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Short communication

Myocardial contractility and heart pharmacokinetics of adriamycin following a single administration in rat*

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Summary. A single administration of adriamycin (DXR) 6.0 mg/kg i. v. to rats brings about a biphasic impairment of the maximal myocardial contractile performance, measured as dF/dt of ex vivo isolated atria incubated in the presence of calcium concentrations varying up to 12 m*M*. The initial impairment of the contractile performance peaks 1 week after DXR administration and recovers within 3 weeks (acute phase of cardiotoxicity). After this time and up to the end of the observation period (8 weeks after treatment), delayed cardiotoxicity occurs, showing a progressive and irreversible impairment of the contractile performance of the atria. This behaviour parallels the previously shown ECG and morphological abnormalities. Tissue determinations of DXR showed that the drug is present in myocardium during the acute phase of cardiotoxicity, while the metabolite adriamycinol is not detectable 1 week after DXR administration. These data show that the presence of DXR and/or metabolites in heart muscle is not necessary for the delayed form of cardiotoxicity to become apparent and suggest that this form of cardiotoxicity is related to a mechanism different from that involved in acute cardiotoxicity.

Introduction

Adriamycin (DXR) has been shown to induce in isolated guinea pig atria a negative inotropic effect related to a decrease of cell calcium exchangeability [13], whereby the fast-exchanging compartment is mainly involved [7] in a non-competitive fashion and at sites different from the Ca slow-channels [8, 12, 14]. This form of cardiotoxicity appears to be quite different from that occurring in humans; in fact, human cardiotoxicity consists in a transient impairment of the myocardial contractility and in a late-developing cardiomyopathy which becomes apparent when a cumulative dose higher than 550 mg/m^2 is administered. An experimental model which might be similar to clinical cardiotoxicity was described in rat. It was shown that a single or divided i. v. dose of DXR brings about ECG changes during 1–2 weeks (acute phase), followed by an almost complete recovery; finally, a late-developing cardiotoxic manifestation occurs, worsening up to the end of the observation period of 8 weeks [15]. At this time, morphological alterations are also present.

The aim of this investigation was to observe whether DXR produces modifications of the contractile properties of the heart muscle similar to those described for ECG and morphology, and to study the relationship between the functional impairment and the presence of the drug in myocardial tissue. Attention has been devoted to the presence of metabolites, since some authors [11] have claimed that adriamycinol is more probably involved in the acute cardiotoxicity than the parent compound.

Methods

Female Charles River Sprague-Dawley rats of 120 g body weight (initial value) were used. Groups of six animals received a single i. v. administration of 6.0 mg/kg DXR and were killed 2 days, and 1, 2, 3, 4, 6 and 8 weeks after treatment.

The hearts of survivors were isolated. Spontaneously beating atria were incubated in Tyrode's solution containing calcium concentrations ranging from 0.1 to 12 m*M* and the isometric contractile force was measured (dF/dt) according to the previously described experimental model [14]. The stepwise addition of calcium brings about a parallel increase of contractile force up to a maximum, which was taken as the maximal contractile performance of the atria. Tissue determinations of DXR and of its metabolites were carried out by repeated extractions of the homogenized tissue at room temperature with *n*-butanol/ethyl acetate 3:2 and HPLC analysis of the extract with a fluorometric detector in order to detect all molecules bearing an anthracycline backbone. This method was validated by determining DXR and adriamycinol after addition of a known amount of the pure compounds (kindly supplied by Farmitalia C. Erba, Milan) to the tissue homogenate; the results were confirmed by adding ^{14}C-DXR and comparing the results of the chemical and radiochemical determinations (limit of detection 0.5 ng, recovery 95%–98%). The quantitative determination of aglycones was also performed by a different method, based on the elution of a Sep-Pak C18 cartridge (Waters Ass.) with MeOH/H_3PO_4

