Erythropoietin Pretreatment Protects Against Acute Chemotherapy Toxicity in Isolated Rat Hearts

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The use of chemotherapeutic agents, such as anthracycline or trastuzumab, in oncology is limited by their cardiac toxicity. Recent experimental studies suggest that recombinant human erythropoietin (rhEPO) can be considered as a protective agent because its administration protects against cardiac ischemic injury, improving functional recovery, and reducing cell death. The aim of this study was to investigate whether pretreatment by rhEPO protects against acute cardiotoxicity induced by doxorubicin and trastuzumab, using the isolated rat heart model. Rats were treated with rhEPO (5000 IU/kg, intraperitoneally [ip]) or vehicle. One hour later, hearts were isolated and retrogradely perfused at constant flow. Following 20 mins of stabilization, hearts were perfused for 60 mins with modified-Krebs solution containing 6 mg/l doxorubicin or 10 mg/l trastuzumab. Hearts receiving doxorubicin were paced; those receiving trastuzumab were unpaced. Control hearts were perfused with modified-Krebs solution only. Doxorubicin exposure decreased left ventricular developed pressure (LVDP; approximately −40% of baseline) and increased end diastolic pressure (EDP; approximately +390% of baseline) and coronary perfusion pressure (CPP; approximately +70% of baseline). Incidence of ventricular tachycardia or fibrillation (VT/VF) was also significantly enhanced (86% vs. 0% in control group). Trastuzumab exposure increased CPP and EDP (approximately +70% of baseline for the both) without affecting LVDP. Prior rhEPO treatment significantly prevented doxorubicin-induced deleterious effects on LVDP, EDP, and VT/VF incidence. rhEPO administration also prevented trastuzumab-induced deleterious effects on CPP and EDP. This study shows that pretreatment by rhEPO protects myocardium against functional damage and electrophysiologic injury induced by acute doxorubicin or trastuzumab exposure. Further investigations are required to elucidate the precise mechanisms involved. Exp Biol Med 233:76–83, 2008

Key words: erythropoietin; doxorubicin; trastuzumab; cardiotoxicity; isolated rat heart

Introduction

Although rare, cardiotoxicity is a serious complication of cancer treatment. The incidence and severity of cardiotoxicity are notably dependent on the type of drugs used (1). Indeed, the use of anthracycline antibiotics as anticancer agents is limited by the late and irreversible cardiomyopathy they produce. In humans, nonspecific electrocardiographic changes, characteristic modifications in nuclear and cytoplasmic structure, decreased ejection fraction and congestive heart failure with increased mortality have been reported (2–5). Animal studies have described altered nuclear and cytoplasmic structure (6), increased coronary resistance (7), electrocardiographic abnormalities (8), evidence of left ventricular failure (9, 10), and increased mortality (11, 12) following doxorubicin administration. Although the mechanisms responsible for these changes remain undefined, several hypotheses have been suggested. Drug exposure may cause cellular damage through lipid peroxidation by intracellular free-radical production (12–14), intercalation of doxorubicin into nuclear and mitochondrial DNA (15), cellular calcium overload (11), and release of histamine (16, 17). Finally, it seems that the chronic cardiomyopathy induced by doxorubicin may result, at least in part, from acute hemodynamic and metabolic effects accompanying each drug exposure (1, 18).

In the past decade, the new drug trastuzumab has yielded impressive results in the treatment of metastatic

This work was supported by a supply of rhEPO and doxorubicin from Dr. E. Brudieu and a supply of trastuzumab from Dr. M.-J. Robein-Dobremez from the Pharmacie du CHU de Grenoble, France.

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Received June 1, 2007.
Accepted September 7, 2007.

DOI: 10.3181/0706-RM-152
1535-3702/08/2331-0076$15.00

breast cancer but also unexpected cardiotoxicity (19, 20). This molecule is a recombinant monoclonal antibody targeted against human epidermal growth factor receptor-2 protein (HER2), which is overexpressed in approximately 30% of breast cancer (21). As a single agent, the cardiotoxicity rate of trastuzumab varies between 3% and 7%, expressing in congestive heart failure. When trastuzumab is associated to standard chemotherapy, a decrease in left ventricular function is observed in almost 30% of women (22). Several hypotheses regarding the pathogenesis of trastuzumab cardiotoxicity have been proposed. They include both immune-mediated and direct toxicity (23).

