The Interval-Force Relationship: A Technique for Evaluating the Cardiac Toxicity of Anthracycline Analogs

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SUMMARY

The most serious side effect of the anthracycline derivatives is dose-related cardiac toxicity induced during repeated administration. An in vitro method is described which assesses the interval-force relationship in evaluating the contractility of the rat and rabbit heart. Repeated administration of Adriamycin resulted in a progressive decrease in contractility which correlated closely with the cumulative dose administered. This model offers a reliable method to evaluate the effect of new anthracycline analogs on the heart and to study the potential of other drugs or agents to protect against cardiac toxicity.

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Although anthracycline antibiotics have proved to be among the most effective antitumor agents, the cardiac toxicity of this group of drugs remains a major obstacle to prolonged application by medical oncologists. An anthracycline derivative with antitumor activity but without a detrimental effect on the heart is still the main goal of investigators in the field of anthracycline research. The persistent search for nontoxic derivatives has provided >500 analogs, each of which deserves adequate testing for antitumor activity and, if therapeutically active, evaluation of its effect on the heart. A method to assess the cardiotoxicity of anthracycline analogs in laboratory animals should be reliable and workable. The predictive value of the available techniques is controversial.

The mechanical function of the heart is to pump blood to allow the delivery of oxygen and the removal of waste products. Neither quantification of light- and electron-microscopic alterations nor electrophysiologic phenomena reflect the impairment of the mechanical activity of the heart after prolonged exposure to anthracyclines.

There is a need for a reliable parameter for the contractile behavior of the heart (1). Unfortunately, no simple in vivo method can satisfactorily reflect myocardial contractility without being affected by the preload and/or afterload of the heart. Assessment of right and/or left ventricular function in intact animals given a myocardial depressant is therefore subject to some uncertainty (2).

Extracardiac factors such as the lungs, pericardium, and peripheral circulation can strongly influence cardiac pump function irrespective of myocardial contractility. In fact, anthracyclines do cause disturbances in body fluids, which means that variation in the preload and/or afterload of the left ventricle may occur (3).

The isolated heart perfused according to Langendorff is an accepted model for the study of cardiac effects of pharmacologic agents. It offers the advantages of stability and allows the exclusion of extracardiac factors. The apicobasal shortening of the preparation can be considered a parameter for contractility.

The known relationship between the heart rate and the intrinsic contractile behavior must be defined. Electrical stimulation of an isolated heart makes it possible to analyze the whole appropriate frequency range. The curve depicting the so-called interval-force relationship was first described by Kruta in 1937 (4).

This paper describes an interval-force relationship for the hearts of two animal species. All animals had been repeatedly exposed to Adriamycin up to a cumulative dose known to cause chronic cardiac toxicity in both the
rabbit and the rat (1,5). The model consists of perfused isolated artificially stimulated hearts, avoids preload and afterload, and uses perfusates of standardized composition. This approach provides a reliable "endpoint parameter" (1) for the evaluation of the effects of anthracyclines on the contractile function of the heart.

MATERIAL AND METHODS

Animals

Male New Zealand white rabbits weighing 2.9–3.9 kg were maintained in a climatized environment isolated from other animals. Rabbits were given water and pelleted rabbit food ad libitum. The food contained 89 mg of α-tocopherol per kg supplemented with α-tocopherol-acetate and 0.45 ppm of selenium. One week after a predetermined cumulative dose of Adriamycin (400 mg/m²) had been reached, the animals were killed as described below.

Wistar rats weighing 190–220 g were kept in Perspex cages, four to a cage, and were given water and pelleted rat food ad libitum. After 6- and 12-week treatment periods, animals were randomly taken from the population and used for the assessment of contractile function. For each treatment period, four rats that did not receive Adriamycin served as controls.

Drug Administration

Fourteen male rabbits received Adriamycin at a dose of 2 mg/kg/week given in two injections into the marginal ear vein. The control rabbits received equal volumes of 0.9% NaCl solution iv twice a week.

Sixteen female Wistar rats were given Adriamycin via the tail vein at a dose of 2.0 mg/kg/week after being lightly anesthetized with ether. Before and after the injection the vein was flushed with 0.1 ml of 0.9% NaCl to avoid toxic necrosis of the tail. The seven control rats received equal volumes of 0.9% NaCl solution.

Preparation of the Animals

Prior to the perfusion of the isolated hearts the rabbits were anesthetized with hypnorm (1 ml/kg im) and the rats were anesthetized with ether. When sufficient anesthesia had been obtained, the animals were heparinized. The chest was then opened, the thymus dissected, and the aorta presented. Next the pericardium was opened and a ligature was placed around the aorta. A Perspex cannula was introduced into the aorta and the heart was excised from the pericardium and connected to the perfusion system via the cannula.

Perfusion System

The perfusion according to Langendorff’s technique was performed with an apparatus developed by Zimmerman et al (6) (fig 1). Constant perfusion pressure is obtained by means of a water lock. For the rabbits a pressure of 80 cm H₂O was maintained and for the rats a pressure of 100 cm H₂O was maintained. The temperature of the perfusate was kept constant at 37.5°C. The composition of the perfusate (in mmols/liter) was sodium, 149; potassium, 8.4; calcium, 5.2; magnesium, 4.2; chloride, 138; HCO₃⁻, 20; HPO₄²⁻, 1.6; and glucose, 11. These constituents were dissolved in twice-distilled water just before the start of the experiment. The perfusate was saturated with 95% O₂ and 5% CO₂ at the final temperature and pressure. This provided a pH of 7.42. The apex moves in the direction of the base of the heart at every contraction. The vertical displacement of the apex can be regarded as a parameter of contractile function. The mechanical displacement was converted to an electrical signal by means of a displacement transducer. Displacement was recorded on a Hewlett Packard stripchart recorder (HP 7848A).

