Pro-Oxidantive Effect of Dehydroepiandrosterone on Indomethacin-Induced Acute Gastritis in Rats

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Abstract − This study examined whether or not a pretreatment with dehydroepiandrosterone (DHEA) has an effect on indomethacin-induced gastric mucosal damage. The DHEA group, male Sprague-Dawley rats, was administrated with DHEA orally at a dose of 4 mg/day for one week before inducing gastritis with indomethacin (50 mg/kg, p.o.). Histological assay, lipid peroxidation assay, superoxide dismutase (SOD), glutathione peroxidase (GPx) and Catalase activities were determined. Interestingly, it was found that the DHEA pretreatment attenuated the gastric lesion area induced by indomethacin. Rather, the pretreatment with high dose of DHEA led to submucosal edema, leukocyte infiltration in submucosa and mucosal necrosis. The levels of MDA in the DHEA pretreatment were also higher than those in the rats given with vehicle pretreatment. This suggests that the DHEA pretreatment deteriorates severe inflammation in indomethacin-induced gastritis. DHEA supplementation significantly increased SOD activity in the gastric mucosa. However, the catalase and GPx activities were not altered by DHEA. The co-administration of DHEA with an indomethacin might not offer a protective effect against the acute gastritis induced by indomethacin.

Keywords: Dehydroepiandrosterone (DHEA), Antioxidant effect, Indomethacin-induced gastritis, Superoxide dismutase activity (SOD)

INTRODUCTION

Dehydroepiandrosterone (DHEA) is a C19 steroid that is also known as 5-androsten-3β-ol-17-one. It is the most abundant steroid hormone in circulation and is secreted by adrenal cortex (Kroboth *et al*., 1999). DHEA is secreted synchronously with cortisol in response to corticotropin-releasing hormone (CRH) and adrenal corticotropin- releasing hormone (ACTH) (Nieschlag *et al*., 1973). There have been literally thousands of reports published on the metabolic and health properties of DHEA over the last 5 decades. It has been reported that the administration of DHEA has many beneficial effect against many diseases (Green *et al*., 2001; Kuebler *et al*., 2001; Williams *et al*., 2002; Shilkaitis *et al*., 2005). The ameliorative effect of a DHEA against oxidative stress have been suggested as a key mechanism for its beneficial effect (Khalil *et al*., 1998; Khalil *et al*., 2000). Other studies have demonstrated that DHEA had antioxidant effect in various acute and chronic oxidative stress associated disorders (Aragno *et al*., 2000a; Brignardello *et al*., 2000; Aragno *et al*., 2001). However, DHEA antioxidant effect has been controversial because DHEA has also been observed to exert pro-oxidant effect (Hayashi *et al*., 1994). Several evidences emerges that DHEA treatment increases reactive oxygen species (ROS) and some enzymes relevant to oxidative stress, i.e. cytochrome P450 and catalase, suggesting that DHEA might possess pro-oxidantive effect (Prough *et al*., 1994).

ROS has been well known as an oxidative stress factor in the gastric mucosa as well as the gastric epithelium in the immediate vicinity (Ernst, 1999). The decreasing risk for gastric cancer or gastritis with the dietary supplementation of antioxidants like beta-carotene and vitamin E support the important role of ROS in the formation of gastric ulcers and cancer (La Vecchia *et al*., 2000a; La Vecchia *et*

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al., 2000b; Wu et al., 2004). Moreover, the levels of these antioxidants are lowered during an infection, which leads to ROS accumulation in the gastric epithelium (Ernst, 1999). This study examined what is a real effect of DHEA on oxidative stress via drug-induced acute gastritis animal model because an influence of DHEA on morphological gastritis and multi-targeted enzyme activities of defense system against oxidative stress might be a clue to the real effect of DHEA.

MATERIALS AND METHODS

Experimental anymals

All animals were treated in strict accordance with the NIH Guide for the Human Care and Use of Laboratory Animals. Male Sprague-Dawley rats weighing approximately 250 grams were housed under standard laboratory conditions. The animals were maintained in a temperature-controlled room ($25^{\circ}C$) and kept on a 12:12 light dark cycle (lights on at 08:00. and off at 20:00.). Food and water were provided ad libitum. The experiments were carried out from 16:00 to 17:00. There were 10 rats per group. The DHEA group was administrated DHEA orally at a dose of 0.5, 1, 2 or 4 mg/day for one week before inducing gastritis with indomethacin.