Very recently, a new cytoprotective property of erythropoietin (EPO) was identified. This cytokine is produced by the adult kidney and is indispensable for proliferation, survival, and differentiation of erythroid progenitor cells (24). Erythropoietin receptors (EPOR) have been identified in nonhematopoietic tissues, including the heart (25), that could explain other cellular response activated following the binding of EPO to its receptor. Indeed, many recent experimental studies suggest that exogenous recombinant human EPO (rhEPO) administration exerts a cardioprotective effect against infarction and ischemia-reperfusion injury (26–28) and could be considered as a preconditioning agent (29). Moreover, two studies in rodents showed that rhEPO is also able to prevent cardiac dysfunction in chronic model of doxorubicin-induced cardiomyopathy (30, 31).

We have thus investigated in this study whether rhEPO preconditioning could protect the myocardium against acute hemodynamic and electrophysiologic damages induced by doxorubicin or trastuzumab, using the isolated rat heart model.

Material and Methods

Animals and Experimental Design. The animal care complied with the Guide for the Care and Use of Laboratory Animals (7th ed.), Washington, DC: National Academy Press, 1996. Male Wistar rats (250–350 g) were provided by Janvier (Le Genest-St-Isle, France).

This study was conducted in two parts. In the first part, rats were treated with either rhEPO or its vehicle. Subsequently, all animals were allowed to recover for 1 h. In the second part, isolated hearts were perfused for 60 mins with or without doxorubicin or trastuzumab added in the perfusion buffer. The dose of doxorubicin (Pfizer, Paris, France) used (6 mg/l of modified-Krebs or 10.3 μ*M*) was in accordance with numerous previous works studying doxorubicin toxicity on isolated rat hearts (7, 32–34). The dose of trastuzumab (10 mg/l of modified Krebs) was determined according to serum level measured in patients receiving the weekly dose (100 mg per week) likely to achieve clinical efficacy (35).

Experimental Groups. The rats were divided into eight experimental groups ($n = 7$ in each group):

Group C1. Hearts from rats treated with saline (1 ml/kg, ip) were perfused with modified-Krebs buffer only and paced throughout the protocol.

Group D. Hearts from rats treated with saline (1 ml/kg, ip) were perfused with modified-Krebs containing 6 mg/l doxorubicin and paced throughout the protocol.

Group E1. Hearts from rats treated with rhEPO (5000 IU/kg, ip) were perfused with modified-Krebs only and paced throughout the protocol.

Group ED. Hearts from rats treated with rhEPO (5000 IU/kg, ip) were perfused with modified-Krebs containing 6 mg/l doxorubicin and paced throughout the protocol.

Group C2. Hearts from rats treated with saline (1 ml/kg, ip) were perfused with modified-Krebs buffer only and unpaced.

Group T. Hearts from rats treated with saline (1 ml/kg, ip) were perfused with modified-Krebs containing 10 mg/l trastuzumab and unpaced.

Group E2. Hearts from rats treated with rhEPO (5000 IU/kg, ip) were perfused with modified-Krebs only and unpaced.

Group ET. Hearts from rats treated with rhEPO (5000 IU/kg, ip) were perfused with modified-Krebs containing 10 mg/l trastuzumab and unpaced.

Because of nonspecific electrocardiographic changes induced by doxorubicin, hearts treated by that anthracyclin were classically paced (7, 33). As no such changes were observed, neither in patient treated with trastuzumab nor in rodent hearts perfused with trastuzumab (preliminary experiments), hearts perfused by that molecule were not paced. The experimental protocol is summarized in Figure 1.

Perfusion Protocol on Isolated Heart. One hour after the administration of rhEPO or its vehicle, rats were heparinized (1000 U/kg, ip) and anesthetized with 60 mg/kg ip sodium pentobarbitone. The heart was rapidly excised and immediately immersed in 4°C modified-Krebs solution (NaCl 118, KCl 4.7, $CaCl_2$ 1.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.2, and glucose 11.0 m*M*). The aortic stump was then cannulated and the heart perfused retrogradely using the Langendorff technique at constant flow (15 ml/min) with oxygenated modified-Krebs solution.

