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European Journal of Pharmacology 414 (2001) 71–78

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Cardioprotective effects of zofenopril, a new angiotensin-converting enzyme inhibitor, on doxorubicin-induced cardiotoxicity in the rat

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Received 4 December 2000; received in revised form 17 January 2001; accepted 23 January 2001

Abstract

We have studied the effect of zofenopril, a new angiotensin-converting enzyme inhibitor in preventing cardiac injury induced by chronic doxorubicin treatment in rats. Cardiac function was assessed by measuring changes in electrocardiogram (ECG) tracings, haemodynamics and cardiac responses in vivo to isoprenaline, 4 weeks after suspension of doxorubicin treatment, in vehicle-treated rats and in animals receiving zofenopril (15 mg/kg/os/day) alone, doxorubicin (1.5 mg/kg i.v. once a week for 5 weeks) or zofenopril + doxorubicin treatment. Doxorubicin induced a significant lengthening of the QαT interval, which was completely prevented by zofenopril treatment. The cardiac positive inotropic effect induced by i.v. isoprenaline was selectively depressed by doxorubicin (no changes in chronotropic responses) and this adverse effect of doxorubicin was also prevented in zofenopril + doxorubicin pretreated rats. Doxorubicin induced a significant increase in relative heart weight, which was likewise prevented in zofenopril + doxorubicin treated rats. In separate experiments, zofenopril did not interfere with the antitumor activity of doxorubicin (inhibition of tumor growth in nude mice xenografted with A2780 human tumor line). In conclusion, the oral administration of zofenopril is able to significantly ameliorate, up to 4 weeks after the end of doxorubicin administration, doxorubicin-induced cardiotoxicity without affecting the antitumor activity of this anthracycline.

Keywords: Anthracycline; Angiotensin-converting enzyme inhibitor; Cardiotoxicity; Inotropic; Electrocardiography

1. Introduction

Anthracyclines such as doxorubicin and epirubicin are among the most widely used cytotoxic drugs for the treatment of a wide spectrum of human tumors (Arcamone, 1981; Hitchcock-Bryan et al., 1986; Fischer et al., 1989; Mouridsen et al., 1990; Bonadonna, 1984): their use in cancer chemotherapy is limited by the occurrence of a severe dose-related cardiomyopathy (Rhoden et al., 1993; Buzdar et al., 1985; Von Hoff et al., 1979; Dardir et al., 1989; Torti et al., 1986), eventually leading to congestive heart failure. Anthracycline-induced congestive heart failure almost invariably develops in patients receiving cumulative doses of doxorubicin over 550 mg/m^2 or epirubicin over 1000 mg/m^2: these patients also become unresponsive to positive inotropic agents within 2–3 months from the start of treatment (Singal and Natasha, 1998; Lefrak et al., 1973).

The efficacy of anthracyclines as cytotoxic against several types of human tumors has prompted intensive efforts in the searching of drug treatments (such as antioxidants and metal chelators), which may reduce or prevent the risk of developing cardiotoxicity and congestive heart failure. However, the cardioprotection afforded by these treatments has not been demonstrated consistently effective (De-Silvestro et al., 1996; Unverferth et al., 1985; Van Vleet et al., 1980). In particular, the difficulty of achieving constant plasma concentrations of antioxidant drugs and their poor uptake at heart level are the major limiting factors of this approach (Myers et al., 1983; Dorr, 1996; Wang and Kang, 1999; Konorev et al., 1999).

A few studies have also suggested that angiotensin-converting enzyme inhibitors, which are widely used for treatment of a number of cardiovascular diseases (hypertension, congestive heart failure, acute myocardium infarction and diabetic nephropathy) may exert a protective role toward anthracycline-induced cardiotoxicity.

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)00782-8

Jensen et al. (1996) reported, in a limited number of patients with advanced breast cancer, that two angiotensin-converting enzyme inhibitors, enalapril and ramipril, ameliorate symptoms of anthracycline-induced congestive heart failure. A few studies about the use of angiotensin-converting enzyme inhibitors treatment to prevent experimental anthracycline-induced cardiotoxicity (Tokudome et al., 2000; Maeda et al., 1997; Al-Shabannah et al., 1998) are available. Two of these studies were either performed in vitro (Maeda et al. 1997), or after single administration of doxorubicin and an angiotensin-converting enzyme inhibitor in vivo (Al-Shabanah et al., 1998). Moreover, none of these studies addressed whether the concomitant administration of an angiotensin-converting enzyme inhibitor may have a detrimental effect on the antitumor efficacy of doxorubicin.

