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SPECIAL REPORT

Curcumin attenuation of acute adriamycin myocardial toxicity in rats

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The protective effect of curcumin on acute adriamycin (ADR) myocardial toxicity was analysed in rats. ADR toxicity, induced by a single intraperitoneal injection (30 mg kg^{-1}), was revealed by elevated serum creatine kinase (CK) and lactate dehydrogenase (LDH). The level of the lipid peroxidation products, conjugated dienes and malondialdehyde, was markedly elevated by ADR. ADR caused a decrease in myocardial glutathione content and glutathione peroxidase activity. In contrast, cardiac catalase activity was increased in ADR rats. Curcumin treatment (200 mg kg^{-1}, seven days before and two days following ADR) significantly ameliorated the early manifestation of cardiotoxicity (ST segment elevation and an increase in heart rate) and prevented the rise in serum CK and LDH exerted by ADR. ADR rats that received curcumin displayed a significant inhibition of lipid peroxidation and augmentation of endogenous antioxidants. These results suggest that curcumin inhibits ADR cardiotoxicity and might serve as novel combination chemotherapeutic agent with ADR to limit free radical-mediated organ injury.

Keywords: Adriamycin; curcumin; cardiotoxicity; electrocardiogram; free radicals; glutathione peroxidase; catalase

Introduction Adriamycin (ADR), an antitumor antibiotic, has been found to be a most effective agent against a variety of human cancers. However, with increasing use, it has been apparent that an acute as well as cumulative dose-related cardiotoxicity have been recognized as a severe complication of ADR chemotherapy (Doroshow, 1991) and measures controlling the toxic side effects of ADR are widely appreciated. An important class of therapeutic targets for ADR cardiotoxicity are reactive oxygen species (ROS) (e.g., superoxide radical, hydrogen peroxide and hydroxyl radical), hypothesized to be a major factor in the toxicity of ADR (Keizer *et al*., 1990). Previous studies have demonstrated that antioxidant compounds have some protective effects in ADR cardiotoxicity (Ciaccio *et al*., 1993; Nowak *et al*., 1995). It was therefore of interest to determine whether curcumin, an anti-inflammatory antioxidant (Sharma, 1976; Srivastava & Srimal, 1985) has an inhibitory action on ADR-induced oxidative damage to myocardium. Because of its proven ability to protect myocardium against isoprenaline-induced oxidative insults (Nirmala & Puvanakrishnan, 1996), I hypothesized that curcumin might be of value in preventing oxidative damage after ADR.

Methods Pathogen-free, male Wistar rats, weighing 200 ± 10 g, were used in the present study. ADR treatment regimen used in this investigation to develop an acute cardiotoxicity has been established by Ciaccio *et al*. (1993). Rats were divided into four groups of six animals each: saline (SA), curcumin (CC), adriamycin (ADR) and curcumin + adriamycin (CC + ADR). ADR was administered as a single intraperitoneal injection (30 mg kg^{-1}, in saline). Control animals received an equal volume of saline only. Curcumin (200 mg kg^{-1} in 1% gum acacia) was administered seven days before and two days following ADR. In a preliminary study, three different doses of curcumin (50, 100 and 200 mg kg^{-1} body weight) were examined and since the maximum protection was seen at a dose of 200 mg kg^{-1} body weight (Figure 1), this treatment protocol was followed in the present study.

Two days after ADR administration, rats were killed by decapitation under light ether anaesthesia. Blood samples were collected and serum separated by centrifugation was analysed for lipid peroxide (Yagi, 1984). Serum CK and LDH were also measured by standard methods. The heart was quickly excised, placed immediately in ice-cold physiological saline to wash it free from blood and homogenized in 0.1 M Tris-HCl buffer, pH 7.4. Homogenates were used for the measurements of lipid peroxide (Yagi, 1984), conjugated dienes (Nowak *et al*., 1995), reduced glutathione (Moron *et al*., 1979), glutathione peroxidase (Paglia & Valentine, 1967) and catalase (Aebi, 1984).

