*Biochimica et Biophysica Acta* 929 (1987) 5–13
Elsevier



BBA 12021

# Effects of the anti-cancer drug adriamycin on the energy metabolism of rat heart as measured by in vivo $^{31}$P-NMR and implications for adriamycin-induced cardiotoxicity

K. Nicolay [a], W.P. Aue [d], J. Seelig [d], C.J.A. van Echteld [b], T.J.C. Ruigrok [c] and B. de Kruijff [a]

[a] *Institute of Molecular Biology and Medical Biotechnology, University of Utrecht,* [b] *Interuniversity Cardiology Institute, and* [c] *Department of Cardiology, University Hospital, Utrecht (The Netherlands) and* [d] *Department of Biophysical Chemistry, Biocenter, University of Basel, Basel (Switzerland)*

(Received 7 July 1986)
(Revised manuscript received 3 February 1987)

Key words: Adriamycin; NMR, $^{31}$P-; Cardiotoxicity; Energy Metabolism; (Rat heart)

**In vivo $^{31}$P-NMR was used to measure the effects of the anti-tumor drug adriamycin on the energy metabolism of rat heart. The exclusive acquisition of NMR signal from cardiac muscle was assured by positioning a solenoidal radio-frequency NMR coil around the heart. Appropriate control experiments verified that $^{31}$P-NMR spectra solely originated from this organ. Acute effects occurring shortly after adriamycin administration are expressed in $^{31}$P spectra as a dose-dependent decline in the cardiac levels of phosphocreatine, after which stabilization at a new steady-state level occurs. These acute effects of a single dose are complete in 30–60 min and no significant further changes take place within 150 min after drug introduction. Longer-term effects of single high doses and of multiple lower doses were measured up to a week after the initiation of treatment. It seemed that at a total dose of 20 mg/kg, drug-induced interference with cardiac energy metabolism was more pronounced than at the same dose in the acute phase. These $^{31}$P-NMR data demonstrate that adriamycin treatment is accompanied by a decrease of the cardiac phosphocreatine/ATP ratio which might be an expression of the well-established cardiotoxicity of the drug.**

## Introduction

The anthracycline antibiotic adriamycin is active against a wide variety of human and experimental tumors [1]. The major side-effect of clinical relevance is the development of a cumulative, dose-dependent cardiotoxicity [2], thereby limiting the total dose that can be delivered safely. There is increasing evidence that adriamycin-mitochondria interactions play a prominent role in the development of this drug-induced cardiomyopathy (for a review see Ref. 3). The function of isolated heart mitochondria in suspension is markedly inhibited upon incubation with adriamycin [3]. A number of mechanisms by which the drug interferes with mitochondrial energy production have emerged from various studies. Thus, the drug inhibits electron transport and oxidative phosphorylation [4,5]. Recently, we have presented evidence suggesting that the inhibition of these functions arises from specific binding of adriamycin to the phospholipid cardiolipin [5,6], which is involved in a number of crucial functions catalyzed by the inner mitochondrial membrane (for a review see Ref. 7).

Correspondence: K. Nicolay, Institute of Molecular Biology and Medical Biotechnology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

Furthermore, it has been shown that activation of adriamycin through respiratory chain activity which requires binding of the drug to the inner mitochondrial membrane leads to the initiation of severe lipid peroxidation in suspensions of isolated heart mitochondria [8,9]. A third mechanism suggested by in vitro studies involves dissociation of the heart mitochondrial isozyme of creatine kinase from the outer surface of the inner mitochondrial membrane [10,11]. It is important to note that pronounced inhibition of respiration and coupled ATP synthesis [12] as well as severe peroxidative damage [13] have also been demonstrated for mitochondria isolated from the heart of adriamycin-treated animals. Moreover, using cytofluorescence techniques we have recently demonstrated that significant binding of adriamycin to mitochondria occurs in the isolated, perfused rat heart [14] under conditions where its energy metabolism is markedly impaired, as evidence by $^{31}$P-NMR measurements (see also Ref. 15). The above observations suggest that inhibition of cardiac high-energy phosphate production through direct adriamycin-mitochondria interactions might play an important role in the drug-induced depression of heart function.

The objective of the present study was to obtain a first insight into the possible influence of adriamycin on energy metabolism of rat heart in vivo, as measured by $^{31}$P-NMR in the living animal. To this aim a solenoidal radio-frequency coil was positioned around the heart of mechanically ventilated rats, thereby ensuring the acquisition of NMR signal from the heart tissue exclusively. Apart from the effects of a single intravenous injection with adriamycin, the effects of multiple doses were also monitored. These preliminary measurements suggest that adriamycin administration leads to a dose-dependent decrease of the cardiac phosphocreatine/ATP concentration ratio.