A water-filled balloon coupled to a pressure transducer was inserted into the left ventricular cavity *via* the left atrium for left ventricular balloon pressure (LVBP) recording. Left ventricular end-diastolic pressure (LVEDP) was adjusted between 8 and 12 mm Hg. Myocardial temperature was measured by a thermoprobe inserted into the left ventricle and was maintained close to a constant 37°C. After 10 mins of stabilization, hearts from C1, D, E1, and ED groups were paced at 300 bpm by means of stainless steel electrodes attached to the apex and to the aortic cannula, which was maintained throughout the protocol. Hearts from C2, T, E2, and ET were not paced. Following a 20-min stabilization period, hearts were perfused for 60 mins with or without doxorubicin or trastuzumab added in the perfusion buffer.

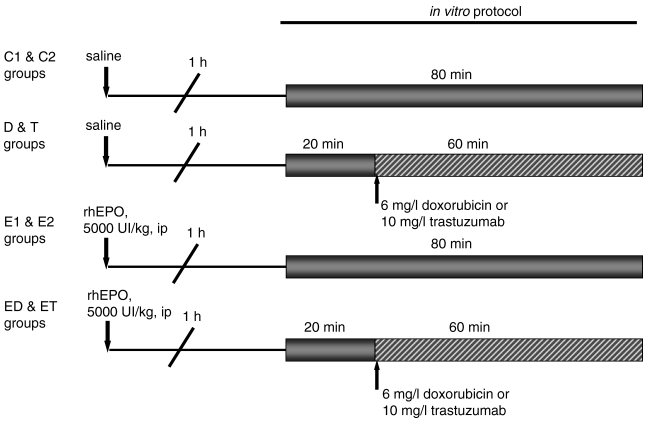

Figure 1. Experimental protocol. Hearts from C1, D, E1 and ED groups were paced at 300 bpm. Hearts from C2, T, E2 and ET were not paced.

Coronary perfusion pressure (CPP), left ventricular developed pressure (LVDP = difference between left ventricular systolic pressure and LVEDP), and heart rate (HR, in unpaced hearts) were continuously recorded. LVBP inflation, measured throughout this protocol, reflected both the compliance of the ventricle and the balloon. However, because the balloon volume was held constant, changes in LVBP represented changes in LVEDP. Moreover, percentages of change in LVBP were due to changes in LVEDP. The cardiac contractility was assessed measuring the first derivative of LVDP, dP/dt max and min indexes. Arrhythmias were classified in accordance with the Lambeth Convention guidelines (36). Pressure recordings were analyzed for the incidence (%) of ventricular tachycardia or fibrillation (VT/VF) occurring during the 60-min perfusion period.

Statistical Analysis of Data. All data are presented as mean ± standard error of the mean (SEM). Baseline hemodynamic data were compared using a one-way analysis of variance (ANOVA). Comparison of CPP, LVEDP, LVDP, and dP/dt data were performed by a two-way repeated-measures ANOVA with *post hoc* multiple-comparison Tukey tests. P values ≤ 0.05 were considered significant. Arrhythmia incidences, expressed as percentages, were compared using Fisher's Exact tests.

Results

Baseline Hemodynamic Data. At the end of the stabilization, LVEDP, LVDP, and CPP data were not different among the C1, D, E1, and ED groups or among the C2, T, E2, and ET groups, as shown in Table 1. Moreover, HR baseline values were not different among C2, T, E2, and ET groups, as shown in Table 3.

Effects of Doxorubicin. *On Coronary Perfusion Pressure.* After 60-min perfusion with doxorubicin, CPP significantly rose to 169% ± 3% of the baseline level, whereas in hearts perfused with normal buffer it was 100% ± 5% of baseline (Fig. 2). The same effect was seen in hearts from rhEPO treated rats (ED: 174% ± 12 vs. E1: 117% ± 10% of baseline CPP). Because coronary perfusion flow rate was held constant, the increase in CPP induced by doxorubicin exposure can be attributed to an increase in coronary resistance.

