Comparative Cardiac Toxicity of Daunomycin in Three Rodent Species*† (33727)

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(Introduced by D. P. Rall)

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Daunomycin is an antibiotic of the anthracycline group isolated from cultures of *S. peucetius* (1) and composed of a pigmented aglycone (daunomycinone) (2) bound in glycosidic linkage to an amino sugar (daunosamine) (3). The compound has demonstrated antitumor activity against a wide spectrum of transplanted rodent tumors (4) and acute leukemia in clinical studies (5). Clinically, total doses greater than 25 mg/kg have been associated with the development of cardiopulmonary symptoms such as tachycardia, with or without arrhythmia, gallop rhythm, congestive heart failure, tachypnea, and cases of dyspnea (5, 6). The present studies were initiated to determine whether daunomycin could induce similar or other types of cardiac toxicity in animals.

Methods and Materials. Three male rats (250–300 g), 5 male guinea pigs (450 g) and 50 male golden hamsters (90–130 g) were anesthesized with 30–45 mg/kg of pentobarbital sodium intraperitoneally. The right carotid artery in the guinea pig and hamster and the right femoral artery in the rat were cannulated and connected to a Sanborn 7722B recorder through a Sanborn 267B transducer. Respiration was monitored after tracheotomy, with a Sanborn 270 transducer. Electrocardiogram (ECG) and heart rate were monitored by means of needle electrodes inserted into the limbs and chest.

Drugs were dissolved in physiological saline and infused into the femoral vein in dose volumes of 0.2 ml or less. The initial drug doses were: daunomycin (NSC 82151), 50 mg/kg; dactinomycin (Lyovac, Cosmegen) (NSC 3053), 0.1 mg/kg; Cyclophosphamide (Cytoxan) (NSC 26271), 50.0 mg/kg; rubidomycin (NSC 83142), 50 mg/kg; ouabain, 1.0 mg/kg. In those experiments where cardiac toxicity was not induced by the initial dose additional injections were given at 15-min intervals.

With the exception of ouabain, all drugs were supplied by Cancer Chemotherapy National Service Center. Ouabain was purchased from K & K Rare Chemicals, Plainview, N. Y.

Results. Species. The initial phase of this study was concerned with finding a species in which acute cardiac toxicity could be induced by daunomycin.

Rat. The cardiovascular system of the rat was relatively resistant to daunomycin. A total of 200–300 mg/kg produced slight decreases in blood pressure and heart rate and some variable changes in the amplitude of ECG waves.

Guinea pig. A total of 75–175 mg/kg daunomycin was given to these animals. After most injections a transient hypotensive effect was seen. Daunomycin had a striking respiratory depressant effect on all animals. Periods of 2–4 min of apnea occurred following certain doses. Usually any changes in the ECG were secondary to these periods of apnea.

Hamster. In contrast to the rat and guinea pig, a slight to moderate pressor response was usually observed during infusion with daunomycin. In addition, alterations in the ECG were usually seen within 10–40 sec followed by a definite cardiac arrhythmia 20–90 sec later.

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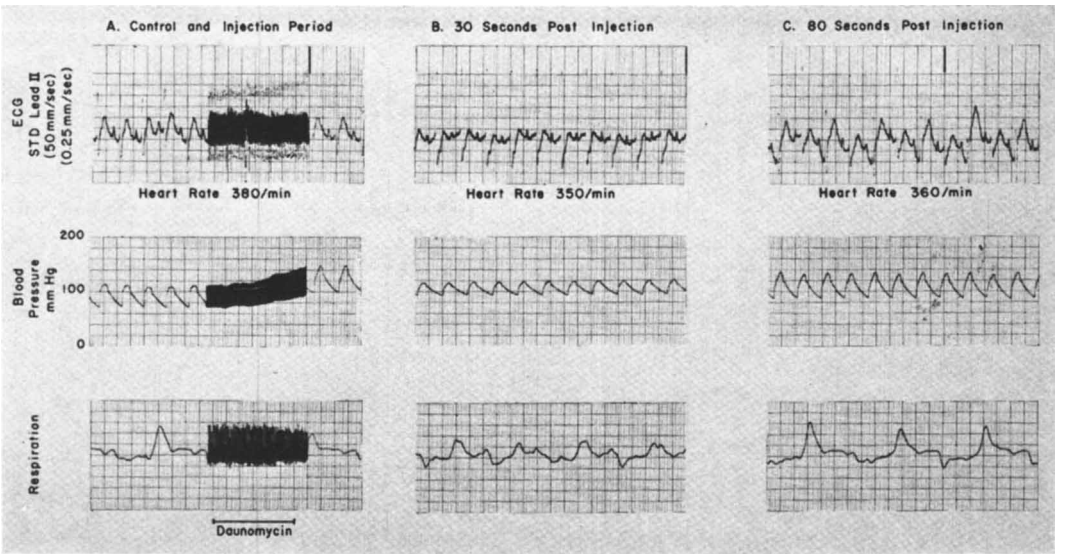