## Materials and Methods

Adriamycin (NSC 123127) was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The purity of the drug was routinely checked by thin-layer chromatography a described previously [5]. Drug samples for injection were prepared by dissolving appropriate amounts of adriamycin in 0.9% NaCl immediately prior to use. Injection volumes of 2 ml/kg were used, delivered via the tail vein. In the experiments aimed at measuring the acute effects of a single dose of adriamycin, the drug solution was injected inside the magnet through a non-magnetic cannula which had been inserted into the tail vein immediately after anaesthesia (see below). Control animals received an equal volume of isotonic saline.

Male Wistar rats (300–350 g) with unlimited access to water and food were used.

Animals were anaesthetized by intraperitoneal injection with sodium pentobarbital (50 mg/kg) and, subsequently, placed on a platform warmed to 40 °C with a circulating water bath by which body temperature was maintained at $37 \pm 1$ °C. After tracheotomy, animals were mechanically ventilated with a $N_2O/O_2$ (2 : 1) mixture by means of a baby lung ventilator (Hoek Loos, Amsterdam). The respiratory rate was set at 50/min while inspiration and expiration times were made equally long.

Access to the heart was gained by a mid-sternal incision after which the ribs were tied back away from the heart, and the pericardial sac was removed. Subsequently, the solenoidal NMR receiver coil was positioned around the heart. Anaesthesia, tracheotomy and thorax surgery usually took in the order of three quarters of an hour, after which the animal was immediately transferred to the magnet.

The solenoidal NMR coil consisted of 5 turns of silver plated high purity copper wire (diameter, 0.8 mm) covered with Teflon tubing. The entire coil was wrapped with Teflon tape. Coil dimensions were: inner diameter, 2.0 cm; height, 1.0 cm. The coil leads (length approx. 2 cm) were soldered to a box containing the tuning and matching capacitors (Johansson type 5761). The quality factor Q of the coil amounted to approx. 200 in air; in the living rat it dropped to about 100. These values compare favorably with literature data [16].

$^{31}$P-NMR experiments were carried out at 32.4 MHz on a Bruker BNT-80 spectrometer which was interfaced to an Oxford Instruments magnet (field strength, 1.9 T) having a horizontal clear bore of 30 cm. Quadrature phase detection was

employed. The 90° pulse was 30 μs, as determined on the heart in vivo. Usually, spectra of 300 scans were acquired with a 5 kHz spectral width and pulse angles of approx. 45°. A recycle time equal to the rate of ventilation (1.2 s) was found to yield optimal resolution. The magnetic field was optimized by using the $^1$H-NMR resonance (at 80.05 MHz) of water from the heart. Shimming appeared to be most reproducible and optimal when the thorax was filled with 2 ml 0.9% NaCl (at 37°C) fully immersing the heart. This phenomenon of magnetic susceptibility matching [17] has also been reported to improve the resolution in $^{31}$P-NMR spectra of isolated, prefused hearts [18]. The line width of the $H_2O$ signal was generally in the range of 40–60 Hz.

Heart rate was routinely measured throughout the entire experiment. Blood gas analysis was performed on samples drawn from the heart after terminating the NMR experiments. Subsequently, animals were killed by the intracardial injection of 1 ml of Triotal (200 mg sodium pentobarbital/ml).

$^{31}$P-NMR spectra of whole rat blood, drawn freshly from an anaesthetized rat, were obtained at 121.5 MHz on a Bruker CXP-300 spectrometer. Routinely, 250 scans were accumulated in 20 min (recycle delay, 5 s). Probe temperature was maintained at 25°C.

Relative concentrations of phosphorylated metabolites in the $^{31}$P-NMR spectra were determined by computer simulation of the experimental spectra. The difference between these raw spectra and the computer-generated spectra was minimized via an iterative procedure which employs Simplex routines.

Where appropriate, the collected data were analyzed by means of a parametric two-tailed Student's *t*-test employing the mean difference between the treated and control group.

The combination of pulse angles and pulse repetition times used here leads to partial saturation of the resonances. Since the spin-lattice relaxation times, $T_1$, differ for phosphocreatine and ATP, their peak areas were corrected for saturation by comparison with spectra obtained under fully-relaxed conditions (recycle delay, 10 s) for each animal. It appeared that, on the average, phosphocreatine and ATP amounted to 69% and 81% of the full intensity.

## Results and Discussion

### *$^{31}$P-NMR of heart in control rats*

A prerequisite for performing in vivo studies is obviously the maintenance of stable physiological conditions throughout the duration of the experiment. This criterion was satisfied in this study as evidenced by the measurement of heart rate, blood gas patterns and body temperature. The above parameters remained normal and stable during the longest NMR experiments (approx. 6 h). Moreover, $^{31}$P-NMR spectra of the heart of control rats did not show detectable changes during this time (data not shown).