The electrocardiogram (ECG) was recorded 48 h after ADR injection. All animals were anaesthetized with thiopentane (30 mg kg^{-1}, intraperitoneally), needle electrodes were inserted under the skin for the limb lead at position II and ECG parameters (heart rate beats min^{-1}) and ST segment (expressed in mv) were measured using an electrocardiograph (Indchem Electronics Company, Madras, India).

Statistics All values are presented as mean ± s.d. of six experiments. All data were subjected to one-way analysis of variance (ANOVA) followed by Bonferroni's test.

Results No deaths were observed in any of the experimental groups during the study period. Animals receiving ADR had an increase in heart rate and ST segment elevation compared to control groups. Curcumin significantly ($P < 0.01$) prevented the ECG abnormalities exerted by ADR (Table 1). Serum concentration of CK and LDH were significantly ($P < 0.001$) higher in ADR rats than in controls. The concentration of CK and LDH increased 2.0 fold compared to control groups. Curcumin (200 mg kg^{-1}) significantly ($P < 0.001$) prevented

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Table 1 Protective effect of curcumin on ADR-induced changes in ECG and biochemical variables

Parameters	*Saline*	*Curcumin*	*Adriamycin*	*Curcumin + adriamycin*
Heart rate (beats min^{-1})	424 ± 17.13	420 ± 18.14	495 ± 22.36*	417 ± 14.34
ST segment (mV)	0.132 ± 0.024	0.128 ± 0.031	0.285 ± 0.046*	0.146 ± 0.042
Serum lipid peroxide (nmol ml^{-1})	1.89 ± 0.45	1.78 ± 0.56	3.46 ± 0.79*	2.04 ± 0.83
Heart TBARS (nmol mg^{-1} protein)	0.41 ± 0.046	0.35 ± 0.065	0.95 ± 0.074*	0.53 ± 0.058
Conjugated dienes (nmol g^{-1} tissue)	4.54 ± 1.21	4.01 ± 1.80	8.76 ± 2.63*	5.21 ± 2.38
Reduced glutathione (nmol g^{-1} tissue)	1.62 ± 0.57	1.78 ± 0.49	0.43 ± 0.075$^{\#}$	1.50 ± 0.43
Glutathione peroxidase†	28.14 ± 5.09	30.35 ± 6.40	17.01 ± 4.56$^{\#}$	25.38 ± 5.77
Catalase‡	18.15 ± 6.31	16.71 ± 4.55	30.33 ± 5.78*	16.14 ± 4.93

TBARS: thiobarbituric acid reactive substances; †μmol NADPH oxidized min^{-1} mg^{-1} protein; ‡μmol H_2O_2 decomposed min^{-1} mg^{-1} protein. Data are presented as mean ± s.d. ($n = 6$). *Significantly ($P < 0.01$) higher than all groups; $^{\#}$significantly ($P < 0.01$) lower than all groups.

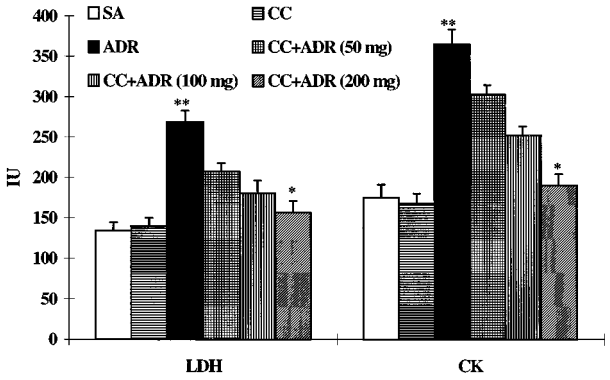

Figure 1 Dose-dependent effects of curcumin (CC) on adriamycin (ADR)-induced changes in serum lactate dehydrogenase (LDH) and creatine kinase (CK). Results are expressed as μmol of pyruvate (LDH) or creatine (CK) formed min^{-1} l^{-1} at 37°C. Values are presented as mean ± s.d. of six rats. **$P < 0.001$ compared with control, *$P < 0.01$ compared to curcumin + adriamycin (100 mg and 50 mg, respectively) and determined by ANOVA followed by a *post-hoc* comparison using Bonferroni. SA = saline.

