Free Radical Research, February 2010; 44(2): 171–180

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healthcare

Compound 48/80 causes oxidative stress in the adrenal gland of rats through mast cell degranulation

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(Received 11 August 2009; revised 28 September 2009)

Abstract
Rats were intraperitoneally treated once with compound 48/80 (C48/80), a mast cell degranulator, (0.75 mg/kg). Serum serotonin, histamine and corticosterone levels increased 0.5 h after C48/80 treatment, but their increases were reduced thereafter. Adrenal total ascorbic acid (ascorbic acid plus dehydroascorbic acid), ascorbic acid and dehydroascorbic acid levels decreased 0.5, 3 or 6 h after C48/80 treatment, adrenal lipid peroxide level increased at 3 and 6 h, adrenal non-protein-SH level decreased at 3 and 6 h and adrenal β-tocopherol level decreased at 3 h. Ketotifen, a mast cell stabilizer (1 mg/kg) administered intraperitoneally at 0.5 h before C48/80 treatment, attenuated all these changes found in the serum and adrenal at 3 h after treatment, while β-tocopherol (250 mg/kg), administered orally at 0.5 h after C48/80 treatment, attenuated all these changes in the adrenal tissue. These results indicate that C48/80 causes oxidative stress in rat adrenal gland through mast cell degranulation.

Keywords: *Compound 48/80, rat adrenal gland, oxidative stress, mast cell degranulation*

Abbreviations: *AA, Ascorbic acid; C48/80, compound 48/80; DHA, dehydroascorbic acid; DTT, dithiothreitol; EDTA, ethylenediaminetetraaceitic acid; GSH, reduced glutathione; LPO, lipid peroxide; NEM, N-ethylmaleimide; NPSH, non-protein SH; ROS, reactive oxygen species; TCA, trichloroacetic acid.*

Introduction

Compound 48/80 (C48/80) is a condensation product of *N*-methyl-*p*-methoxy phenylethylamine and formalin [1]. This compound induces systemic anaphylaxis and inflammation in experimental animals [2–4]. It has been demonstrated that, in rats treated once with C48/80, connective tissue mast cells such as peritoneal mast cells, but not mucosal tissue mast cells, are degranulated, resulting in release of histamine and serotonin from the connective tissue mast cells [5,6].

Nasmyth [7] reported that the adrenal content of ascorbic acid (AA), i.e. reduced ascorbic acid (or vitamin C), in fed Wistar rats with a single subcutaneous injection of C48/80 (5 mg/kg) was less than 50% of that in untreated rats at 1.5 and 3 h after injection and that the decreased adrenal AA content was returned to ~ 70% of the level of untreated rats at 5 h. Bousquet et al. [8] also reported that, in fed Holtzman rats with a single intraperitoneal injection of C48/80 (200 μg/animal), adrenal AA level was decreased with a concomitant increase in plasma corticosterone level at 0.5 h after injection. We have shown in fasted Wistar rats with a single intraperitoneal injection of C48/80 (0.75 mg/kg) that although the serum level of dehydroascorbic acid (DHA), i.e. oxidized ascorbic acid, increases 0.5 h after injection, the serum levels of total ascorbic acid

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ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.3109/10715760903380466

(AA + DHA) and AA increase at 0.5 h and further increase at 3 h, but the increased serum AA + DHA and AA levels decrease at 6 h [9]. In addition, we have suggested that the increase in AA level in the serum of C-48/80-treated rats found at 3 h after treatment is related with the increase in AA level in the liver tissue [9]. These findings may allow us to assume that, in rats treated once with C48/80, AA is released from the adrenal into the bloodstream at the early time of post-treatment. Among the adrenal, liver, brain, heart, kidney, lung, skeletal muscle, spleen and testis of young rats, the adrenal possesses the highest contents of antioxidants such AA, *α*-tocopherol (vitamin E) and reduced glutathione (GSH) and the lowest content of lipid peroxide (LPO) [10]. AA plays a critical role in the adrenal through catecholamine biosynthesis, adrenal steroidogenesis and protection of cytochrome P450 against lipid peroxidation [11]. AA exerts an antioxidant action not only by scavenging reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical, hydrogen peroxide and peroxyl radical by itself [12–15] but also by interacting with GSH or vitamin E [16,17]. Therefore, there is a possibility that oxidative stress associated with AA depletion may be caused in the adrenal of rats treated once with C48/80. However, it is still unclear whether a single C48/80 treatment causes oxidative stress in the adrenal of rats through mast cell degranulation.

