Prevention of the Cardiotoxic Effects of Adriamycin and Daunomycin in the Isolated Dog Heart[1] (36432)

EUGENE H. HERMAN, RAMAKANT M. MHATRE, INSU P. LEE, AND VAMAN S. WARAVDEKAR

Microbiological Associates, Inc. and National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

In many instances, serious cardiopulmonary toxicity has been encountered during clinical use of the antineoplastic anthracycline compounds, daunomycin (NSC-82,151) and adriamycin (NSC-123,127) (1–3). The structures of these compounds are shown in Fig. 1. The mechanism by which these compounds induce cardiac toxicity is not completely understood. Experimentally, the injection of daunomycin or adriamycin has produced acute ventricular arrhythmias in Syrian golden hamsters and rhesus monkeys (4). In other studies both compounds have been observed to increase coronary perfusion pressure in the isolated dog heart (5). Conceivably, similar alterations in coronary blood flow might be responsible for the experimental and clinical cardiac toxicity induced by daunomycin and adriamycin. The present studies were initiated to determine whether pharmacological pretreatment of the isolated heart with a variety of agents could alter the coronary vascular actions of daunomycin and adriamycin.

Methods. Isolated heart preparations. Twenty adult mongrel dogs (10–12 kg) were anesthetized with pentobarbital sodium (30 mg/kg) and the heart was isolated according to a previously described procedure (6). Coronary perfusion pressure was obtained by means of a needle-tipped catheter inserted into the perfusion circuit and attached to a pressure transducer. The electrocardiogram (ECG) and heart rate were determined by means of needle-tipped electrodes inserted into the left and right ventricles. The force of contraction was measured with a Walton-Brodie strain gauge sutured to the left ventricle. All parameters were recorded on a Hewlett-Packard polygraph.

Drugs. In 1 series of experiments adriamycin (50 mg) was given 15 min after pretreatment with saline (2 ml), atropine sulfate (1 mg), *dl*-propranolol hydrochloride (0.5 mg), diphenhydramine hydrochloride (10 mg) or lysergic acid diethylamide (50 μg). Prior to the beginning of each experiment all compounds, calculated as the free base, were dissolved or diluted in physiological saline to a dose volume of 1 to 2 ml. Experiments in this series were repeated twice.

In a second series, the isolated hearts were pretreated with 100 mg of either the disodium salt of ethylenediaminetetraacetic acid (EDTA) or (±) 1,2-di(3,5-dioxopiperazine-1-yl) propane (ICRF 159) (Fig. 2). Both agents were dissolved in physiological saline in volumes of 10 ml (EDTA) and 40 ml (ICRF 159) and administered 30 min prior to daunomycin (50 mg) or adriamycin (50 mg). Drug injections were made directly into the intake line of the perfusion circuit. Samples of blood (5 ml) were collected prior to and at 1, 5, 15, 30 and 60 min after daunomycin or adriamycin administration. Each experiment was repeated in 3 separate hearts.

Daunomycin and ICRF 159 were supplied by Drug Research and Development, Chemotherapy, National Cancer Institute, and adriamycin was obtained from Farmitalia, Milan, Italy.

Paper chromatography and fluorescence studies. After centrifugation of the blood samples, known quantities of plasma were applied to Whatman No. 1 paper and allowed

[1] Supported in part by Contract PH43-68-1283 with Chemotherapy, National Cancer Institute, National Institutes of Health, Bethesda, MD 20014.