Zofenopril is a new angiotensin-converting enzyme inhibitor characterized by a remarkable uptake by cardiac tissue, producing a striking and long-lasting inhibition of cardiac angiotensin-converting enzyme as compared to other drugs of this class (Sun and Mendelsohn, 1991; Subissi et al., 1999). Moreover, owing to the presence of a sulphydryl group, it is also an effective radical scavenger with antioxidant properties (Chopra et al., 1992; Mak et al., 1990; Napoli et al., 1999). Zofenopril has shown to exert a remarkable cardioprotective effect in a number of in vitro and in vivo models of ischaemic myocardial injury (Ferrari et al., 1992; Subissi et al., 1999) and its early administration in patients with myocardial infarction improve their long-term survival (Ambrosioni et al., 1995).

The aim of this study was to assess whether pretreatment with zofenopril can prevent the development of anthracycline-induced cardiotoxicity and heart failure in rats.

With this aim, rats were treated with zofenopril, given orally in the diet, at a nominal dose (15 mg/kg/day) producing a full antihypertensive effect in this species (Mitchell et al., 1996; DeForrest et al., 1989; Cushman et al., 1989; Gonzales et al., 2000). The effect of this dose regimen on doxorubicin-induced cardiopathy was assessed up to 4 weeks from the end of doxorubicin administration.

In addition, to check whether zofenopril treatment determines any interference with the antitumoral activity of doxorubicin, we assessed the efficacy of doxorubicin treatment in nude mice xenografted with the human ovarian carcinoma cell line A2780 and simultaneously receiving zofenopril by oral gavage.

2. Material and methods

2.1. Animals

A total of 32 male Sprague–Dawley rats (Harlan, Corezzana, Bergamo, Italy), 27–29-days old at the start of the study, was used. Animals were divided into four groups of eight rats each and maintained four per polycarbonate cage under continuously monitored environmental conditions. Drinking water and specific powdered diet (Altromin MT, Rieper, Bolzano, Italy) were supplied ad libitum. Environmental conditions, as well as the procedures for housing and handling the animals, were in compliance with EU and Italian Guidelines for Laboratory Animal Welfare.

2.2. Study design

After 2 weeks of acclimatization, animals in zofenopril and zofenopril + doxorubicin groups received zofenopril per os, mixed in powdered diet, at a nominal dose level of 15 mg/kg/day. Animals in control and doxorubicin groups received powdered diet alone. One week after the start of treatment, animals in doxorubicin and zofenopril + doxorubicin groups received, by injection into the tail vein, doxorubicin at a dose level of 1.5 mg/kg once a week for five consecutive weeks (7.5 mg/kg total dose). Animals in control and zofenopril groups received vehicle i.v. These doses and schedules were selected on the bases of previous pharmacological studies for zofenopril (dose level for a clear antihypertensive effect in the rat) and previous our cardiotoxicity studies for doxorubicin (total dose inducing consistent cardiac lesions). Vehicle (1% lactose in saline) and doxorubicin were administered at a dose volume of 10 ml/kg body weight. Intravenous administration of doxorubicin was suspended after 5 weeks, while zofenopril treatment was continued for further 4 weeks after suspension of doxorubicin.

Four weeks after suspension of doxorubicin treatment, animals were anaesthetized with 0.3 ml/kg of Hypnorm (fentanyl citrate, 0.315 mg/ml and fluanisone 10 mg/ml) and 40–60 mg/kg of sodium pentobarbitone, both given intraperitoneally. After the induction of anaesthesia, electrocardiogram (ECG) tracings (lead II) were recorded from each animal by means of subcutaneous needles electrodes. After ECG recording, animals were subjected to surgical preparation to allow the measurement of cardiovascular parameters: systolic blood pressure, diastolic blood pressure, mean blood pressure, heart rate, left ventricular systolic pressure, dP/dt_{MAX} (first derivative of left ventricular systolic pressure), left ventricular end diastolic pressure. The above parameters were measured both as basal values and after intravenous administration of increasing doses of a positive adrenergic inotropic agent (isoprenaline), administered at 10–15-min intervals. This interval was sufficient for return to control values of all parameters. At the end of each experiment, the heart of each animal was excised and weighed.