As can be seen in one of the control spectra of Fig. 1 (e.g., in the absence of adriamycin), the $^{31}$P-NMR spectrum of rat heart is dominated by

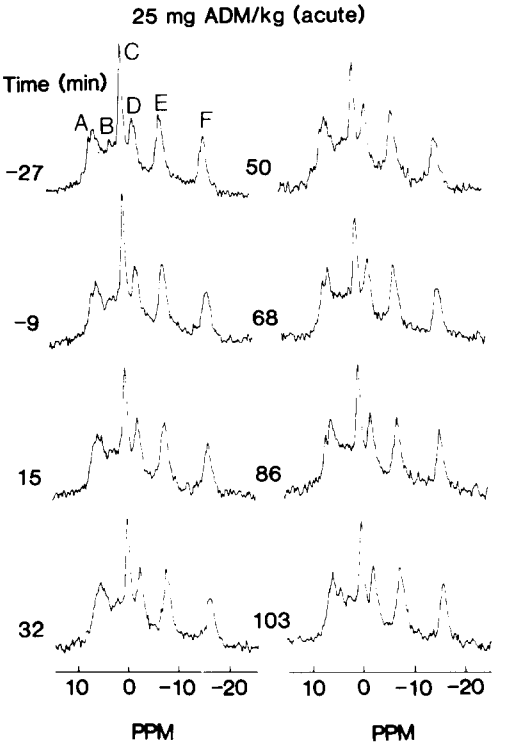


Fig. 1. Acute effects of adriamycin on 32.4 MHz $^{31}$P-NMR spectra of rat heart in vivo. Spectra (600 scans each) were accumulated at the indicated times before and after intravenous injection of 25 mg adriamycin/kg at time zero. Peak assignments are: A, phosphomonoesters, including inorganic phosphate and 2,3-diphosphoglycerate; B, phosphodiesters; C, phosphocreatine; D, γ-ATP and β-ADP; E, α-ATP, α-ADP and NAD; F, β-ATP.

resonances originating from phosphocreatine, ATP and 2,3-diphosphoglycerate. The latter compound resides in blood present inside the heart. By taking $^{31}$P-NMR spectra of blood freshly drawn from the heart of a rat and comparing the relative intensities of the 2,3-diphosphoglycerate and ATP resonances in these whole-blood spectra with those in heart spectra, it could be demonstrated that blood accounts for maximally 5% of the ATP resonance in the heart spectrum, in agreement with data in the literature [19]. Phosphocreatine exclusively originates from the tissue of interest, i.e., heart muscle (see below). Consequently, phosphocreatine/ATP ratios determined from heart spectra are representative of myocardial tissue.

It is clear that solid conclusions concerning adriamycin-induced effects on heart muscle studied by $^{31}$P-NMR can only be drawn if the experimental set-up used here does not detect significant amounts of non-cardiac skeletal muscle (e.g., from chest wall or diaphragm). A number of arguments exclude significant contamination of the heart spectra with spurious signals from non-cardiac origin. First, the ratio of phosphocreatine to ATP was found to be $2.2 \pm 0.1$ ($n = 5$) in control hearts. This value is consistently lower than that seen in resting skeletal muscle. Moreover, it correlates satisfactorily with our $^{31}$P-NMR data (Van Echteld, C.J.A. and Nicolay, K., unpublished data) obtained on isolated perfused rat hearts (phosphocreatine/ATP $= 2.00 \pm 0.07$ ($n = 4$)) as well as with those of Bailey et al. [20] for glucose-perfused rat hearts (phosphocreatine/ATP $= 1.99 \pm 0.19$) and those reported by Jalles et al. [21] for rat myocardium (phosphocreatine/ATP $= 2.0$) in vivo. Secondly, the selectivity of the in vivo receiver coil was assessed experimentally. $^{31}$P-NMR spectra were acquired with the coil positioned normally but after removal of the heart from the animal. No appreciable phosphorus signal, which, due to death of the animal might be expected to originate predominantly from inorganic phosphate, was observed under these conditions (not shown). This indicates that tissue peripheral to the coil does not contribute significantly to the in vivo spectra. Thirdly, the rate of phosphocreatine depletion was measured following respiratory arrest. To this aim, $^{31}$P-NMR spectra were collected before and after turning off the ventilator and clamping the ventilator tubes. Phosphocreatine fell to 50% of its initial value in 5–10 min, which is within the range of values published by others for cardiac muscle phosphocreatine under comparable conditions [21,22]. Skeletal muscle phosphocreatine is known to be considerably less rapidly utilized following death of the animal. [21]. The above observations lead us to conclude that the present coil set-up enables the essentially exclusive acquisition of $^{31}$P-NMR signal from rat heart.