FIG. 1. Changes in ECG (upper traces), blood pressure (middle traces), and respiration (lower traces) following 2 min i.v. injection of 50 mg/kg of daunomycin.

Hamster cardiac toxicity. One of three different patterns noted preceeding the onset of the major arrhythmia is seen in Fig. 1. Figure 1A shows the pressor response observed during daunomycin injection. Approximately 20 sec later, as seen in Fig. 1B, the ST segment was depressed and the T wave greatly reduced in amplitude. Figure 1C, 60 sec. post injection shows alterations in the amplitude of the R waves. Some R waves became small and finally disappeared in abnormal negative complexes preceeding the major arrhythmia.

In 7 other experiments ST segment depression was followed by increases in the amplitude of the T wave before changes in the R wave occured. T wave elevation has been associated both experimentally and clinically with elevated extracellular potassium levels. In the present experiments the serum potassium levels in three animlas were not altered by daunomycin.

Figure 2 demonstrates a pattern seen in 3 experiments in which changes in the R, ST, and T waves were minimal prior to the arrhythmia. The type of arrhythmia induced by daunomycin in the majority of the experiments is seen in the latter portion of Fig. 2B and more completely in Fig. 2C. The arrhythmia appears as a bidirectional ventricular tachycardia in which the QRS complex of one ventricular beat is upright and that of the following is downward. There appears to be complete A–V dissociation since P waves which appear bear no relation to the abnormal QRS complexes.

It should be noted that in those animals followed with serial electrocardiograms the arrhythmia reverted to sinus rhythm within 30–90 min after onset. Death usually occurred 4–5 days later and could be attributed to bone marrow depression and gastrointestinal toxicity.

Five animals were given single i.v. doses of 12.5, 25.0, or 50 mg/kg 24–96 hr prior to rechallenging with graded doses of daunomycin. This prior pretreatment did not significantly alter the arrhythmia threshold.

Specificity. To determine whether the arrhythmia was daunomycin specific, two anticancer drugs without known cardiac toxicity were employed as negative controls.

Dactinomycin (Lyovac, Cosmegen) (NSC 3053), an antibiotic, was administered in a series of doses ranging from 0.1 to 6.4 mg/kg with the total cumulative dose of 13 mg/kg. At these doses dactinomycin had no obserable effect on the ECG. In addition, the threshold for the daunomycin arrhythmia was not changed.