2.3. Cardiovascular measurements

Four weeks after suspension of doxorubicin treatment, animals were anaesthetized as indicated earlier (Hypnorm

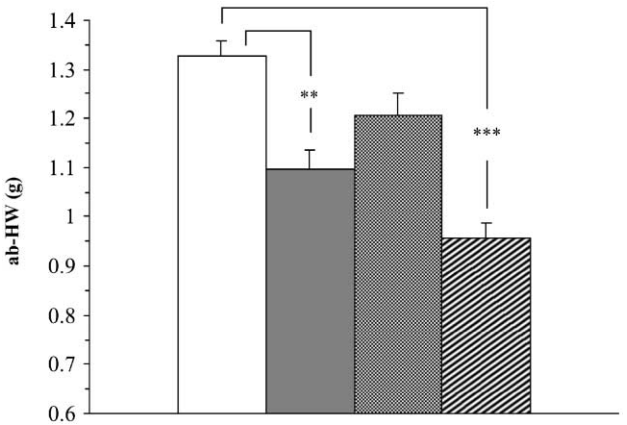

Fig. 1. Changes in absolute heart weight (ab-HW) induced by vehicle (open bars), zofenopril (grey bars), doxorubicin (dotted bars) and zofenopril + doxorubicin (hatched bars). Heart weight was recorded 4 weeks after suspension of doxorubicin treatment. $^{**}P < 0.01$; $^{***}P < 0.001$ vs. vehicle group.

and sodium pentobarbitone) and placed on a homeothermic blanket system to maintain the body temperature at 37°C. The trachea was exposed, and a polyethylene cannula was inserted to allow a good ventilation. A catheter (Angiocath 24 G; Becton Dickinson, Sandy, UT, USA) was inserted into a tail vein for infusion of sodium pentobarbitone (5 mg/ml; flow rate 0.5–3 ml/h) to maintain the anaesthesia adequately and/or to inject other substances.

Blood pressure was measured by means of a polyethylene cannula (PE 50) filled with heparinized saline (25 IU/ml) inserted into the left femoral artery. The cannula was connected to a transducer (Transpack, Abbott, North Chicago, USA) and the signal was amplified by means of a BM 614/2 amplifier (Biomedica Mangoni, Pisa, Italy).

Left ventricular systolic pressure was measured by means of a Millar mikro-tip® transducer catheter inserted into the left ventricle via the right carotid artery and connected to a BM 614/2 amplifier (Biomedica Mangoni). Heart rate, dP/dt_{MAX} and left ventricular end diastolic pressure signals were obtained from primary signals (left ventricular systolic pressure and blood pressure) by means of an acquisition data system (Ponemah, Gould G.N. Sistemi, Milano, Italy). All the above signals were continuously recorded by means of the acquisition data system indicated above.

2.4. Electrocardiographic measurements

Four weeks following the suspension of doxorubicin treatment, ECG tracings (lead II) were recorded by means of an electrocardiograph connected to subcutaneous needle electrodes in the anaesthetized animals. A BM 613 electrocardiograph (Biomedica Mangoni) connected to an acquisition data system was used to record and monitor ECG tracings. Analysis of ECG tracings was carried out by means of an acquisition data system (Ponemah), which measured the duration of the QαT (from the beginning of R wave to the apex of T wave) and QRS intervals, QRS voltage and heart rate. These EGC parameters are considered to be predictive of cardiac damages induced by doxorubicin treatment.

2.5. Evaluation of antitumor activity

Female athymic nude mice (Harlan, Italy), 6–8 weeks old, were used throughout the study. Mice were maintained in laminar flow rooms, according to the UK Coordinating Committee on Cancer Research guidelines (UKCCCR, 1988). Human ovarian carcinoma A2780 cells were maintained in RPMI 1640 (Gibco/BRL, Gaithersburg, MD) supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units penicillin and 100 μg streptomycin at 37°C in a 5% CO_2, 95% air humidified incubator. Tumor lines originated from subcutaneous (s.c.) in vivo injection of tumor cell (10×10^6 cells/flank/0.2 ml) in the right flank of athymic female nude mice. Human tumor lines were maintained by serial s.c. passages of tumor fragments. Doxorubicin was administered in vivo at a dose level of 7 mg/kg body weight (dose volume of 10 ml/kg) given i.v. every 7 days for three times (q7d × 3). Zofenopril was administered in vivo at a dose level of 10 mg/kg body weight (dose volume of 10 ml/kg) given daily, by oral gavage, for 2 weeks (qd × 14). Treatment started, with both drugs, when tumors were approximately 50 mg in weight (day 13). Tumor growth was followed by caliper measurement of length and width at predetermined (twice/week) times. Tumor weight (TW) was calculated using the formula: mg = volume in $mm^3 = width^2 \times$