Table 1. Baseline Hemodynamic Data[a]

Groups	CPP (mm Hg)	LVEDP (mm Hg)	LVDP (mm Hg)
C1	72 ± 7	10 ± 1	90 ± 9
D	69 ± 4	10 ± 1	94 ± 8
E1	65 ± 3	10 ± 1	79 ± 9
ED	74 ± 5	11 ± 2	82 ± 6
C2	60 ± 3	11 ± 1	94 ± 4
H	70 ± 4	10 ± 1	111 ± 4
E2	59 ± 3	9 ± 2	110 ± 10
EH	57 ± 2	12 ± 4	99 ± 8

[a] Coronary perfusion pressure (CPP), left ventricular end-diastolic pressure (LVEDP), and left ventricular developed pressure (LVDP) measured at the end of the 20 min-stabilization period in groups C1 (paced hearts from saline-treated rats perfused by normal buffer), D (paced hearts from saline-treated rats perfused by buffer containing 6 mg/l doxorubicin), E1 (paced hearts from rhEPO-treated rats perfused by normal buffer), ED (paced hearts from rhEPO-treated rats perfused by buffer containing 6 mg/l doxorubicin), C2 (hearts from saline-treated rats perfused by normal buffer), T (hearts from saline-treated rats perfused by buffer containing 10 mg/l trastuzumab), E2 (hearts from rhEPO-treated rats perfused by normal buffer), and ET (hearts from rhEPO-treated rats perfused by buffer containing 10 mg/l trastuzumab). Data are mean ± SEM. No statistical difference was seen among the four corresponding groups for each hemodynamic parameter.

On Left Ventricular Function. During 60-min perfusion with doxorubicin, LVBP rose markedly (to 489% ± 80% of the baseline level in D group vs. 139% ± 12% in C1 group) and LVDP steadily decreased (to 60% ± 5% of the baseline level in D group vs. 93% ± 3% in C1 group). Prior rhEPO treatment significantly prevented both these deleterious effects induced by doxorubicin perfusion (ED: 189% ± 30 vs. E1: 107% ± 8% of baseline LVBP, and ED: 93% ± 10% vs. E1: 100% ± 13% of baseline LVDP) (Fig. 2). By the same manner, the cardiac contractility (shown by dP/dt max and min indexes) was identically affected by doxorubicin exposure, and that deleterious effect was also prevented by prior rhEPO treatment (data not shown).

On Ventricular Arrhythmias. Table 2 presents the incidence of VT/VF recorded throughout the 60-min perfusion period in the four experimental groups. A high incidence of VT/VF was observed in the presence of doxorubicin (six of seven rats), which was attenuated by prior rhEPO treatment (three of seven rats).

Effects of Trastuzumab. *On Coronary Perfusion Pressure.* After 60-min perfusion with trastuzumab, CPP significantly rose to 169% ± 15% of the baseline level, whereas in hearts perfused with normal buffer it was 141% ± 5% of baseline (Fig. 3). Prior rhEPO treatment significantly prevented that deleterious effect (ET: 135% ± 7% vs. E2: 130% ± 5% of baseline CPP; Fig. 3). Because coronary perfusion flow rate was held constant, the

Table 2. Ventricular Arrhythmias Data[a]

Groups	C1	D	E1	ED
VT/VF (%)	0	86*	0	43

[a] Incidence of ventricular tachycardia or fibrillation (VT/VF) recorded throughout the 60-min perfusion period in groups C1 (paced hearts from saline-treated rats perfused by normal buffer), D (paced hearts from saline-treated rats perfused by buffer containing 6 mg/l doxorubicin), E1 (paced hearts from rhEPO-treated rats perfused by normal buffer), and ED (paced hearts from rhEPO-treated rats perfused by buffer containing 6 mg/l doxorubicin).
* $P \leq 0.05$ vs. C1 group.

increase in CPP induced by trastuzumab exposure can be attributed to an increase in coronary resistance.

