VASODILATOR EFFECTS OF β-AGONIST ISOPRENALINE IN DOXORUBICIN-INDUCED MODEL OF HEART FAILURE

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Abstract- ***We investigated the vasodilatory effect of isoprenaline at large vessels (aorta, renal and saphenous arteries, vena cava, renal and saphenous veins) in doxorubicin-induced model of heart failure. Thirty saline-treated (normal group) and thirty doxorubicin treated rabbits (1 mg/kg administered intravenously twice weekly for 8 weeks) were studied after 16 weeks of treatment. Chronic heart failure was confirmed by histopathology. Arteries and veins were cut as rings and so bathed in Krebs maintained at 37°C and gassed with 95% O_2 and 5% $C0_2$. Then all tissues were placed under different resting tensions and allowed to equilibrate for 1 hour. Then all the tissues were contracted with U-46619 (O.1 μM) nearly ten minutes before initial application of isoprenaline. When the U-46619 (O.1 μM) -induced contraction reached a plateau, concentration-response curves to isoprenaline were obtained. Isoprenaline was chosen as vasodilator resulting from stimulating beta-receptors in blood vessels. Maximum effect (Ema) and median effective concentration (EC50) were determined from each concentration-response curve and pD_2 was calculated as -log (EC50). Isoprenaline induced relaxations in all vessels. Aorta and renal artery were the most sensitive ones and had the maximum relaxations (15-20 %). In relaxation due to β-adrenoceptor agonist isoproterenol, the aorta and renal artery were the most sensitive vessels. Compared with control, in doxorubicin treated rabbits, E_{max} of isoprenaline was not modified in all the studied vessels. Relaxation responses were negligible and maximum responses in vena cava, and renal vein were only 5-10 percent. Of all vessels there was no significant difference between control and doxorubicin induced group of heart failure in response to isoprenaline.***

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INTRODUCTION

Doxorubicin (DXR) is one of the most widely used broad-spectrum anticancer agents (1). However, because this agent produces a chronic and dose-related cardiomyopathy as its principal side effect (2,3), its clinical utility is limited. DXR can intercalate with DNA and changes DNA functions. It inhibits DNA and RNA synthesis (4) and causes strand breaks and induces apoptosis (5). These cytotoxicities are believed to be mediated either by an inhibition of DNA topoisomerase II or by the generation of free radicals (4,6). The production of superoxide radical anions by oxidation-reduction cycling of DXR is of critical importance in mediating the chronic cardiotoxicity associated with the clinical use of DXR (7). Imbalances of the circulatory system such as decreases in blood pressure are often observed in clinical situations during repetitive treatment with DXR (8,9). Such imbalances have been revealed also in in vivo studies (10,11), and it is considered that these circulatory imbalances are also caused by cardiac dysfunction induced by DXR. In vitro studies have suggested that DXR acutely induces a release of Ca^{2+} from intracellular storage sites of vascular smooth muscle and causes direct vasoconstriction (12) and vasodilatation (13). During the course of intravenous (IV) injection of DXR, the vasculature is exposed to high levels of DXR. Prior to the present study, however, no in vitro investigations had been carried out on the responses mediated by beta adrenoceptors in small and large peripheral vessels following heart failure induced by DXR have been carried out. The aim of our study was to investigate the possibility of changed sensitivity of beta-adrenoceptors in this model of heart failure.

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MATERIALS AND METHODS

Sixty male New Zealand White rabbits, weighing 2.5 to 3 Kg were obtained (Institute of Pasture, Tehran, Iran). The animals were housed individually under identical conditions with free access to food and water. The animals were randomly divided into two groups: Thirty saline-treated (normal group) and thirty DXR-treated rabbits (l mg/kg administered intravenously twice weekly for 8 weeks) were studied after 16 weeks treatment. Congestive heart failure (CHF) was confirmed by histopathology. Animals were killed by an overdose of pentobarbitone sodium. Arteries and veins were carefully removed with as little connective tissue as possible and placed in cold physiological salt solution (PSS). Three pairs of arteries and veins (thoracic aorta, left renal and lateral

