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Effect of Curcumin on Cetrain Lysosomal Hydrolases in Isoproterenol-Induced Myocardial Infarction in Rats

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ABSTRACT. The effect of curcumin on lysosomal hydrolases in serum and heart was studied by determining the activities of β-glucuronidase, β-*N*-acetylglucosaminidase, cathepsin B, cathepsin D, and acid phosphatase. Rats treated with isoproterenol (30 mg/100 g body weight) showed a significant increase in serum lysosomal hydrolase activities, which were found to decrease after curcumin treatment. Isoproterenol administration to rats resulted in decreased stability of the membranes, which was reflected by the lowered activity of cathepsin D in mitochondrial, lysosomal, and microsomal fractions. Curcumin treatment returned the activity levels almost to normal, showing that curcumin restored the normal function of the membrane. Histopathological studies of the infarcted rat heart also showed a decreased degree of necrosis after curcumin treatment. BIOCHEM PHARMACOL 51;1:47–51, 1996.

KEY WORDS. isoproterenol; heart; myocardial infarction; curcumin; lysosome; cathepsin D

Considerable attention has been focused on lysosomal alterations that might accompany ischemic or hypoxic myocellular damage. Formation of autophagic vacuoles, disruption of lysosomes, and spread of lysosomal enzymes throughout the cell have been observed in ischemic hearts [1, 2]. Irreversible necrosis is mediated by abnormal degradation of cellular constituents by lysosomal hydrolases, and this could indicate that some of the latent hydrolases that are labilized by ischemia may originate from other membrane-bound sites in addition to lysosomal vacuoles [3]. A decrease in lysosomal stability increases the levels of lysosomal enzymes, leading to altered metabolism of different connective tissue constituents, viz. glycosaminoglycan, glycoprotein [4], and collagen [5], in experimentally induced myocardial infarction.

ISO†-induced myocardial infarction results in increased lysosomal hydrolase activities that may be responsible for tissue damage and infarcted heart [6]. Decreased activity of lysosomal enzymes in particulate fractions of ischemic heart homogenates has been a common finding [7, 8]. One approach to ameliorate the damage due to myocardial injury is to stabilize the membranes of ischemic myocytes, including lysosomal membranes, and to protect the cells from autolytic and heterolytic damage. Curcumin (diferuloyl methane), an important constituent of *Curcuma longa*, possesses antioxidant [9], antiinflammatory [10], and anticancer [11] actions, in addition to antiaggregatory and antithrombolytic properties [12, 13] and is observed to prevent benzo[a]pyrene-induced DNA damage at both the target (mouse forestomach) and non-target (mouse bone marrow) sites [14]. Several mechanisms [15, 16] have been proposed to explain the action of curcumin. Curcumin is known to have a membrane-stabilizing action [17], and it is possible that stabilization of myocardial cell membranes, particularly the lysosomal membranes, may prolong the viability of ischemic cardiac muscle. The purpose of this study was to investigate the action of curcumin on the lysosomal hydrolase activities in heart and serum and on the histopathological changes taking place in the heart during curcumin treatment. Distribution of the major cardiac lysosomal proteinase, cathepsin D, which is localized predominantly in myocytes rather than in interstitial cells [18], also was studied.

MATERIAL AND METHODS

Isoproterenol-HCl, *p*-nitrophenol, *p*-nitrophenyl-β-D-glucuronide, *p*-nitrophenyl phosphate, hemoglobin, *N*-α-benzoyl arginine *p*-nitroanilide HCl, *p*-nitrophenyl-β-D-*N*-acetyl-glucosaminide, and bovine serum albumin were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. Curcumin was received as a gift from CFTRI, Mysore, India. All other chemicals used were of analytical grade.

Female rats (Wistar, inbred at the CLRI animal facility), weighing approximately 100 g, were housed in solid-bottomed polypropylene cages. The animals received a commercial rat diet (Hindustan Lever, Bombay) and water *ad lib*. The animals were divided into four groups as follows: (1) normal control group; (2) ISO-administered group (30 mg/100 g body weight, subcutaneously, twice at an interval of 24 hr) as described by Wexler and Kittinger [19]; (3) curcumin-treated control group; and (4) ISO-administered group treated with curcumin.

