Korean J Physiol Pharmacol
Vol 10: 79–83, April, 2006

The Effect of Dehydroepiandrosterone on Isoproterenol-induced Cardiomyopathy in Rats

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We evaluated therapeutic and preventive properties of dehydroepiandrosterone (DHEA), a weak androgenic steroid, against isoproterenol-induced cardiomyopathy. The cardiomyopathy was induced by daily i.p. administration of isoproterenol to rats for five days. One group of rats were given with daily s.c. for 5 days during isoproterenol and the other group with daily s.c. DHEA for total 10 days, including 5 days before and during isoproterenol. The animals were killed after each treatment, and cardiac muscle failure was evaluated using histopathologic examination and biochemical indices. DHEA was found to reduce the damaged area and inhibit the elevation in the serum levels of glutamic oxaloacetic transaminase (SGOT), lactate dehydrogenase (LDH), skeletal muscle creatine kinase (CK) and heart creatine kinase (CK-MB) induced by isoproterenol. We also assayed widely used oxidative stress parameters, including thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), catalase and glutathion peroxidase (GPx). DHEA decreased the escalated level of TBARS and enhanced the anti oxidant defense reaction with an increase in Mn-SOD and Cu/Zn-SOD. On the other hand, the treatment with DHEA did not affect catalase and GPx activity. The present study indicates that DHEA has a therapeutic and preventive effect against isoproterenol-induced cardiomyopathy and its effects may depend largely on the increase in SOD activity.

Key Words: Dehydroepiandrosterone, Isoproterenol, Cardiomyopathy, Oxidative stress

INTRODUCTION

Cardiomyopathy is a serious disease in which the heart muscle becomes inflamed and doesn't work as well as it should. In most cases, the causes of cadiomyopathy are not unknown, nevertheless, oxidative stress has been implicated in the onset and progression of cardiovascular diseases such as congestive heart failure and cardiomyopathy (Belch et al, 1991). Therefore Dhalla et al (1996) attempted to investigate ameliorative effects of certain antioxidants, such as vitamin E, dimethylthiourea and probucol on oxidative stress and inflammation in heart failure.

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) are prehormones that are produced in large quantities by the zona reticularis of the human adrenal gland and are converted to androgens or estrogens in peripheral tissues (Ebeling & Koivisto, 1994), and DHEA, a weak androgenic steroid, has been associated with enhancing immune responses and upregulating resistance against viral, parasitic, and bacterial infections. The natural concentration of DHEA(S) in the circulation is known to reach a peak after puberty and gradually decreases thereafter. Recently, there have are several reports to recommend the use of DHEA in the treatment of a wide range of disorders and diseases (Svec & Porter, 1998). The ameliorative effects of DHEA against oxidative imbalance have been proposed as the key mechanism for its beneficial effects (Aragno et al, 2000). The reported antioxidant properties of DHEA led us to investigate its therapeutic and protective effect in an isoproterenol-treated rat model of cardiomyopathy.

METHODS

Animals

The animal care and handling were approved by the animal institutional ethics committee in School of Medicine, Chung Ang University. Ten to twelve male Sprague-Dawley rats weighing between 200 g and 220 g were used for the study. They were fed with commercial pellet diet and given water ad libitum. They were maintained under standard environmental condition of temperature (23±2°C) and humidity (50±5%).

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ABBREVIATIONS: DHEA, dehydroepiandrosterone; SGOT, serum levels of glutamic oxaloacetic transaminase; LDH, lactate dehydrogenase; CK, creatine kinase; TBARS, thiobarbituric acid reactive substances; SOD, superoxide dismutase; GPx, glutathion peroxidase.

Experiments

Animal were divided into 4 groups as shown in Table 1, and were separately evaluated for the cardioprotective and protective activity of DHEA.

At 11th day, the animals were killed by decapitation. The blood was collected by cardiac puncture, the sera were separated by centrifugation at 5,000 rpm for 10 min and frozen at −70°C for estimation of lactate dehydrogenase (LDH) (Preus et al, 1988), creatine kinase isoenzyme (CK-MB) (Preus et al, 1988), and glutamic oxaloacetic transaminase (GOT) (Roberts et al, 1987).