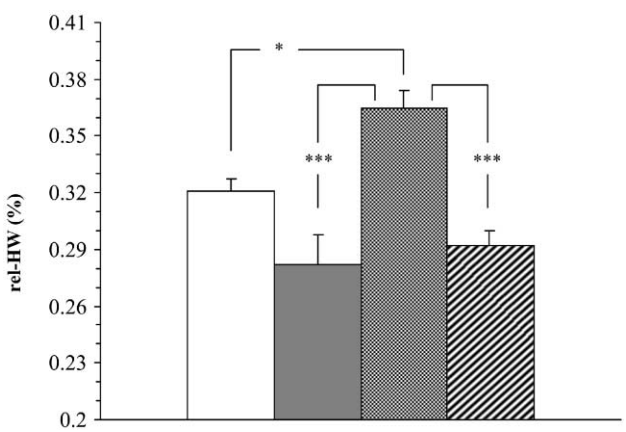

Fig. 2. Changes in relative heart weight (rel-HW) induced by vehicle (open bars), zofenopril (grey bars), doxorubicin (dotted bars) and zofenopril + doxorubicin (hatched bars). Heart weight/body weight ratio was calculated 4 weeks after suspension of doxorubicin treatment. $^{*}P < 0.05$ vs. vehicle group; $^{***}P < 0.001$ vs. zofenopril and zofenopril + doxorubicin groups.

Table 1
Effects of zofenopril and doxorubicin on ECG 4 weeks after suspension of doxorubicin treatment

	HR (b/min) ($n = 8$)	QαT (ms) ($n = 8$)	QRS (ms) ($n = 8$)	QRS (mV) ($n = 8$)
Control	329 ± 10	32.7 ± 0.8	23.7 ± 0.5	0.453 ± 0.05
Zofenopril	343 ± 9	29.1 ± 0.9	22 ± 0.8	0.471 ± 0.03
Doxorubicin	328 ± 9	48.1 ± 1.5[a,b,c]	24.3 ± 1	0.516 ± 0.05
Zofenopril + doxorubicin	327 ± 9	37.2 ± 1.7	22.2 ± 0.5	0.438 ± 0.02

n = number of animals.
[a] $P < 0.001$ vs. control group.
[b] $P < 0.001$ vs. zofenopril group.
[c] $P < 0.001$ vs. zofenopril + doxorubicin group.

length/2 (Geran et al., 1972). The following effects achieved by the drug treatments were evaluated.

• *Tumor Weight Inhibition %* (TWI%) in treated versus control mice, determined 7–10 days after the last drug treatment;

• *Log Cell Kill* (LCK) in treated mice according to the formula: $T - C/DT \times 3.32$, where T and C are the days taken by the tumors in treated (T) and control (C) mice to reach 1 g of weight and the DT is the tumor Doubling Time calculated from semilogarithmic best fit curve of tumor weight in the control mice, plotted versus time, when the growth of tumor was in the exponential phase (Teicher, 1997).

2.6. Drugs

Doxorubicin (Adriblastina™) in clinical formulation was purchased from Pharmacia, Milano, Italy. Doxorubicin was dissolved in the vehicle (1% lactose in sterile saline) under sterile conditions, at a concentration of 0.15 mg/ml. Zofenopril (batch no. 9721302E), synthesized at the Chemistry Department of Laboratori Guidotti, Pisa, Italy, was suspended in carboxymethylcellulose 0.5% at a concentration of 1 mg/ml. Hypnorm was from Janssen-Cilag, Saunderton, Buckinghamshire, UK. Pentobarbital and isoprenaline were from Sigma, St. Louis, MO, USA.