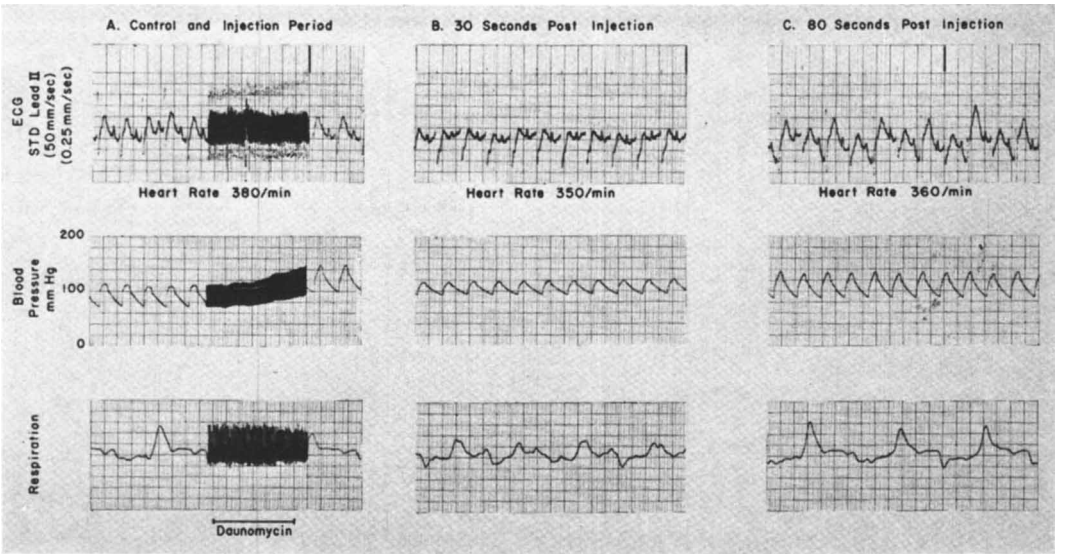

FIG. 2. Effects of the i.v. administration of a single dose (50 mg/kg) of daunomycin on ECG (upper traces), blood pressure (middle traces), and respiration (lower traces). Duration of the daunomycin injection is approximately 2 min.

Cyclophosphamide (Cytoxan) (NSC 26271), an alkylating agent, was given in doses from 50 to 300 mg/kg with a total cumulative dose of 650 mg/kg. Even at the highest dose there was relatively little change in the ECG, although heart rate was decreased by 17%. Again the threshold for daunomycin induced arrhythmias was not altered by cyclophosphamide.

In contrast, rubidomycin (NSC 83142) an antibiotic isolated by Dubost *et al.* (7) and thought to be structurally similar to daunomycin did alter the ECG. A single dose of 50 mg/kg produced an arrhythmia identical to that observed with daunomycin.

Ouabain cardiac toxicity. In order to compare qualitatively the daunomycin induced arrhythmia with that of a digitalis glycoside, ouabain was administered intravenously to 15 hamsters at doses of 1–32 mg/kg. A wide spectrum of ECG abnormalities was observed, including T wave elevation, bundle branch block, ventricular premature contractions, ventricular tachycardia and fibrillation. The overall pattern was not qualitatively similar to that of daunomycin and there were no cases of bidirectional ventricular tachycardia.

Discussion. During the course of daunomycin therapy of acute leukemia certain lethal cardiopulmonary complications were observed (5, 6) and generally occured in patients who received 25 mg/kg or more daunomycin cumulatively over a period of several weeks. In the present studies with hamsters, daunomycin and rubidomycin were found to induce a definite cardiac toxicity which was specific since both dactinomycin and cyclophosphamide had little if any cardiac effect.

The type of toxicity induced by daunomycin in the hamster differs from that observed clinically because it is acute and appears predominately as an arrhythmia. Clinically, small doses ranging from 1 to 2 mg/kg have been employed with a duration of several days while in the present experiments only a single large dose was administered. It is conceivable that if comparable doses were given clinically acute arrhythmias might occur.

Chronic studies in the hamster might provide a more comparable picture of toxicity

but technical difficulty in giving serial i.v. injections was a limiting factor. In addition, daunomycin has been shown to cause severe peritonitis when given intraperitoneally. In the present experiments, however, i.v. pretreatment using graded doses and time intervals did not significantly alter the threshold for acute cardiac toxicity thus suggesting that the drug does not permanently influence cardiac rhythm.