A separate experiment was carried out to determine the antioxidant potential of DHEA in animals divided into four groups, as mentioned in Table 1. The heart of each animal was dissected out immediately after each treatment. One portion of the heart was used for histological examination; biopsies of the heart were fixed in paraformaldehyde (4% wt/vol.), embedded in paraffin, cut into 4-mm section, dewaxed, and stained with hematoxylin-eosin. The remaining portion was washed in ice-cold saline and homogenized in Tris-HCl buffer (0.1 M), pH 7.4. The homogenate was used for TBARS (Buege & Aust, 1978), SOD (Mn and Cu/Zn) (Marklund & Marklund, 1974), catalase (Imre et al, 1984) and GPx (Sinet et al, 1975) assays.

Table 1. The treatments profile

Day	1	2	3	4	5	6	7	8	9	10
Group 1	Ss	Ss	Ss	Ss	Ss	Ss+Si	Ss+Si	Ss+Si	Ss+Si	Ss+Si
Group 2	Ss	Ss	Ss	Ss	Ss	Ss+I	Ss+I	Ss+I	Ss+I	Ss+I
Group 3	Ss	Ss	Ss	Ss	Ss	D+I	D+I	D+I	D+I	D+I
Group 4	D	D	D	D	D	D+I	D+I	D+I	D+I	D+I

Ss: saline s.c., Si: saline i.p., D: DHEA 40 mg/kg s.c., I: isoproterenol 5 mg/kg i.p.