2.7. Statistical analysis

All the data presented in the text, tables and figures are means ± standard error of the mean (S.E.M.). Statistical comparisons were performed using, when appropriate, Student's *t*-test or a one-way analysis of variance followed by Bonferroni's test.

3. Results

3.1. Effects on body and heart weights

Chronic doxorubicin treatment induced a progressive reduction in body weight gain, starting from the fourth week of treatment, as compared to control animals. The real delay in body weight gain was probably underestimated in this group since all doxorubicin-treated animals showed a remarkable ascite at the end of the experiment. Rats receiving zofenopril + doxorubicin showed a similar delay in body weight gain, but a lower incidence of ascite and a lesser degree in its severity. Treatment with zofenopril alone produced a significant reduction in body weight gain versus control group starting from fifth week of treatment, as observed with angiotensin-converting enzyme inhibitors in general (Buikema et al., 2000). However, this

Table 2
Effects of zofenopril and doxorubicin on basal cardiovascular parameters 4 weeks after the suspension of doxorubicin treatment

Parameters	Control ($n = 7$)	Zofenopril ($n = 7$)	Doxorubicin ($n = 7$)	Zofenopril + doxorubicin ($n = 8$)
SBP (mm Hg)	157 ± 3.7	140 ± 6.6	133 ± 7.7	139 ± 6.2
DBP (mm Hg)	102 ± 2	91 ± 5.1	84 ± 8.5	97 ± 5.5
MBP (mm Hg)	124 ± 2.9	109 ± 5.8	105 ± 8.7	114 ± 6.1
HR (b/min)	324 ± 14	332 ± 11	310 ± 17	329 ± 11
LVSP (mm Hg)	144 ± 4.3	128 ± 6.6	128 ± 8.9	133 ± 6.7
dP/dt_{MAX} (mm Hg/s)	7085 ± 290	7042 ± 328	5704 ± 538	6758 ± 390
LVEDP (mm Hg)	5.8 ± 1	5.6 ± 0.5	6 ± 0.5	5.6 ± 0.7
CI (dP/dt_{MAX}/LVSP)	86 ± 2.6	94 ± 2.2	81 ± 3.8	90 ± 1.6

n = number of animals.
SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HR, heart rate; LVSP, left ventricular systolic pressure; dP/dt_{MAX}, maximum first derivative of LVSP; LVEDP, left ventricular end diastolic pressure; CI, contractility index.

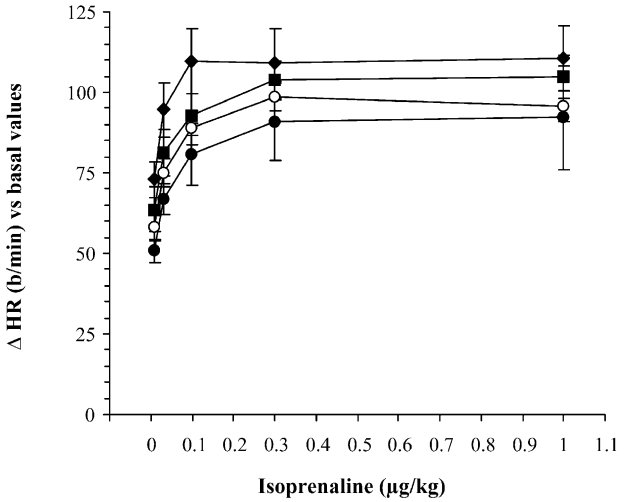

Fig. 3. Effect of vehicle (○, $n=7$), zofenopril (■, $n=7$), doxorubicin (●, $n=5$) and zofenopril + doxorubicin (♦, $n=8$) on heart rate responses elicited by increasing doses of isoprenaline (0.01–1 μg/kg) in anaesthetized rats 4 weeks after suspension of doxorubicin treatment.

