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Review

Doxorubicin and mechanical performance of cardiac trabeculae after acute and chronic treatment: a review

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Abstract

Doxorubicin, a very potent and often used anti-cancer drug, has a wide spectrum of biological activity. Classic studies have demonstrated that doxorubicin and other members of the anthracycline family intercalate with DNA and partially uncoil the double-stranded helix. Doxorubicin has a high affinity for cell nuclei: as much as 60% of the total intracellular amount of doxorubicin is found in the nucleus. Once binding to DNA occurs, several consequences may ensue. The binding of anthracyclines to DNA inhibits DNA polymerase and nucleic acid synthesis. In addition, anthracyclines are known to stabilize the otherwise cleavable complex between DNA and homodimeric topoisomerase II enzyme subunits, resulting in the formation of protein-linked DNA double strand breaks. In tumor cells, these anthracycline-induced perturbations are believed to result in a final common pathway of endonucleolytic DNA fragmentation known as apoptosis. Because proliferation is an important determinant of tumor growth, interference with the genome is regarded as the primary cause of the anti-tumor action of doxorubicin. Intercalation with DNA may not be important in the cardiotoxicity associated with doxorubicin therapy (see next section), because cardiac cell proliferation in humans stops after 2 months of age. This review is focussed on the effects of doxorubicin on mechanical performance in skinned cardiac trabeculae after acute and chronic administration of doxorubicin. We look especially at the mechanical performance and the molecular changes observed and related to mechanical performance.

Keywords: Doxorubicin; Cardiac trabeculae; Cardiotoxicity; Transduction pathways

1. Cardiotoxicity of doxorubicin

The prolonged clinical use of doxorubicin is largely limited by the occurrence of a dose-related cardiomyopathy resulting in congestive heart failure (Doroshow, 1991). Both acute and chronic cardiovascular changes have been described in patients undergoing doxorubicin chemotherapy (Lefrak et al., 1973; Olson and Mushlin, 1990). Acute side-effects may develop within minutes after intravenous administration of the drug and include hypotension, tachycardia and various arrhythmias (Singal et al., 1987). These early effects are usually transient and clinically manageable, and do not form a major concern. However, life-threatening chronic effects may develop several weeks or months after repetitive doxorubicin administration. Cardiovascular signs indicative of chronic cardiotoxicity include hypotension, tachycardia, cardiac dilation and ventricular failure (Singal et al., 1987).

The incidence of doxorubicin-related cardiotoxicity increases sharply above a cumulative dose of 550 mg/m^2 body surface area, and once the dose administered exceeds this level the therapy is generally excluded from the chemotherapeutic regimen. Unfortunately, this implies that patients may be deprived of an effective agent in order to reduce the risk of cardiotoxic side-effects. Evaluation of the degree of cardiac damage in patients is crucial for further management, and can be done by both invasive and non-invasive techniques. Myocardial damage as a result of chronic doxorubicin treatment can be studied in vitro by endocardial biopsy techniques (Bristow et al., 1978). Histological evaluation reveals characteristic myocardial changes that have been evaluated in humans (Bristow et al., 1978) and in experimental animals (Bertazzoli et al.,

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1979). Alternatively, doxorubicin cardiotoxicity can be evaluated in vivo by measuring electrocardiogram (ECG) changes. For example, ST-segment widening and T-wave flattening have been reported following doxorubicin therapy (Cortes et al., 1975). In humans, the current mainstay of monitoring chronic doxorubicin cardiotoxicity is still, with all its limitations, the measurement of cardiac ejection fractions. The precise biochemical mechanism of doxorubicin cardiotoxicity remains the subject of debate.

2. Mechanisms of cardiotoxicity by doxorubicin

In the past years, several mechanisms have been proposed to account for the anthracycline-induced cardiotoxic side effects. The most popular ones will be discussed below.

2.1. Free radical hypothesis

There are two major pathways by which doxorubicin causes free radical formation (Olson and Mushlin, 1990). First, there is a range of flavin-centered, NADPH-dependent reductases capable of producing a one-electron reduction of anthracyclines to anthracycline semiquinone free radicals. Semiquinones are stable under anaerobic conditions, but can readily donate the extra electron to molecular oxygen under aerobic conditions, generating superoxide anion radicals. Second, anthracycline free radicals may arise via a non-enzymatic mechanism involving reactions of anthracyclines and iron. For example, iron(III) readily interacts with doxorubicin. This is followed by a redox reaction, wherein the iron atom accepts an electron, generating an iron(II)-doxorubicin free radical complex. This radical complex can easily reduce oxygen, thereby leading to the generation of oxygen free radicals. The principal reactive oxygen species are superoxide radicals and hydroxyl radicals which have the potential to initiate damage to various intracellular components, including nucleic acids, lipids and proteins (Cheeseman and Slater, 1993). The heart is particularly susceptible to free radical injury, because it contains less free radical detoxifying substances (superoxide dismutase, glutathion, and catalase) than do metabolic organs such as liver or kidney (Olson et al., 1981; Olson and Mushlin, 1990). Moreover, doxorubicin is known to have a high affinity for cardiolipin, a major phospholipid component of the mitochondrial membrane in heart cells, resulting in selective accumulation of doxorubicin inside cardiac cells (Goormaghtigh and Ruysschaert, 1984).