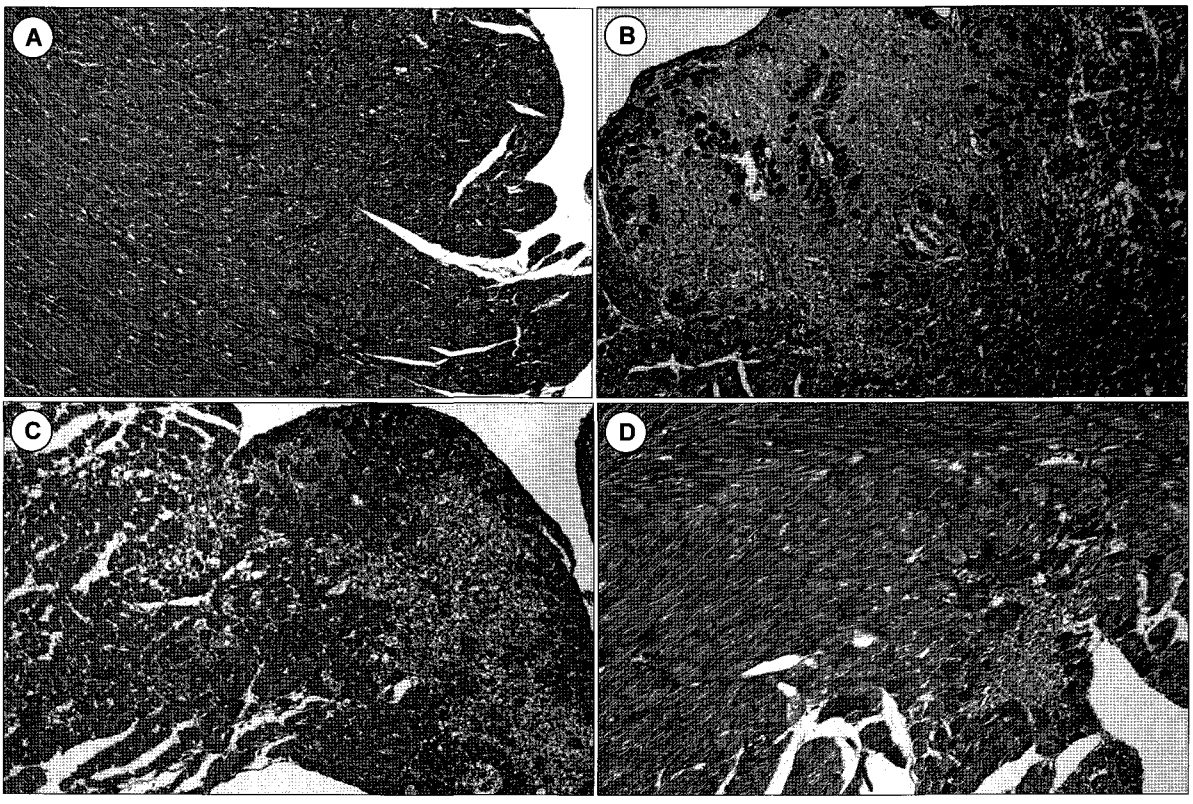

Fig. 1. Photomicrographs of horizontal sections of heart in rats, (A) treated with saline (Group 1), demonstrating there is no damage in heart (hematoxylin-eosin staining, enlargement×100), (B) treated with isoproterenol (Group 2), demonstrating wide range of damaged area follicles heart (hematoxylin-eosin staining, enlargement×100), (C) co-treated with DHEA and isoproterenol, showing the reduction of the damaged area heart (hematoxylin-eosin staining, enlargement×100), and (D) pretreated with DHEA and co-treated with DHEA and isoproterenol,.indicating that there is almost no damaged area heart (hematoxylin-eosin staining, enlargement×100).

Statistical analysis

The significance between the treatments was determined using Student's *t*-test for cardiotoxicity and one-way analysis of variance (ANOVA) for antioxidant activity. Data were presented as mean±S.E.M. Differences were considered statistically significant if a value of $P<0.05$ was obtained.

RESULTS

Histopathology

Fig. 1 shows the effect of DHEA on isoproterenol-induced tissue damage in rat cardiac muscle. As seen in figure 1B, treatment of rats with isoproterenol (group 1) for 5 days always were wide damaged area of cardiac muscle. However, co-administration of isoproterenol and DHEA (group 3) reduced the damage of cardiac muscle induced by isoproterenol (Fig. 1C). Pretreatment of rats with DHEA for 5 days and consecutive co-exposure of the animals to DHEA and isoproterenol (group 4) for 5 days almost completely protected the isoproterenol-induced cardiac muscle damage with little necrotic areas (Fig. 1D).

Cardioprotection

Figs. 2~5 indicate that DHEA has a therapeutic and protective effect on isoproterenol-induced cardiomyopathy. Rats treated with isoproterenol had significantly elevated levels of aspartate transaminase, LDH, CK and CK-MB, compared to control, indicating that isoproterenol caused cardiotoxicity. The daily s.c. administration of DHEA once for 5 consecutive days together with i.p. isoproterenol significantly inhibited the elevation of GOT, LDH and CK-MB, whereas the level of CK did not show any significant reduction by this treatment. Pretreatment of rats with DHEA for 5 days and subsequent 5 days of co-exposure to DHEA and isoproterenol had the most inhibitory effect on the escalation of SGOT, LDH, and CK-MB, and even CK.

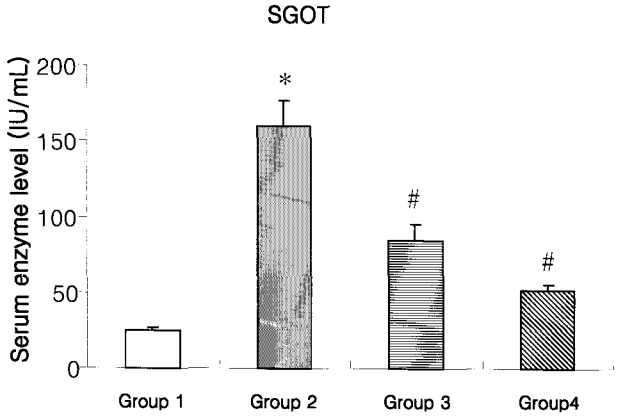

Fig. 2. Effect of DHEA on isoproterenol-induced elevation of SGOT. Values are the mean±SEM. * and # designate significant differences ($P<0.05$) compared to control (Group 1) and Group 2, respectively.

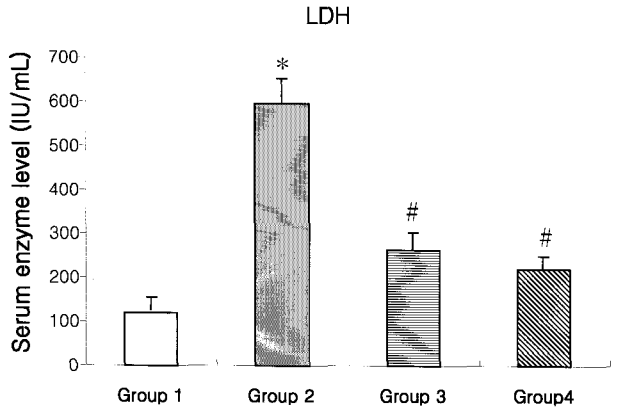

Fig. 3. Effect of DHEA on isoproterenol-induced elevation of LDH. Values are the mean±SEM. * and # designate significant differences ($P<0.05$) compared to control (Group 1) and Group 2, respectively.