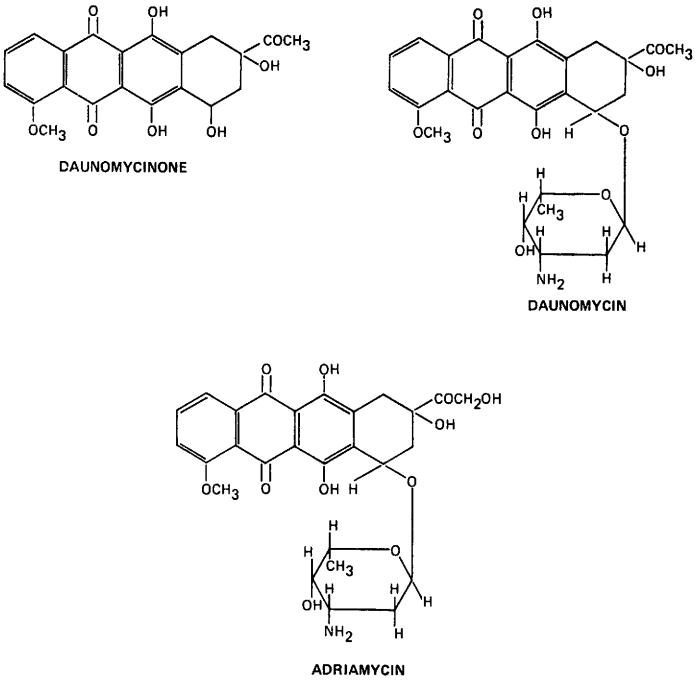

FIG. 1. Structures of daunomycin, adriamycin and daunomycinone.

to run overnight for separation of the components (5). The solvent system used was butanol:acetic acid:H_2O in the proportion of 2:1:1.

Fluorescent spots on the chromatograms were cut and added separately to centrifuge tubes. The extracts with dimethyl sulfoxide were read directly in an Aminco-Bowman spectrophotofluorometer (5). The emission spectra for all compounds was 585 nm with an excitation wavelength of 480 nm.

Results. Pretreatment with various blocking agents. The effect of 50 mg adriamycin on the control isolated heart preparation is shown in the left-hand portion of Fig. 3. This dose produced a gradual increase in pump perfusion pressure which reached maximal intensity within 30 min. Initially the force of contraction remained relatively constant; however, after 30 min a significant diminution in this parameter occurred. By 60 min, the contractions of the heart were reduced from 25 to 40% of control. At 60 min areas of hemorrhage were apparent in most hearts. Heart rate and ECG remained constant until perfusion pressure reached 150 to 200 mm Hg at which time a bradycardia and diminution in amplitude of the ECG waves were noted. Daunomycin (50 mg) produced a similar pattern of activity.

Pretreatment with either *dl*-propranolol (0.5 mg), atropine (1.0 mg), diphenhydramine (10 mg) or lysergic acid diethylamide (50 μg) did not alter the course of adriamycin-induced alterations in coronary perfusion pressure (Table I).

Pretreatment with EDTA or ICRF 159. EDTA or ICRF 159, when administered to the isolated heart, caused marked ECG ab-

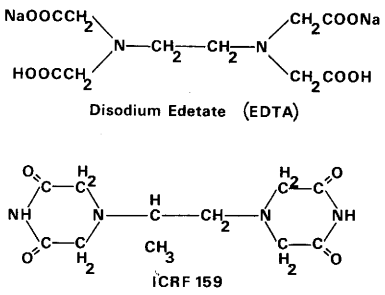

FIG. 2. Structures of ethylenediaminetetraacetic acid (EDTA) and (±) 1,2-di(3,5-dioxopiperazine-1-yl) propane (ICRF 159).