Spectra with sufficient signal-to-noise ratio could be obtained in the order of 5 min. This enables dynamic, acute changes occurring upon injection with adriamycin to be monitored with sufficient time-resolution, as will be discussed next.

*Acute effects of a single dose*

Fig. 1 shows a typical series of in vivo $^{31}$P-NMR spectra of rat heart taken before and after the intravenous injection of 25 mg adriamycin/kg.

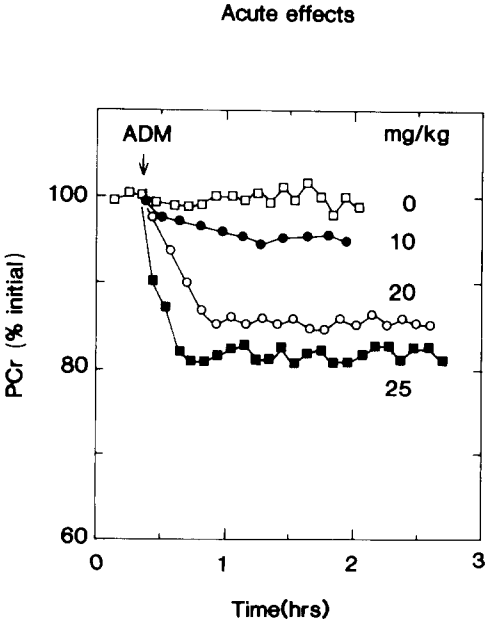


Fig. 2. Dose and time dependence of acute effects of adriamycin (ADM) on phosphocreatine (PCr) levels of rat heart, as determined from in vivo $^{31}$P-NMR spectra. Relative phosphocreatine concentrations are plotted as the percentage of the initial value, present before drug administration. Areas of the phosphocreatine resonances were determined by computer simulation of the $^{31}$P spectra. For simplicity, data points for only one animal per dose are shown.

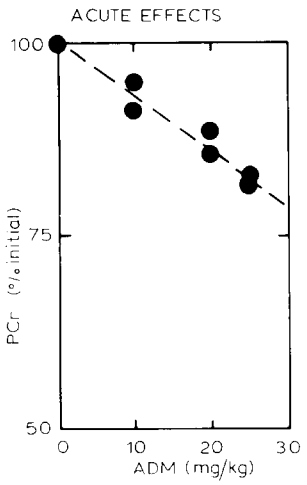


Fig. 3. Steady-state levels of phosphocreatine (PCr) reached after a single injection, as a function of dose. The average phosphocreatine content (relative to the control value) was determined from a series of spectra acquired during the period in which it had stabilized at the lower level (see Fig. 2). Each separate point represents the result for one individual animal. ADM, adriamycin.

Quantitative evaluation of the spectra reveals that only phosphocreatine levels experience slight, but significant changes (see below) upon drug administration. ATP concentrations and heart rate remained unchanged within experimental error. The latter observation is in line with that of Zbinden et al. [23] who reported that heart rate remained virtually constant in adriamycin-treated rats, except for in very late stages when the appearance of bradycardia heralded imminent death of the animal.

Fig. 2 is a compilation of the time dependence of the cardiac phosphocreatine concentration before and after injection of various doses of adriamycin. Acute effects show up as a dose-dependent decrease in cardiac phosphocreatine concentration which is complete in approx. 30–60 min. Thereafter, phosphocreatine peak intensities remained stable at the lower level for at least 2.5 h which was the longest time tested in these acute experiments. It is interesting to note, that the time dependence of the adriamycin-induced decrease in heart phosphocreatine levels is similar to the time-dependence of drug clearance from plasma of rats after an intravenous bolus injection of comparable doses [24,25]. Half-times of plasma clearance and uptake into the various tissues, including heart, were reported to be 15–20 min. Since metabolism of adriamycin is slow and very limited in the rat [26], it is likely that the interference with heart energy metabolism is due to direct, acute effects of adriamycin itself.

Fig. 3 demonstrates that the steady-state levels of phosphocreatine reached after adriamycin administration in the present acute experiments depend linearly on the magnitude of the injected dose. At 25 mg/kg, the maximal dose used, phosphocreatine dropped to $82 \pm 1\%$ of its original intensity. An evaluation of the statistical significance of the change in the mean phosphocreatine level reached for each dose, as compared to the mean phosphocreatine level in control animals, showed that the drug-induced effects are statistically significant ($P < 0.5\%$ at 10 mg/kg; $P < 0.1\%$ at 20 and 25 mg/kg). Effects on cardiac phosphocreatine of a similar magnitude as those described here have been reported by Tamatsu et al. [27] upon administration of single, high doses of 5-fluorouracil to guinea pigs, as measured by $^{31}P$ topical NMR.