Although anthracyclines can readily generate oxygen radicals in several ways, only few free radical scavengers have been reported to protect the heart from doxorubicin-induced toxicity. Among them are dexrazoxane and flavonoids. Dexrazoxane (ICRF-187) is currently the only cardioprotective agent in clinical use. It has been reported to ameliorate the cardiotoxicity associated with doxorubicin in both preclinical (Imondi et al., 1996) and clinical studies (Speyer et al., 1988; Swain et al., 1997). Its mechanism of action appears to be the prevention of free radical formation by doxorubicin, probably through binding of iron (Hasinoff, 1994). Flavonoids are naturally occurring derivatives that are ubiquitous in photosynthesizing cells (Hertog et al., 1993). There is general agreement that flavonoids act as both scavengers of reactive oxygen species (Haenen et al., 1993) and iron chelators (Van Acker et al., 1996). Flavonoids have been found to protect the heart from doxorubicin-induced cardiotoxicity when co-administered with doxorubicin in mice (Van Acker et al., 1993), which suggests that these compounds are potential cardioprotectors against doxorubicin-induced chronic cardiotoxicity. There is still controversy regarding the relevance of free radical formation by doxorubicin in doxorubicin-related cardiotoxicity (Keizer et al., 1990). Probucol, a promotor of endogeneous antioxidants, provides only partial protection against the doxorubicin-induced cardiomyopathy (Siveski et al., 1994). It has been reported that free radical scavengers such as vitamin E and *N*-acetylcysteine decrease both lethality and severity of cardiac histological lesions in rodents injected with doxorubicin (Olson et al., 1981). In contrast, other studies have shown that vitamin E and *N*-acetylcysteine failed to attenuate doxorubicin-induced cardiotoxicity in rats and dogs (Herman et al., 1985; Julicher et al., 1986; Van Vleet et al., 1980). Possible explanations could be the different experimental setups, timing of measurement, etc. Also, the reserve in antioxidant defense plays an important role (Dalloz et al., 1999).

However, from the point of view of the free radical hypothesis, the study of Arai et al. (2000) is noteworthy because this study showed how the formation of hydrogen peroxide and the down-regulation of the sarcoplasmic reticulum calcium pump are connected, explaining the pathway whereby the two could be related.

2.2. Ca^{2+} overload hypothesis

According to the Ca^{2+} overload hypothesis, anthracyclines induce an excessive rise in intracellular calcium. Support for this hypothesis came from studies on rabbits that were treated chronically with doxorubicin (Olson et al., 1974). The results showed a substantial accumulation of calcium in ventricular myocardium and calcium inclusions in mitochondria. A doxorubicin-induced increase of the Ca^{2+} accumulation in mitochondria is generally at the expense of ATP production by oxidative phosphorylation, thereby resulting in depletion of high-energy phosphates (Ohhara et al., 1981). Many studies have reported doxorubicin-related Ca^{2+} transport abnormalities in cardiac tissue. For example, doxorubicin alters the *trans*-sarcolemmal Ca^{2+} influx by effects on the Na^+/K^+-ATPase (Boucek et al., 1997) and on the Na^+/Ca^{2+} exchanger

(Caroni et al., 1981). Furthermore, doxorubicin alters the Ca^{2+} release function of the sarcoplasmic reticulum by effects on the Ca^{2+}-ATPase and the Ca^{2+} release channel (Boucek et al., 1987; Ondrias et al., 1990; Boucek et al., 1993; Dodd et al., 1993). This hypothesis was recently supported by the observation that doxorubicin induces down-regulation of the sarcoplasmic reticulum Ca^{2+}-ATPase 2 (Arai et al., 2000). The authors also provided evidence that this down-regulation was mediated by hydrogen peroxide, thereby interconnecting the free radical and the Ca^{2+} overload hypothesis. They also showed that in the signal transduction pathway, mitogen-activated protein kinases and the early response gene Erg-1 are activated. In this respect, it is noteworthy that the mitogen-activated protein kinases and the early response gene Erg-1 are also involved in the development of hypertrophy (Schaub et al., 1997)). The situation is, however, more complicated because of the existence of non-free radical damaging pathways (see below). Moreover, the study of Arai et al. (2000) is based on primary cultures of neonatal heart cells. The main toxic effect of doxorubicin is the development of a cardiomyopathy long after ending of the treatment.