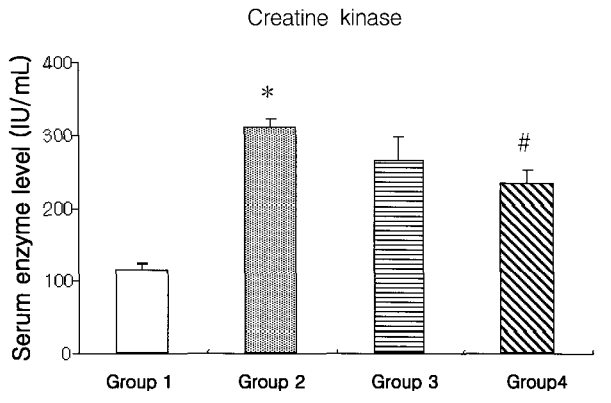

Fig. 4. Effect of DHEA on isoproterenol-induced elevation of CK. Values are the mean±SEM. * and # designate significant differences ($P<0.05$) compared to control (Group 1) and Group 2, respectively.

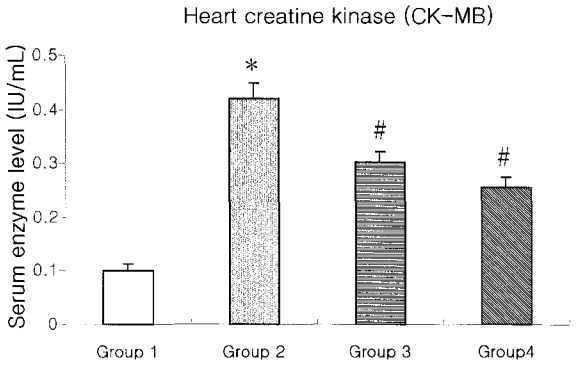

Fig. 5. Effect of DHEA on isoproterenol-induced elevation of CK-MB. Values are the mean±SEM. * and # designate significant differences ($P<0.05$) compared to control (Group 1) and Group 2, respectively.

Table 2. Alteration of the isoproterenol-induced antioxidant status by DHEA

Group	TBARS	SOD (Cu/Zn)	SOD (Mn)	Catalase	GPx
1	35.65±1.43	34.78±0.41	30.59±0.34	79.10±3.21	24.77±0.86
2	42.10±1.92*	30.41±0.18*	27.93±0.20*	49.39±2.16*	22.43±0.92
3	28.24±1.44**	31.55±0.19**	29.05±0.24**	47.92±1.98*	21.75±0.58
4	29.09±1.55**	32.36±0.25**	29.61±0.29**	48.98±2.40*	22.87±0.41

Values are mean±SEM of four groups. TBARS: nmol/mg protein, SOD: unit/mg protein, Catalase: μM H_2O_2 composed/min/mg protein, GPx: unit/mg protein. *$P<0.05$ vs. Group 1, **$P<0.05$ vs. Group 2.

Antioxidant activity

Table 2 shows antioxidant activity of DHEA on isoproterenol-induced oxidative stress. The treatment with isoproterenol induced lipid peroxidation in the heart tissue of rats measured as TBARS. ; treatment with isoproterenol for 5 days resulted in significantly higher level of TBARS than control. The important antioxidant enzyme activities were all affected by isoproterenol with significantly lowered activity of Cu/Zn-SOD and Mn-SOD, while caltalase and GPx activity were not significantly reduced.

The increase in TBARS by isoproterenol was completely blocked by DHEA. The pretreatment and co-exposure with DHEA (group 4) did not show any more reduction in TBARS than co-exposure alone (group 3). The decrease of two types of SOD activity was all reversed by isoproterenol in group 3 and group 4. We, however, did not observe any change of caltalase and GPx activity in both group 3 and group 4.