Chemicals

DHEA, Indomethacin, thiobarbituric acid, trichloroacetic acid, malonaldehyde bis (dimethyl acetal), bovine serum albumin, o-phthalaldehyde, diethylenetriaminepentaacetic acid, ascorbic acid, N-ethylmaleimide and glutathione were purchased from Sigma (St. Louis, MO, USA). Potassium phosphate (dibasic) and potassium dihydrogenphosphate were purchased from Showa (Tokyo, Japan). The protein assay kits were purchased from BioRad (Richmond, CA, USA).

Histological examination

The gastric lesions were induced by the oral administration of 50 mg/kg indomethacin, which was suspended in a 3% solution of sodium bicarbonate ($NaHCO_3$). Five hours after administering the indomethacin, the animals were sacrificed by an overdose of ether. The stomach was opened along the greater curvature and spread out with pins on a corkboard. The area (mm^2) of the mucosal erosive lesions was measured using a dissecting microscope with a squared grid (X10; Olympus, Tokyo, Japan). For the histological examination, segmental biopsies of the stomach were fixed in formaldehyde (10% wt/vol.), embedded in paraffin, cut into 4-mm sections, de-waxed, and stained with hematoxylin-eosin.

Lipid peroxidation assay

The level of lipid peroxidation, which is a marker of oxidative stress, was determined using the method reported by Austin et. al, which involved measuring the formation of the thiobarbituric acid-reactive substances (TBARS) spectrophotometrically (Austin et al., 1995). Briefly, each gastric mucosa was harvested, and sonicated in 1 ml of Tris-HCl buffer (pH 7.0). After centrifuging at 600×g for 10 min at $4^{\circ}C$ (Micro17TR, Hanil, Korea), 0.9 ml of trichloroacetic acid (8%) was added to 0.3 ml of the supernatant. The mixture was then centrifuged at 10,000×g for 5 min at $4^{\circ}C$. 0.25 ml of TBA (1%) was then added to 1 ml of the supernatant and the resulting solution was heated at $100^{\circ}C$ for 20 min. The tubes were cooled, 2 ml of n-butanol was added to each tube and the resulting mixture was vortexed for 90 s. After centrifuge at 3,000×g for 5 min at $4^{\circ}C$, 1 ml of the butanol phase was used for the TBARS assay at 532 nm (UV-160A, Shimadzu, Japan) against malondialdehyde (MDA, dimethyl acetal) standards. The results are expressed as nmol/mg protein. The protein level was determined using the Bradford method.

Enzyme analysis

A spectrophotometric assay was used to quantify the total (Cu, Zn and Mn) SOD activity (Oberley et al., 1984). Each protein extract (10 mg) was assayed and compared with 1 Unit of bovine Cu and Zn SOD activity. The assay was carried out for 2 min after adding the radical producer, xanthine oxidase. The SOD activity is expressed as the percentage inhibition of the formazan produced in the control reaction without SOD or the protein extracts. 100% formazan product formation is the amount of Nitro Blue Tetrazolium reduced by the radicals formed by xanthine oxidase over a 2 min period. The assays were performed either in duplicate or triplicate. The GPx activity was measured according to the method described by Sinet with tert-butyl-hydroperoxide (t-butyl-HPx) as the substrate (Sinet et al., 1975). The gastric mucosal tissue were homogenized in a buffer solution (1 mg/5 ml) containing PBS 0.05 M (pH 7.4). After homogenization, 30 mg of the gastric mucosal protein was added to 500 ml of phosphate-buffered saline (PBS) pH 7.0 containing 10^{-3} M reduced glutathione, 2 units of yeast glutathione reductase (Sigma type III) and 2×10^{-4} nicotinamide adenine dinucleotide phosphate (NADPH). After 10 min at $37^{\circ}C$, the reaction was initiated by adding t-butyl-HPx to a final concentration of 10^{-3} M under constant agitation. The level of NADPH oxidation was calculated using the extinction co-

efficient for NADPH of 6.22×10^3 at 340 nm. The reaction was carried out for 5 min. One enzyme unit was defined as 1 mM NADPH/mU per mg of protein. The catalase activities were measured using kits purchased from Cayman Chemicals (Ann Arbor, MI, USA) according to the manufacturer's instructions.