On Left Ventricular Function. During 60-min perfusion with trastuzumab, LVBP rose markedly (to 173% ± 47% of the baseline level in T group vs. 83% ± 16% in C2 group). Prior rhEPO treatment significantly prevented that deleterious effect induced by trastuzumab perfusion (ET: 84% ± 7% vs. E2: 96% ± 13% of baseline LVBP; Fig. 3). LVDP was not modified following 60-min perfusion with trastuzumab, and that parameter was not statistically different among C2 (122% ± 8% of baseline), T (104% ± 4% of baseline), E2 (100% ± 8% of baseline), and ET (111% ± 5% of baseline) groups. HR was also not modified following 60-min perfusion with trastuzumab, and that parameter was not statistically different among the four groups, as shown in Table 3.

On Ventricular Arrhythmias. No arrhythmias were seen in hearts perfused with trastuzumab, indicating no deleterious electrophysiologic effect of this drug.

A specific comment about CPP in C1 and C2 groups can be made. Indeed, CPP were statistically different among those groups, with an increase in CPP over time in the C2 group. Both groups were identical except for pacing (doxorubicin treated hearts were paced, whereas trastuzumab treated hearts were unpaced). The increase in CPP observed in C2 group could thus be related to the uncontrolled heart rate.

Discussion

In this study, we observed that acute doxorubicin or trastuzumab exposure induced cardiotoxicity in terms of hemodynamic and electrophysiologic damages. Prior rhEPO administration was able to prevent that chemotherapy-induced cardiotoxicity.

Cardiotoxic Effects Induced by Doxorubicin. In accordance with previous works (7, 32–34), we observed that an acute exposure to doxorubicin induced a rise in CPP related to an increase in coronary resistance, and alterations in systolic function and diastolic properties. Elevation of coronary resistance could result from a direct coronary vasoconstriction or by an alteration in ventricular compliance with compression of intramural arteries (7). We also observed that doxorubicin exposure induced ventricular electrophysiologic damages and arrhythmia. Indeed, a high incidence of ventricular fibrillation and tachycardia were observed during doxorubicin perfusion, which is in accordance with previous works showing a proarrhythmic effect of this anthracycline (32, 33, 37). There is evidence that acute cardiotoxicity of doxorubicin depends on oxygen-derived free radical generation and lipid peroxidation (12, 14, 15) Indeed, many compounds possessing antioxidant properties are able to protect the myocardium against doxorubicin-induced toxicity (38–42). Finally, free radical–mediated damage induced by doxorubicin may lead to cellular membrane alteration, resulting in myocyte death, which, in turn, could cause the biochemical and functional changes observed, that is, the rise in EDP and the fall in LVDP, phosphocreatine, and ATP content (7).

Prevention of Doxorubicin-Induced Cardiotoxicity by EPO Pretreatment. Many recent experimental studies have highlighted the cytoprotective role of rhEPO in ischemia-reperfusion injury independent of its hematopoietic action (26–28). Indeed, rhEPO (administered at the same dose used in this study) improves ventricular function, reduces infarction, and prevents apoptosis following ischemia-reperfusion in the rat heart (28, 43). Numerous other recent works in different animal species have shown that rhEPO enhances the survival of ischemic cardiomyocytes (44–47). Finally, it seems that rhEPO could have anti-

Table 3. Heart Rate Data[a]

Groups	C2	T	E2	ET
Stabilization	271 ± 11	281 ± 13	260 ± 12	250 ± 10
Perfusion 10 mins	276 ± 11	238 ± 11	254 ± 13	253 ± 12
Perfusion 20 mins	273 ± 9	240 ± 12	252 ± 11	243 ± 4
Perfusion 30 mins	261 ± 8	245 ± 6	258 ± 9	239 ± 4
Perfusion 40 mins	255 ± 6	242 ± 8	257 ± 10	231 ± 5
Perfusion 50 mins	253 ± 6	256 ± 11	247 ± 12	236 ± 5
Perfusion 60 mins	238 ± 8	254 ± 10	230 ± 10	230 ± 7

[a] Heart rate (in bpm) measured at the end of the 20-min stabilization period and throughout the perfusion protocol in groups C2 (hearts from saline-treated rats perfused by normal buffer), T (hearts from saline-treated rats perfused by buffer containing 10 mg/l trastuzumab), E2 (hearts from rhEPO-treated rats perfused by normal buffer), and ET (hearts from rhEPO-treated rats perfused by buffer containing 10 mg/l trastuzumab). Data are mean ± SEM. No statistical difference was seen among the four groups.