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† Abbreviation: ISO, isoproterenol.
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TABLE 1. Effect of curcumin on lysosomal hydrolase activities in serum in experimentally induced myocardial infarction

Group	β-Glucuronidase*	β-*N*-Acetylglucosaminidase*	Cathepsin B†	Cathepsin D‡	Acid phosphatase§
Control	8.63 ± 0.69	17.23 ± 0.96	10.32 ± 0.96	10.46 ± 1.02	5.06 ± 0.36
Isoproterenol	12.60 ± 0.75‖	22.03 ± 1.56‖	14.88 ± 1.20‖	15.99 ± 1.13‖	9.25 ± 0.60‖
Curcumin	8.76 ± 0.88	17.46 ± 0.86	10.52 ± 1.02	10.95 ± 1.69	4.89 ± 0.56
Curcumin + isoproterenol	9.06 ± 0.65	18.42 ± 1.02	11.56 ± 1.23	11.79 ± 1.17	5.22 ± 0.87

Values are means ± SD of six determinations.
* Expressed in μmol *p*-nitrophenol/hr/100 mg protein.
† Expressed in μmol *p*-nitroaniline/hr/100 mg protein.
‡ Expressed in μmol tyrosine/hr/100 mg protein.
§ Expressed in nmol *p*-nitrophenol/min/mg protein.
‖ $P \leq 0.001$, compared with control.

Curcumin (200 mg/kg body weight) suspended in 1% gum acacia in water was given to each animal orally for 2 days prior to ISO administrations, and the same dose was continued during ISO administration.

After the experimental period (24 hr after the second dose of ISO), the rats were killed by cervical decapitation, and blood was collected to obtain serum.

Separation of subcellular fractions

The heart tissue samples, obtained 24 hr after the second dose of ISO, were cut open and placed in isotonic saline to remove the blood. Then the heart tissues were rinsed in ice-cold 0.25 M sucrose, blotted, weighed, and minced. The enzyme extracts were prepared by homogenizing the tissue samples in 0.25 M sucrose at 4°. A portion of this preparation was used to determine the total activity. Another portion of the homogenate was subjected to differential centrifugation, and the different fractions were separated as follows: structural proteins, nucleus, and cell debris at 600 *g* for 10 min; mitochondria at 5,000 *g* for 10 min; lysosomes at 15,000 *g* for 10 min; microsomes at 120,000 *g* for 30 min, and supernatant cytosol.

Measurement of enzymatic activities

Myocardial subfractions were treated with Triton X-100 (final concentration 0.2%, v/v) in ice for 15 min prior to the determination of enzymatic activities. β-Glucuronidase activity was determined according to the method described by Kawai and Anno [20] and β-*N*-acetylglucosaminidase by the method of Moore and Morris [21]. Cathepsin D activity was assayed by the method of Sapolsky *et al.* [22] using 1.5% hemoglobin in 0.1 M acetate buffer, pH 3.0, and estimating the amount of tyrosine liberated. The activity of cathepsin B was determined as described by Barret [23] using *N*-α-benzoyl arginine *p*-nitroanilide HCl as the substrate. Acid phosphatase activity was assayed by the method of Barrett and Heath [24] using *p*-nitrophenyl phosphate as substrate. Activities of these enzymes were expressed as total activity/hr/100 mg protein. Protein in all the enzyme extracts and in serum was estimated by the method of Lowry *et al.* [25] using crystalline bovine serum albumin as the reference standard.

Histological studies

Tissues taken for histological examination (groups 1, 2, 3 and 4) were removed as quickly as possible at autopsy and placed in 10% buffered neutral formalin solution. After fixation was complete, tissues were embedded in paraffin, serial sections were cut at 5 μm, and stained with hematoxylin and eosin. The sections were examined under light microscope, and photomicrographs were taken.

The results were statistically evaluated using Student's *t*-test.