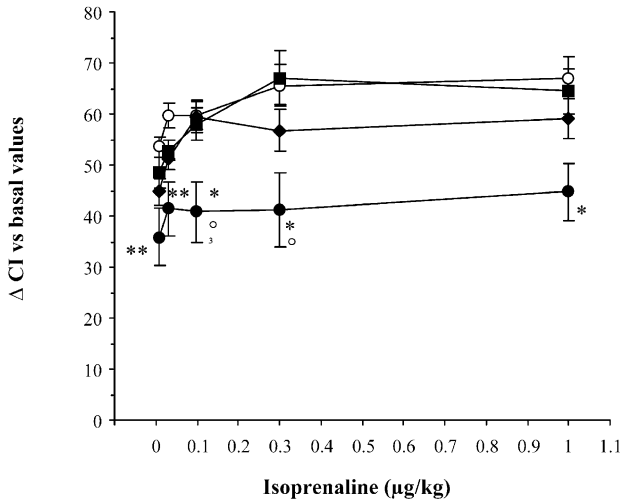

Fig. 5. Effect of vehicle (○, $n=7$), zofenopril (■, $n=7$), doxorubicin (●, $n=5$) and zofenopril + doxorubicin (♦, $n=8$) on contractility index (dP/dt_{MAX} /left ventricular systolic pressure) responses elicited by increasing doses of isoprenaline (0.01–1 μg/kg) in anaesthetized rats 4 weeks after suspension of doxorubicin treatment. $^{*}P<0.05$, $^{**}P<0.01$ vs. vehicle; $^{\circ}P<0.05$ vs. zofenopril group; $^{\diamond}P<0.05$ vs. zofenopril + doxorubicin group.

reduction was of minor degree as compared both to doxorubicin and zofenopril + doxorubicin groups (data not shown).

A significant reduction in absolute heart weight was observed at the end of the experiment in the group receiving zofenopril alone and in rats receiving zofenopril + doxorubicin, while animals that received doxorubicin alone showed only a slight reduction in absolute heart weight as compared to controls (Fig. 1). On the other hand, only a slight reduction in relative heart weight was observed in the zofenopril and zofenopril + doxorubicin groups as compared to controls, while a significant increase in relative heart weight was observed in doxorubicin alone treated animals versus controls, zofenopril and zofenopril + doxorubicin groups (Fig. 2). In addition, this latter difference was likely underestimated, owing to the remarkable ascite observed in doxorubicin-treated animals.

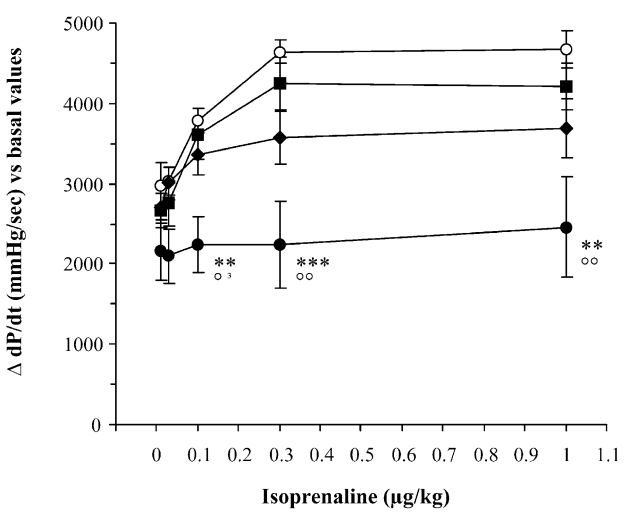

Fig. 4. Effect of vehicle (○, $n=7$), zofenopril (■, $n=7$), doxorubicin (●, $n=5$) and zofenopril + doxorubicin (♦, $n=8$) on the first derivative of left ventricular systolic pressure (dP/dt_{MAX}) responses elicited by increasing doses of isoprenaline (0.01–1 μg/kg) in anaesthetized rats 4 weeks after suspension of doxorubicin treatment. $^{**}P<0.01$, $^{***}P<0.001$ vs. vehicle; $^{\circ}P<0.05$, $^{\circ\circ}P<0.01$ vs. zofenopril group; $^{\diamond}P<0.05$ vs. zofenopril + doxorubicin group.

3.2. Electrocardiographic evaluations

At 4 weeks after the end of treatment, doxorubicin-treated animals showed significant changes in the repolarization phase of the ECG: doxorubicin induced a significant lengthening of QαT interval but not in QRS interval as compared to controls, whereas no significant changes were observed in rats receiving zofenopril or zofenopril + doxorubicin treatments (Table 1). Therefore, zofenopril pretreatment completely prevented changes in ECG repolarization induced by doxorubicin.