It has been suggested that calcium accumulation may be a manifestation rather than a cause of anthracycline cardiomyopathy (Jensen, 1986). The author challenged the concept that an elevated calcium concentration is the primary cause of injury and provided compelling evidence that doxorubicin-induced cardiac dysfunction is associated with a deficiency rather than an excess of calcium in the myofibrillar apparatus. Similarly, Rabkin et al. (1983) noted that the negative inotropic effects of doxorubicin could be antagonized by increasing the calcium concentration of the perfusate. These observations suggest that the pathogenesis of anthracycline-induced cardiotoxicity is initially associated with depletion of myocardial calcium rather than with an excessive increase of the intracellular calcium concentration. The decreased intracellular calcium concentration may eventually result in the development of congestive heart failure. One of the consequences of heart failure may be accumulation of calcium inside cardiac cells, making the increased calcium levels observed after chronic doxorubicin administration a consequence, rather than a cause, of anthracycline-induced cardiotoxicity.

2.3. Anthracycline metabolite hypothesis

Doxorubicin and other anthracyclines with a C-13 carbonyl functional group can be metabolized to C-13 alcohols in the heart (Young and Raymond, 1986). Doxorubicinol, the primary C-13 alcohol metabolite of doxorubicin, has been found to accumulate in cardiac tissue in a time- and dose-dependent manner (Del et al., 1985; Rossini et al., 1986), and there is evidence that doxorubicinol plays an important role in doxorubicin-associated cardiotoxicity (Danesi et al., 1987; Olson et al., 1988). From a pharmacokinetic point of view, the metabolite theory offers a likely explanation for the delayed cardiotoxicity of anthracycline treatment. For example, 1 h after injection of doxorubicin, the concentration of doxorubicin in the heart reaches its peak value whereas doxorubicinol is nearly undetectable at that time. Over the next 24 h, however, the doxorubicinol concentration in the heart begins to increase, reaching levels that are two- or three-fold higher those than in the liver, despite similar levels of doxorubicin in the two tissues (Peters et al., 1981). The metabolite theory is furthermore supported by the observation that doxorubicinol is much more toxic than doxorubicin in isolated cardiac preparations. At concentrations that are relatively low when compared to those of doxorubicin, doxorubicinol has been found to compromise both systolic and diastolic function of isolated heart preparations and to block ATPase activity of the sarcoplasmic reticulum, mitochondria, and sarcolemma (Olson et al., 1988).

2.4. Prostaglandins and platelet activating factor (PAF) hypothesis

Anthracyclines alter arachidonic acid metabolism, which suggests that prostaglandins, thromboxanes and leukotrienes may be involved in anthracycline toxicity (Das, 1981). Doxorubicin stimulates the production of prostaglandins (Robison and Giri, 1987) and thromboxanes (Remuzzi et al., 1985) in rats. Other data suggest that PAF may also contribute to anthracycline toxicity. PAF receptor antagonists have been reported to attenuate histological lesions in doxorubicin-treated rats (Bristow et al., 1983).

2.5. Histamine hypothesis

According to Bristow et al. (1983), anthracyclines stimulate the release of histamine from mast cells, which raises the possibility that histamine may be a mediator of anthracycline cardiotoxicity. Because free radicals are also known to stimulate the release of histamine, free radicals generated by anthracyclines could conceivably mediate histamine release. This concept is supported by the observation that free radical scavengers inhibit doxorubicin-induced histamine release and attenuate cardiac histological lesions in rodents (Klugmann et al., 1986). How histamine causes cardiotoxicity and histological lesions is unclear. Histamine has been found to facilitate calcium influx via histamine H_2 receptors and to evoke arrhythmias via H_1 receptors, which suggests that calcium may be involved (Olson and Mushlin, 1990).

2.6. Direct interaction with the actin–myosin contractile system

Most contraction-related studies concerning the inotropic effect of doxorubicin involve whole hearts or cardiac preparations with functionally intact membranes. However, the use of intact preparations makes it impossible to separate direct effects of doxorubicin on the actin–

myosin contractile system from effects mediated by interaction with other cellular components. Few studies focus on contractile alterations that are directly caused by interaction of doxorubicin with the contractile apparatus. It has been reported that doxorubicin has a high affinity for cardiac actin in vitro, probably the result of the high affinity of the daunosamine moiety in the anthracycline molecule for macromolecules (Lewis et al., 1982). Contractile changes caused by doxorubicin and that result from a direct interaction with the contractile apparatus can be studied by using preparations in which both inner and outer membranes of the preparation have been permeabilized. It was shown in our laboratory that doxorubicin exerts a positive inotropic effect when added to permeabilized skeletal muscle preparations of rabbits (De Beer et al., 1992). Because all membranes were permeabilized in these preparations, the contractile effect could only be caused by a direct interaction with the contractile system.