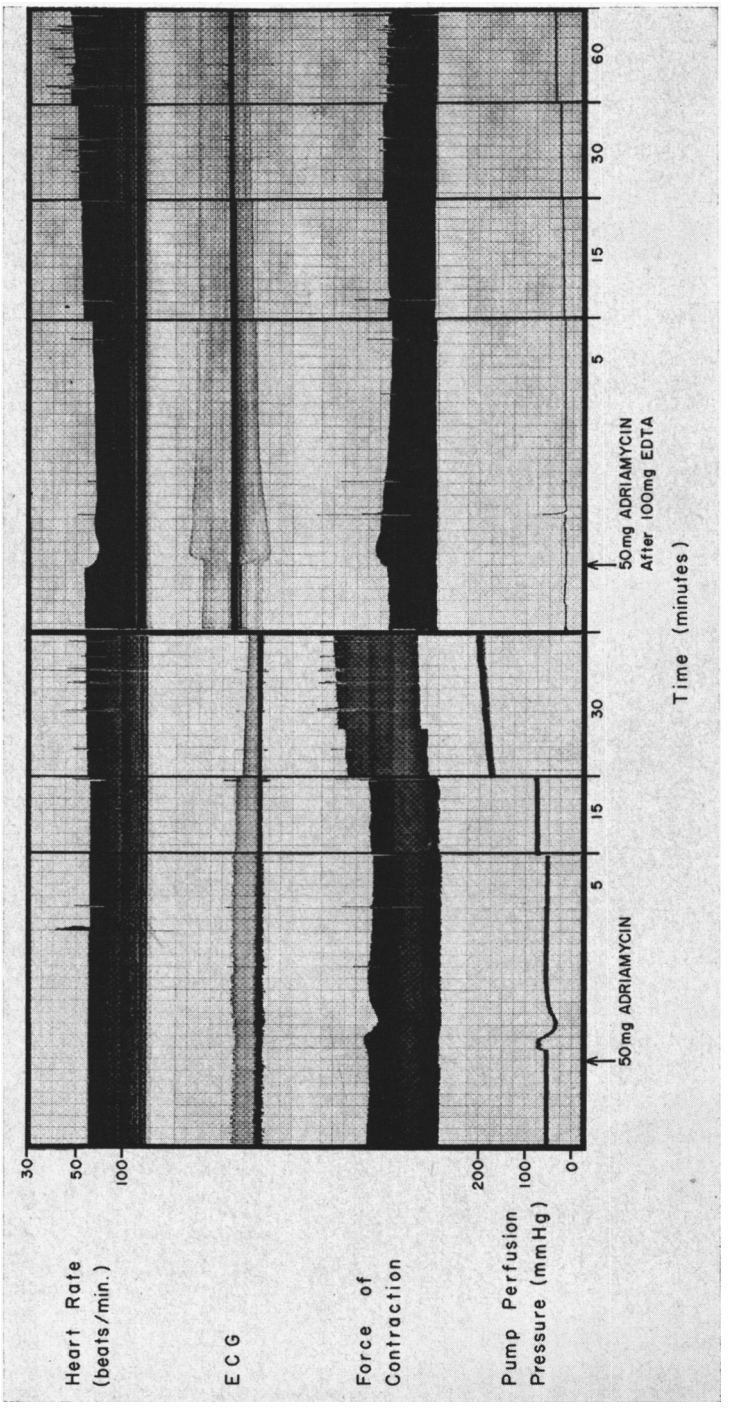

FIG. 3. The effect of 50 mg adriamycin on heart rate (beats/min), ECG, force of contraction and pump perfusion pressure (mm Hg) in the isolated, blood-perfused dog heart pretreated with saline (left portion) or 100 mg EDTA (right portion). Chart speed 0.25 mm/sec.

TABLE I. Effect of Pretreatment with Various Blocking Agents on the Increase in Coronary Perfusion Pressure Induced by 50 mg Adriamycin in the Isolated Blood-Perfused Dog Heart.

	No. of expts.	Control perfusion pressure (mm Hg)	Maximal change in perfusion pressure (mm Hg)	Time for maximal change in perfusion pressure (min)
Saline (0.5 ml)	3	20–35	165–175	25–30
Atropine (1 mg)	2	10–15	180–185	35–45
dl-Propranolol (0.5 mg)	2	10–15	185–215	30–50
Diphenhydramine (10 mg)	2	15–20	175–200	40–50
Lysergic acid diethylamide (50 μg)	2	15–25	150–225	25–45

normalities including in a few instances, ventricular fibrillation and a decrease in the force of contraction. These effects were less pronounced if the agents were given slowly over a 15-min period. The administration of adriamycin or daunomycin was not attempted in any heart unless all parameters had returned to a stabilized condition.