3.3. Effects on haemodynamics at rest and after isoprenaline stimulation

No significant alterations of basal haemodynamic parameters were observed in animals receiving doxorubicin

Table 3
Antitumor activity of doxorubicin (7 mg/kg, q7d×3 i.v.) alone or in combination with zofenopril (10 mg/kg, qd×14 os) on human ovarian carcinoma A2780 xenotransplanted in nude mice

Compounds	TWI%	LCK
Doxorubicin	89[a]	2.0
Zofenopril	24	0
Zofenopril + doxorubicin	96[a]	2.3

TWI = tumor weight inhibition; LCK = log cell kill.
[a] $P<0.01$ by Student's t-test vs. zofenopril-treated mice.

alone, zofenopril alone or zofenopril + doxorubicin as compared to control group. Animals receiving doxorubicin alone showed a remarkable decrease, although not statistically significant, in dP/dt_{MAX} as compared to the other groups (Table 2).

The administration of increasing doses of isoprenaline induces a dose-dependent increase in ventricular inotropic and chronotropic performance (heart rate, dP/dt_{MAX} and contractility index; Figs. 3, 4 and 5). In animals receiving doxorubicin alone, the inotropic responses to isoprenaline were severely depressed as compared to controls and zofenopril + doxorubicin-treated rats (Figs. 4 and 5). No differences were observed in the chronotropic responses to isoprenaline in all groups (Fig. 3).

The above results indicate that the administration of zofenopril is able to markedly ameliorate the impairment of positive inotropic responses to isoprenaline in animals that received chronic doxorubicin treatment: in animals receiving zofenopril + doxorubicin, the isoprenaline-induced increases in dP/dt_{MAX} and contractility index were near to control values.

3.4. Effects on antitumoral activity of doxorubicin

The antitumor activity study on human tumor A2780 was carried out to compare the effect on the tumor growth of doxorubicin administered alone or in combination with zofenopril. This tumor model was characterized by a good response to the doxorubicin treatment, with 89% of tumor growth inhibition and a value of LCK = 2.0 (Table 3).

No inhibitory effects on the growth of the ovarian carcinoma A2780 was detected with zofenopril at the dose tested. Nevertheless, some relevant features were obtained by the simultaneous administration of doxorubicin and zofenopril. The data indicated that the antitumor efficacy of doxorubicin was not affected by zofenopril: if any, a slight, although not statistically significant, increase of antitumor activity was observed in mice that received zofenopril + doxorubicin as compared to those treated with doxorubicin alone (Fig. 6).

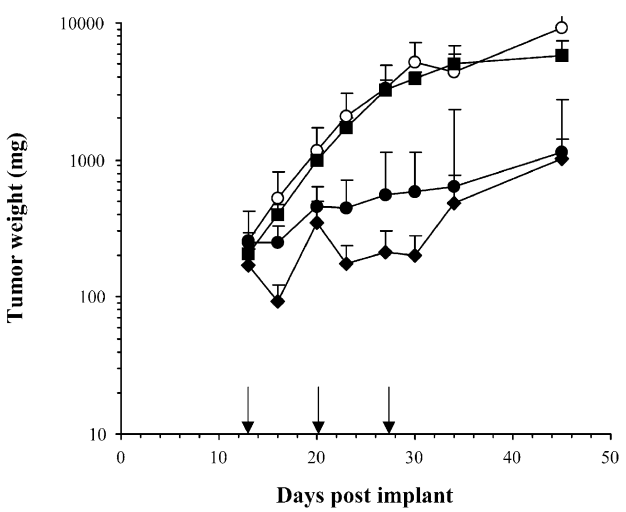

Fig. 6. Response of human ovarian carcinoma A2780 to doxorubicin treatment alone or in combination with zofenopril: (○, $n = 6$) control; (●, $n = 6$) doxorubicin, 7 mg/kg i.v. q7d×3 (arrows in the figure); (■, $n = 6$) zofenopril, 10 mg/kg os daily qd×14, from 13th to 27th day; (◆, $n = 6$) zofenopril + doxorubicin at the same dosage and schedule indicated before. Each point on the graph indicated the average volume of six tumor ± S.E.

4. Discussion

Chronic anthracycline treatment induces a progressive and severe deterioration of the repolarization phase in rats ECG (Jensen et al., 1984). QαT interval is considered one of the most sensitive markers of doxorubicin-induced ECG alterations (Villani et al., 1986). We showed previously that a positive correlation exists between degree and number of doxorubicin-induced histological cardiac lesions, as assessed histologically, and concomitant lengthening of QαT interval in this species (Cirillo et al., 2000).

