration have been advised by some authors [2–8].

This report presents the effects of PEG 400 on the toxicity induced by a chronic or acute treatment of ADR in mice. The possible interference between ADR and PEG 400 on the antineoplastic activity of the antibiotic has also been examined.

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MATERIALS AND METHODS

One hundred and fifty CD1 mice (25–30 g), divided in 30 per group, were used. ADR was administered i.p. in 0.9% NaCl solution at a dose of 15 mg/kg on day 1 (groups 1 and 2) or 5 mg/kg on days 1, 8, 15 and 22 (groups 3 and 4). PEG 400 for gas chromatography (MERCK) (15% v/v in distilled water) was administered i.p. 3 hr prior to ADR in a dose corresponding to a volume of 20 ml/kg (3.45 g/kg) (groups 2 and 4). In group 5 PEG 400 was injected i.p. on days 1, 8, 15 and 22 without following ADR treatment. Animals were weighed weekly and inspected daily for survival and general toxicity. An autopsy was performed in all animals shortly after death, or at the end of the experiment (10 weeks).

The heart, liver and small bowel were removed and fixed in 3% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4 and embedded in Epon 812. Sections were cut at 1 μm, stained with 1% toluidine blue and observed by light microscopy. Five sections per heart were examined by a double-blind method.

The effect of PEG 400 on the antitumor activity of ADR was studied in BDF1 mice implanted i.p. with 10^5 L1210 leukemia cells. ADR was administered i.p. on day 1 at dosages of 2.9 and 6.6 mg/kg. In addition, the effect of the solvent was also studied in CD1 mice implanted i.p. with 10^6 Ehrlich ascites tumor cells. ADR was administered i.p. on day 1 at dosages of 7.5, 3.75 and 1.87 mg/kg. Also, in antitumor studies PEG 400 (15% in distilled water) was administered i.p. 3 hr before ADR at the dosage of 20 ml/kg.

RESULTS

Figure 1 depicts the changes in mean body weight of animals treated acutely or chronically with ADR or ADR + PEG 400. It can be noted that the severe drop in body weight is greatly limited by the pretreatment.

The cumulative mortality data for all groups of animals are presented in Fig. 2.

The cardiac lesions observed in this study were similar to those described previously in other studies in animals [9–12] and man [13]. They consisted in cytoplasmic vacuolization, cellular atrophy, nuclear pyknosis, myofibrillar loss and lymphocitic infiltration (Fig. 3). These lesions were virtually absent in PEG-400-pretreated animals (Fig. 4).

The light microscopic examination of liver tissue showed a slight vacuolization of the hepatocytes, with centrolobular congestion and necrosis. Centrolobular congestion and necrosis were almost completely absent in PEG-400-pretreated animals.

Table 1 shows the influence of PEG 400 on the antitumor activity of ADR in L1210 leukemia and in Ehrlich ascites carcinoma. The administration of PEG 400 3 hr before ADR had no influence on the antitumor acitivity of the antibiotic.

DISCUSSION

PEGs are water soluble, moderately viscous, colorless liquids with efficacious solvent properties which, for their low toxicity and wide range of

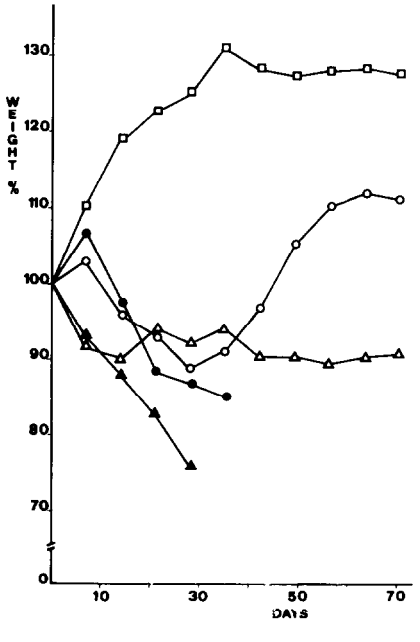

Fig. 1. Mean body weight changes expressed as percentages of initial weight of mice receiving (△) ADR 15 mg/kg, (△) ADR 15 mg/kg + PEG 400, (●) ADR 5 mg/kg/week for 4 weeks, (○) ADR 5 mg/kg/week for 4 weeks + PEG 400 and (□) PEG 400 alone.