It is interesting to note that a comparison of our data with those obtained by Ng et al. [15] on Langendorf perfused hearts suggests that the acute effects exerted by similar doses of adriamycin are more pronounced in vitro than in vivo. These authors showed that butylated hydroxytoluene, an anti-oxidant, minimizes the spectral changes induced by adriamycin.

In summary, acute effects of adriamycin are reflected in the $^{31}P$-NMR spectra of rat heart in vivo as a significant, dose-dependent decline in phosphocreatine levels. Hence, a single injection with adriamycin significantly decreases the energy-buffering capacity of the heart muscle.

*Semi-acute effects of single and multiple doses*

Apart from measuring the effects of adriamycin on the concentrations of high-energy phosphates in the heart immediately after injection of a single dose, longer term effects of a single, relatively high dose and of multiple lower doses were examined. The total doses used here (10–25 mg adriamycin/kg) are chosen such as to cover the range of cumulative doses (15–20 mg/kg) reported to produce signs of cardiotoxicity in rats [23]. Due to the

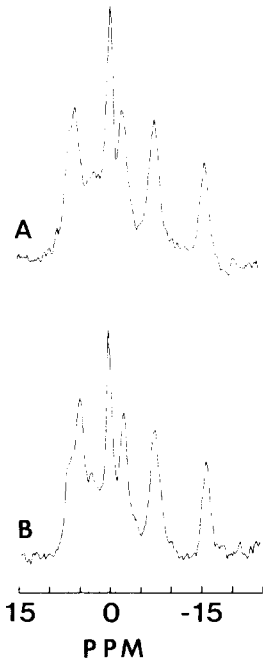


Fig. 4. Semi-acute effects of a single dose of 20 mg adriamycin/kg on in vivo $^{31}$P-NMR spectra of rat heart. Note that spectra (900 scans each) of individual animals are shown. A, spectrum obtained 2 days after injection; B, 4 days after injection.

surgery necessary to expose the heart, the development with time of adriamycin-induced effects on heart occurring over periods of days cannot be measured on a single animal but requires separate animals to be taken per time point.

Thus, Fig. 4 displays $^{31}$P spectra of the heart of two different animals which had both received a single injection of 20 mg adriamycin/kg on day 0 and were examined by NMR on day 2 (Fig. 4A) and day 4 (Fig. 4B), respectively. From a quantitative evaluation of the spectra (Table I), it appeared that at day 4 the adriamycin-induced effects on the cardiac phosphocreatine/ATP ratio were larger than the acute effects of the same dose (i.e., 20 mg/kg) detailed above. There is no significant decrease in the phosphocreatine/ATP ratio at a dose of 10 mg/kg (Table I) when compared to the control phosphocreatine/ATP ratio of $2.2 \pm 0.1$.

Next, we measured the effects on cardiac energy metabolism of multiple, lower doses of

TABLE I

SEMI-ACUTE EFFECTS OF A SINGLE HIGH DOSE OF ADRIAMYCIN ON THE CARDIAC PHOSPHOCREATINE/ATP RATIO

Rats were injected with a single dose of either 10 or 20 mg adriamycin/kg at day 0. At day 2, 3 and 4, two rats in each group were taken for $^{31}$P-NMR. Typically, ten $^{31}$P-NMR spectra were measured (300 scans each) for each animal. Computer simulation of this series of spectra enabled the determination of the (mean) phosphocreatine/ATP ratio for each individual animal. S.E. in this mean phosphocreatine/ATP ratio (per animal) ranged from 0.08 to 0.18. The phosphocreatine/ATP ratios given in the table represent mean ± S.E. for two animals.

| Day | Phosphocreatine/ATP | |
|---|---|---|
| | 10 mg/kg | 20 mg/kg |
| 2 | $2.15 \pm 0.20$ | $2.05 \pm 0.16$ |
| 3 | $2.08 \pm 0.17$ | $1.81 \pm 0.19$ |
| 4 | $1.89 \pm 0.23$ | $1.65 \pm 0.15$ |