TABLE 2. Effect of curcumin on heart lysosomal hydrolase activites in experimentally induced myocardial infarction

Group	β-Glucuronidase*	β-*N*-Acetylglucosaminidase*	Cathepsin B†	Cathepsin D‡	Acid phosphatase§
Control	20.32 ± 0.72	44.13 ± 4.18	27.32 ± 1.87	24.68 ± 1.67	22.72 ± 1.42
Isoproterenol	32.00 ± 1.24‖	56.68 ± 5.01¶	36.98 ± 2.90‖	32.62 ± 2.32‖	30.55 ± 1.05‖
Curcumin	21.26 ± 0.95	42.68 ± 4.23	25.92 ± 2.02	23.24 ± 1.64	21.98 ± 1.56
Curcumin + isoproterenol	21.29 ± 1.08	44.30 ± 4.94	28.78 ± 2.52	26.23 ± 1.25	23.89 ± 1.86

Values are means ± SD of six determinations.
* Expressed in μmol *p*-nitrophenol/hr/100 mg protein.
† Expressed in μmol *p*-nitroaniline/hr/100 mg protein.
‡ Expressed in μmol tyrosine/hr/100 mg protein.
§ Expressed in nmol *p*-nitrophenol/min/mg protein.
‖ $P \leq 0.001$, compared with control.
¶ $P \leq 0.01$, compared with control.

TABLE 3. Effect of curcumin on the subcellular distribution of heart cathepsin D in experimentally induced myocardial infarction

Group	Cathepsin D (μmol tyrosine liberated/hr/100 mg protein)					A*	B†
	Nuclear fraction	Mitochondrial fraction	Lysosomal fraction	Microsomal fraction	Cytosolic fraction		
Control	12.05 ± 1.63	13.29 ± 1.67	26.41 ± 1.36	7.39 ± 0.66	16.18 ± 1.23	0.613	0.655
Isoproterenol	14.45 ± 1.04	8.44 ± 1.02‡	16.04 ± 0.94‡	3.22 ± 0.35‡	26.78 ± 2.48‡	1.669	0.816
Curcumin	11.95 ± 1.75	13.94 ± 1.35	25.77 ± 1.46	7.69 ± 0.71	16.07 ± 1.97	0.624	0.691
Curcumin + isoproterenol	12.37 ± 1.19	14.25 ± 1.42	26.91 ± 1.69	6.13 ± 0.83	18.49 ± 1.75	0.687	0.705

Values are means ± SD of six samples.
* A: ratio of cytosol (free) to lysosomal (bound) activity.
† B: ratio of cytosol (free) to total activity.
‡ $P \leq 0.001$, compared with control.

RESULTS

The activities of lysosomal hydrolases in serum are presented in Table 1. Serum lysosomal hydrolases (β-glucuronidase, β-*N*-acetylglucosaminidase, cathepsin B, cathepsin D, and acid phosphatase) were found to be elevated significantly following ISO-induced myocardial infarction. The levels of these enzymes remained at near normal levels in rats that received both curcumin and ISO.

A significant increase in the activity of β-glucuronidase, cathepsin B, cathepsin D, β-*N*-acetylglucosaminidase and acid phosphatase in the heart was noticed in rats treated with ISO (Table 2) curcumin treatment caused a decrease in the enzyme activity to near control levels.

Table 3 shows the activities of cathepsin D in the subcellular fractions of the heart. The data presented clearly indicate that significant changes were not observed in cathepsin D activity in the nuclear fraction of the heart in rats treated with ISO or with curcumin and ISO. However, there was a significant decrease in the activity of cathepsin D in mitochondrial, lysosomal, and microsomal fractions of the heart in rats treated with ISO. Cathepsin D activity in the cytosolic fraction of the heart was found to be elevated significantly in ISO-treated rats, but this increase in activity was observed to be reduced markedly in rats treated with curcumin and ISO. ISO-administered groups showed an increased ratio of cytosol to bound lysosomal activities and cytosol to total activity for cathepsin D in the heart. Treatment of ISO-administered rats with curcumin showed a marked decrease in the ratio of cytosol to bound lysosomal and cytosol to total activities, which were found to be near normal values.

Figure 1 represents the normal architecture of heart upon histological examination. A massive necrosis of the heart muscle fibers with disruption of muscle bundles was observed in ISO-treated groups (Fig. 2). The curcumin-treated control group revealed normal cardiac muscle fiber architecture (Fig. 3). In the ISO-administered group treated with curcumin, a decreased degree of necrosis was observed (Fig. 4).

