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DETECTION OF ANTINEOPLASTIC AGENT INDUCED CARDIOTOXICITY BY ^{31}P NMR OF PERFUSED RAT HEARTS

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Development of dose dependent chronic irreversible cardiotoxicity is a key problem encountered in chemotherapy with adriamycin. Here it has been demonstrated that infusion of this agent produced distinct and largely irreversible changes in levels of phosphate metabolites and substantial acidosis that are detected by ^{31}P NMR of the Langendorf perfused heart. Administration of the antioxidant, butylated hydroxytoluene minimizes these spectral changes but does not substantially diminish the antineoplastic activity of adriamycin. Bisantrene (CL 216,942), a noncardiotoxic anthracene with antineoplastic activity, produces only minor perturbations of the ^{31}P spectrum of the perfused rat heart. These studies demonstrate the potential utility of employing ^{31}P NMR to monitor acute or chronic cardiotoxicity in the perfused rat heart and for developing noninvasive in vivo NMR techniques for monitoring cardiotoxicity in experimental animals and humans.

Adriamycin (ADR) is one of the most widely used antineoplastic agents. The clinical utility of this and other anthracyclines is limited by the development of a dose-dependent cardiotoxicity that may result in congestive heart failure (1). The problem is further aggravated by the fact that a number of procedures employed in cancer therapy have been reported to enhance ADR induced cardiotoxicity: x-radiation of the mediastinum (2), and chemotherapy with mitomycin C (4), cyclophosphamide, actinomycin D, mithramycin (2), vincristine or bleomycin (3).

Four general approaches have been adopted to limit this cardiotoxicity: 1) preparation of suitable analogs (5), 2) administration of agents to block the cardiotoxicity of ADR (2,6-16), 3) development of novel methods for delivering this drug to the tumor (17,18), and 4) schedule manipulation (3,19,20).

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Evaluation of the effectiveness of these procedures requires the availability of suitable methods for detecting cardiotoxicity in experimental models. Currently available methods include electrocardiography (21), echocardiography (21,22), radionuclide cineangiography (23-25), measurement of systolic time intervals (26), the interval-force technique (27), treatment of cultured cardiocytes (29), and endocardial biopsy (29,30). Some of these procedures can also be employed to monitor cardiotoxicity in humans. Biopsy is generally considered the most reliable procedure; it often detects lesions before any physiological manifestations of cardiomyopathy can be detected by any of the other methods. However the biopsy technique has certain limitations (31): 1) it is a dangerous procedure, 2) specialized training is required, 3) a subjective choice is made as to which lesions are significant, and 4) the sampled region may not be representative of the entire organ.

Phosphorus-31 NMR spectroscopy of perfused rat hearts incorporates many of the desirable characteristics of the detection techniques listed above and offers some unique advantages. This method has proven to be a very sensitive monitor of the bioenergetic state of the entire heart (32,33) or of specific regions of this organ (34).

MATERIALS AND METHODS

The male Sprague-Dawley rat has been demonstrated to develop anthracycline induced cardiomyopathy (35). In our studies these animals were anesthesized with pentobarbital (60mg/kg), their hearts were surgically removed, and retrograde Langendorf perfusion with Krebs-Henseleit buffer saturated with 95% O_2 and 5% CO_2 was initiated by suturing a cannula to the aorta (36,37) (perfusion rate 20 ml/min). The perfused organ was inserted into a 20 mm O.D. NMR tube and 80.96 MHz ^{31}P NMR spectra were measured without sample spinning on a Bruker CXP-200/300 spectrometer.

RESULTS AND DISCUSSION

The spectrum of the perfused rat heart (Fig. 1) was similar to those previously reported by other investigators (32,33) and qualitatively resembled that of the *in vivo* rat heart (38). Infusion of ADR (1 μg/ml, 20 ml/min, 33.2 min, corresponding to an overall dose of 2.2 mg/kg) led to a slight decrease in the phosphocreatine (PCr) peak and no significant change in the levels of the other abundant phosphate metabolites or in the apparent internal pH measured from

the chemical shift of the inorganic phosphate (P_i) peak (39,40). The drug concentration employed in this study was comparable to the peak serum concentrations (0.42-11 μg/ml) in rats inoculated with ADR in doses ranging between 2 mg/kg and 5 mg/kg (41-43). After 33.2 min perfusion with ADR-free buffer was reinitiated. Progressive deterioration in the metabolic state of the heart ensued. This was reflected in a decrease in the levels of high energy phosphate metabolites (PCr and ATP), an increase in P_i and a decrease in the apparent pH. By 1.11 h (Fig. 1) the apparent pH had decreased to 6.7 and the spectrum resembled that of a nearly dead heart (which exhibits only a P_i resonance (43)). Subsequently some recovery of cardiac phosphate metabolism occurred (see 1.74-1.82 h spectrum in Fig. 1). The apparent pH increased to 7.2. However the spectrum never returned to its control level (which in the absence of ADR could be maintained for more than 2 h of perfusion).