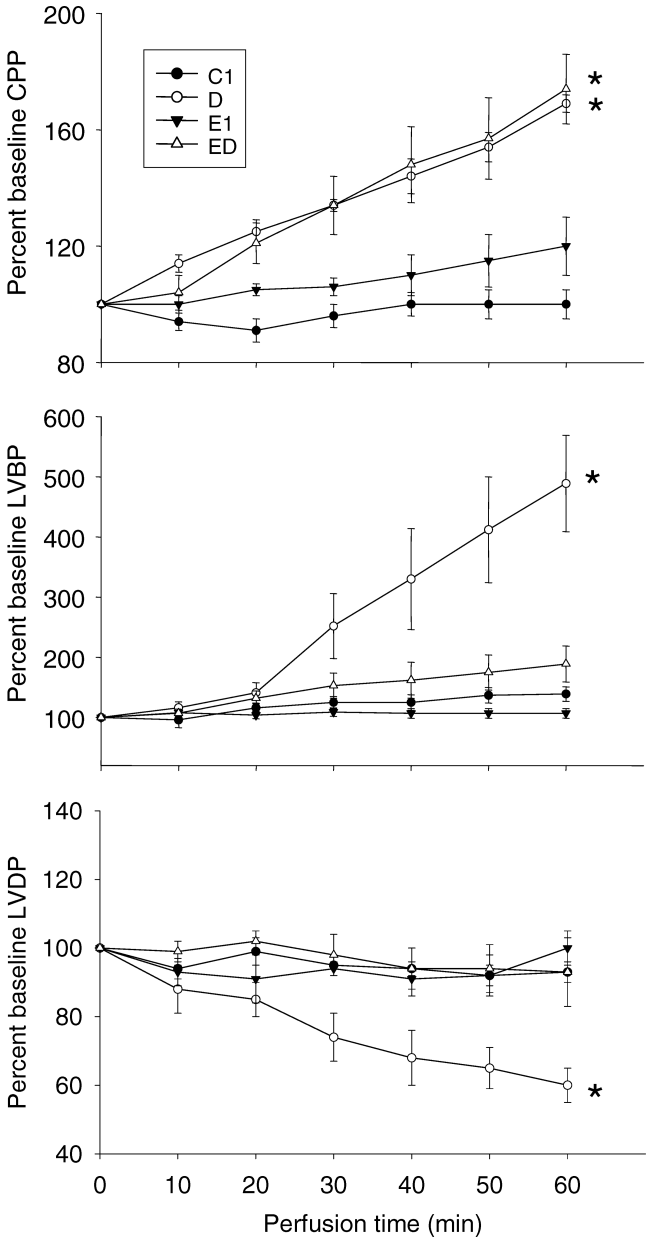

Figure 2. CPP, LVBP, and LVDP, expressed as percentage of baseline values, measured during the 60 mins of perfusion in groups C1 (paced hearts from saline-treated rats perfused by normal buffer), D (paced hearts from saline-treated rats perfused by buffer containing 6 mg/l doxorubicin), E1 (paced hearts from rhEPO-treated rats perfused by normal buffer), and ED (paced hearts from rhEPO-treated rats perfused by buffer containing 6 mg/l doxorubicin). Data are mean ± SEM. * $P \leq 0.05$ vs. the other groups (without *), globally.