saphenous arteries, vena cava, left renal and lateral saphenous veins) of the normal rabbits and DXR-treated rabbits were studied. The arterial and venous rings were mounted in 20 ml isolated organ baths, bathed in Krebs maintained at 37°C and gassed with 95% O_2 and 5% CO_2. Blood vessels were used immediately. The rings were then placed under different resting tensions that were determined by contraction to noradrenaline (1 μM) from some preliminary experiments. After initial application of tension, tissues were left to equilibrate for at least 45-min period. Isoprenaline was chosen as agonist of beta-adrenoreceptors that produces relaxation. In vascular beds after initial application of tension tissues were left to equilibrate for a 60 min period, during that time the tension was re-adjusted to a set value that was maintained constant throughout the rest of the experimental day. Then all tissues were precontracted with U-46619 (0.1 μM) nearly ten minutes before initial application of isoprenaline. U-46619 was chosen as precontractor since it has no effect on beta adrenoreceptors. This induced submaximal contraction in all vessels. When the U-46619-induced contraction reached a plateau, cumulative concentration-response curves (CCRC) to isoprenaline was obtained by increasing the concentration of the isoprenaline in half-log increments. Relaxation to isoprenaline in each concentration allowed five minutes to reach maximum. All concentrations are expressed as the final concentration in the organ bath fluid. Results are expressed as mean±standard error of mean (S.E.M). Comparisons between two groups were performed using unpaired Student's t-test with values. Comparisons among several groups were performed using one-way analysis of variance. A value of $p<0.05$ was taken as statistically significant. Vessel sensitivity was expressed as the negative logarithm of agonist concentration that elicited a PD_2 calculated using Prism program (Graphpad Software Inc San Diego, CA). Solutions and Drugs Reagents were obtained from Sigma Chemical CO (St Louis, MO, USA). The composition of the modified Krebs-Henselite solution was as follows: (in mM): NaCl 118.4, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.6, $MgSO_4$ 0.6, $CaCl_2$ 2.5 and glucose 11. All drugs except U-46619 were dissolved in distilled water. U-46619 was initially dissolved in high- performance liquid chromatography-grade absolute ethanol, with subsequent dilutions made in distilled water. They were prepared on the day of study in normal saline and kept at 4°C until use, at which time they were re-warmed to 37°C.

RESULTS

U- 46619 (9, 11- dideoxy- IIa, 9 α-epoxymetha-nopros-taglandin F 2α) produced submaximal contraction in all vessels. Isoprenaline induced relaxations in both arteries and veins (Fig. 1). Aorta and renal artery were the most sensitive ones and produced the maximum relaxation (15-20%). In relaxation to β-adrenoceptor agonist isoproterenol, there was no significant difference between control and doxorubicin induced model of heart failure.

DISCUSSION

Since circulating catecholamines exert a tonic vasodilatory effect on peripheral vessels via β-adrenoceptors, we investigated the role of β-adrenoceptors in doxorubicin treated rabbits. It is now well established that β-adrenoceptor function is abnormal in the myocardium of patients with adriamycin-induced congestive heart failure (14). In failing ventricles, there are decreased numbers of β-adrenoceptors, reduced isoprenaline-stimulated adenylate cyclase activity, and depressed inotropic and chronotropic responsiveness to β-adrenoceptor agonists. Bristow and colleagues examined isolated cardiac tissue using radioligand-binding techniques and reported that β_1-adrenoceptor down regulation occurred in the failing left ventricle, whereas there was no change in β_2-adrenoceptors (15). The loss of β-adrenoceptors were highly correlated with severity of heart failure symptoms. The fact that noradrenaline has greater β_1 -than β_2-adrenoceptor effects may explain the selective down-regulation of β_1-adrenoceptors (16). In spite of the known down-regulation of β_1-adrenoceptors in the failing left ventricle, few data in peripheral vessels are available. Since the trigger for β-adrenoceptor desensitisation is thought to be chronically elevated circulating catecholamines (15,16), this condition should also induce desensitisation of peripheral β-adrenoceptors as well as those in the heart. In the present study we found no change in isoprenaline-induced relaxations in doxorubicin treated rabbits compared with a normal control population. To explain the lack of changes of β-adrenoceptors in vascular beds of heart failure group, there are two main explanations. Down regulation of β-adrenoceptors is more selective for cardiac β_1-adrenoceptors and vascular beds containing β_2-adrenoceptors (17). Thus a β_2-adrenoceptor resistance to down regulation could explain the absence of β_2-adrenoceptors of vascular changes, such as found in our study, as well as consistent with prior studies (18,19), investigating this problem which found no peripheral β-adrenoceptor desensitisation or down-regulation in animal models of heart failure. An alternative explanation for the lack of β-adrenoceptor changes in our study is that these changes may occur in long time in human heart failure. We allowed the model to develop over sixteen week similar to other animal models of heart

failure (20,21). Since in animal models time that heart failure develops through is very short related to human, results obtained in our model of heart failure have to be interpreted and extrapolated to humans with caution.