There was a substantial diminution in the adenine nucleotide pool. This was reflected in the decreased intensities of ATP and ADP and the absence of any significant AMP resonances. Ohhara et al. (12) have observed similar changes (decrease in adenine nucleotides as well as in NAD and in the developed contractile tension) in rat hearts perfused for 60 min with 10 μg/ml ADR. At lower concentrations of the drug such as those employed in our study no decrease in cardiac tension was detected.

The spectral changes induced by ADR treatment were similar to those observed after prolonged global ischemia of rat hearts (32,33,44,45). In that case substantial depletion of the adenine nucleotide pool was also detected, and this effect was minimized by administration of adenosine deaminase inhibitors (44,45). Ohhara et al. (12) observed a similar protective effect with coenzyme Q_{10}. Reports that treatment with adenosine decreases ADR-induced cardiotoxicity (9,10) are also consistent with ADR-induced depletion of the adenine pool.

Antioxidants such as α-tocopherol (6) and various sulfhydryls (7,8) are known to inhibit ADR-induced cardiotoxicity. One of us has recently reported that butylated hydroxytoluene (BHT) also blocks ADR toxicity in mice and does not significantly diminish the antineoplastic activity of this agent (46).

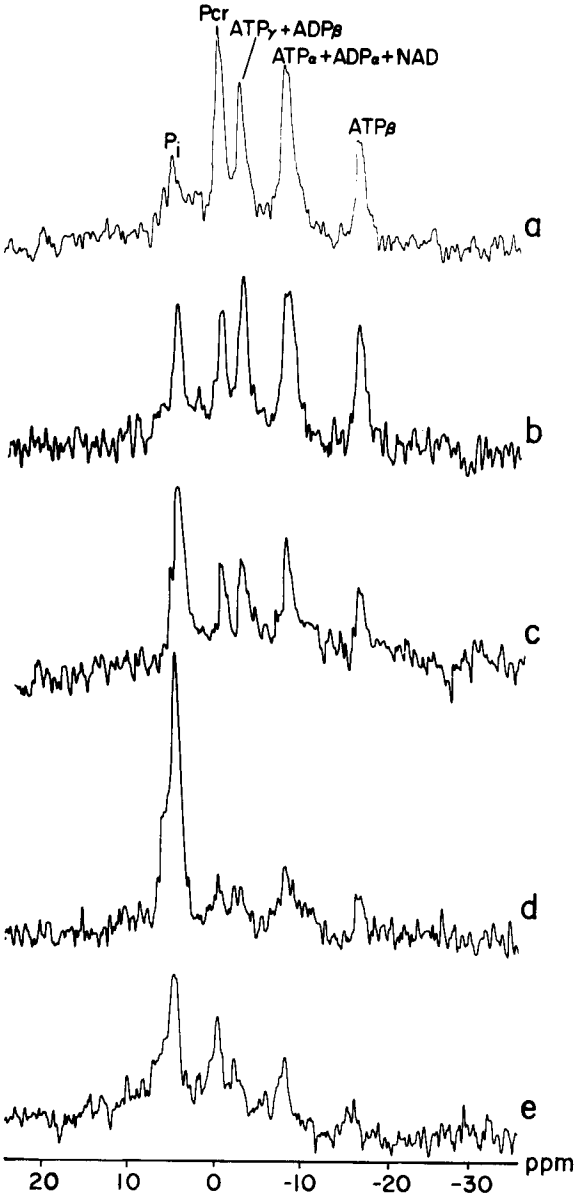

Figure 1: 80.96 MHz ^{31}P NMR spectra (128 scans, 4K data points, 45 μsec pulse (45°); 2 sec repetition) of a perfused rat heart. (a) untreated control, pH = 7.3; (b) 33 minutes after exposure to ADR, pH = 7.3. Infusion of ADR was terminated after 33 minutes and the subsequent spectra were recorded during perfusion with drug-free buffer: (c) 66 minutes, pH = 7.0; (d) 76 minutes, pH = 6.7; (e) 109 minutes, pH = 7.2.