Statistical analysis

Within each group, the mean concentration ± standard errors were compared. The significance of the differences between the groups was determined using a Student t test or ANOVA after verifying the fitness for parametric analysis. A p value < 0.05 was considered significant.

RESULTS

Body weight

The administration of DHEA did not affect the normal weight gain during treatment (data not shown). There was no significant difference between the control (4% dimethyl sulfoxide, DMSO) and DHEA group.

Histological damage

The lesion of the gastric mucosal injury was measured 5 hours after administering indomethacin, which was given to the rats after they had received a 7-day pretreatment with the vehicle or DHEA. The total area of the gastric mucosal injury was measured by the naked eye and under a dissecting microscope with a squared grid. The results are shown in Table I. The severity of gastric damage was more evident ($p < 0.05$) in the high dose (4 mg/day) of DHEA, even though there was no significant difference in the lower doses of DHEA. Therefore, we applied the highest dose of DHEA in further studies.

In the hematoxylin-eosin stained sections, there were differences in the cellular architecture between the control and DHEA group. Focal mucosal erosion was observed in the control group. However, the DHEA group showed submucosal edema, leukocyte infiltration in the submucos and mucosal necrosis (Fig. 1).

Oxidative stress and enzyme activity

Table II shows the results of oxidative stress parameters. The TBARS concentration was measured to determine the level of malondialdehyde (MDA) production, which is the end-product of lipid peroxidation by reactive oxygen species. The contents of TBARS in the DHEA group were significantly higher than those in the control group (1.75 ± 0.03 vs. 2.07 ± 0.06, $p < 0.05$, Table II). This suggests that the DHEA pretreatment caused oxidative damage in the indomethacin-induced gastritis model instead of having a protective effect against oxidative stress. The SOD activity was significantly higher in the gastric mucosa of the DHEA group than the control (100 ± 12.6 in the control vs. 150.7 ± 16.9% the DHEA group). The DHEA pretreatment before administering indomethacin did not alter the catalase (103.6 ± 19.3 vs. 101.5 ± 21.1 mmole/min/mg protein) and GPx activities (0.62 ± 0.05 vs. 0.65 ± 0.08 units/mg protein) compared with the control group treated with indomethacin alone.
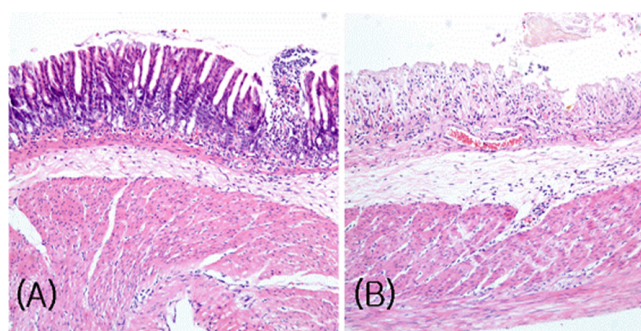
Fig. 1. Representative photomicrographs of hematoxylinl-eosin stained sections. (A) Control group showing focal mucosal erosion with inflammatory cells infiltration. (B) DHEA (4 mg/day) group showing diffuse mucosal necrosis and edema.

Table I. Effect of DHEA on the area of gastric lesion in rats treated with indomethacin. The data is reported as the mean ± S.E.M., n=10. *$p < 0.05$ vs. control (indomethacin alone) group

	Gastric lesion (mm^2 ± SEM)
Indomethacin	49.3 ± 5.3
DHEA (0.5 mg/day)+Indomethacin	53.6 ± 9.1
DHEA (1 mg/day)+Indomethacin	51.6 ± 5.9
DHEA (2 mg/day)+Indomethacin	54.2 ± 9.8
DHEA (4 mg/day)+Indomethacin	65.2 ± 5.1*

Table II. Effect of DHEA on oxidative stress parameters in rats treated with indomethacin. The data is reported as the mean ± S.E.M., n=10. *$p < 0.05$ vs. control (indomethacin alone) group