In the present study, therefore, we attempted to clarify whether a single C48/80 treatment causes oxidative stress in the adrenal gland of rats through mast cell degranulation. Namely, we examined the effect of a single C48/80 treatment on serum AA + DHA, DHA, non-protein-SH (NPSH), *α*-tocopherol, LPO, histamine, serotonin, corticosterone levels and adrenal AA + DHA, AA, DHA, NPSH, *α*-tocopherol and LPO levels in rats. In addition, the effect of *α*-tocopherol administration on the levels of these components in the serum and adrenal of C48/80-treated rats was examined in order to confirm the occurrence of C48/80-induced oxidative stress in the adrenal gland. As vitamin E is known to inhibit C48/80-evoked mast cell degranulation *in vitro* [18], *α*-tocopherol was administered to rats after the appearance of C48/80-evoked mast cell degranulation. The effect of the pre-administration of ketotifen, a connective tissue mast cell stabilizer [19], on the levels of these components in the serum and adrenal gland of C48/80-treated rats was examined in order to confirm the involvement of mast cell degranulation in C48/80-induced oxidative stress in the adrenal gland.

Materials and methods

Chemicals

α-Tocopherol, C48/80, ketotifen and methyl serotonin were purchased from Sigma Chemical Co. (St. Louis, MO); AA (L-form), corticosterone, 2,2'-dipyridyl, 5,5'-dithiobis(2-nitrobenzoic acid) (Ellman's reagent), dithiothreitol (DTT), *δ*-tocopherol, ethylendiaminetetraacetic acid (EDTA), *N*-ethylmaleimide (NEM), GSH, *o*-phthalaldehyde, 2-thiobarbituric acid, trichloroacetic acid (TCA), Tween 80 and other chemicals from Wako Pure Chemical Industries (Osaka, Japan). All chemicals were used without further purification.

C48/80 treatment and α-tocopherol and ketotifen administrations

C48/80 (0.75 mg/kg), dissolved in distilled water, was intraperitoneally injected to 7-week-old Wistar male rats fasted for 24 h, which were purchased from Nippon SLC Co. (Hamamatsu, Japan), under non-anaesthesia as described in our previous report [9]. Untreated rats received an intraperitoneal injection of an equal volume of distilled water. *α*-Tocopherol (250 mg/kg), dissolved in 5% Tween 80, was orally administered to C48/80-treated rats at 0.5 h after C48/80 treatment. Ketotifen (1 mg/kg), dissolved in 0.9% NaCl, was intraperitoneally injected to C48/80-treated rats at 0.5 h before C48/80 treatment. Each vehicle was administered in the same manner at the same time point. The doses of *α*-tocopherol and ketotifen and their administration routes used in the present study were determined based on our previous reports [20,21]. There was no difference in the levels of all serum and adrenal components determined between rats administered with 5% Tween 80 alone and 0.9% NaCl alone. Therefore, the data obtained from both vehicle-administered rats were combined. All animals were maintained with free access to water and without food during the experiment. All animals received humane care in compliance with the guidelines of the Management of Laboratory Animals in Fujita Health University. This animal experiment protocol was approved by the Institutional Animal Care and Use Committee.

Sample collection and determinations of serum and adrenal components

In the time course study, C48/80-treated and untreated rats were killed under ether anaesthesia at 0.5, 3 or 6 h after treatment. In the study of *α*-tocopherol or ketotifen administration, C48/80-treated rats, C48/80-treated rats administered with either *α*-tocopherol or ketotifen and untreated rats were killed under ether anaesthesia at 3 h after C48/80 treatment. During killing, blood was collected from the inferior vena cava. Serum was obtained from the collected blood by centrifugation. Immediately after killing, both adrenals of each rat were removed, washed in ice-cold 0.9% NaCl, wiped on a paper

filter and then weighed. Each isolated adrenal was frozen on dry ice. The serum and adrenals obtained were stored at –80°C until used.