3. Contractile effects of doxorubicin

In in vitro studies on intact cardiac preparations, opposite directed inotropic effects following acute doxorubicin administration have been described (Azuma et al., 1981; Singal et al., 1987). Both positive inotropic effects (Kim et al., 1980; Temma et al., 1992; Van Boxtel et al., 1978) and negative inotropic effects (De Jong et al., 1990; Hofling and Bolte, 1981; Singal and Pierce, 1986; Politi et al., 1985; Voest et al., 1994) have been reported for heart preparations following acute doxorubicin incubation. Positive inotropic effects may result from direct stimulation of the cyclic AMP system or indirectly from release of catecholamines, whereas negative inotropic effects may result from suppression of mitochondrial respiratory function and loss of ATP and creatine phosphate from the myocardium (Singal and Pierce, 1986). In addition to acute inotropic effects, chronic treatment of animals with doxorubicin has been associated with contractile alterations in isolated heart preparations (Boucek et al., 1997; De Wildt et al., 1985; Jensen, 1986). These studies clearly demonstrate that doxorubicin affects the contractile response of heart muscle preparations. However, the results regarding direction and magnitude of the inotropic effect are inconsistent. Doxorubicin has a variety of subcellular actions inside cardiac cells (Singal et al., 1987) and many processes inside cardiac cells may be affected by doxorubicin before the binding of Ca^{2+} to Troponin C on the thin filament occurs. Therefore, the use of intact preparations makes it difficult to separate direct inotropic effects of doxorubicin on the actin–myosin contractile system from indirect effects. To study the direct effect of doxorubicin on contractile proteins, one can use skinned fibre segments. In such preparations, contractile changes resulting from a direct interaction of doxorubicin with the contractile apparatus can easily be investigated.

Evidence has now been accumulating that, besides the well-accepted mechanism for cardiotoxicity of doxorubicin, the drug itself affected the properties of the contractile machinery.

Most contractile studies concerning the inotropic effect of doxorubicin make use of whole hearts or cardiac preparations with membranes still functionally intact. However, the use of intact preparations makes it impossible to separate direct effects of doxorubicin on the actin–myosin contractile system from effects that are mediated by interaction with other cellular components. Few studies focus on contractile alterations that are caused directly by interaction of doxorubicin with the contractile apparatus. It has been reported that doxorubicin has a high affinity for cardiac actin in vitro, probably as a result of the high affinity of the daunosamine moiety in the anthracycline molecule for macromolecules (Lewis et al., 1982). Contractile changes induced by doxorubicin and resulting from a direct interaction with the contractile apparatus can be studied by using preparations with both inner and outer membranes permeabilized. It was shown in our laboratory that doxorubicin exerts a positive inotropic effect when added to permeabilized skeletal muscle preparations of rabbits (De Beer et al., 1992). Because all membranes in these preparations were permeabilized, the contractile effect could only be caused by a direct interaction with the contractile system.

3.1. Dynamic properties of the contractile machinery

A method for investigating the mechanism of muscle contraction is to impose sudden length changes on a muscle preparation and to follow the resulting mechanical transient. Adaptation of the mechanical transient to the sudden length change reflects processes such as conformational changes within a cross-bridge while attached, or either the attachment or detachment of cross-bridges. Depending on the amplitude of the length change the dynamics of the head or the dynamics of the attachment and detachment can be studied. Small changes ($< 1\%$) mainly induce an alteration in the conformation of the head, which can be followed during the several ms. The subsequent hundreds of ms of the force transient reflect (de)attachment of cross-bridges. The whole transient can be described by the sum of three exponential functions with time constants τ_1, τ_2, and τ_3 (De Beer et al., 2000). τ_1 describes the transition rate of the conformational change in the head, τ_2 the detachment rate of the cross-bridge and τ_3 the rate of re-attachment. Detachment and reattachment rates of the cross-bridges can be studied more easily with the "slack"-test (Edman, 1979). With this test the maximal velocity of unloaded shortening V_0 and the rate constant of force redevelopment τ_r are determined (Brenner, 1988). Both parameters are related to muscle type (Edman et al., 1988).

The ultimate step in excitation–contraction coupling is the generation of force by actin–myosin interactions. Although there are several reports an impaired cardiac contraction after chronic doxorubicin treatment, studies on the direct interaction of doxorubicin with the actin–myosin contractile system are scarce. Direct effects on the actin–myosin interaction can be studied by using preparations in which both inner and outer membranes have been permeabilized. The contractile function of such skinned preparations remains fully intact and the striated appearance of the myofibrils is not altered, as can be seen from electron micrographs of skinned preparations (De Winkel et al., 1993). An earlier report from our laboratory showed that doxorubicin has a positive inotropic effect in permeabilized skeletal muscle fibers, which is best explained by a direct interaction of doxorubicin with the actin–myosin contractile system (De Beer et al., 1992). Because doxorubicin is associated with alterations of cardiac contractile performance, we now review the direct effects of doxorubicin on the contractile system of heart muscle preparations, especially trabeculae.

Experiments with skinned fibres are hampered by diffusion limits of ions like Ca^{2+} within the specimen. Even in thin muscle preparations, such as trabeculae, diffusion limitations might interfere with processes involving muscle contraction, and might therefore influence the magnitude of the tension response (Kentish and Jewell, 1984; Stienen et al., 1990). Therefore, it is essential to use very thin preparations to avoid this complication.