After these measurements, a total atrioventricular block was induced at the atrial septum, thus disturbing the bundle conduction.

Fifteen minutes later, after a steady state had been established, the heart was stimulated by a 1-msec square-wave pulse from a current source at twice the threshold value, usually 0.1 mA. The initial pacing frequency was chosen just above the idioventricular rhythm. The stimulation frequency was increased in 25-msec steps for the rat hearts and in 50-msec steps for the rabbit hearts, both of 60 seconds' duration, until no adequate reaction to pacing occurred. Displacement at each frequency was calculated and expressed in percentages of displacement at the lowest frequency. Displacement was plotted against stimulation interval, thus providing a Kruta curve (4). After the experiment, the hearts were perfused with formalin or glutaraldehyde for light- and electron-microscopic examination of the morphologic features.

Gross pathologic examination was performed after each experiment.

RESULTS

During the treatment period, five of the rabbits receiving Adriamycin died of hematologic toxicity and intercurrent infection. Three rabbits were not evaluable because of technical problems during the surgical procedure. Three rats died immediately after the administration of Adriamycin and two died in the tenth week of treatment, probably due to hematologic toxicity.

Pathologic examination resulted in similar findings in both of the animal species studied. The alterations were
Figure 1.—Perfusion apparatus. A: Reservoir in which perfusate is preheated. B: Heating reservoir in which perfusate level can be regulated. P: Hydrostatic pressure. K₁: Stopcock to close the outlet of the reservoir (reservoir can be filled during the experiment). K₂: Accessory stopcock. Opening removes pressure above B. K₃: Stopcock for formalin or glutaraldehyde reservoir. K₄: Stopcock to connect the heart with the perfusate supply. K₅: Water column. S: Rubber tube. h₁: Depth of the tube in the water column. h₂: Difference between the levels in the heating reservoirs and coronary ostia, which determines perfusion pressure.
characterized by overt signs of cardiac failure: pleural effusions, ascites, nutmeg appearance of the liver, anasarca, and hydropericardium. These alterations were less pronounced in the rats exposed to six doses of Adriamycin than in those given 12 doses. No further histologic investigation was performed. Analysis of the contractile function of the control rabbits and rats showed a gradual increase of displacement with increasing stimulation rate. Maximal apical displacement occurred at a stimulation interval of 250 msecs in the rabbit hearts and at 100 msecs in the rat hearts (figs 2 and 3). The increase was up to 120% of the initial value for the rabbit hearts and up to 135% for the rats. Alternating pulse or fibrillation occurred at stimulation intervals of 200 and 75 msecs in the rabbit and rat respectively. However, the Kruta curve of Adriamycin-treated animals showed signs of impaired contractile function: in the rabbit, maximum displacement was reduced to 103% of the initial value, and the maximum occurred at a longer stimulation interval than in the control rabbits. The isolated rat heart function showed similar abnormalities. Maximal displacement seems to decrease gradually in relation to the dose of the drug. There was no definite stimulation interval at which peak force developed. Alternating pulse or fibrillation occurred at higher stimulation frequencies (fig 4). These factors indicate impaired functional integrity.

DISCUSSION

Adriamycin-induced cardiotoxicity is characterized by electrophysiologic, morphologic, and functional abnormalities which may ultimately result in clinically recognizable and life-threatening congestive heart failure (7). Studies in the rat done by Zbinden and Brändle (8) have shown that there is a relationship between electrocardiographic abnormalities and the total dose administered. In the rabbit, Jaenke found a strong relationship between the dose and the morphologic alterations in the heart tissue (5); histologic examination revealed overt signs of congestive heart failure. These findings have been confirmed by others (9), but the studies did not include electrocardiographic evaluations of cardiotoxicity.

Recently, the rat has been shown to develop morphologic alterations similar to those described in the rabbit (10), and congestive heart failure also occurs in the rat. However, no clear relationship has been found between the electrocardiographic and morphologic alterations (8).

Left ventricular performance may be considered the sum of preload or muscle fibril length, afterload or impedance to the injection of the blood, and the contractile state of the myocardium, which refers to the intrinsic ability of the heart to generate force independent of such factors.
as low-loading and catecholamines. Assessment of the functional integrity of the heart after in vivo exposure to drugs, ie, assessment of contractility under standardized conditions of preload and afterload, and with the use of a standardized perfusion medium, provides a valuable and reliable parameter with predictive value for the cardiotoxic potency of anthracyclines. Interval-force curves have been shown to reflect this contractile function accurately (11). Although a variety of methods for the assessment of contractility have been described (12), the present method deserves preference because of the relatively simple procedure. The rat has proved to be an almost ideal animal model for cardiotoxicity studies. Moreover, this species has recently been shown to offer a particularly useful system for the monitoring of objective tumor response as well as of development of cardiotoxicity by new anthracycline analogs (13). This combination of qualities has not been described in the rabbit. The results of the present study suggest that the decline of contractile properties in the hearts of both species is dose-related. These findings are consistent with the dose-related electrocardiographic and morphologic alterations described for the rat and the dose-related morphologic alterations described for the rabbit. Our findings suggest that the decline in contractility is progressive. In the search for analogs with equal oncolytic action and fewer cardiotoxic side effects, characterization of contractile decline is of paramount importance.

Evaluation of the functional integrity of isolated perfused hearts on the basis of Kruta curves provides a unique possibility to combine electrophysiologic, morphologic, and functional criteria for the screening of anthracyclines with respect to cardiotoxicity.

REFERENCES