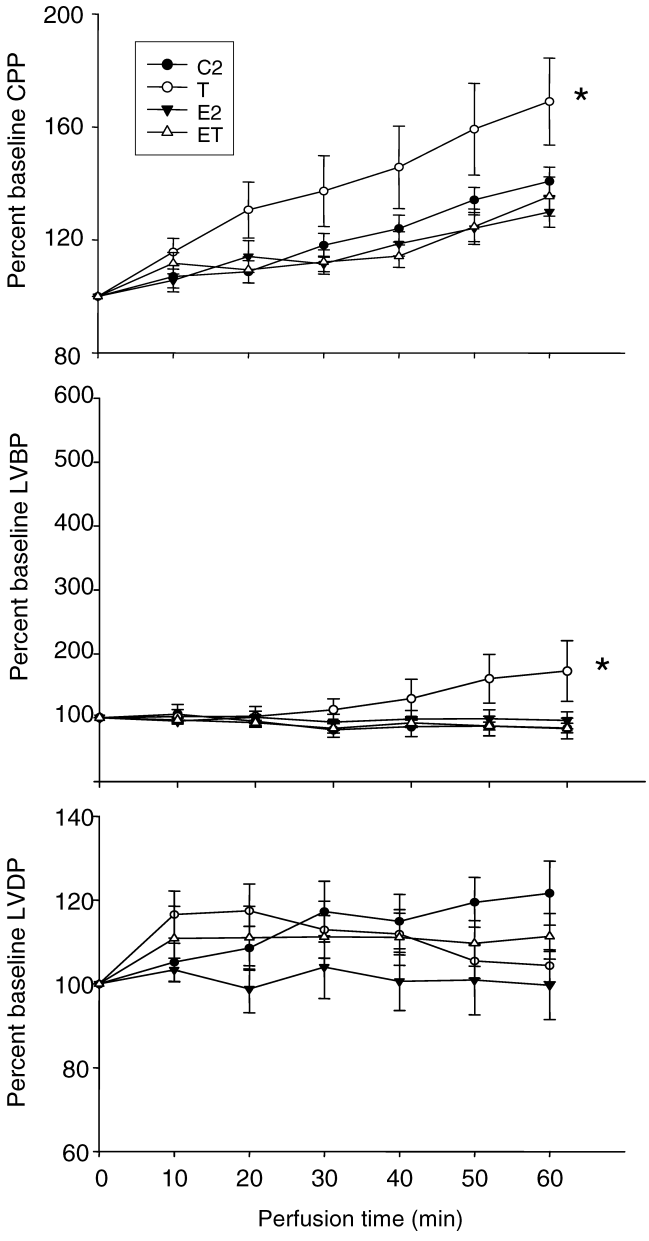

Figure 3. CPP, LVBP, and LVDP, expressed as percentage of baseline values, measured during the 60 mins of perfusion in groups C2 (hearts from saline-treated rats perfused by normal buffer), T (hearts from saline-treated rats perfused by buffer containing 10 mg/l trastuzumab), E2 (hearts from rhEPO-treated rats perfused by normal buffer), and ET (hearts from rhEPO-treated rats perfused by buffer containing 10 mg/l trastuzumab). Data are mean ± SEM. * $P \leq 0.05$ vs. the other groups (without *), globally.

inflammatory and antioxidant properties because rhEPO reduces inflammation and cytokine production and attenuates oxidative stress when chronically administered during heart failure (48).

In this study, we showed that prior rhEPO administration was able to protect the myocardium against acute doxorubicin-induced contractile dysfunction, preventing EDP increase as well as LVDP decrease, but was unable to prevent CPP increase. The doxorubicin-induced proarrhythmic effect was also significantly attenuated by rhEPO administration. It has been shown in canine heart that rhEPO administration could also prevent arrhythmias triggered by an ischemia-reperfusion sequence (49).

Thus, rhEPO-induced protection against doxorubicin cardiotoxicity could be due to the antioxidant and cytoprotective properties of this compound, preventing arrhythmia occurrence and ventricular function alteration.

Because the rise in CPP induced by doxorubicin was not prevented by rhEPO administration, it seems that cardiotoxic effect is independent of the others, perhaps depending on coronary flow disturbances. Indeed, when coronary resistance is altered, it may result in an abnormal distribution of coronary flow, potentially resulting in localized areas of ischemia and functional damage (7). That hypothesis remains to be tested.