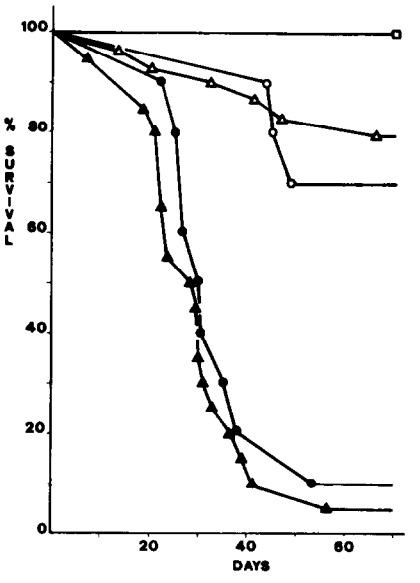

Fig. 2. Cumulative mortality data for mice receiving (▲) ADR 15 mg/kg, (△) ADR 15 mg/kg + PEG 400, (●) ADR 5 mg/kg/week for 4 weeks, (○) ADR 5 mg/kg/week for 4 weeks + PEG 400 and (□) PEG alone.

compatibilities, have found considerable uses as pharmacological vehicles for water-insoluble drugs. However, during recent years many investigators have observed that PEGs and various other vehicles possess pharmacological and toxicological properties of their own [2–8]. In particular, PEG 400 exerts an anticonvulsant activity in monkeys [4] and sedative and muscle relaxant properties in mice [5]. Heilman *et al.* observed that PEGs 200, 300, 400 and 600 appeared to have no effect on the blood pressure of the anesthethized dog, but they enhanced the blood pressure responses to epinephrine, norepinephrine and acetylcholine. Although the mechanisms for the enhancing effect were not definitely established, the authors suggested that an effect both on the peripheral vasculature and on the heart were present [7]; these substances also have immunostimulant properties [14, 15] and exert some important actions on cell membranes [16, 17]. In addition, the diglycolic and triglycolic acids, possible metabolites of PEGs [3], are reported to be excellent chelators of calcium [18], forming a stable but soluble complex.

The molecular mechanisms involved in these biological actions have been neither considered nor investigated. It is unclear which biological action of PEG 400 is involved in the significant reduction of ADR toxicity observed in our study. On the other hand, this antineoplastic drug possesses a wide spectrum of biochemical actions, such as intercalation with DNA [19], generation of free radical compounds [20], interference with oxidative phosphorylation [21] and modification of electrolyte composition and movements in the

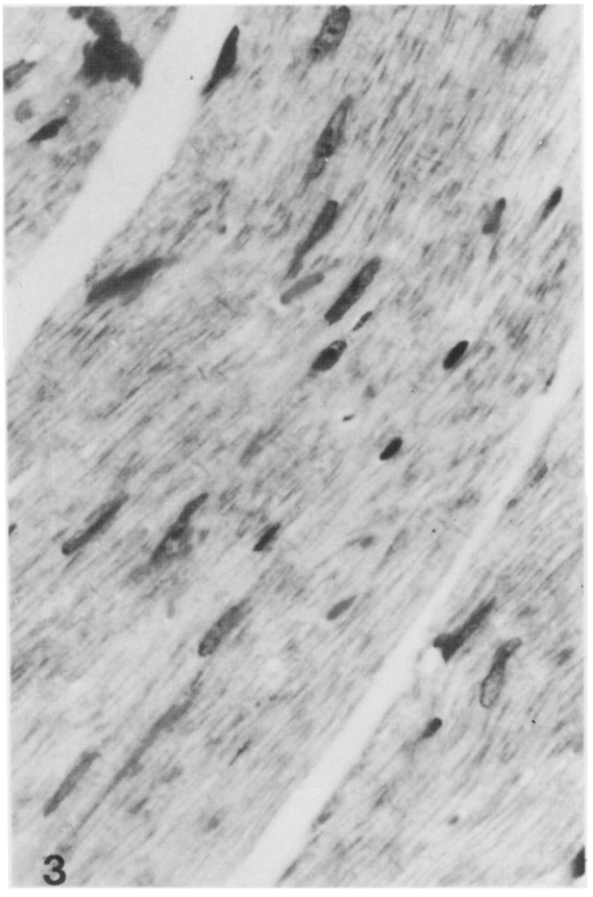

Fig. 3. Severe damage of ventricular tissue from a mouse given ADR 15 mg/kg (×28.8).

