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The activities of tissue xanthine oxidase and adenosine deaminase and the levels of hydroxyproline and nitric oxide in rat hearts subjected to doxorubicin: protective effect of erdosteine

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Abstract

The aim of this experimental study was to investigate the effects of erdosteine, an antioxidant agent, on doxorubicin (DXR)-induced cardio-toxicity through nitric oxide (NO) levels, collagen synthesis, xanthine oxidase (XO) and adenosine deaminase (ADA) activities in rats. Rats were treated with erdosteine (10 mg/kg b.wt. per day, orally) or saline starting 2 days before administrating a single dose of DXR (20 mg/kg i.p.) or saline. At the 10th day of the DXR administration, hearts were removed under anesthesia for biochemical measurements. Enzyme activities as well as OH-proline and NO levels were found to be significantly increased in DXR group compared with the control group. All of the parameters studied except ADA activity were decreased significantly approximating to the control levels upon erdosteine administration. In conclusion, erdosteine seems to be an alternative agent for protection of cardiac tissue against DXR-induced cardio-toxicity through its regulatory effect on XO activity and NO level.

Keywords: Doxorubicin; Erdosteine; Xanthine oxidase; Adenosine deaminase; Nitric oxide; Hydroxyproline

1. Introduction

Doxorubicin (DXR), an anthracycline antibiotic that causes severe cardiotoxicity, has been believed to induce the toxic effects via oxidative mechanisms (Dalledonne et al., 1993; Quiles et al., 2002; Venditti et al., 1998). The results of DXR-induced cardiotoxicity are disarrangement of the Z-disc structure, the lack of the thin filaments and the disruption of the cytoskeleton architecture (Molinari et al., 1990). DXR causes cellular injury through detrimental effects on DNA, proteins, lipids and other cellular structures. It causes lipid

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peroxidation, oxidation of adjacent organelles, and DNA (Zhou and Kang, 2000). The myocardial side effect of DXR has been attributed to the ability of the drug to interact with the genome or to promote lipid peroxidation and generation of free radicals (Doroshow, 1983; Myers, 1998).

It was demonstrated that cardiac NO are increased during the development of DXR-induced cardiomyopathy (Sayed-Ahmed et al., 2001). Recent studies suggest that nitric oxide (NO) may have different roles in cardiac function and disease. Basal production of NO modulates contractility of myocardium and regulates blood flow distribution (Varin et al., 1999). In contrast, high levels of NO production, particularly by inducible NO synthase (iNOS), are associated with numerous types of myocardial disease (Haywood et al., 1996; Vejlstrup et al., 1998; Weinstein et al., 2000). The high levels of NO by iNOS induction may further participate in myocardial oxidative damage through peroxynitrite formation by reacting with superoxide anion (Adams et al., 1999). Peroxynitrite is a potent and aggressive cellular oxidant and causes the formation of 3-nitro-L-tyrosine. This modification has been occupied in a diverse array of disease settings, including myocarditis (Kooy et al., 1997; Weinstein et al., 2000).

It was demonstrated that interstitial accumulation collagen in myocardium was high in DXR-treated rats and decreasing the interstitial collagen accumulation was beneficial in preventing DXR-induced myocardial damage (Tokudome et al., 2000). Hydroxylation of proline is one of the important steps in collagen formation, and assessment of hydroxyproline level may give the clue about collagen accumulation in the extracellular matrix (Monboisse and Borel, 1992).

Erdosteine [*N*-(Carboxymethylthioacetyl)-homocysteine thiolactone], a mucolytic agent, contains two blocked sulfhydryl groups which are released following its metabolic process (Braga et al., 2000; Dechant and Noble, 1996; Yildirim et al., 2003). It is widely used orally in the clinics due to its mucolytic and expectorant properties. Erdosteine itself does not have free thiole group, but its metabolization produces active metabolite with SH group. Only metabolite I of erdosteine reaches high blood levels, and so the activity of erdosteine is generally attributed to metabolite I (Braga et al., 2000). Its active metabolites exhibit free radical scavenging activity through these –SH groups (Dechant and Noble, 1996; Gazzani et al., 1989). The aim of this study, therefore, is to investigate the in vivo effects of erdosteine against DXR-induced cardiotoxicity through purin catobolism, NO system and hydroxyproline (OH-P) formation, as an index of collagen synthesis.