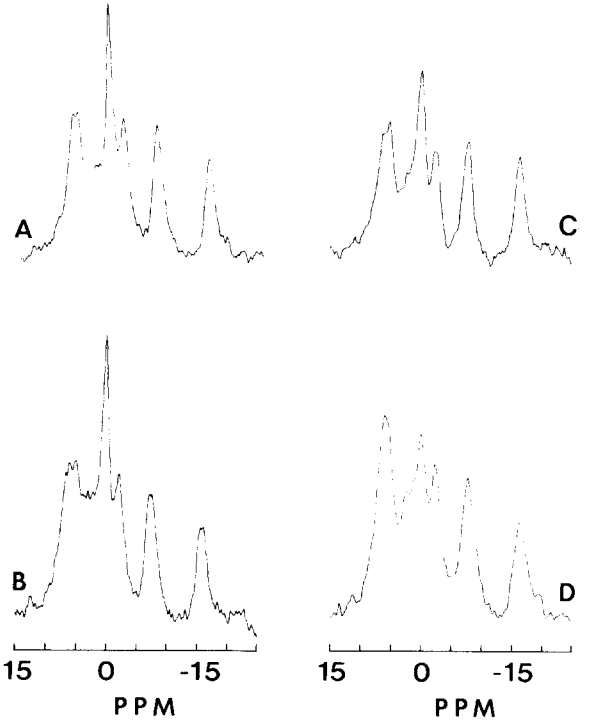


Fig. 5. Semi-acute effects of five injections of 2 mg adriamycin/kg (A, B) and 4 mg adriamycine/kg (C, D) on $^{31}$P-NMR spectra of in vivo rat heart. Injections were given daily on 5 consecutive days. Spectra (900 scans each) of individual animals are displayed. Spectra A and C were obtained on day 5 after the first injection, while spectra B and D were collected on day 7.

adriamycin. One group of rats received five injections of 2 mg adriamycin/kg on 5 consecutive days and were examined on day 5, 6 and 7 after starting the treatment at day 0. Another group was treated and measured according to the same protocol, except that five 4 mg/kg injections were given. Two animals were examined by $^{31}$P-NMR per time point in each group. Fig. 5 shows $^{31}$P-NMR spectra obtained from the heart of two animals from each group at day 5 (Fig. 5A, C) and at day 7 (Fig. 5B, D). A quantitative evaluation of the phosphocreatine/ATP ratio in spectra of all animals examined by NMR is presented in Table II. From these data it appears that: (i) there are pronounced effects of adriamycin on cardiac energy status in the 4 mg/kg group only; (ii) prolonged exposure to the drug results in a gradual decrease in the phosphocreatine/ATP concentration ratio in the 4 mg/kg group.

Klarasch and Novak [28] have also reported adriamycin induced alterations in the energy metabolism of rat heart. These authors examined perchloric acid extracts of freeze-clamped rat heart tissue after a single dose of 15 mg/kg by $^{31}$P-NMR. It was found that phosphocreatine and ATP significantly decreased at day 4 post adriamycin and declined to 55% and 45% of controls, respectively, at day 12. Although these data seem to differ from our results in quantitative terms, they confirm the significant effects on high-energy phosphates described here.

To our knowledge, there is only one other report on in vivo effects of adriamycin on cardiac energy metabolism describing preliminary observations made by Nunnally, R.L. (quoted in Ref. 29) applying $^{31}$P topical NMR to rabbits. In that study, the animals were treated with adriamycin on 5 consecutive days, and monitored daily by NMR. The major effects which were evident within 4 days of treatment were a severe broadening of the spectral lines and a marked reduction of the myocardial phosphorus levels. The phenomenon of line broadening was tentatively ascribed to the generation of free radical species by adriamycin. No substantial changes in the relative concentrations of phosphocreatine and ATP were evident from the $^{31}$P-NMR spectra, in contrast to our findings in the rat. In addition, no consistent line-broadening effects were evident under our experimental conditions.

TABLE II

SEMI-ACUTE EFFECTS OF MULTIPLE INJECTIONS OF ADRIAMYCIN ON THE CARDIAC PHOSPHOCREATINE/ATP RATIO

Rats were injected with either 2 or 4 mg adriamycin/kg on day 0, 1, 2, 3 and 4. At day 5, 6 and 7, two rats in each treatment group were taken for $^{31}$P-NMR. For further details see legend to Table I.

| Day | Phosphocreatine/ATP | |
|---|---|---|
| | 5·2 mg/kg | 5·4 mg/kg |
| 5 | 1.91 ± 0.18 | 1.46 ± 0.19 |
| 6 | 1.70 ± 0.23 | 1.35 ± 0.28 |
| 7 | 1.83 ± 0.20 | 1.14 ± 0.34 |

*Mechanism of adriamycin-induced interference with cardiac energy metabolism*

Before discussing the possible implications of the present data, it is important to mention that the rats treated with a single large dose (20 mg/kg) and those treated with five lower doses (in particular the 4 mg/kg group) produced signs of general toxicity such as anorexia and diarrhea within a few days. This might partly explain the body weight loss which was observed, in agreement with observations made by others [30]. As a consequence, the effects on cardiac energy status as measured by $^{31}$P-NMR might be a combination of specific cardiotoxic effects of adriamycine and of nonspecific effects which cause deterioration of the overall condition of the animal. The interpretation of the acute effects seen immediately after drug administration (Figs. 1–3) most probably is not complicated by the above consideration.