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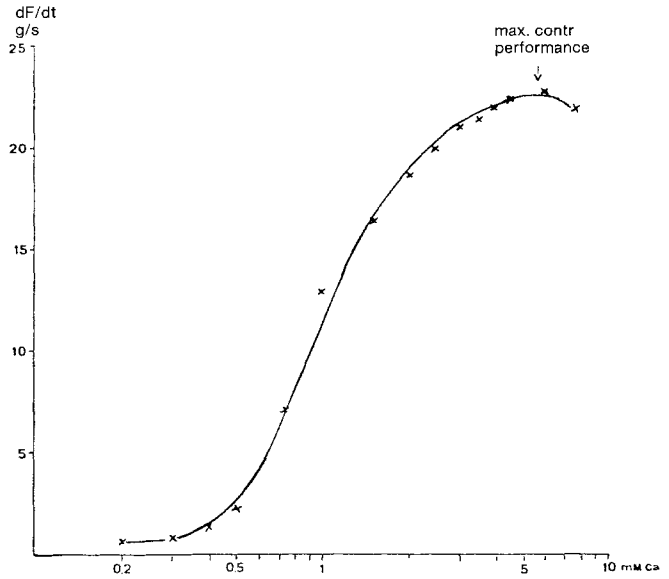

Fig. 1. Contractile force (dF/dt of the isometric tension) of spontaneously beating isolated rat atria incubated in the presence of different calcium concentrations

[1]. In rat heart obtained from animals treated i. v. at the mentioned dose, aglycones were occasionally found in trace amounts.

Results

The present experiments in isolated rat atria show that the maximal contractile performance is developed in the presence of 5–7 m*M* calcium concentrations (Fig. 1). This is in agreement with the results of previous investigations carried out in guinea pig atria [14].

Organs ex vivo isolated from animals treated i. v. with 6.0 mg/kg DXR show that the maximal contractile force is impaired by about 12% during the 1st week after treatment (acute phase of cardiotoxicity), while a recovery occurs within 3 weeks. A further decrease of this parameter occurs at later times, about −25% at the 6th week and even more, −40%, at the 8th week after DXR administration, when the experiment was stopped (Fig. 2). These values are significantly different from controls using Student's *t*-test.

The presence of DXR and its metabolites was evaluated in samples derived from the same hearts which were used for contractile measures. Adriamycinol is the only detectable metabolite; aglycones were detected only occasionally in trace amounts and were considered to be irrelevant in the development of cardiotoxic symptoms. Figure 2 shows that myocardial DXR peaks shortly after treatment and is present in a large amount when the contractile signs of acute cardiotoxicity are present. The drug level drops rapidly, reaching a very low value within 3 weeks, when the contractile force has recovered. DXR is not detectable at later times, when the delayed contractile impairment appears. Adriamycinol is present immediately after treatment and is no longer detectable 1 week later.

Discussion

The contractile performance developed by isolated atria in the presence of calcium concentrations beyond the physiological value was shown to undergo a dose-related impairment in acute experiments of DXR-induced cardiotoxicity in guinea pig [14]. The present investigations confirm this parameter as a valuable index of DXR cardiotoxicity also in rat: the maximal contractile force drops after a single i. v. DXR administration (acute cardiotoxicity), recovers within 3 weeks and undergoes a progressive, significant