2. Materials and methods

Male Sprague–Dawley rats (60 days old) were used in the experiments. The animals were housed in quiet rooms with 12:12-h light–dark cycle (07:00–19:00 h) and the experiments were performed in accordance with "*Guide for the Care and Use of Laboratory Animals*, DHEW Publication No. (NIH) 85–23, 1985" and approved by local ethical committee at Medical School of Inonu University.

Rats were randomly assigned to one of the three groups: control untreated rats ($n = 8$); animals treated with single i.p. injection of DXR (20 mg/kg b.wt.) (Carlo Erba, Turkey) ($n = 7$) (Venditti et al., 1998); animals treated for 12 days with oral administration of erdosteine (Ilsan, Turkey) (10 mg/kg b.wt. per day) (Dechant and Noble, 1996) beginning 2 days before single i.p. injection of DXR ($n = 10$). Three rats from DXR group (formerly $n = 10$) were excluded from the study because they dead after i.p. DXR injection in following days.

At the 10th day of DXR-treatment, the animals were anesthetized with urethane (i.p. 1.2 g/kg), and then hearts were rapidly excised and stored at −70 °C until the study. After weighing the heart, homogenate and supernatant were prepared as described elsewhere (Irmak et al., 2001), and the following determinations were made on the samples using commercial chemicals supplied by Sigma. Xanthine oxidase (XO) (Prajda and Weber, 1975) and ADA (Choong and Humphrey, 1987) activities were determined in the supernatant, tissue NO level (Cortas and Wakid, 1990) in homogenate according to the methods described

elsewhere. Protein measurements were made at all stages according to the Lowry's method (Lowry et al., 1951). The heart tissue hydroxyproline (OH-P) levels were determined by the method of Woessner (1961) after some pieces of samples were dried, weighed, digested in nitric acid/perchloric acid solution for 3 h.

Data were analyzed by using a commercially available statistics software package (SPSS® for WINDOWS v. 9.0, Chicago, USA). One-way Analysis of Variance (ANOVA) test was performed. Post Hoc multiple comparisons were done with LSD. Results were presented as means ± S.E.M. P-values < 0.05 were regarded as statistically significant.

3. Results

The results are summarized in the Table 1. The activities of XO and ADA were increased in DXR group in comparison with control group ($P < 0.05$). Erdosteine treatment with DXR decreased XO activity significantly in comparison with DXR alone group ($P < 0.05$). On the other hand, the activity of ADA was not affected from erdosteine treatment significantly. The level of NO production was higher in DXR group than the other groups ($P < 0.001$). The level of OH-proline was increased in DXR group in comparison with control and DXR plus erdosteine groups ($P < 0.001$).

4. Discussion

We have found that DXR caused increase in all the parameters studied (hydroxyproline, ADA, XO and NO), and erdosteine showed its protective effect by reversing their measurements almost to the control levels except ADA. Under aerobic conditions, the reduction of DXR through redox cycling results in the formation of superoxide anion radicals, which can later generate other reactive oxygen species. Reactive oxygen species generation, through the production of hydroxyl radicals, can lead to cellular damage through macromolecules including DNA (Cummings et al., 1991; Yee and Pritsos, 1997). XO utilizes molecular oxygen as an electron acceptor, with negligible reactivity toward NAD^+. Consequently, XO is reoxidized under physiological conditions through two, one-electron reductions of molecular oxygen to generate two superoxide anion radicals, which can subsequently form hydrogen peroxide. Conversely, xanthine dehydrogenase prefers to utilize NAD^+ as an electron acceptor to produce NADH through a direct two-electron reduction (Pritsos and Gustafson, 1994; Hille and Nishino, 1995; Parks and Granger, 1986). The present study demonstrated that DXR administration alone resulted in high XO activities. It was similar to the literature that DXR induces reactive oxygen species production by increasing XO activities. Furthermore, DXR is a toxic agent to all cellular components including genetic material. During DXR-induced cardiotoxicity, purines are degraded to hypoxanthine, and