The question arises as to why the hamster of the 3 rodent species was susceptible to daunomycin cardiotoxicity. It is possible that the myocardium of the hamster may selectively take up the drug or that the drug in some way is selectively activated by enzymes in the hamster heart. A second species difference observed following daunomycin injection was a transient pressor response in the hamster as contrasted to either no change or a depresser response in the rat and guinea pig. Whether these alterations in blood pressure have any relationship to the selective occurrence of the bidirectional ventricular arrhythmia in the hamster is not known at the present time. The possibility that the two abnormalities arise from a release of catecholamines is currently undergoing investigation. Though not observed in the present studies with hamsters, the bidirectional ventricular ECG pattern was reported to occur in dogs, (8) and humans (9) as a sign of digitalis intoxication. At least two explanations for this type of arrhythmia were proposed. In one, there may be a single focus situated in the A–V node or in the bundle above its bifurcation sending out rhythmic impulses which travel alternately along the right and left branches of the bundle. The alternate explanation is that there are two foci sending out impulses, one in either ventricle and the tachycardia is composed of a rapid succession of ectopic beats arising alternately in the right and left ventricles. Digitalis glycosides exert a number of cardiac actions which might be responsible for initiating the bidirectional rhythm. For example, digitalis has profound effects upon automaticity especially in the ventricles and may expose idioventricular impulse generation, by permitting time for diastolic depolarization and eventual firing of spontaneously active foci. At the present time it is not known whether daunomycin exerts similar direct cardiac actions but the inability of ouabain to induce a bidirectional ventricular tachycardia would seem to indicate that another mechanism might be responsible for the arrhythmia seen in the hamster. Further experiments to better define the daunomycin arrhythmia physiologically and pharmacologically are in progress.

Summary. Daunomycin, an antibiotic used in the treatment of acute leukemia has been implicated in the development of a lethal cardiopulmonary syndrome. Rats, guinea pigs, and hamsters were studied to determine whether cardiac toxicity would be induced in animals. The rat was resistant to high doses of daunomycin (200–300 mg/kg) while fatal respiratory depression occurred in the guinea pig (75–175 mg/kg). In contrast 50 mg/kg induced acute cardiac alterations in the hamster consisting initially of changes in the ST segment, R and T waves followed by a bidirectional ventricular tachycardia. The arrhythmia in the hamster was daunomycin specific since both dactinomycin and cyclophosphamide has little cardiac effect whereas rubidomycin (50 mg/kg) produced comparable ECG effects. Bidirectional ventricular arrhythmias have been reported to occur in animals and humans as a sign of digitalis intoxication, however, this was not seen in the hamster after varying doses of ouabain. Both digitalis and daunomycin are glycosides but the present experiments tend to indicate they may induce cardiac toxicity in the hamster by different mechanisms.

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Moloney Lymphoma Antibodies from Mice; Localization in Spleens of Moloney Lymphoma Bearing Mice* (33728)

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Moloney virus induced lymphomas have been shown to contain tumor specific transplantation antigens (1–3). The existence of these antigens has been demonstrated by transplantation methods (1) and by various tests involving specific humoral antibodies induced by injecting heavily irradiated lymphoma cells, into syngeneic mice (2). This paper describes studies of the *in vivo* localization properties of the latter type antibodies. Antibodies were demonstrated which were fixed in the spleen when injected intravenously into tumor bearing mice but showed no such fixation in normal mice. The fixation was demonstrated using radioactive iodine as a label for the antibodies. This was interpreted as showing that the spleen contains antigens associated with the Moloney tumor or virus.

Materials and Methods. The following Moloney lymphomas were used: (i) YAC (an ascites tumor) in transplant generation 155 in male A/Sn mice and in generation 156 in male A/Sn, A/Hc and A/St mice; (ii) the YBA tumor (solid) in generation 45 in female CBA mice; (iii) the YLI tumor (solid) in generation 31 in male C57 Leaden mice.

The syngeneic antiserum for the detection of antigens associated with Moloney lymphoma was produced by repeated inoculation of irradiated lymphoma cells into syngeneic recipients (2). It had high cytotoxic activity against the Moloney lymphoma cells in the presence of complement *in vitro* and showed a high degree of membrane fluorescence against the same targets in the indirect test. Normal serum of the appropriate mouse strains was used as control.

The globulin fraction of each serum (from either normal or immunized mice) was prepared by precipitation with Na_2SO_4 as described previously (4), but with the modification that the initial Na_2SO_4 concentration was 18% (w/v) rather than 13.5% and the final Na_2SO_4 concentration was 13.5% rather than 12%.

Tumor sediments were prepared as described previously. Washed tissue fragments were homogenized and the insoluble sediment was washed thoroughly with borate buffer (pH 8.0) and water, and subsequently lyophilized (4). The globulin fraction from the Moloney antiserum was iodinated with iodine labeled with ^{125}I and globulin from normal isogeneic serum was iodinated with iodine labeled with ^{131}I was described previously (4). The paired-labeled mixture was purified with the corresponding tumor sediment as described below.

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