Moreover, two studies recently showed that rhEPO is able to prevent cardiac dysfunction in a model of cardiomyopathy induced by chronic doxorubicin exposure. Thus, chronic administration of rhEPO attenuates left ventricular dysfunction and cardiomyocytes atrophy and degeneration, potentially, through anti-inflammatory or proangiogenic mechanisms (30, 31). However, the cardiotoxic effects of acute doxorubicin exposure differ from those of chronic administration (where vascular density and histologic modifications appear). Therefore, it is important to study the acute cardiotoxicity of doxorubicin, reflecting what happens during each administration and to identify potential protective agent, such as rhEPO. Finally, in our isolated rat heart model of acute doxorubicin toxicity, it is important to note that rhEPO protection occurred independent of rhEPO hematopoietic action.

Trastuzumab-Induced Cardiotoxicity and Prevention by EPO Pretreatment. In this study, we observed for the first time that acute trastuzumab exposure induced cardiotoxicity in terms of contractile dysfunction, using the isolated rat heart model. Indeed, we showed here that trastuzumab perfusion induced a rise in CPP related to an increase in coronary resistance. This could result from a direct effect of trastuzumab causing coronary vasoconstriction. The increase in EDP observed during trastuzumab exposure could be related to a change in the diastolic properties of the ventricle consistent with a decrease in ventricular compliance. We also observed that systolic function was not altered by trastuzumab exposure.

Trastuzumab specifically binds to the extracellular portion of HER2. That receptor family is involved in cell-cell and cell-stromal communication, primarily through a process known as signal transduction, in which external growth factors or ligands affect the transcription of various genes by phosphorylating or dephosphorylating a series of transmembrane proteins and intracellular signaling intermediates, many of which possess enzymatic activity (50). It has been shown that adult mutant mice carrying a cardiac-restricted deletion of HER2 exhibited multiple independent parameters of dilated cardiomyopathy, suggesting the essential role of the HER2 signaling in the cardiomyopathy prevention (51). Moreover, HER2 signaling seems to be implicated in apoptosis prevention because activation of that receptor promotes survival and inhibits apoptosis *in vitro* in cardiomyocytes (52), whereas its inhibition induces apoptotic activation with cytochrome C release, an increase in DNA fragmentation, and significant mitochondrial dysfunction (53, 54).

Prior rhEPO administration conferred myocardial protection against trastuzumab-induced toxicity by preventing coronary vasoconstriction and the increase in LVEDP. Because rhEPO prevents apoptosis in various situations, it could represent one of the mechanisms underlying its protective effect against trastuzumab cardiotoxicity. Further investigations are needed to confirm that hypothesis.

Clinical Perspectives. The increasing used of doxorubicin and trastuzumab as adjuvant breast cancer therapy and the growing population of long-term pediatric cancer survivors mean that, more than ever, cardiotoxicity is an important issue for oncology. Cardiomyopathy induced by chronic chemotherapy may result, at least in part, from acute cardiotoxic effects accompanying each drug exposure (1, 18). Therefore, it is of interest to identify new protective agents that prevent those deleterious cardiac effects during chemotherapy administration. Today, rhEPO is known to protect cells against various stresses. Here, we observed, for the first time, that the drug prevents cardiac damage induced by acute doxorubicin or trastuzumab exposure. In this study, doses of doxorubicin or trastuzumab used in isolated rat hearts correspond to those measured in serum level of patients after each chemotherapy administration. rhEPO administration could, therefore, be used during each chemotherapy administration to reduce acute cardiotoxic effects accompanying each drug exposure and, potentially, to prevent long-term development of cardiomyopathy. Further clinical investigations are now needed to explore the potential benefit of rhEPO in oncology.

Finally, because one isolated rhEPO administration has no hematopoietic consequence, the therapeutic scheme of rhEPO administration proposed here (before each chemotherapy exposure only) does not present the adverse consequences associated with chronic rhEPO administration, such as hypertension or thrombosis (26).

In summary, this study suggests that rhEPO preconditioning can protect the myocardium against hemodynamic damages and electrophysiologic disturbances induced by an acute doxorubicin or trastuzumab exposure. The exact mechanisms involved in the cardioprotection conferred by rhEPO against chemotherapy toxicity, and in particular, the antioxidant role of this compound, should be determined. This study opens the way to clinical applications where prevention of acute cardiotoxic effects of chemotherapy could be envisaged.

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