The effect of BHT on ^{31}P spectra of the perfused rat heart is shown in Fig. 2. Inclusion of BHT (1 μg/ml) in the perfusion buffer produced only a slight diminution of the PCr peak, a slight increase of P_i and a slight decrease in the

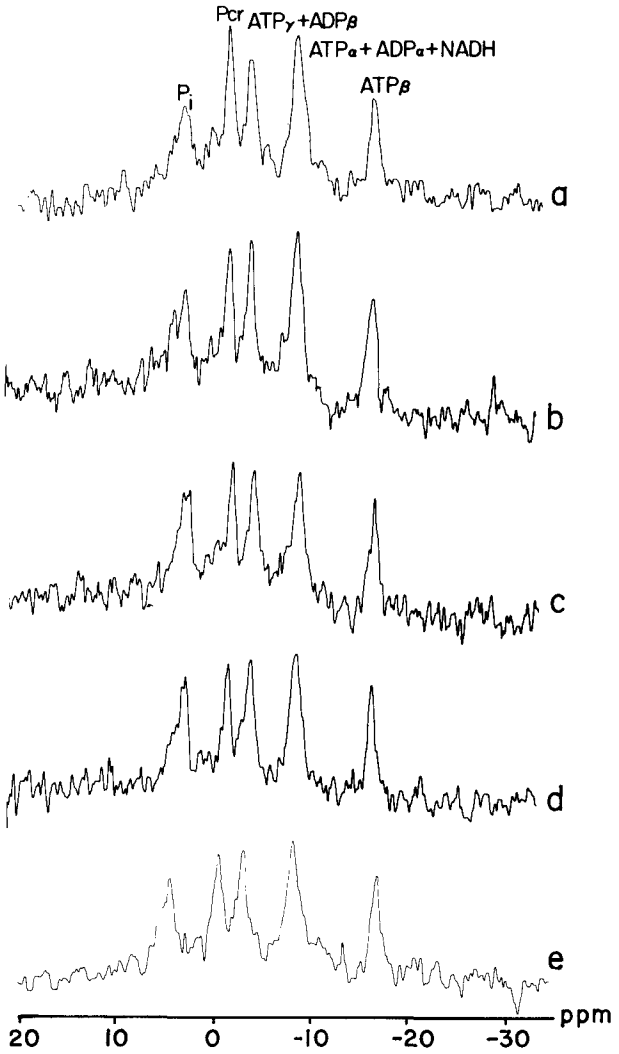

Figure 2: 80.96 MHz ^{31}P NMR spectra of a perfused rat heart (same spectral parameters as in Figure 1): (a) untreated control; (b) 19 minutes after infusion of BHT; infusion with BHT was terminated after 23 minutes and infusion with ADR was initiated (t = 0); (c) spectrum measured at t = 28.5-33.2 min, pH = 7.3; ADR infusion was terminated at t = 33.2 min and perfusion was continued with drug-free buffer; (d) spectrum measured at t = 38.0-42.7 min, pH = 7.2; (e) spectrum measured at t = 1.11-1.19 h.

apparent internal pH (from 7.4 to 7.1). After 23 minutes perfusion with BHT was terminated and the same series of experiments as depicted in Fig. 1 was performed. There was no further change in the ^{31}P spectrum.

Similarly treatment of the rat heart with bisantrene (1 μg/ml, 20 ml/min, 60 min), a noncardiotoxic anthracene with antitumor activity (CL 216,942, Lederle