DISCUSSION

Cardiomyopathy is a structural or functional abnormality of muscle of the heart, causing weakening of heart muscle, which results in inability of heart to efficiently pump blood. Although there is no known cause of cardiomyopathy, several studies suggested that oxidative stress might be associated with the etiology of cardiomyopathy. Singal et al (2001) suggested that diabetic cardiomyopathy might be relevant to an antioxidant deficit, and that antioxidant therapy might be useful in improving cardiac function in diabetes. Another study surmised that Mn-SOD deficiencies, resulting from the increased release of mitochondrial free radicals, lead to sustained oxidative stress that exceeds the cardiac antioxidant defense capacity and contribute to persistent oxidative damage in myocardium (Wen et al, 2004). These previous investigations led us to undertake the present study to evaluate the effect of an antioxidant substance, DHEA, on cardiomyopathy in an animal model.

Isoproterenol-induced cardiomyopathy has been used as a model for the evaluation of cardio protective agents (Tanaka et al, 1980; Lushnikova et al, 2001). The induction of cardiomyopathy by isoproterenol has been reported to result from an increase in lipid peroxidation through enhanced free radical formation (Sushamakumari et al, 1989). In the present study, isoproterenol induced cardiotoxicity with increase in damaged area, revealed by histopathologic examination. The reliable indicators of cardiomyopathy measured were found to be all elevated in agreement with the histopathologic observation, indicating that the biochemical indicators of the sera show evidence that isoproterenol was a cause of cardiotoxicity.

Data presented here demonstrate that the administration of DHEA was able not only to decrease the damaged area in cardiac muscle histopathologically, but also to lower serum cardiotoxicity indices such as SGOT, LDH, CK, and CK-MB. Moreover, we found that the regimen of pretreatment with DHEA and consecutive co-exposure to DHEA and isoproterenol has shown more therapeutic and preventive effect on cardiac muscle damage by isoproterenol than that of co-exposure to DHEA and isoproterenol. Since the pretreatment of DHEA sustained the level of DHEA in blood and tissue before isoproterenol administration, the effect of DHEA might be more apparent in this group than that of co-exposure group.

Several earlier studies indicate that antioxidant status is associated with the etiology, therapy and protection for cardiomyopathy (Demirbag et al, 2005; Liang et al, 2005; Zacks et al, 2005). Oxidants are increased and antioxidants are decreased in patients with idiopathic dilated cardiomyopathy and there is a significant correlation between the potency of oxidative stress and the severity of idiopathic dilated cardiomyopathy (Demirbag et al, 2005). Several studies report the anti-oxidant effect of DHEA in a variety of tissues (Bastianetto et al, 1999; Aragno et al, 2003; Pelissier et al, 2004; Tunez et al, 2005). The offensive oxidative stress is largely counteracted by an intricate antioxidant defense system including enzymatic scavengers such as SOD, catalase and glutathione peroxidase. Of them, SOD plays a pivotal role in attenuating oxidative stress in mitochondria. SOD reacts with superoxide to speed its conversion into hydrogen peroxide. There are two intracellular SOD including mitochondrial Mn-SOD and cytosolic Cu/Zn-SOD, and Mn-SOD is largely related to protecting mitochondria from superoxide attack (Floyd & Carney, 1992).

We observed anti-oxidant effect of DHEA in cardiac muscle damaged by isoproterenol. The elevated TBARS by isoproterenol was abolished by the administration of DHEA. In addition, the activity of Mn-SOD and Cu/Zn-SOD was significantly recovered to the level before isoproterenol treatment. In contrast, there was no statistically significant alteration in catalase and GPx activity with DHEA. We suggest that Mn-SOD deficiency-triggered free radicals released from mitochondria exceed the antioxidant capacity of the heart, thus leading to sustained oxidative stress-induced damage in myocardium. Our findings provide us a clue that the effect of DHEA on cardiomyopathy might be due to anti-oxidant effect through the major role played by Mn-SOD.

In summary, we found in the present study that isoproterenol-induced cardiomyopathy was attenuated by DHEA

treatment. The elevated biological markers, such as LDH, CK, CK-MB and SGOT in cardiomyopathy are all recovered to normal values with DHEA treatment. Additionally, our study demonstrates that the use of DHEA might be an effective therapeutic and preventive modality, since it exerts anti-oxidant activity largely through Mn-SOD activation among innate anti-oxidant defense systems.

ACKNOWLEDGEMENT

This work was supported by Research Grant of Chung-Ang University (2005).

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