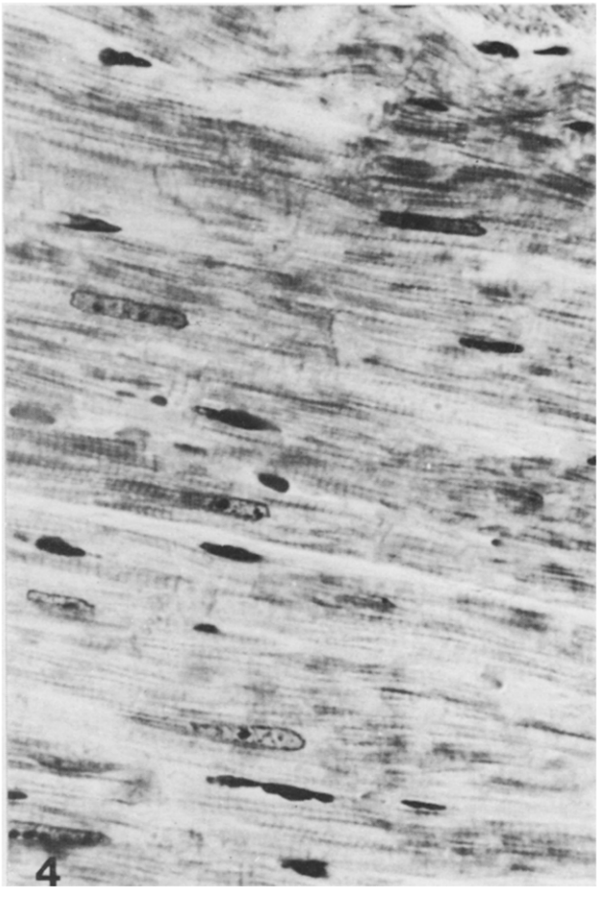

Fig. 4. Light microscopic appearance of ventricular tissue from a mouse receiving ADR 15 mg/kg + *PEG* 400 (×28.8).

Table 1. Effect of ADR and ADR + PEG 400 against L1210 leukemia and Ehrlich ascites carcinoma

Coumpound	ILS*	LTS†
L1210 leukemia		
ADR 2.9 mg/kg	117	—
ADR 2.9 mg/kg + PEG 400 3.45 g/kg	113	—
ADR 6.6 mg/kg	136	—
ADR 6.6 mg/kg + PEG 400 3.45 g/kg	136	—
Ehrlich ascites carcinoma		
ADR 7.5 mg/kg	—	8/10
ADR 7.5 mg/kg + PEG 400 3.45 g/kg	—	9/10
ADR 3.75 mg/kg	—	8/10
ADR 3.75 mg/kg + PEG 400 3.45 g/kg	—	9/10
ADR 1.87 mg/kg	—	9/10
ADR 1.87 mg/kg + PEG 400 3.45 g/kg	—	10/10

*Percentage increase in median survival time.
†Long-term survivors (>90 days)/No. of treated mice.

cell [22]; whether just one or many of these factors are relevant to the antineoplastic activity or to the toxicity of the drug is still unclear.

It is noteworthy that both the antitumor activity and the acute toxicity of ADR were not counteracted by PEG; the early weight loss is in fact similar in all groups of treated animals. In addition, we found in previous experiments (data not shown) that the acute mortality rate caused by ADR was significantly more severe when young mice (18–20 g) were used; this systemic acute effect was not prevented by PEG.

The protection against ADR cardiotoxicity exerted by PEG and the lack of reduction of the antitumor effect and of the systemic acute toxicity suggests that ADR may have at least two mechanisms of tissue damage, one resulting in cardiac toxicity, which is counteracted by PEG, and the other responsible for antitumor activity, which is not antagonized by this solvent.

Additional work is necessary to further clarify the pharmacological properties of PEG and its interaction with ADR and to determine whether this combination would be useful in cancer chemotherapy.

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