Table 1
The activities of XO, ADA, and the levels of NO and hydroxyproline (OH-P) in control, DXR and DXR plus erdosteine groups

	OH-P (mg/g dry tissue)	XO (U/g prot)	ADA (U/g prot)	NO (μmol/g wet tissue)
(1) Control ($n = 8$)	0.975 ± 0.050	0.210 ± 0.027	0.082 ± 0.009	0.594 ± 0.052
(2) DXR ($n = 7$)	1.575 ± 0.126	0.583 ± 0.029	0.129 ± 0.011	1.041 ± 0.119
(3) DXR plus erdosteine ($n = 10$)	1.056 ± 0.062	0.241 ± 0.033	0.095 ± 0.014	0.611 ± 0.044
P-values				
1–2	0.0001	0.0001	0.020	0.0001
1–3	N.S.	N.S.	N.S.	N.S.
2–3	0.0001	0.0001	N.S.	0.0001

N.S., not significant.

xanthine dehyrogenase is converted to XO. XO catalyzes the conversion of hypoxanthine to uric acid with superoxide anion production and indirectly hydroxyl radical formation. The treatment of DXR toxicity by erdosteine prevented the high XO activity and formation of free radicals, which are toxic to cellular structures. On the other hand, high purine catabolism can be detected by high activity of another enzyme, ADA. Parallel to XO activity, ADA also showed high activity in DXR administered rats. These two enzymes may be considered as the indicators of DNA catabolism. DXR is a highly toxic to genetic material and these high enzyme activities may indicate that there might be genetic destruction in myocardial tissue.

It has been demonstrated that high NO production, especially by iNOS activity in myocardium, was present during DXR therapy (Sayed-Ahmed et al., 2001). It reflects that DXR-induced myocardial injury may be contributed by high NO production as supported by our study results. Under aerobic conditions, NO reacts with superoxide anion and forms a radical, peroxynitrite. Peroxynitrite oxides cellular structures and causes lipid peroxidation (Weinstein et al., 2000; Sayed-Ahmed et al., 2001). Our previous study showed that DXR causes lipid peroxidation in myocardial tissue (Fadillioglu et al., 2003). Our colleagues showed marked elevation in NO level in damaged kidney tissue of the cisplatin-treated rats and erdosteine significantly attenuated this increment (Yildirim et al., 2003). In the present study, erdosteine treatment markedly decreased NO production thereby preventing tissue injury. Previous studies demonstrated that erdosteine treatment results in prevention of lipid peroxidation in myocardium and plasma of DXR administered rats (Fadillioglu et al., 2003; Fadillioglu and Erdogan, 2003). Therefore, erdosteine may exert its inhibitory effect on NO production by inhibiting iNOS. This study investigated the in vivo protective activity of a drug with a –SH group, the metabolite I of erdosteine, against toxic effect of DXR induced by free radicals. Part of the toxicity can also be attributed to NO release, which itself is generally not very toxic, but when produced in the presence of superoxide anion, leads to peroxynitrite which is toxic for many biomolecules especially those with protein and non-protein SH group.

Increased interstitial collagen accumulation in myocardium is related with myocardial damage (Tokudome et al., 2000). DXR caused high OH-P level, which may possibly indicate enormous collagen accumulation in cardiac tissue. Simultaneous administration of temocapril, an angiotensin-converting enzyme inhibitor, with DXR was found to be beneficial in preventing DXR-induced myocardial damage and inhibited interstitial collagen accumulation (Tokudome et al., 2000). We found that erdosteine treatment can prevent OH-P formation due to DXR-induced toxicity in cardiac tissue. Metabolite I of erdosteine also seems to protect against the OH-P formation due to DXR.

