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Tween 80 Increases Plasma Adriamycin Concentrations in Mice by an Apparent Reduction of Plasma Volume*

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Abstract—*Coadministration of Tween 80 enhances the activity of adriamycin against selected experimental tumors in mice. Although some investigators have suggested a direct effect of Tween 80 on tumor cells, we wished to determine whether altered plasma concentrations of adriamycin occurred which might account for the apparent therapeutic synergism. Male* CDF_1 *mice were treated i.p. with* 6.7 mg/kg *of adriamycin alone or combined with* 5000 mg/kg *of Tween 80 in physiologic saline. Fluorometric determination of adriamycin equivalents in plasma revealed significantly higher adriamycin concentrations* 1 *and* 2 hr *post treatment in mice that had received Tween 80. In three separate experiments, a significant, reversible increase in packed cell volume occurred in the peripheral blood of mice treated i.p. with Tween 80. This increase was maximal at* 1–2 hr *post treatment. The magnitude of the apparent plasma volume reduction accounted quantitatively for the increase in drug concentration.*

INTRODUCTION

As REPORTED by others [1, 2] and confirmed at our institution, Tween 80 enhances the chemotherapeutic activity of adriamycin against selected experimental tumors in mice. Because this might be a pharmacokinetic phenomenon rather than a direct effect of Tween 80 on tumor cells, we compared the plasma concentrations of adriamycin equivalents in mice treated with adriamycin alone or with adriamycin plus Tween 80. Preliminary experiments revealed that i.p. injection of Tween 80 resulted in an unusual accumulation of fluid in the peritoneal cavities of treated mice. Our investigation of this phenomenon suggests that Tween 80 produces a transient reduction of plasma volume in these mice.

MATERIALS AND METHODS

Adriamycin was supplied by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Tween 80 (J. T. Baker Chemical Co.) was obtained commercially. Solutions were prepared in aqueous NaCl (0.9 g/100 ml) so that injection of 0.2 ml/10 g of body weight provided the dosages desired for mice.

Male CDF_1 mice (20–22 g) were obtained from Southern Animal Farms, Prattville, AL. They were housed in individual stainless steel cages with hardwood bedding (Betta-Chip, Northeastern Products Corp., Warrensburg, NY) and received Wayne Lab-Blox F6 (Allied Mills, Chicago, IL) and tap water *ad libitum*. Experimental chemotherapy studies with murine leukemia P388 were carried out as described by Schabel *et al.* [3].

To study plasma concentrations of adriamycin, groups of 10 mice were treated with single doses of adriamycin (6.7 mg/kg) or adriamycin (6.7 mg/kg) plus Tween 80 (5000 mg/kg). Mice used as a source of plasma for controls in the adriamycin measurement received no treatment. Food and water were withheld during the observation period in this and subsequent experiments. At 1 and 2 hr post-treatment, 5 mice from each group were killed by cardiac puncture and exsanguination under chloroform anesthesia. Plasma samples from individual mice were analyzed spectrophotofluorometrically for to-

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tal adriamycin equivalents by an adaptation of the method of Finkel *et al.* [4]. The applicability of this method for measurement of adriamycin has been discussed [5]. We used an Aminco-Bowman spectrophotofluorometer (American Instrument Co., Silver Spring, MD). Fluorescence emission was measured at 550 nm when the sample was excited at 475 nm.

In each of 3 subsequent experiments, mice were treated i.p. with single doses of Tween 80 (5000 mg/kg). At 0.25, 0.5, 1, 2 and 4 hr post treatment, 5 mice were killed, and blood samples were collected for determination of packed cell volume by microcapillary centrifugation [6]. Total protein concentrations in plasma were measured colorimetrically by the Biuret method (No. 540, Sigma Chemical Co., St. Louis, MO).

Statistical analyses using two-way analysis of variance were carried out as described by Steel and Torrie [7].