The present study demonstrates that the major consequence of adriamycin administration for the energy metabolism of rat heart in vivo is a dose-dependent decrease in the cardiac phosphocreatine/ATP ratio. It is important to note that the rat may be limited as a model for the chronic cardiotoxicity induced by adriamycin in humans. For that reason, the clinical significance of the present findings cannot be defined.

The exact mechanism by which adriamycin causes an acute depression of cardiac phosphocreatine in vivo (Figs. 1–3) is not known. Acute decreases in cardiac output, expressed in

hemodynamic parameters like left-ventricular pressure and heart-minute volume, have been reported to occur in rats [31]. These changes, however, may not be expected to be the cause of decreased phosphocreatine levels. Especially since it has been demonstrated by in vivo $^{31}$P-NMR in rats that, rather, increased cardiac work, induced by infusion of catecholamines, leads to a transient depression of phosphocreatine to 80% of control in 15–20 min, after which phosphocreatine begins to rise again, reaching control values in approx. 30 min [21]. Therefore, we favor the view that adriamycin directly decreases the phosphocreatine content of the heart, thereby eventually depressing cardiac output, instead of indirectly affecting phosphocreatine levels through initiation of changes in output.

It might well be that the early effects of adriamycin on cardiac energy metabolism are the manifestation of direct drug-mitochondria interactions. Heart mitochondria are known to be affected by adriamycin in an early stage of treatment [3,23,30]. Thus, Zbinden et al. [23] showed that prolongation of the duration of ventricular depolarization (i.e., the QRS complex) in the electrocardiogram was the earliest and most consistent change observed in rats after treatment with adriamycin. This change in the electrocardiogram coincided with deterioration of heart mitochondrial function and with morphologic alterations involving, in particular, the mitochondria. Porta and colleagues [30] studied hearts of rats acutely treated with adriamycin by electron microscopy and also found that the earliest ultrastructural changes were seen at the level of the cardiac mitochondria. Furthermore, we have recently shown by cytofluorescence techniques that adriamycin becomes associated with mitochondria in the isolated, Langendorf-perfused rat heart [14] under conditions where the phosphocreatine levels of the isolated organ are significantly affected as measured by $^{31}$P-NMR (Van Echteld, C.J.A. and Nicolay, K., unpublished data; see also Ref. 15). As pointed out in the Introduction, acute and direct adriamycin-mitochondria interactions might be expected to result in a depressed capacity to synthesize ATP via inhibition of respiration and coupled ATP production [4,12]. The action of creatine kinase would then lead to ATP homeostasis and decreased phosphocreatine levels [32], in line with the acute effects observed in this study. Another factor which possibly contributes is the dissociation by adriamycin of the inner mitochondrial membrane-associated isozyme of creatine kinase [10,11] which might result in a reduced efficiency of phosphocreatine synthesis coupled to ATP production [32].

At the higher doses tested, the longer-term effects of adriamycin are more pronounced than the acute effects. Although a mechanistic interpretation of this observation is clearly preliminary, a progressive inhibition of mitochondrial energy production due to adriamycin-induced lipid peroxidation [8,9,13] might well contribute to the pronounced effects of adriamycin on cardiac energy status seen at longer times after the initiation of treatment.

The present preliminary study suggests that in vivo $^{31}$P-NMR is sensitive to adriamycin-induced cardiomyopathy. Future work on the potential of the technique to serve as a diagnostic tool for monitoring heart status on a regular basis during adriamycin therapy will have to concentrate on two aspects. First, extensive studies comparing the NMR data with independent methods to assess cardiac function and heart cell morphology will have to be conducted. Secondly, NMR technology will have to be further developed to enable a fully non-invasive and non-destructive measurement of high-resolution NMR spectra of heart in the living subject. Recent reports by Aue et al. [33] and Bottomley et al. [34,35] employing magnetic field gradients to define the sensitive volume detected by NMR make it feasible that high-quality, localized spectra of deeply buried regions can be routinely obtained in the near future, thereby fully circumventing the surgery used in this study to position the NMR receiver coil around the heart.

**Acknowledgements**

The generous financial support by the Netherlands Cancer Foundation (Koningin Wilhelmina Fonds) which made this study possible is gratefully acknowledged. We are indebted to Dirk de Moes from the Department of Cardiology of the University Hospital of Utrecht for his essential support in training handling the animals. We are

grateful to Klaas Dijkstra (Department of Physical Chemistry, University of Groningen) for giving access to the spectrum simulation routines.