These results are in line with the activity of other agents bearing a SH group, such as *N*-acetylcysteine (Venditti et al., 1998). In the light of these results, DXR administration resulted in cardiotoxicity and this toxicity was thought to be related to the abnormal purine catabolism as well as high NO and OH-proline production. Erdosteine may exert cardio-protective effects on DXR-induced-cardiotoxicity through not only by its antioxidant effects but also by the inhibitory effect of it on purine catabolism, NO production and OH-proline production in myocardial tissue. However, it can not be ruled out that erdosteine also effects the antitumor effects of DXR. This should be investigated in later studies in the rat cancer models in which DXR and erdosteine will be used together. In addition, it should be emphasized that we need further investigation to improve the knowledge of the protective effects of erdosteine in molecular level against chemotherapeutic agents. The protective activity of increasing doses of erdosteine should be further investigated in a subsequent study in order to see whether there is a dose–effect relationship, and whether the findings correlate with the blood levels of metabolite I.

References

Adams, V., Jiang, H., Yu, J., Mobius-Winkler, S., Fiehn, E., Linke, A., Weigl, C., Schuler, G., Hambrecht, R., 1999. Apoptosis in skeletal myocytes of patients with chronic

heart failure is associated with exercise intolerance. J. Am. Coll. Cardiol. 33, 959–965.

Braga, P.C., Sasso, M.D., Zuccotti, T., 2000. Assessment of the antioxidant activity of the SH metabolite I of erdosteine on human neutrophil oxidative bursts. Drug Res. 50, 739–746.

Choong, Y.S., Humphrey, S.M., 1987. Differences in the regional distribution and response to ischaemia of adenosine-regulating enzymes in the heart. Basic Res. Cardiol. 82, 576–584.

Cortas, N.K., Wakid, N.W., 1990. Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clin. Chem. 36, 1440–1443.

Cummings, J., Anderson, L., Willmott, N., Smyth, J.F., 1991. The molecular pharmacology of doxorubicin in vivo. Eur. J. Cancer 27, 532–535.

Dalledonne, I., Milzani, A., Colombo, R., 1993. DXR depresses the alpha-actinin-induced formation of actin bundles. Cancer Biochem. Biophys. 13, 245–254.

Dechant, K.L., Noble, S., 1996. Erdosteine. Drugs 52, 875–881.

Doroshow, J.H., 1983. Effect of antracycline antibiotics on oxygen radical formation in rat heart. Cancer Res. 43, 460–472.

Fadillioglu, E., Erdogan, H., 2003. Effects of erdosteine treatment against doxorubicin-induced toxicity through erythrocyte and plasma oxidant/antioxidant status in rats. Pharmacol. Res. 47, 317–322.

Fadillioglu, E., Erdogan, H., Sogut, S., Kuku, I., 2003. Protective effects of erdosteine against doxorubicin-induced cardiomyopathy in rats. J. Appl. Toxicol. 23, 71–74.

Gazzani, G., Fregnan, G.B., Vandoni, G., 1989. In vitro protection by erdosteine against oxidative inactivation of alpha-1-antitrypsin by cigarette smoke. Respiration 55, 113–118.

Haywood, G.A., Tsao, P.S., von der Leyen, H.E., Mann, M.J., Keeling, P.J., Trindade, P.T., Lewis, N.P., Byrne, C.D., Rickenbacher, P.R., Bishopric, N.H., Cooke, J.P., McKenna, W.J., Fowler, M.B., 1996. Expression of inducible nitric oxide synthase in human heart failure. Circulation 93, 1087–1094.

Hille, R., Nishino, T., 1995. Flavoprotein structure and mechanism. 4. Xanthine oxidase and xanthine dehydrogenase. FASEB J. 9, 995–1003.

Irmak, M.K., Koltuksuz, U., Kutlu, N.O., Yagmurca, M., Ozyurt, H., Karaman, A., Akyol, O., 2001. The effect of caffeic acid phenethyl ester on ischemia-reperfusion injury in comparison with alpha-tocopherol in rat kidneys. Urol. Res. 29, 190–193.

Kooy, N.W., Lewis, S.J., Royall, J.A., Ye, Y.Z., Kelly, D.R., Beckman, J.S., 1997. Extensive tyrosine nitration in human myocardial inflammation: evidence for the presence of peroxynitrite. Crit. Care Med. 25, 812–819.

Lowry, O., Rosenbraugh, N., Farr, L., Rondall, R., 1951. Protein measurement with the folin-phenol reagent. J. Biol. Chem. 183, 265–275.