For serum serotonin and histamine determinations, serum samples were deproteinized by adding perchloric acid at a final concentration of 3% and then centrifuged at 4°C for 10 min (10 000 x g). Serum serotonin was measured by the method of Shibata et al. [22] using high-performance liquid chromatography with electrochemical detection, except that 40 mM sodium dihydrogenphosphate used for the mobile phase was replaced by 0.1 M citric acid-0.1 M sodium acetate (0.7:1.0, v/v). Methyl serotonin was used as an internal standard. Serum histamine was measured by the methods of Lorenz et al. [23] and Shore et al. [24]. Histamine was reacted with *o*-phthalaldehyde and the intensity of the resultant fluorescence was measured spectrofluorometrically (the excitation wavelength, 360 nm; the emission wavelength, 450 nm). Serum corticosterone was measured by the method of Mattingly [25] using authentic corticosterone as a standard. Corticosterone in serum was extracted with dichloromethane and the resultant extract was mixed with a fluorescence reagent (ethanol and concentrated H_2SO_4 in the ratio of 3:7 v/v). The resulting fluorescence of the acid layer was measured spectrofluorometrically (the excitation wavelength 470 nm; the emission wavelength 530 nm). Each adrenal tissue was homogenized in nine volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.5) containing 1 mM EDTA to prepare 10% homogenate. This homogenate was used for the assays of AA + DHA, AA, DHA, NPSH, *α*-tocopherol and LPO. AA + DHA, AA and DHA in adrenal tissue or serum were determined by the methods of Zannoni et al. [26] and Okamura [27] as follows: for the determination of AA + DHA, 0.3 ml of 10% adrenal homogenate or serum was incubated with 0.1 ml of 10 mM DTT at 37°C for 30 min to convert all DHA to AA in the homogenate or serum and then the excess DDT was removed with 0.1 ml of 0.5% NEM. An aliquot of the supernatant obtained after deproteinization with 0.5 ml of ice-cold 10% TCA was used for the assay of the resultant AA plus the original AA. For the determination of AA, 0.3 ml of 10% adrenal homogenate or serum was mixed with 0.2 ml of a solution of 10 mM DDT-5%NEM. An aliquot of the supernatant obtained after deproteinization with 0.5 ml of ice-cold 10% TCA was used for the assay of AA. AA in each sample was measured by the 2,2'-dipyridyl method. The concentration of AA was determined using the standard curve of authentic L-AA. The concentration of DHA in adrenal tissue or serum was estimated from the difference between the concentrations of AA + DHA and AA determined. NPSH in adrenal homogenates or serum was assayed by the method of Sedlak and Lindsay [28] using Ellman's reagent and GSH as a standard. *α*-Tocopherol in adrenal homogenates or serum was measured by the high-performance liquid chromatographic method with electrochemical detection using authentic *δ*-tocopherol as an internal standard as described in our previous report [29]. LPO in serum was spectrofluorometrically measured by the thiobarbituric acid method of Yagi [30] using tetramethoxypropane as a standard. LPO in adrenal homogenates was spectrofluorometrically measured by the thiobarbituric acid method of Ohkawa et al. [31] using tetramethoxypropane as a standard except that 1 mM EDTA was added to the reaction mixture. In both LPO assays, the excitation and emission wavelengths were 515 and 553 nm, respectively. The amount of LPO in adrenal homogenates or serum is expressed as that of malondialdehyde (MDA) equivalents.

Statistical analysis

All results obtained are expressed as means ± SD. The statistical analyses of the results were performed using a computerized statistical package (StatView). Each mean value was compared by one-way analysis of variance (ANOVA) and Fisher's protected least significant difference (PLSD) for multiple comparisons as the post-hoc test. The significance level was set at $p < 0.05$.

Results

Serum histamine and serotonin concentrations in rats treated once with C48/80 were significantly higher than those in untreated control rats at 0.5 h after treatment (Figures 1A and B). The increased serum histamine and serotonin concentrations in C48/80-treated rats decreased time-dependently thereafter, although the serum histamine and serotonin concentrations in the C48/80-treated group at 3 or 6 h were significantly higher than those in the control group (Figures 1A and B). Serum corticosterone concentration in C48/80-treated rats was significantly higher than that in untreated control rats at 0.5 or 3 h after treatment and there was no significant difference in serum corticosterone concentration between the treated and untreated rats at 6 h (Figure 1C).

