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Comparison of acute effects of anthracyclines on cardiac electrophysiological parameters of isolated guinea-pig hearts*

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Summary. This study was performed to evaluate the acute effects of two anthracycline derivatives, doxorubicin and 4′*O*-tetrahydropyranyl-doxorubicin [(THP)-doxorubicin], on the conduction intervals, heart rate and refractoriness of isolated spontaneously beating guinea-pig hearts using a high-resolution ECG recording technique (SST-ECG). Doxorubicin as well as (THP)-doxorubicin were added to the perfusate in increasing concentrations of 0.1, 1 and 10 μM. Doxorubicin did not significantly alter the heart rate or conduction intervals. Only the rate-dependent QT interval was significantly shortened under the influence of 10 μM doxorubicin. In contrast, 10 μM (THP)-doxorubicin led to a significant reduction in the heart rate (−13% ± 3%; $P < 0.01$, $n = 7$) and to a prolongation of atrioventricular conduction time (24% ± 10%; $P < 0.05$, $n = 7$). The rate-dependent repolarization period (QT interval) was only insignificantly shortened in the presence of 10 μM (THP)-doxorubicin. The maximal following frequencies of each part of the conduction system were not changed by 10 μM doxorubicin. In the presence of (THP)-doxorubicin, the maximal following frequency of the ventricular myocardium was increased by as much as 36% ± 8% ($P < 0.01$, $n = 7$), indicating a shortening of the effective refractory period of the ventricular myocardium (V-ERP). These results show that the activation of (THP)-doxorubicin resembles the effects of Ca-antagonistic compounds on the heart (i.e. decrease in the spontaneous sinus rate and prolongation of the AV-nodal conduction interval). Changes in the QT interval exerted by doxorubicin and the shortening of the ventricular effective refractory period by (THP)-doxorubicin may indicate an alteration of the K^+-conductance of the membrane. As the acute electrophysiological effects of doxorubicin and (THP)-doxorubicin are modest and occur only at excessive concentrations (10 μM), a direct influence on the generation of arrhythmias in healthy hearts is unlikely.

Introduction

Anthracycline derivatives are widely used in the treatment of acute leukemia and various types of solid tumors [15, 22, 23, 26]. Although these compounds have a high therapeutic efficacy, their use is often limited by severe toxic side effects such as bone marrow suppression, cardiac arrhythmias or alopecia. Among cardiac side effects, electrical abnormalities have been described that may occur immediately or up to weeks after the beginning of treatment [19]. Especially for doxorubicin, an increase in the frequency of ventricular premature beats as well as nonspecific repolarization abnormalities and even sudden death attributable to arrhythmias have been reported [7, 25]. In addition to these arrhythmias, cardiomyopathy develops during treatment with doxorubicin and often limits its use [2, 4, 24]. The newly developed anthracycline derivative 4′ *O* - tetrahydropyranyldoxorubicin [(THP)-doxorubicin] has been found to be as potent as, if not superior to, doxorubicin in tumor therapy but causes less cardiotoxicity [13, 20]. The present study was designed to evaluate the direct effects of doxorubicin and (THP)-doxorubicin on the cardiac electrical activity and refractorines of isolated Langendorff-perfused guinea-pig hearts. An increased susceptibility to cardiac arrhythmias caused by direct electrophysiological effects during anthracycline therapy can be discussed upon these results.

Materials and methods

Guinea pigs of either sex, weighing 300–400 g and fed ad libitum, were injected intraperitonially with 250 IU heparin sulfate 1 h before being sacrified by a blow to the neck, resulting in its dislocation. The chest was then quickly opened and the heart was removed and attached to a modi-

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Table 1. Effects of doxorubicin and (THP)-doxorubicin on heart rate and conduction intervals