Molinari, A., Calcabrini, A., Crateri, P., Arancia, G., 1990. Interaction of anthracyclinic antibiotics with cytoskeletal components of cultured carcinoma cells (CG5). Exp. Mol. Pathol. 53, 11–33.

Monboisse, J.C., Borel, J.P., 1992. Oxidative damage to collegen. EXS 62, 323–327.

Myers, C., 1998. The role of iron in doxorubicin-induced cardiomyopathy. Semin. Oncol. 25, 10–14.

Parks, D.A., Granger, N.D., 1986. Xanthine oxidase: biochemistry, distribution and physiology. Acta Physiol. Scand. 548, 87–99.

Prajda, N., Weber, G., 1975. Malign transformation-linked imbalance: decreased XO activity in hepatomas. FEBS Lett. 59, 245–249.

Pritsos, C.A., Gustafson, D.L., 1994. Xanthine dehydrogenase and its role in cancer chemotherapy. Oncol. Res. 6, 477–481.

Quiles, J.L., Huertas, J.R., Battino, M., Mataix, J., Ramirez-Tortosa, M.C., 2002. Antioxidant nutrients and adriamycin toxicity. Toxicology 180, 79–95.

Sayed-Ahmed, M.M., Khattab, M.M., Gad, M.Z., Osman, A.M., 2001. Increased plasma endothelin-1 and cardiac nitric oxide during doxorubicin-induced cardiomyopathy. Pharmacol. Toxicol. 89, 140–144.

Tokudome, T., Mizushige, K., Noma, T., Manabe, K., Murakami, K., Tsuji, T., Nozaki, S., Tomohiro, A., Matsuo, H., 2000. Prevention of doxorubicin (adriamycin)-induced cardiomyopathy by simultaneous administration of angiotensin-converting enzyme inhibitor assessed by acoustic densitometry. J. Cardiovasc. Pharmacol. 36, 361–368.

Varin, R., Mulder, P., Richard, V., Tamion, F., Devaux, C., Henry, J.P., Lallemand, F., Lerebours, G., Thuillez, C., 1999. Exercise improves flow-mediated vasodilatation of skeletal muscle arteries in rats with chronic heart failure: role of nitric oxide, prostanoids, and oxidant stress. Circulation 99, 2951–2957.

Vejlstrup, N.G., Bouloumie, A., Boesgaard, S., Andersen, C.B., Nielsen-Kudsk, J.E., Mortensen, S.A., Kent, J.D., Harrison, D.G., Buse, R., Aldershvile, J., 1998. Inducible nitric oxide synthase (iNOS), in the human heart: expression and localization in congestive heart failure. J. Mol. Cell Cardiol. 30, 1215–1223.

Venditti, P., Balestrieri, M., De Leo, T., Di Meo, S., 1998. Free radical involvement in doxorubicin-induced electrophysiological alterations in rat papillary muscle fibres. Cardiovasc. Res. 38, 695–702.

Weinstein, D.M., Mihm, M.J., Bauer, J.A., 2000. Cardiac peroxynitrite formation and left ventricular dysfunction following doxorubicin treatment in mice. J. Pharmacol. Exp. Ther. 294, 396–401.

Woessner, J.B., 1961. The detemination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. Arch. Biochem. Biophys. 93, 440–447.

Yee, S.B., Pritsos, C.A., 1997. Comparison of oxygen radical generation from the reductive activation of doxorubicin, streptonigrin, and menadione by xanthine oxidase and xanthine dehydrogenase. Arch. Biochem. Biophys. 347, 235–241.

Yildirim, Z., Sogut, S., Odaci, E., Iraz, M., Ozyurt, H., Kotuk, M., Akyol, O., 2003. Oral erdosteine administration attenuates cisplatin-induced renal tubular damage in rats. Pharmacol. Res. 47, 149–156.

Zhou, Z., Kang, Y.J., 2000. Immunocytochemical localization of metallothionein and its relation to doxorubicin toxicity in transgenic mouse heart. Am. J. Pathol. 156, 1653–1662.