3.2. Acute inotropic effect of doxorubicin

3.2.1. Steady response

Bottone et al. (1997) showed that acute incubation with doxorubicin increases both the maximal Ca^{2+}-activated and rigor tension in skinned cardiac trabeculae. The inotropic effect on Ca^{2+}-activated tension proved to be dose- and time-dependent, and was already manifest 3 min after drug exposure. There was a slight increase in Ca^{2+} sensitivity only after incubation with the highest doxorubicin concentration (20 μM). Because of the rapid onset of the effect and because all membranes were made permeable by skinning, the authors concluded that the effect is likely to be caused by a direct interaction of doxorubicin with the force-generating system. The increase in both the Ca^{2+}-activated tension and the rigor tension can be explained by at least two hypotheses that may not be mutually exclusive: (1) the population of strongly bound cross-bridges is increased, or (2) the force per strongly bound cross-bridge is increased during contraction.

In order to gain an insight into the relative inotropism of anthracycline drugs in skinned trabeculae, Bottone et al. (1997) also measured the acute inotropic effect of several commonly used members of the anthracycline family. At a concentration of 20 μM, doxorubicin and epirubicin were the most potent to enhance the maximal tension of trabeculae. Idarubicin and daunorubicin also had a positive inotropic effect, but did not significantly increase maximal tension. Randomized clinical trials have shown that doxorubicin is the most cardiotoxic anthracycline, and that epirubicin and idarubicin are less cardiotoxic (Jain et al., 1985; Cersosimo, 1992). The position of daunorubicin as a cardiotoxic anthracycline is less clear but it may be considered as slightly less or as cardiotoxic as doxorubicin (Gilladoga et al., 1976; Jaenke et al., 1980). The non-anthracycline anti-cancer drugs, taxol and 5-fluoro-uracil, did not show any positive inotropic action. These results suggest that a relation exists between the severity of the clinically observed cardiotoxicity of anthracyclines and their potency to increase the maximal tension of skinned cardiac preparations in the experimental setup the authors used.

3.2.2. Transient response

De Beer et al. (2000) performed experiments to unravel the underlying mechanism of the acute positive inotropism of doxorubicin. Acute incubation of skinned trabeculae with 20 μM doxorubicin resulted in clear attenuation of the cross-bridge cycling rate. This conclusion was based on the following results of the slack-test: The rate of force redevelopment (τ_r) is increased and the velocity of unloaded shortening (V_0) is decreased. It was shown that acute doxorubicin incubation increases both the maximal Ca^{2+}-activated tension and the rigor tension under static isometric conditions (see also Bottone et al., 1997), which is best explained by an increase in the population of strongly bound cross-bridges. Accumulation of cross-bridges in the strongly bound states can be caused by either an increased rate of cross-bridge attachment or by a decreased rate of cross-bridge detachment or by both. Based on the decrease of V_0 together with the increase of τ_r and on the higher isometric tension response, the authors conclude that a decreased rate of cross-bridge detachment underlies the lower cross-bridge cycling rate in skinned cardiac trabeculae on acute doxorubicin incubation. A decreased uncoupling rate of cross-bridges explains the higher isometric tension level of trabeculae on acute doxorubicin exposure. In addition, the observation that the time constant describing the net detachment of cross-bridges (τ_2) in the quick-release protocol is slightly increased supports the likelihood of decreased relaxation on doxorubicin incubation.

3.3. Chronic inotropic effect of doxorubicin

3.3.1. Steady response

Because doxorubicin-induced cardiomyopathy is associated with repeated administration of this drug, Bottone et al. (1998) studied the effect of chronic doxorubicin treatment on the contractile response of isolated skinned trabeculae. In this study, rats were injected for 4 weeks with

doxorubicin at a weekly dose of 2 mg/kg body weight, and the animals were used in experiments up to 6 weeks after the last doxorubicin infusion. The maximal Ca^{2+}-activated tension was found to be progressively decreased upon chronic doxorubicin treatment, starting from week 2 after the final doxorubicin administration. The decreased tension response is likely to have resulted from chronic damage to the myocardium, because doxorubicin treatment was discontinued at least 2 weeks before the negative inotropic effect became manifest. Although the authors found characteristic morphological changes of heart cells, such as microvacuoles and edema, they did not observe cardiac cell loss or fibrosis, and the dimensions of the trabeculae did not differ between control and doxorubicin preparations. Furthermore, the normalized passive stiffness of the doxorubicin-treated preparations was similar to the control values. The negative inotropic effect of doxorubicin can therefore not be explained by a loss of myocytes. Because rats suffered severe weight loss as a result of the doxorubicin treatment, a starved group with restricted food intake was included in order to test whether a loss of body weight might have interfered with the contractile alterations observed. No effect of food deprivation on the active tension and the rigor tension response of trabeculae was found, indicating that, in these experiments, the doxorubicin-induced impairment of contractile performance is a specific and direct action that cannot be explained by body weight loss. Therefore, the negative inotropic effect is best explained by a direct interaction of the drug with the contractile system or by physiological changes and/or adaptations of the contractile apparatus in response to doxorubicin treatment.