The effect of pretreatment with 100 mg EDTA 30 min prior to 50 mg adriamycin is shown in the right-hand portion of Fig. 3. In this experiment coronary perfusion pressure increased from 15 mm Hg to only 30 mm Hg at the end of 60 min. Initially, the amplitude of the ECG and heart rate had increased but after 15 min these parameters returned to control levels. Similar effects were noted after administration of ICRF 159; these results, together with the control and EDTA pretreatment experiments, are summarized in the top portion of Fig. 4.

Metabolic studies. In each heart pretreated with saline, EDTA or ICRF 159, blood samples were collected at various time intervals for detection of metabolic products. The results of these determinations are summarized in the lower portion of Fig. 4. In all experiments only 1 metabolic product was detected with an R_f value (0.92) similar to the aglycone, daunomycinone. The R_f values for daunomycin and adriamycin were 0.70 and 0.65, respectively. It was observed in hearts pretreated with saline that during the period of increasing coronary perfusion pressure there was a simultaneous increase in the appearance of metabolic product from adriamycin or daunomycin. The formation of the aglycone occurred quite rapidly during the first 5 min and subsequently more gradually over the remaining 55 min of the experiment. In contrast, when hearts were pretreated with either EDTA or ICRF 159, little change in coronary perfusion pressure was seen and less aglycone metabolite was formed. Detectable amounts of adriamycin aglycone were found in only 1 of 3 hearts pretreated with EDTA.

Discussion. Daunomycin and adriamycin cause an increase in coronary perfusion pressure in the isolated, blood-perfused dog heart. This effect was produced by 50 mg of either compound, whereas earlier studies indicated that doses as low as 12.5 mg also caused similar responses (7). The changes in the coronary perfusion pressure observed in the isolated heart appear to result from a direct increase in vasomotor tone since alterations in various blood components did not occur. A direct vasoconstriction of hamster cheek-pouch vessels has been previously reported (8). The increase in perfusion pressure was not influenced by pretreatment with agents blocking the vasomotor effects of histamine, epinephrine, norepinephrine, 5-hydroxytryptamine and acetylcholine. These results indicate that daunomycin and adriamycin alter coronary tone by a nonautonomic mechanism.

The injection of daunomycin or adriamycin did not produce an immediate increase in perfusion pressure. Significant changes occurred after 5 min suggesting that some modification of the molecule may be necessary before coronary vascular tone is altered. During the period of increasing perfusion pressure both compounds were converted to an aglycone metabolite similar to daunomycinone. Small amounts of this compound, in

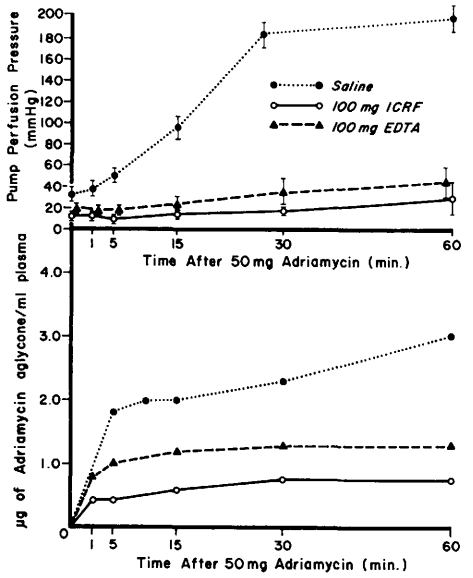

FIG. 4. Top portion, change in pump perfusion pressure (mm Hg) at various times after administration of 50 mg adriamycin in control hearts and hearts pretreated with 100 mg EDTA or ICRF 159. Each point represents mean ± SE of 3 experiments. Circulating blood volume was approximately 500 ml. Bottom portion, recovery of aglycone metabolites by paper chromatography at 1, 5, 15, 30 and 60 min from experiments described above for the isolated heart. Each point represents the mean of 3 experiments except those hearts pretreated with EDTA where detectable amounts of aglycone were obtained from 1 experiment.