		Doxorubicin ($n = 7$)	P	(THP)-Doxorubicin ($n = 7$)	P
AH interval (ms)	Control	48.0 ± 3.1		55.6 ± 2.6	
	0.1 μM	48.7 ± 3.2	NS	55.6 ± 2.3	NS
	1 μM	50.5 ± 4.7	NS	57.3 ± 1.9	NS
	10 μM	58.4 ± 8.2	NS	67.5 ± 3.8	<0.05
HV interval (ms)	Control	11.6 ± 0.7		10.7 ± 0.6	
	0.1 μM	11.1 ± 0.8	NS	10.5 ± 0.5	NS
	1 μM	11.4 ± 0.7	NS	10.7 ± 0.5	NS
	10 μM	11.9 ± 0.8	NS	10.7 ± 0.5	NS
QRS interval (ms)	Control	22.9 ± 0.6		22.2 ± 1	
	0.1 μM	22.9 ± 0.5	NS	22.5 ± 0.9	NS
	1 μM	22.4 ± 0.5	NS	22.9 ± 0.8	NS
	10 μM	23.3 ± 0.7	NS	23.3 ± 0.9	NS
Heart rate (beats/min)	Control	222 ± 6		212 ±12	
	0.1 μM	215 ± 7	NS	192 ± 8	NS
	1 μM	205 ±10	NS	192 ±11	NS
	10 μM	204 ±14	NS	183 ± 7	<0.01

All values represent the mean ± SEM. NS, not significant

fied Langendorff-type recirculating perfusion system (Anton Paar KG, Graz, Austria). Tyrode's solution, gassed with a mixture of oxygen (95%) and carbon dioxide (5%) and warmed to 36°C, was used as a perfusate (in mM: NaCl, 132.1; KCl, 2.7; $CaCl_2$, 2.5; $MgCl_2$, 1.15; $NaHCO_3$, 24; NaH_2PO_4, 0.42; D-glucose, 5.6). Depending on the size of the heart, the perfusion rate was adjusted to 4–6 ml/min.

Bipolar ECG signals were recorded from the epicardiac surface of the spontaneously beating hearts. Two silver-wire electrodes were placed on the epicardiac surface, one posterior to and near the valve plane and the other in the opposite position near the initial part of the anterior interventricular artery. In addition to the common ECG signals, the recorded unfiltered bipolar signals contained information about the early atrial and His bundle activity and were amplified by a factor of 100, monitored on a digital storage oscilloscope and stored on a tape recorder [16, 18]. The equilibration period was 30 min; if any irregularities occurred during this time, the heart was discarded.

Either doxorubicin hydrochloride (Adriblastin, Behring, FRG) or 4′*O*-tetrahydropyranyl-doxorubicin [THP)-doxorubicin; Pirarubicin, Farm Italia Carlo Erba AG, Switzerland] was added to the perfusate at 15-min intervals at concentrations of 0.1, 1 and 10 μM. In the present experiments, drug concentrations of 0.1 and 1 μM corresponded to plasma levels previously reached in patients at 5 min and 24 h after the administration of either drug [12]. The highest concentration was used to evaluate side effects at toxic concentrations. At 15 min after the administration of the highest concentration (10 μM) of either compound, the sinus-node recovery time (SNRT) and the maximal rate of pacing at 1 : 1 conduction or the so-called upper border frequency of the sinoatrial (SABF) and atrioventricular conduction (anterograde Wenckebach periodicity, AWP and of the atrial (ABF) and ventricular myocardium (VBF) were determined using programmed stimulation. Rectangular pulses of 2 ms duration and a constant current of twice the diastolic threshold were used for programmed stimulation.

SNRT was evaluated by pacing the heart for 20 s at a pacing rate of 300 beats/min. SNRT was defined as being the interval between the onset of the last stimulus-induced P-wave and the onset of the first spontaneously occurring P-wave. For evaluation of the maximal rate of pacing, the stimulation rate was increased in a stepwise fashion. With each step, the pacing interval was shortened by a maximum of 10 ms, beginning at a stimulation rate 10 beats faster than the spontaneous sinus rate. At each step the heart was paced for 10 s. The maximal rate of pacing was defined as being the longest pacing interval that failed to evoke a response of the sinoatrial and atrioventricular conduction or of the atrial or ventricular myocardium. For determinations of SABF and AWP as well as SNRT, pacing electrodes were positoned in the sinus-node area, whereas for determinations of the maximal rate of pacing of the atrial and ventricular myocardium, they were placed on the left atrium and left ventricle, respectively. Statistical analysis was performed using a two-sided t-test. All values represent the mean ± SEM. Results were considered to be significant at $P < 0.05$.