Because the sarcoplasmic reticulum plays an important role in regulating the intracellular Ca^{2+} concentration and because impaired Ca^{2+} homeostasis has been associated with doxorubicin cardiotoxicity (Olson and Mushlin, 1990), we (Bottone et al., 1998) studied the functionality of the sarcoplasmic reticulum in preparations of doxorubicin-treated rats. We found no effect on the ratio between the peak tension level of the caffeine transient and the maximal Ca^{2+}-activated tension level in saponin skinned trabeculae. Therefore, in our preparations, both function and volume of the sarcoplasmic reticulum remained in equilibrium with the contractile performance. Our results do not seem to be consistent with those of other studies reporting an alteration of the sarcoplasmic reticulum function after both acute and chronic doxorubicin exposure (Ondrias et al., 1990; Dodd et al., 1993). Although the capacity of the calcium pump, storage capacity, and calcium release by the sarcoplasmic reticulum all seemed to be functioning normally in our preparations, we cannot exclude the possibility that the rate of Ca^{2+} uptake by the sarcoplasmic reticulum may have been altered. The Ca^{2+}-loading time was 3 min. We cannot exclude the possibility that the sarcoplasmic reticulum could sequester the maximum amount of Ca^{2+} within 3 min.

3.3.2. Transient response

To elucidate the mechanism underlying the negative inotropic effect after chronic doxorubicin treatment, dynamic experiments were performed. De Beer et al. (2000) provided evidence that chronic doxorubicin treatment gives rise to attenuation of the cross-bridge cycling rate. All three time constants describing adaptation of strongly bound cross-bridges to the new length after a quick releasing step (τ_1, τ_2, and τ_3) were significantly increased. In addition, V_0 was significantly decreased and τ_r was significantly increased in the slack-test. Chronic doxorubicin treatment of rats led to a marked decrease of the maximal isometric tension of isolated trabeculae. Moreover, no myofibrillar loss or formation of fibrosis in preparations of chronically treated rats was observed and the passive stiffness of trabeculae remained unchanged (see also Bottone et al., 1998). Together, these results show that the decrease in maximal tension is most likely caused by changes in the actin–myosin interaction. Both attachment and detachment processes in the cross-bridge cycle are impaired, resulting in a decrease of the isometric tension level.

Cardiac cells express functionally different isoforms of myosin heavy chain encoded by two different genes designated as α and β. The α-myosin heavy chain isoform has a greater ATPase activity than the β-myosin heavy chain isoform and the relative expression of these two myosin isoforms has a considerable effect on myocardial function. De Beer et al. (2000) observed a significant shift in myosin heavy chain composition towards the β-myosin heavy chain isoform in left and right ventricles of doxorubicin-treated rats compared to those of controls. The altered cross-bridge kinetics with chronic doxorubicin treatment in their experiments may therefore be explained by an increase of the β-myosin heavy chain to α-myosin heavy chain ratio in ventricles of the doxorubicin-treated rats. Their results are well consistent with those reported by Cappelli et al. (1989) who also found an isomyosin shift in doxorubicin-treated rats. There are no studies on the effect of doxorubicin on α-myosin heavy chain and β-myosin heavy chain composition in human ventricles. Several sets of conditions have been reported to induce increased β-myosin heavy chain levels in ventricles of experimental animals: chronic caloric restriction (Morris et al., 1989; Haddad et al., 1993), chronic endurance efforts (Fitzsimons et al., 1990), and aging (Hoh et al., 1978). Preparations that have a relatively high expression of the β-myosin heavy chain isoform are characterized by a lower intrinsic contractility (Holubarsch et al., 1985). An increased expression of the β-myosin heavy chain isoform has also been associated with a decreased maximal shortening velocity (Ebrecht et al., 1982). A shift towards the β-myosin heavy chain isoform allows the heart to adopt a slower contraction velocity and is correlated with an improved economy of contraction (Swynghedauw, 1995). According to the literature, a reduced food intake has been associated

with up-regulation of the β-myosin heavy chain isoform and concomitant down-regulation of the α-myosin heavy chain isoform (Morris et al., 1989; Haddad et al., 1993). Because doxorubicin treatment results in a loss of body weight, it cannot be ruled out that the increased expression of the β-myosin heavy chain isoform in doxorubicin-treated rats might represent a consequence of an altered nutritional state. There is no information as to whether, in the experiments of De Beer et al. (2000), a shift in the myosin heavy chain isoform composition occurred in starved rats. However, Bottone et al. (1998) found no effect of food deprivation on the isometric tension response of isolated trabeculae of rats that were starved in order to obtain a body weight loss similar to that in doxorubicin-treated rats. Because the tension response of trabeculae of starved rats was normal, the loss of body weight per se seems to be unrelated to the cardiac contractile response in this latter study. This implies that the contractile alterations seen on doxorubicin treatment are caused by a chronic action of doxorubicin rather than being a non-specific consequence of a reduction in body weight. Another way to investigate the effect of body weight on mechanical performance could be to stimulate a gain in weight, for instance by administration of insulin-like growth factor. Indeed insulin-like growth factor-I results in a transient increase in body weight, when administered from week 8 (Ambler et al., 1993). However, insulin-like growth factor will also prevent apoptosis, a prerequisite for correct working mechanism of cytostatics.