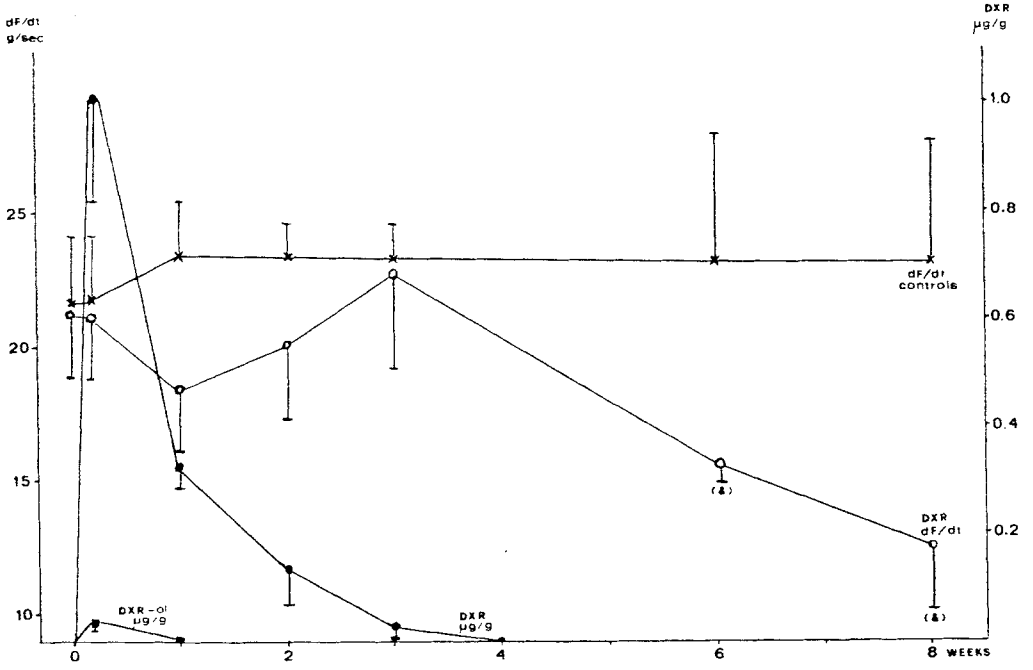

Fig. 2. Effect of a single dose of DXR (6.0 mg/kg i. v.) on the maximal cardiac isometric contractility of ex vivo isolated rat atria and on their DXR content. Contractile force (dF/dt) is given in g/s, DXR content in heart tissue as µg/g fresh tissue. Mean ± SE; (&) $P < 0.05$

and irreversible impairment several weeks later (delayed cardiotoxicity).

The time course of this functional parameter parallels that of ECG indexes measured in similar experiments of delayed cardiotoxicity in rat [15]. QαT and SαT duration were also found to be prolonged immediately after treatment, to recover partially within the following 3 weeks, and to increase again by the 6th week after DXR administration and even more by the 8th week; by this time, morphological alterations were also present in myocardial tissue. Hence the results of previous investigations and of the present study concur in demonstrating that DXR brings about an acute and reversible form of cardiotoxicity which is followed by a delayed, irreversible cardiotoxic pattern: this is apparent from the evaluation of morphological and ECG observations as well as from functional data.

As to the origin of the cardiotoxicity, the hypothesis was formulated that DXR and/or its metabolites act as electron carriers from electron-transferring enzymes to oxygen, thus promoting formation of oxygen free radicals [2, 3, 9, 10]. This might produce membrane lipoperoxidation, leading to an impairment of Ca transport by the subcellular structures involved in calcium homeostasis [5]. This is an attractive hypothesis, although the experimental data are still controversial; in fact, generation of $O_2^{\cdot-}$ and $OH^{\cdot}$ free radicals was demonstrated in simplified in vitro systems, while a study dealing with whole heart homogenate does not report exact figures for production of free radicals in this preparation [3], thus suggesting that rescue systems against oxygen toxicity exist in myocardial cells. Moreover, the involvement of free radicals in DXR cardiotoxicity was questioned by Jackson [6] on the basis of acute and chronic experiments, while lipoperoxidation by DXR was recently denied by Zidenberg-Cherr [16].

Anyway, the involvement of free radicals applies to the acute, reversible form of cardiotoxicity; however, due to the short biological life of free radicals and of the peroxides which are consequently produced, the mechanism could account for the delayed, irreversible cardiotoxicity only if the drugs producing oxygen free radicals, i. e. DXR and metabolites, were taken up and stored in the myocardial cells until the delayed morphofunctional cardiotoxic signs become apparent.

In our experiments DXR and adriamycinol were found in cardiac tissue during the acute phase of cardiotoxicity, but were no longer detectable in heart tissue several weeks before the ECG, functional and morphological signs of delayed cardiotoxicity became apparent. In particular, adriamycinol was shown to be far less persistent than DXR, contradicting the hypothesis that this metabolite is involved in cardiotoxicity to a greater degree than the parent compound [11].

Heart tissue was also assayed for the presence of aglycones, which might be of interest in the genesis of cardiotoxicity, since their lipophilic properties might allow better cell penetration than is the case with the more hydrophilic parent compounds. However, these derivatives were detected in only few of the samples and in very limited amounts: therefore, they were assumed to be uninvolved in the processes leading to cardiotoxicity.

Thus, the present data show that the presence of anthracyclines, either DXR or its metabolites, is not necessary for the delayed cardiotoxic effects to develop. This is at variance with the results of the acute experiments of Doroshow et al. [4], and suggests that the mechanism underlying the delayed DXR-induced pathology is probably different from that involved in the acute form.

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