Results

Heart rate and conduction times

After the administration of 0.1 and 1 μM doxorubicin as well as (THP)-doxorubicin, no significant changes in the heart rate (HR), AV-nodal (AH-interval), His-bundle (HV-interval) or intraventricular (QRS-interval) conduction were detectable. Even under the influence of 10 μM doxorubicin, the HR and conduction intervals remained within control values. In contrast, 10 μM (THP)-doxorubicin led to a marked prolongation of atrioventricular conduction (24% ± 10%, $P < 0.05$, $n = 7$) as well as to a significant reduction of the HR (−13% ± 3%, $P < 0.01$, $n = 7$) (Table 1). The frequency-dependent repolarization period (QT interval) was slightly but significantly shortened by doxorubicin and only insignificantly altered by (THP)-doxorubicin (Fig. 1).

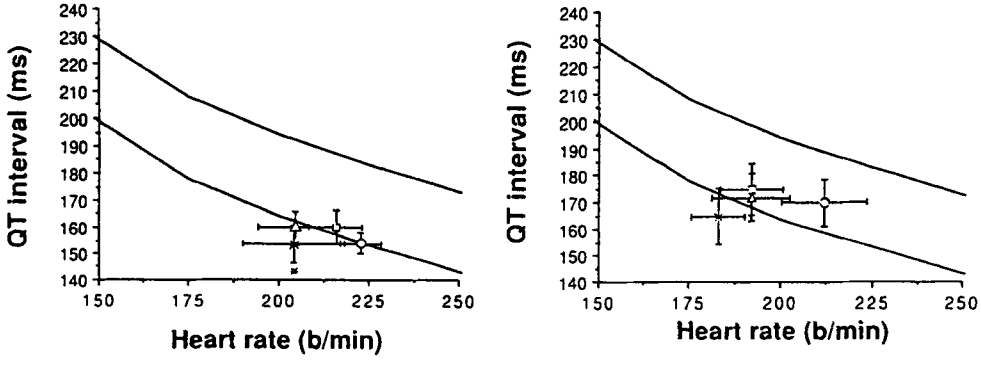

Fig. 1. Effects of doxorubicin (*left panel*) and (THP)-doxorubicin (*right panel*) on the frequency-dependent QT duration. The area between the two lines indicates the range of normal behaviour of the QT interval at different heart rates under control conditions [13, 14]. Drug concentrations: □, 0.1 μM; △, 1 μM; ×, 10 μM. ○, control; b/min, beats per minute. Values represent the mean ± SEM. * $P < 0.05$, $n = 7$ for 1, 2, 3, and 4

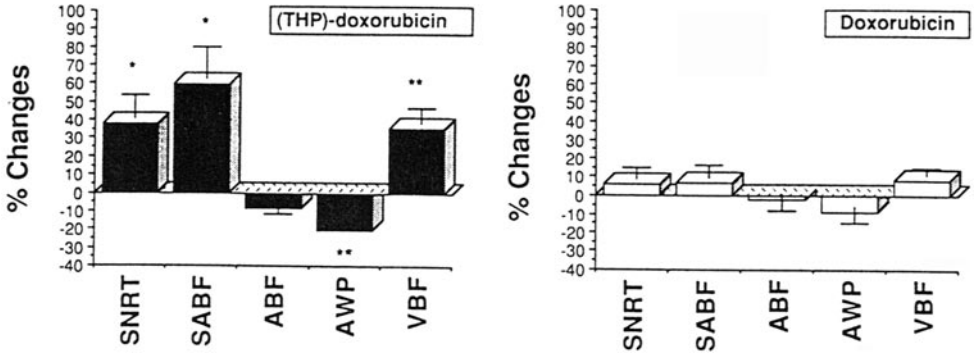

Fig. 2. Percentages of change in sinus node recovery time (*SNRT*), maximal following or border frequencies of the sinoatrial conduction (*SABF*) and of the atrial (*ABF*) and ventricular myocardium (*VBF*), and the anterograde Wenckebach periodicity of the AV node (*AWP*). Values represent the mean ± SEM. * $P < 0.05$, ** $P < 0.01$, $n = 7$