4. Contractile adaptation in ventricular myocardium

It is well established that heart failure is associated with changes in gene expression coding for sarcomeric proteins, thereby affecting mechanical and energetic properties of the heart. An altered genetic expression of the heart may serve as adaptation mechanism to improve cardiac function and recover a normal economy of contraction (Swynghedauw, 1995).

Doxorubicin is known to affect muscle-specific gene expression in rat heart cells (Cappelli et al., 1989; Ito et al., 1990; Kurabayashi et al., 1993). One important question arises: Is doxorubicin-induced cardiotoxicity in patients also associated with a shift in the myosin heavy chain composition in the ventricular myocardium? Although a decrease in ventricular myofibrillar ATPase activity in failing human hearts has been reported (Swynghedauw, 1986), the physiological relevance of an isomyosin shift in human ventricular myocardium is controversial. Only low levels of α-myosin heavy chain (~ 10%) were found in human ventricular myocardium and no significant changes in the ventricular myosin heavy chain composition could be detected during heart failure (Gorza et al., 1984). Recently, however, using a quantitative reverse transcription Polymerase Chain Reaction, it was shown that substantial levels of α-myosin heavy chain mRNA (~ 35%) are present in ventricular myocardium (Lowes et al., 1997; Nakao et al., 1997). In two types of myocardial failure, the α-myosin heavy chain expression was markedly down-regulated and this myosin heavy chain exhibited reciprocal up-regulation. The reason for the controversial results concerning the existence of significant myosin heavy chain shifts in human ventricles may lie in the different detection methods used. Coumans et al. (1997) reported that increased mRNA levels for myosin heavy chain do not necessarily correspond with an increased amount of their corresponding proteins. However, Nakao et al. (1997) suggested that when immunohistochemical techniques are used, it is difficult to discriminate between myosin heavy chain isoforms at the protein level because the α- and β-myosin heavy chain in humans are highly homologous (Matsuoka et al., 1991; Jaenicke et al., 1990), which may easily lead to underestimation of the amount of α-myosin heavy chain. In summary, based on presently available data, it cannot be ruled out that genes coding for myosin heavy chain isoforms are candidates as molecular base of both doxorubicin-induced and other types of myocardial failure in humans. In studies of the decreased ventricular myofibrillar ATPase activity in the failing human heart, most of the attention has focused on alterations in the expression of myosin heavy chain isogenes. However, changes in the expression of other contractile genes have also been associated with contractile failure. For example, the expression of myosin light chains was altered in ventricles of patients suffering from heart failure (Hirzel et al., 1985; Trahair et al., 1993), an effect which was related to a lower Ca^{2+} sensitivity of cardiac preparations (Morano et al., 1997). In addition, an altered expression of cardiac troponin T isoforms has been found in heart failure (Solaro, 1992), which may affect cardiac contractile properties (Martin et al., 1991). Mutations in cardiac actin (Olson et al., 1998) and titin (Hein et al., 1994) also have been related to heart failure.

5. Relation between acute and chronic inotropic effects

In the previous sections we have considered the interference of acute and chronic treatment with doxorubicin with the actin–myosin contractile system. An important question is whether the chronic effects of doxorubicin are the result of cumulative acute effects or whether separate acute and chronic cardiotoxic mechanisms are involved. There are arguments for one common progressive mechanism, while some findings favor different mechanisms.

Indications for the existence of one common cardiotoxic mechanism can be found in the results of a chronic study (Bottone et al., 1998). At 1 week post-treatment, the maximal tension of trabeculae was slightly increased as compared to that of controls. After 1 week, however, a progressive decrease of the maximal tension was observed

for up to 6 weeks post-treatment. Continuous overstimulation of the contractile machinery during the acute phase of doxorubicin cardiotoxicity may have led to changes resulting in a decreased contractile performance on the longer term. These results point to a biphasic effect of doxorubicin, i.e. an early effect characterized by increased contractile performance, and a delayed effect with attenuation of the contractile performance. One single mechanism is favored when acute and chronic effects of doxorubicin on the cross-bridge kinetics are compared. Acute doxorubicin incubation gives rise to a significant decrease of the cross-bridge cycling rate in skinned trabeculae. De Beer et al. (2000) provided evidence that this acute impairment is caused by a decreased uncoupling rate of attached cross-bridges, while the attachment rate of cross-bridges remained unaffected. Chronic doxorubicin administration, however, results in clear and significant alterations in both activation and relaxation phase. Comparing these acute and chronic effects, we hypothesize that doxorubicin has a progressive deleterious action on cross-bridge kinetics, thereby first impairing uncoupling processes and in a later stage also impairing coupling processes of cycling cross-bridges.