Moreover, previous studies have established that—much alike the development of cardiac lesions—the doxorubicin-induced lengthening of QαT interval undergoes a time-dependent worsening, which extends beyond the end of anthracycline administration (Jensen et al., 1984; Mettler et al., 1977; Olson and Capen, 1978). This feature mimics the time course of anthracycline-induced congestive heart failure in patients, a life-threatening event, which often occurs up to several months after the administration of these cytotoxic drugs (Von Hoff et al., 1979; Buzdar et al., 1985; Rhoden et al., 1993).

The results of this study indicate that, without interfering with the antitumor activity of the anthracycline, oral zofenopril treatment prevents the cardiotoxic effects of doxorubicin in rats. ECG alterations induced by doxorubicin were almost totally prevented.

An aggravating feature of anthracycline-induced congestive heart failure is the developing unresponsiveness to inotropic agents (Singal and Natasha, 1998; Lefrak et al., 1973). This feature of anthracycline-induced congestive heart failure was mimicked—in the present rat model—by a selective impairment of cardiac inotropic effects induced by isoprenaline.

Zofenopril treatment significantly ameliorated the deterioration induced by doxorubicin on myocardial inotropic responses to β-adrenergic stimulation. In addition, zofenopril was able to prevent the increase in relative heart weight observed in animals that received doxorubicin alone. This parameter, which can be assumed to be indicative of doxorubicin-induced cardiomyopathy, was completely prevented in rats that received zofenopril + doxorubicin.

The mechanism(s) through which doxorubicin treatment induces cardiotoxicity is still debated. Free radical hypothesis, Ca^{2+} homeostasis disturbances, anthracycline metabolite formation are some of the hypotheses that have been proposed to explain the possible mechanism of dox-

orubicin-induced cardiac dysfunction (Olson and Mushlin, 1990; Minotti et al., 1995).

The cardioprotective effects of zofenopril could be mainly accounted by the inhibition of the renin–angiotensin system, which plays a central role in the worsening of cardiac injury of ischaemic origin (Fleetwood et al., 1991; Li and Chen, 1987). The antihypertensive effects of zofenopril could also ameliorate and/or preserve the cardiac performance by reducing the coronary and vascular resistances and then reducing myocardial afterload.

Zofenopril, owing to the presence of a sulphydryl group in its structure, can also act as an oxygen free radicals scavenger and this component of the mechanism of action is likely involved in cardioprotection afforded toward cardiac injuries induced by oxidative stress (Liu et al., 1992; Mak et al., 1990; Li et al., 2000). An increase in free radicals formation has been well documented in doxorubicin-induced cardiotoxicity (Keizer et al,. 1990; Xianhua et al., 1998); therefore, a contribution of scavenger action of zofenopril in preventing doxorubicin-induced cardiotoxicity appears likely.

Disturbances of Ca^{2+} regulation have been reported to be associated with doxorubicin-induced cardiotoxicity (Wang and Korth, 1995; Kusuoka et al., 1991). Increases of the binding of ryanodine to Ca^{2+} channel and alterations of the Ca^{2+} homeostasis in the sarcoplasmic reticulum are two of the major findings ruled out by several author in this matter (Zucchi et al., 1997; Pessah et al., 1990, 1992). Since zofenopril has shown to preserve and maintain Ca^{2+} homeostasis in rabbits subjected to ischaemia/reperfusion experiments (Ferrari et al., 1992), zofenopril could play a significant role in the preservation and regulation of Ca^{2+} homeostasis in cardiomyocytes affected by doxorubicin treatment too, although the Ca^{2+} downregulation induced by doxorubicin could be produced by mechanism different from those involved in ischaemia.

In conclusion, the present findings demonstrate that zofenopril, without interfering with the anticancer activity of this anthracycline, effectively prevents the development of doxorubicin-induced cardiotoxicity. These data suggest a possible usefulness of zofenopril as a cardioprotective agent contributing to a safer use of anthracyclines in patients subjected to chemotherapy.

Acknowledgements

We thank Ms. Clelia Irrissuto and Mr. Giuseppe Lopez for their expert technical support.

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