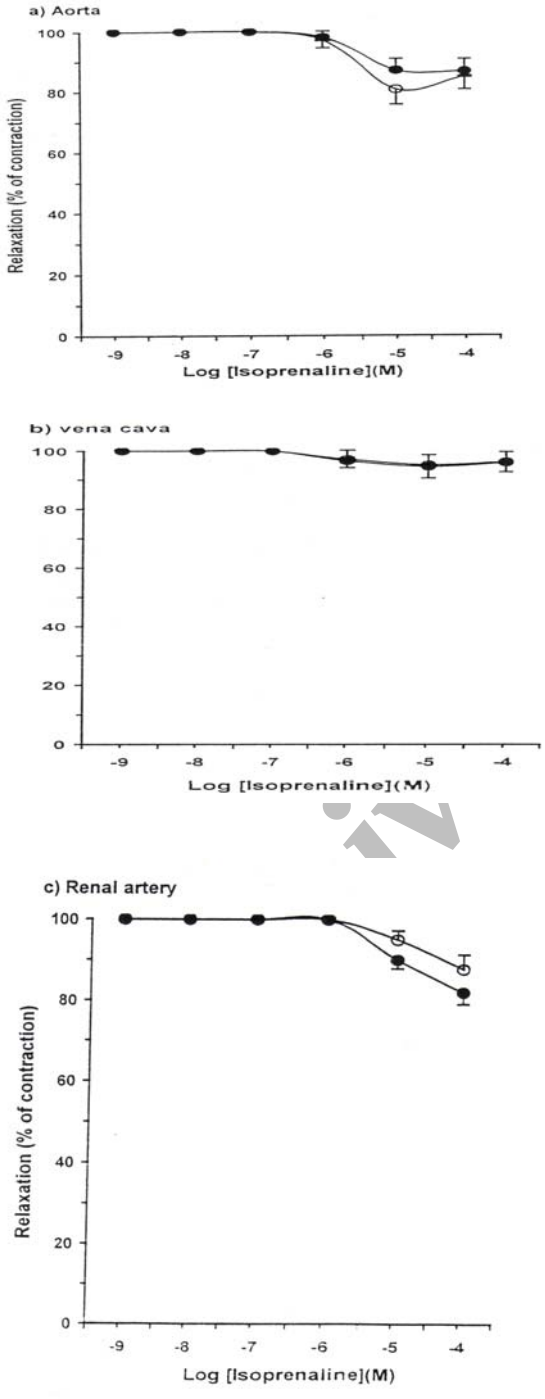

Fig. 1. Relaxation in response to isoprenaline in the three isolated arteries and veins of control animals (O) and doxorubicin treated rabbits (●). Results are expressed as % of maximum response to U-46619 (0.1 μM) used for inducing tone. Each point represents the mean= S. E. (n=7). Statistically, there are no significant differences between the two group using unpaired students t-test and one-way ANOVA.

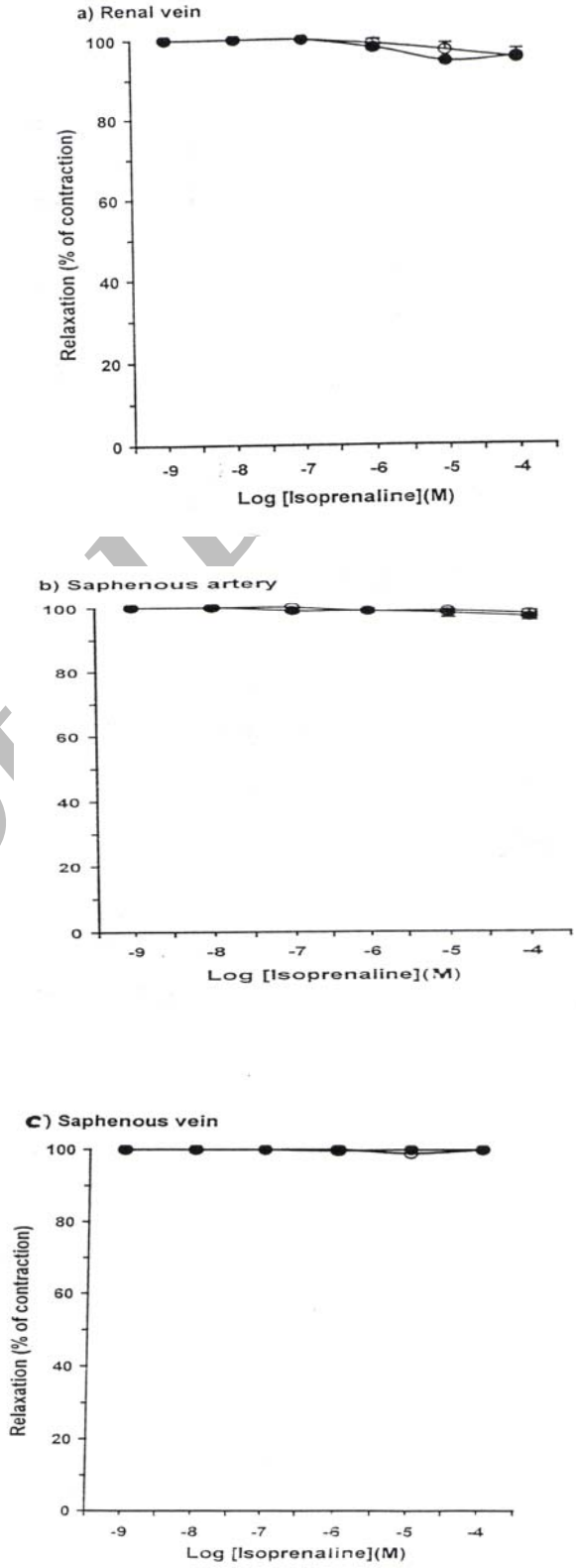

Fig. 2. Relaxation in response to Isoprenaline in the three isolated arteries and veins of control animals (O) and doxorubicin treated rabbits (●). Results are expressed as % of maximum response to U-46619 (0.1 μM) used for inducing, tone. Each point represents the mean±SEM. (n=7). Statistically. there are no significant differences between the two group using unpaired Students t-test and one-way ANOVA.

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