Indications in favor of different acute and chronic mechanisms were found in experiments where doxorubicin (20 μM) was added to preparations of chronically doxorubicin-treated rats at 4 weeks post-treatment. A positive inotropic effect was not observed when trabeculae of chronically treated rats were incubated with doxorubicin (unpublished data). This indicates that acute doxorubicin incubation cannot neutralize the impaired contractile response resulting from chronic doxorubicin treatment, suggesting that different mechanisms underlie the opposed inotropic effects of doxorubicin.

It is tempting to relate the appearance of the mitogen-activated protein kinases and early response genes such as Egr-1 with the long-term development of the doxorubicin-induced cardiomyopathy. The early response genes are activated within 30–60 min after exposure to a hypertrophic stimulus and the mitogen-activated protein kinases play a major role in the transduction pathways (Schaub et al., 1997). Hypertrophy is consider to be the first step in the process that ends in heart failure (Swynghedauw, 1999).

Based on the present experimental data, however, no specific evidence can be provided as to whether a similar or different mechanism(s) can account for the acute and chronic inotropic doxorubicin effects.

5.1. Intervention by dexrazoxane (ICRF-187)

The cardiotoxicity associated with chronic doxorubicin therapy has prompted a search for potential antidotes that prevent the development of cardiac damage. One such compound, dexrazoxane (ICRF-187), provides substantial cardioprotection when co-administered with anthracycline drugs, in both animal studies and in clinical trials. It is believed that this protection is mediated by the prevention of doxorubicin-induced free radical formation. Because chronic doxorubicin treatment in rats is associated with an impaired cross-bridge cycle, De Beer et al. (2000) investigated whether the cardioprotective action of dexrazoxane involves preservation of the normal cross-bridge kinetics. They showed that treatment of rats with dexrazoxane offers significant and substantial protection against the impairment of cross-bridge kinetics in isolated cardiac trabeculae. In this latter study, dexrazoxane (40 mg/kg body weight) was administered 30 min prior to doxorubicin (2 mg/kg body weight) infusion. In trabeculae of rats that received both dexrazoxane and doxorubicin during the 4-week treatment period, all three time constants in the quick release protocol (τ_1, τ_2, and τ_3) remained unchanged. In addition, both V_0 and τ_r in the slack-test remained virtually unaffected.

Based on these findings, it appears that both the coupling and decoupling processes within the cross-bridge cycle remain virtually normal when rats receiving doxorubicin are pretreated with dexrazoxane. An additional beneficial effect of dexrazoxane was the prevention of body weight loss following doxorubicin treatment. When doxorubicin-treated rats are pretreated with dexrazoxane, the otherwise observed decline in body weight is absent and 'only' stagnation of the body weight gain is observed. These results are consistent with those obtained by others (Villani et al., 1990). Moreover, all the dexrazoxane + doxorubicin rats appeared healthy and showed no signs of diarrhea or edema. The authors concluded that pre-administration of dexrazoxane to rats substantially prevents the deleterious effects of chronic doxorubicin treatment on the trabecular actin–myosin interaction. Also, the α-myosin heavy chain to myosin heavy chain isoform shift was markedly depressed after dexrazoxane treatment, indicating that the cardiomyopathy was also depressed on the molecular level.

6. Summary and conclusion

Over the past years, there have been only few studies of changes in the cardiac actin–myosin interaction in response to doxorubicin treatment. The present review concerns a novel mechanism by which doxorubicin induces cardiac failure: alteration of the actin–myosin cross-bridge interaction. Acute doxorubicin exposure results in a delayed relaxation of attached cross-bridges, while chronic doxorubicin treatment is followed by impairment of processes involved in both the attachment and detachment of cross-bridges. We provided evidence that chronic doxorubicin treatment results in a significant shift towards the "slow ATPase" β-myosin heavy chain isoform in ventricular tissue. Although we did not specifically show that the changes in cross-bridge kinetics on chronic doxorubicin treatment resulted from the concomitant shift in myosin

heavy chain isoform composition, this area warrants further investigation. Because dexrazoxane prevented doxorubicin-related impairments in the cross-bridge kinetics from occurring together with inhibition of the shift in isoforms, treatment regimes that focus directly on precluding or diminishing the drug-related effects on cardiac actin–myosin interaction may constitute a promising strategy for fighting anthracycline cardiotoxicity. Although this is still under debate, potential therapeutics for doxorubicin-induced heart failure in patients might include agents that induce α-myosin heavy chain gene expression.

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