## References


1 Young, R.C., Ozols, R.F. and Myers, C.E. (1981) N. Engl. J. Med. 305, 139–153
2 Ferrans, V.J. (1978) Cancer Treat. Rep. 62, 955–961
3 Goormaghtigh, E. and Ruysschaert, J.M. (1984) Biochim. Biophys. Acta 779, 271–288
4 Muhammed, H., Ramasarma, T. and Kurup, C.K.R. (1982) Biochim. Biophys. Acta 722, 43–50
5 Nicolay, K., Timmers, R.J.M., Spoelstra, E., Van der Neut, R., Fok, J.J., Huigen, Y.M., Verkleij, A.J. and De Kruijff, B. (1984) Biochim. Biophys. Acta 778, 359–371
6 Nicolay, K., Van der Neut, R., Fok, J.J. and De Kruijff, B. (1985) Biochim. Biophys. Acta 819, 55–65
7 Daum, G. (1985) Biochim. Biophys. Acta 822, 1–42
8 Demant, E.J.F. and Jensen, P.K. (1983) Eur. J. Biochem. 132, 551–556
9 Demant, E.J.F. (1983) Eur. J. Biochem. 137, 113–118
10 Newman, R.A., Hacker, M.P. and Fagan, M.A. (1982) Biochem. Pharmacol. 31, 109–111
11 Müller, M., Moser, R., Cheneval, D. and Carafoli, E. (1985) J. Biol. Chem. 260, 3839–3843
12 Bachmann, E., Weber, E. and Zbinden, G. (1975) Agents Actions 4, 383–393
13 Praet, M., Pollakis, G., Goormaghtigh, E. and Ruysschaert, J.M. (1984) Cancer Lett. 25, 89–96
14 Nicolay, K., Fok, J.J., Voorhout, W., Post, J.A. and De Kruijff, B. (1986) Biochim. Biophys. Acta 887, 35–41
15 Ng, T.C., Daugherty, J.P., Evanochko, W.T., Digerness, S.B., Durant, J.R. and Glickson, J.D. (1983) Biochem. Biophys. Res. Commun. 110, 339–347
16 Murphy-Boesch, J. and Koretsky, A.P. (1983) J. Magn. Reson. 54, 526–532
17 Stoll, M.E. and Majors, T.J. (1982) J. Magn. Res. 46, 283–288
18 Ingwall, J.S. (1982) Am. J. Physiol. 242, H729–H744
19 Kantor, H.L., Briggs, R.W. and Balaban, R.S. (1984) Circ. Res. 55, 261–266
20 Bailey, I.A., Radda, G.K., Seymour, A.-M. and Williams, S.R. (1982) Biochim. Biophys. Acta 720, 17–27
21 Jalles, N., Koretsky, A.P., Ebert, C., Karczmar, G. and Weiner, M.W. (1985) Proceedings of the 4th Annual Meeting of the Society of Magnetic Resonance Medicine, pp. 485–486
22 Grove, T.H., Ackermann, J.J.H., Radda, G.K. and Bore, P.J. (1980) Proc. Natl. Acad. Sci. USA 77, 299–302
23 Zbinden, G., Bachmann, E. and Holderegger, C. (1978) Fundament. Cancer Chemother. Antibiotics Chemother. 23, 255–270
24 Yesair, D.W., Schwartzbach, E., Schuck, D., Denine, E.P. and Asbell, M.A. (1972) Cancer Res. 32, 1177–1183
25 Wilkinson, P.M. and Mawer, G.E. (1974) Br. J. Clin. Pharmacol. 1, 241–247
26 Tavaloni, N. and Guarino, A.M. (1980) Pharmacology 21, 244–255
27 Tamatsu, H., Nakazawa, M., Imai, S. and Watari, H. (1984) Japan. J. Pharmacol. 34, 375–379
28 Kharasch, E.D. and Novak, R.F. (1983) Fed. Proc. 42, 259
29 Carswell, H. (1982) J. Am. Med. Assoc. 248, 814
30 Porta, E.A., Joun, N.S., Matsumura, L., Nakasone, B. and Sablan, H. (1983) Res. Commun. Chem. Path. Pharmacol. 41, 125–137
31 Höfling, B., Mendler, N., Heimisch, W., Böning, L., Kolb, H.J. and Bolte, H.-D. (1981) Beitr. Onkol. 9, 49–63
32 Jacobus, W.E. (1985) Ann. Rev. Physiol. 47, 707–725
33 Aue, W.P., Müller, S., Cross, T.A. and Seelig, J. (1984) J. Magn. Reson. 56, 350–354
34 Bottomley, P.A. (1985) Science 229, 769–772
35 Bottomley, P.A., Herfkens, R.J., Smith, L.S., Brazzamano, S., Blinder, R., Hedlund, L.W., Swain, J.L. and Redington, R.W. (1985) Proc. Natl. Acad. Sci. USA 82, 8747–8751