	Indomethacin	Indomethacin+DHEA
MDA (nmole/mg protein)	1.75 ± 0.03	2.07 ± 0.06*
SOD activity (%control)	100 ± 12.6	150.7 ± 16.9*
Catalase activity (mole/min/mg protein)	103.6 ± 19.3	101.5 ± 21.1
GPx activity (Units/mg protein)	0.62 ± 0.05	0.65 ± 0.08

DISCUSSION

This study examined the effect of DHEA on gastric damage in rats treated orally with indomethacin. We demonstrated that DHEA had a deleterious effect on indomethacin-induced acute gastritis. Histological damage with increasing MDA concentration was observed in the gastric mucosal tissue of the rats exposed to indomethacin. Pre-administration of DHEA can accelerate the infiltration of neutrophils into the gastric mucosal tissue of rats, resulting in deteriorated gastric mucosal. This result might, in turn, lead to increase the rate of lipid peroxidation resulting from the production of reactive oxygen species (ROS).

Although the mechanisms of NSAID-induced gastrointestinal damage are complex and not fully understood, there is a correlation between NSAID-induced injury and the inhibition of prostaglandin synthesis. ROS production from neutrophils was also reported to be a major factor in the injury (Wallace 1997; Beck *et al.*, 2000; Bandyopadhyay *et al.*, 2002), indicating that ROS plays important roles in the formation of gastric lesions induced by non-steroidal anti-inflammatory drugs. Our study is in an agreement with the previous investigations. In a few hours after indomethacin administration, there was a high level of gastric ulceration, with a significant increase in the level of lipid peroxidation and vascular permeability, and a decrease in the glutathione and catalase concentration.

DHEA has been reported to have numerous beneficial effect including protection against many age-related diseases (Kroboth *et al.*, 1999; Green *et al.*, 2001; Williams *et al.*, 2002). The ameliorative effect of a DHEA treatment against an oxidative imbalance have been suggested to be the key mechanism for its beneficial effect (Khalil *et al.*, 1998; Aragno *et al.*, 1999, 2000a, 2000b). However, there is still some controversy regarding the ability of DHEA to protect against lipid peroxidation, probably because DHEA supplementation in rodents has been reported to increase the level of reduced nicotinamide-adenine dinucleotide phosphate (NADPH)-dependent microsomal and mitochondrial lipid peroxidation, which is measured as the level of TBARS production in the rat liver and some extrahepatic tissues (Swierczynski *et al.*, 1996a; Swierczynski *et al.*, 1996b; Swierczynski *et al.*, 1997). One study suggested that the main mechanism for the pro-oxidant effect is the induction of the proliferator-activated receptor alpha (PPARα), which appears only when the tissue concentration of the steroid reaches high pharmacological levels. DHEA is unable to activate PPARα at lower levels. Mastrocola *et al.* (2003) reported that DHEA acts as a PPARs inducer by increasing the acyl-CoA-oxidase activity and the cytochrome P450 content. Cytochrome P450 enzyme activation, which results in the formation of superoxide anion radicals and hydrogen peroxide, increases the level of reactive oxygen species in the liver of rats treated with high doses of DHEA (Morgan, 2001).

In agreement with the investigations, a pretreatment with DHEA before administering indomethacin induced severer gastric tissue damage with higher levels of lipid peroxidation than with the indomethacin treatment alone. Among anti-oxidant systems, co-exposure of DHEA and indomethacin increased the activity of SOD without increasing the activity of catalase and GPx. The increase in SOD activity suggests that exposing the stomach to indomethacin after a DHEA pretreatment leads to the hyper-activation of the endogenous antioxidant system against the offensive ROS. The reason for the unique increase in SOD and the unchanged activity of catalase and GPx with indomethacin and DHEA might be that SOD serves as a key component of the protective mechanism against ROS during oxidative stress. This suggests that SOD plays a key role in attenuating the increased oxidative stress with DHEA and indomethacin.

In summary, DHEA has been known to be effective against oxidative stress induced tissue damages. However, its effect is controversial because there have been several reports that DHEA accelerated tissue damage by exerting an oxidative stress factor. DHEA did not show a protective effect against the acute gastritis induced by the NSAID. Indeed, DHEA with NSAID deteriorated NSAID induced gastric tissue damages and lipid peroxidation. These results indicate that DHEA might be a pro-oxidative effect on NSAID induced tissue damages.

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