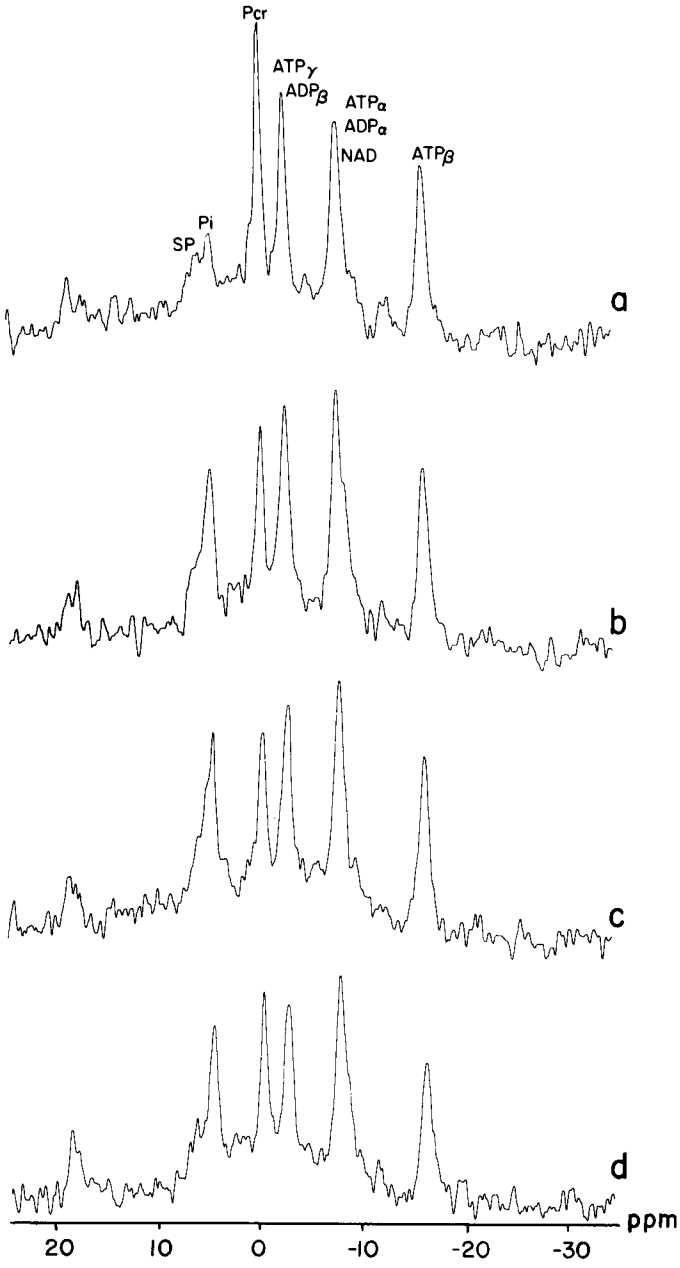

Figure 3: 80.96 MHz ^{31}P NMR spectra of a perfused rat heart (spectral parameters as in Fig. 1): (a) untreated control, pH = 7.1; (b) spectrum measured at 0.95-1.03 h after infusion of the drug was initiated (t = 0), pH = 7.1; treatment with bisantrene was terminated at t = 1.03 h and drug-free buffer was administered; (d) spectrum measured at t = 1.50-1.58 h, pH = 7.1.

Laboratories, Pearl River, NY), produced no sign of extensive deterioration of phosphate metabolism in the heart (Fig. 3). The drug concentration employed in

this study is about half the peak serum concentration in rats given 20 mg/kg, i.v. (47). Only a slight decrease in PCr and decrease in P_i accompanied administration of this analog.

These experiments suggest that ^{31}P NMR spectroscopy of perfused rat hearts may provide a very facile, rapid and relatively economical method for screening new agents for cardiotoxicity and for evaluating blocking agents, delivery systems, dosage schedules and other procedures for diminishing the cardiotoxic effects of ADR and other anthracyclines. Ultrastructural evaluation of cardiac tissue, evaluation of mechanical properties of the heart and chemical analysis of the perfusate and of cardiac tissue homogenates can be incorporated into the procedure.

These experiments have monitored the early response of hearts to ADR and therefore primarily reflect acute rather than chronic cardiotoxicity even though the latter effect is of primary clinical significance. However, it should be noted that significant incidence of cardiomyopathy and congestive heart failure has been reported in rats given only a slightly higher chronically administered cumulative dose of ADR (1 mg/kg/wk for 11-14 weeks) (48). Chronic toxicity could be studied by excising hearts from animals given chronic doses of the drug. However, such an approach is more time consuming and expensive. Although the exact relationship between chronic and acute cardiotoxicity is not yet understood, there are some indications of a correlation between these effects (49). Screening procedures employing acute exposure of mice (50) and rats (51) have been employed to evaluate anthracycline analogs.

Recent development of techniques for noninvasively monitoring high resolution NMR spectra of internal organs and tissues (52-57) suggests that it may soon be possible to monitor the in vivo spectrum of the hearts of experimental animals and humans. Such an approach would be most appropriate for monitoring chronic exposure to these and other antineoplastic agents. This study illustrates the potential utility of such an approach to the noninvasive clinical monitoring of cardiotoxicity.

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