RESULTS AND DISCUSSION

The dosages of adriamycin and Tween 80 used in these studies were chosen because their therapeutic activity against murine leukemia P388 in CDF_1 mice was greater when the two were combined than when adriamycin was administered alone (Table 1). We looked for evidence of a pharmacokinetic effect of Tween 80. Table 2 presents plasma concentrations of adriamycin equivalents. The fluorometric assay used did not distinguish between adriamycin and its fluorescent metabolites. At 1 and 2 hr post treatment, mice that received Tween 80 with adriamycin exhibited significantly higher ($P<0.01$) plasma concentrations of adriamycin equivalents. Routine necropsy revealed residual fluid in the peritoneal cavities of mice that received Tween 80. Semiquantitative aspiration yielded 1.2–1.7 times more fluid than was injected. This fluid may reflect osmotic dilution of the injected Tween 80. If that occurred, the plasma volume would be reduced accordingly.

Table 2. Concentration of adriamycin equivalents (μg/ml) in plasma of mice treated i.p. with adriamycin alone or adriamycin plus Tween 80

Adriamycin diluent*	Time post treatment (hr) 1	2
Saline	0.35 ± 0.02†	0.33 ± 0.04
Saline + Tween 80	0.57 ± 0.04	0.42 ± 0.05

*Adriamycin (6.7 mg/kg) was prepared in aqueous NaCl (0.9 g/100 ml) alone or containing 250 mg of Tween 80/ml (Tween 80 dosage = 5000 mg/kg) as indicated.
†Adriamycin equivalents are presented as the calculated mean ± S.D. of values from 5 individual mice.

In three identical experiments, we measured packed cell volume as an indicator of plasma volume in mice treated i.p. with Tween 80. Our results are presented in Table 3. At 1 and 2 hr post treatment when plasma concentrations of adriamycin equivalents had been elevated by coadministration of Tween 80, the packed cell volumes of mice treated with Tween 80 were significantly elevated. Total plasma protein concentrations at 1 and 2 hr post treatment were 5.00 ± 0.35 and 5.32 ± 0.30 g/dl compared to untreated controls of 4.78 ± 0.25 g/dl (mean ± S.D., $N=5$). Assuming a blood volume of 1.32 ml for a 20 g mouse [8], the reduction of plasma volume

Table 1. Adriamycin plus Tween 80 treatment of advanced P388 leukemia in CDF_1 mice

Treatment schedule	Dosage (mg/kg, i.p.) Adriamycin	Tween 80	No. of tumor cells at time of treatment*	No. of cells surviving treatment	Approximate $\log_{10}$ reduction
Day 5 only	4.4	—	1.8×10^8	2.1×10^7	1
	4.4	5000		2.2×10^5	3
	6.7	—		1.0×10^6	2
	6.7	5000		2.2×10^5	3
	10	—		2.2×10^5	3
	10	5000		4.9×10^3	5
Day 7 only	4.4	—	3.2×10^8	9×10^8	0
	4.4	5000		9×10^8	0
	6.7	—		9×10^8	0
	6.7	5000		4.3×10^8	0
	10	—		9×10^8	0
	10	5000		1.1×10^4	4

*Mice received 10^6 viable cells i.p. on Day 0 in this single, internally controlled experiment.

Table 3. *Packed cell volumes of mice after i.p. treatment with* 5000 mg/kg *of Tween* 80 *in physiologic saline*

Experiment number	Time posttreatment (hr)				
	0.25	0.5	1	2	4
1	44.6±1.7*	43.8±4.6	49.0±1.9	47.8±2.7	46.2±1.1
2	45.6±2.7	49.6±1.9	49.8±2.2	52.2±3.7	46.8±2.6
3	42.8±3.3	42.6±5.0	45.9±1.3	46.8±2.5	43.2±2.6

*Packed cell volumes are presented as the calculated mean±S.D. of values from 5 individual mice.

reflected in the increased proportion of packed cells accounted quantitatively for the increased drug and total protein concentrations 1 and 2 hr post treatment.

Several possible explanations of the chemotherapeutic effect of Tween 80 have been suggested by others [1, 9]. That i.p. administration of Tween 80 to mice results in a transient accumulation of peritoneal fluid and evidence of a concomitant reduction of plasma volume has not been reported previously. Whether this effect may have accounted for the therapeutic synergism of Tween 80 and adriamycin reported by others is open to question. Pretreatment i.p. with olive oil also increases adriamycin concentrations in the blood of mice [10]. Tween and mineral oil concentrations have an important influence on the antitumor activity of various mycobacteria preparations [11]. How common these effects may be, whether they are similar mechanistically, and what implications they have for experimental therapeutics remain to be determined.

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