Programmed stimulation

During programmed stimulation, the SNRT was prolonged by 38% ± 13% in the presence of 10 μM (THP)-doxorubicin ($P < 0.05$, $n = 7$); the SABF was increased (60% ± 17%; $P < 0.05$, $n = 7$) by this drug. Doxorubicin did not significantly alter SNRT (6% ± 6%, $n = 7$) or SABF (7% ± 6%, $n = 7$) values. In the presence of (THP)-doxorubicin, the AWP of the AV node was reached at a significantly lower pacing rate than that used under control conditions (–20% ± 3%; $P < 0.01$, $n = 7$), whereas this parameter remained within control values under the influence of 10 μM doxorubicin (–9% ± 9%, $n = 7$). Neither compound exerted influence on the maximal following frequency of the atrial myocardium (doxorubicin: –8% ± 6%, $n = 7$; (THP)-doxorubicin: 2% ± 9%, $n = 7$), whereas the maximal rate of pacing of the ventricular myocardium was significantly increased by (THP)-doxorubicin (36% ± 8%; $P < 0.01$, $n = 7$). Doxorubicin had no acute effect on this parameter (8% ± 4%, $n = 7$; Fig. 2).

Discussion

Cardiac side effects of anthracycline derivatives have often been reported during tumor therapy [2, 4, 7, 13, 19, 20, 24, 25] and have been well documented in animal experiments [5, 6, 8, 9, 11, 14, 20, 21]. In vivo studies in the dog [8] showed that ectopic atrial and ventricular arrhythmias and AV-nodal conduction blocks were present after 2 weeks of doxorubicin administration. In studies on hamsters, significant changes in heart rate, conduction blocks and arrhythmias were inducible by increasing the doxorubicin concentrations; all of these effects were less marked under the influence of (THP)-doxorubicin [20, 21]. In vitro studies in rat papillary muscles showed a prolongation of the action-potential duration [9]. These changes were suggested to be related either to a reduction of the slow inward current or to an alteration in membrane conductance of potassium ions, both of which influence the duration of the action potential. Azuma et al. [1] found effects similar to those caused by calcium antagonists only under the influence of high concentrations of doxorubicin, whereas at low concentrations the calcium influx into the cell seemed to be enhanced.

The results of the present study show that significant alterations in sinus and AV-nodal activity (i.e. reduction of the spontaneous sinus rate, prolongation of the SNRT, reduction of the AWP and prolongation of the AH-interval) were observed only after the acute administration of 10 μM (THP)-doxorubicin. These results indicate a slight inhibition of transmembrane calcium inflow. The shortening of the frequency-dependent QT interval by doxorubicin is also similar to the influence of nifedipine [17]. An effect on the Ca-dependent slow responses by doxorubicin has also been discussed by Le Marec et al. [10] and Binah et al. [3]. These authors suggested that the suppression of ouabain-induced post-depolarizations may result in reduced influx of calcium into the cell [10]. Comparable with the results obtained by Lazarus et al. [9] in rat papillary muscles, in the present study the sodium-dependent parts of the conduction system, such as the His-bundle and intraventricular conduction, remained unaffected. In contrast, the maximal following frequency of the ventricular myocardium was increased under the influence of both drugs but reached significance only with (THP)-doxorubicin. This might be due to an improvement in potassium conductance. In our study, arrhythmias did not appear spontaneously, nor were they inducible during high rate pacing. The changes that are likely to reflect effects on the different ionic channels were so modest that an increased likelihood of arrhythmias or of an arrhythmogenic effect is unlikely. However, prolonged drug exposure results in ultrastructural changes and cardiomyopathy [2, 4, 5, 20, 23] that is correlated with cardiac arrhythmias. These arrhythmias are caused by conduction disturbances as a result of structural changes and do not result from changes in ion channels.

Therefore, we suggest that rather than a direct blocking effect on ionic channels, structural changes in the myocardium, which are more pronounced in the presence of doxorubicin [2, 4, 13] than during exposure to (THP)-doxorubicin [11, 13], may be responsible for the electrophysiological changes causing arrhythmias.

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