DISCUSSION

It was reported previously that the localization of acid hydrolases in cardiac myocytes is in the lysosome and that the release of these enzymes from the lysosome to the cytosol leads to myocardial cellular injury and death in the ischemic state of the heart [2, 7, 26]. This is in agreement with our findings showing that in ISO-administered rats the activities of serum lysosomal acid hydrolases increased, lysosomal bound acid hydrolases decreased, and cytosol enzyme activities increased. Ravens and Gudbjarnason [27] observed that the release of hydrolytic enzymes from lysosomes after coronary occlusion may be a causative factor for the development of myocardial cellular destruction. Using the immunofluorescence method, Decker *et al.* [2] have also shown the release of cathepsin D from lysosome to cytosol 15–30 min after coronary occlusion.

FIG. 1. Heart tissues of control rats under light microscope showing normal architecture (200×).

FIG. 2. Rat heart showing massive necrosis of muscle fibers 1 day after ISO-induced myocardial infarction (200×).

FIG. 3. Heart tissues of curcumin-treated control rats showing normal muscle fiber architecture (200×).

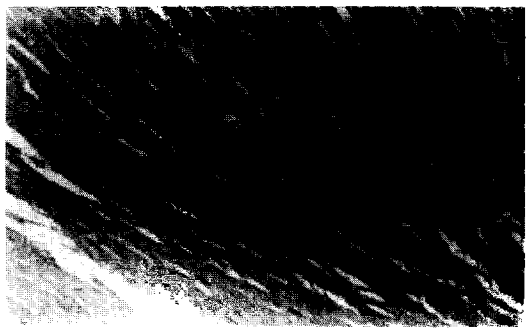

FIG. 4. Heart tissues of ISO-administered rats treated with curcumin, showing a decreased degree of necrosis (200×).

Furthermore, these cytosolic acid hydrolases released from lysosomes and from the sarcoplasmic reticulum induce the dysfunction and disruption of mitochondria, sarcolemma, and other organelle in the same way as reported earlier [28, 29].

In a previous study carried out in dogs [30], activities of acid hydrolases such as β-glucuronidase, acid phosphatase, and cathepsin D were observed in the microsomal fraction, which is composed mainly of sarcoplasmic reticulum, and that activation of these acid hydrolases inside sarcoplasmic reticulum was important for the evolution of ischemic myocardial injury and death. In our study, cathepsin D in the microsomal fraction was found to decrease in ISO-treated rats. Curcumin treatment of ISO-administered rats could inhibit the release of enzymes from the lysosomal and microsomal fractions, which could be due to the stabilizing effect of curcumin on the lysosomal and microsomal membranes. It is interesting to note that the distribution of enzyme activity between the cytosol to lysosomal and cytosol to total activities reported in our studies indicates decreased lysosomal stability in ISO-treated rats. In addition, our studies demonstrated that curcumin might inhibit the release of lysosomal enzymes as well as decrease the activity of the total lysosomal acid hydrolases, thereby enhancing the stability of the lysosomes. Histological studies carried out on the heart samples of different groups (Figs. 1–4) also support our findings, showing that curcumin pretreatment decreases the degree of necrosis.

It is possible that the release of endogenous corticoids by curcumin may help indirectly in stabilizing lysosomal membranes, since this property of glucocorticoids is well known [31]. It has been reported that small doses of ibuprofen suppress lysosomal enzyme release by stabilizing the lysosomal membrane [32]. Curcumin is found to be more potent than ibuprofen as a stabilizer of lysosomal membrane and as an uncoupler of oxidative phosphorylation [17]. Kalra and Prasad [33] have suggested that oxygen free radicals generated during ischemia, in addition to the direct myocardial damaging effect, may also be responsible for the cardiac damage through the release of lysosomal enzymes. The beneficial effect of curcumin may also be mediated by the scavenging of oxygen free radicals [34, 35] with the resultant preservation of cellular viability serving secondarily to preserve lysosomes as well.

The results of this study illustrate that curcumin, due to its stabilizing action on the lysosomal membrane and the consequent diminution in the liberation of hydrolytic enzymes in ISO-induced myocardial infarction in rats, exercises a protective role. Thus, pretreatment with curcumin could preserve lysosomal integrity and delay signs of necrosis in severely ischemic hearts. This protective action is all the more interesting since curcumin, at a dose range of 0.05 to 2 g/kg body weight, is non-toxic and non-mutagenic [36, 37] in rats.

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