the effect induced by ADR (Figure 1). ADR significantly ($P < 0.01$) increased the serum lipid peroxide and the level of lipid peroxide remained 183% higher than in control groups. However, curcumin significantly ($P < 0.01$) reduced the rise in serum lipid peroxide (Table 1). Results from the thiobarbituric acid reactive substances (TBARS) showed a significant effect of ADR on lipid peroxidation in heart homogenate. There were significant ($P < 0.01$) increases in heart conjugated dienes, up to 1.9 times the control levels in ADR rats. Pre- and co-treatment of ADR rats with curcumin remarkably inhibited the rise in lipid peroxidation products. As shown in Table 1, reduced glutathione levels were significantly ($P < 0.01$) decreased in the heart of ADR rats, whereas the glutathione level was markedly increased in CC + ADR groups. There was a significant ($P < 0.01$) decrease in cardiac GP activity in ADR groups as compared with animals received normal saline or curcumin. However, the GP activity was restored to normal values in curcumin-treated ADR rats. ADR administration led to an increase in catalase activity, which is in agreement with previous studies (Ciaccio *et al*., 1993). However, the catalase activity fell to control values in ADR rats that received curcumin.

Discussion

Previous studies have shown that ADR toxicity is associated with oxidative damage (Keizer *et al*., 1990). Since therapeutic strategies are aimed to limit free radical-mediated cardiac injury by ADR, I hypothesized that curcumin treatment would alter cardiotoxicity induced by ADR. The results clearly indicate this is true as curcumin treatment protected against acute ADR toxicity, as assessed by ECG changes and indicators of oxidative injury. Compared to ADR animals, ADR rats treated with curcumin responded with normal cardiac function and decreased lipid peroxidation and conjugated dienes formation. Thus, the first (CD) and last (MDA) products of lipid peroxidation (Nowak *et al*., 1995) were significantly decreased by curcumin treatment. It is interesting to note that in ADR rats, antioxidant enzyme (GP and catalase) levels are also modulated in the heart following curcumin treatment. Of note, cardiac catalase activity was elevated following ADR treatment, which is in agreement with previous studies (Ciaccio *et al*., 1993). The increase in catalase activity might be an adaptive response to protect the heart against the deleterious effects of hydrogen peroxide.

Although the mechanism(s) by which curcumin ameliorates ADR toxicity remains to be elucidated, available evidence documents that multiple molecular mechanisms may contribute to its protective action. Firstly, curcumin inhibits lipid peroxidation by scavenging free radicals and thus blocking the lipid chain reaction, similar to α-tocopherol (Sharma, 1976). The inhibitory action on lipid peroxidation in the present study was reflected in the decrease in levels of CD and TBARS in the curcumin-treated groups. Secondly, the observation that curcumin treatment was accompanied by an increase in cardiac glutathione content, suggests that this treatment may augment the action of these naturally occuring sulphhydryl groups to maintain membrane integrity and help promote the non-enzymatic detoxification of hydroxyl radicals and lipid peroxides. Thirdly, the possible protection against cardiac injury by curcumin through a membrane-stabilizing effect is supported by measurements of ECG parameters, serum lipid peroxides, and serum CK and LDH. The observation that curcumin treatment was accompanied by a decrease in serum lipid peroxides, CK and LDH suggests that this treatment exerted a membrane stabilizing effect. An altered membrane function due to ADR-induced lipid peroxidation is held responsible for the ECG changes, most notably ST segment prolongation (Danesi *et al*., 1991). Thus, membrane stabilization would affect the propogation phase of lipid peroxidation, in that the mobility of lipid peroxyl radicals would be prevented and thus their freedom to interact with adjacent membrane polyunsaturated fatty acids would be restricted.

In conclusion, the present findings demonstrate that curcumin treatment protects against acute ADR cardiotoxicity. Further studies in my laboratory also revealed the protective effects of curcumin in a chronic model of ADR

cardiomyopathy (unpublished observations). Thus, curcumin may be considered as a potentially useful candidate in the combination chemotherapy with ADR to limit free radical-mediated organ injury.

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