other experiments, produced an immediate and sustained increase in perfusion pressure (5). In contrast, when hearts treated with daunomycin were perfused with a physiological-buffered solution, the increase in perfusion pressure and formation of daunomycinone were both minimal (5). Thus, the increase in perfusion pressure appears to be related to the formation, in the red blood cells, of aglycone metabolites of daunomycin and adriamycin.

EDTA and ICRF 159 attenuated the increase in coronary vascular tone and at the same time significantly retarded the formation of aglycone metabolites. These compounds were equally effective although ICRF 159 is less polar and would be more likely to accumulate intracellularly. The exact mechanism for the protective action has not been determined but both compounds are capable of chelating divalent cations (9, 10). An extracellular loss of calcium or magnesium might alter membrane permeability and thereby decrease the uptake of daunomycin or adriamycin. Likewise, a simultaneous decrease in intracellular cation levels might at some point interfere with the metabolic breakdown of daunomycin and adriamycin. Although EDTA and ICRF 159 appear to reduce toxicity in the isolated heart by decreasing adriamycin and daunomycin metabolism, it is not known what effect this action will have on clinical toxicity or antineoplastic activity of the compounds. Studies have shown that ICRF 159 itself exerts both clinical and experimental antineoplastic activity (9, 11). Therefore, the administration of ICRF 159 with adriamycin or daunomycin might be a useful drug combination in cancer chemotherapy.

Summary. In the isolated, blood-perfused dog heart daunomycin (50 mg) or adriamycin (50 mg) induced significant increases in coronary perfusion pressure. This effect appeared to be related to the conversion of the parent compounds to aglycone metabolites similar to daunomycinone. Pretreatment of the heart with agents blocking the vasoactive actions of epinephrine, histamine, serotonin or acetylcholine did not prevent the alteration in coronary vascular resistance. In contrast, 100 mg of either EDTA or ICRF 159 prevented the marked increase in perfusion pressure and reduced the formation of aglycone metabolites. Chelation is a common property of the 2 agents suggesting that removal of certain cations may alter metabolism and reduce the toxicity of adriamycin and daunomycin.

1. Tan, C., Tasaka, H., Yu, K.-P., Murphy, L., and Karnofsky, D. A., Cancer **20**, 333 (1967).

2. Middleman, E., Luce, J., and Frei, E., III, Cancer **28**, 844 (1971).

3. Bonadonna, G., Monfardini, S., DeLena, M., and Fossati-Bellani, F., Brit. Med. J. **2**, 503 (1969).

4. Herman, E., Mhatre, R., Lee, I. P., Vick, J., and Waravdekar, V. S., Pharmacology **6**, 229 (1971).

5. Mhatre, R. M., Herman, E., Huidobro, A., and Waravdekar, V., J. Pharmacol. Exp. Ther. **178**, 216 (1971).

6. Vick, J. A., and Herman, E. H., Pharmacology **6**, 290 (1971).

7. Herman, E. H., and Vick, J. A., Pharmacology **3**, 291 (1970).

8. Arthur D. Little, Inc., "Annual Report to CCNSC," p. 233. ADL, Cambridge, MA, 1967.

9. Creighton, A. M., Hellmann, K., and Whitecross, S., Nature (London) **222**, 384 (1969).

10. Seven, M. J., and Johnson, L. A., "Metal-Binding in Medicine," p. 154. Lippincott, Philadelphia (1960).

11. Hellmann, K., Newton, K. A., Whitmore, D. N., Hanham, I. W. F., and Bond, J. V., Brit. Med. J. 1, 822 (1969).

Received Jan. 20, 1972. P.S.E.B.M., 1972, Vol. 140.