Serum AA + DHA, AA and DHA concentrations were significantly higher in C48/80-treated rats than in untreated control rats at 0.5 h after treatment (Figures 2A–C). The C48/80-treated rats had further increased serum AA + DHA and AA concentrations at 3 h, but the increased serum AA + DHA and AA concentrations in the C48/80-treated group were reduced near the levels of the control group at 6 h (Figures 2A and B). There was no significant difference in serum DHA concentration between the C48/80-treated and control groups at 3 or 6 h (Figure 2C). There was no significant difference in serum NPSH

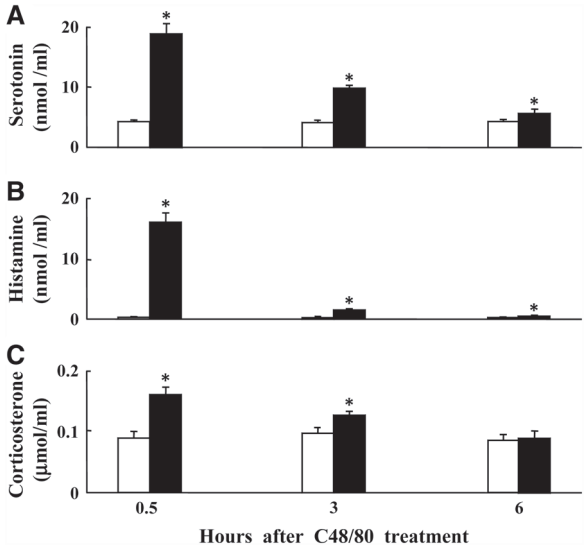

Figure 1. Effect of a single C48/80 treatment on serum serotonin (A), histamine (B) and corticosterone (C) concentrations in rats. C48/80 (0.75 mg/kg) was intraperitoneally injected to fasted rats. C48/80-treated and untreated rats were killed 0.5, 3 or 6 h after treatment. Serum serotonin, histamine and corticosterone were assayed as described in Materials and methods. Open bar, untreated control rats; closed bar, C48/80-treated rats. Each value is a mean ± SD (n = 8 per group). $^{*}p < 0.05$ (vs untreated control rats).

concentration between C-48/80-treated and untreated rats at 0.5, 3 or 6 h after treatment (Figure 2D). Serum α-tocopherol concentration in the C48/80-treated group was significantly lower than that in the control group at 3 h, but not 0.5 or 6 h, after treatment (Figure 2E). Serum LPO concentration in the C48/80-treated group was significantly higher than that in the control group at 3 h, but not 0.5 or 6 h, after treatment (Figure 2F).

There was no significant difference in adrenal weight between both C-48/80-treated and untreated control rats at 0.5, 3 or 6 h after treatment (data not shown). Adrenal AA + DHA, AA and DHA contents in C48/80-treated rats were significantly less than those in untreated control rats at 0.5, 3 or 6 h after treatment (Figures 3A–C). However, adrenal AA + DHA content in the C48/80-treated group was 55, 32 and 43% of that in the corresponding control group at 0.5, 3 and 6 h, respectively (Figure 3A). Adrenal AA content in the C48/80-treated group was 55, 26 and 45% of that in the corresponding control group at 0.5, 3 and 6 h, respectively (Figure 3B). Adrenal DHA content in the C48/80-treated group was 50, 77 and 36% of that in the corresponding control group at 0.5, 3 and 6 h, respectively (Figure 3C). Thus, the reduction of adrenal AA + DHA or AA content was larger at 3 h than at 0.5 or 6 h, while the reduction of adrenal DHA content was less at 3 h than at 0.5 or 6 h (Figures 3A–C). Adrenal NPSH content was significantly higher in the C48/80-treated group than in the control group at 0.5 h after treatment, but the increased adrenal NPSH content was reduced to 55% of the level of the control group at 3 h (Figure 3D). The reduction of adrenal NPSH content in the C48/80-treated group was significantly recovered at 6 h ($p < 0.05$), although the recovered adrenal NPSH content was below the level of the control group (Figure 3D). Adrenal α-tocopherol

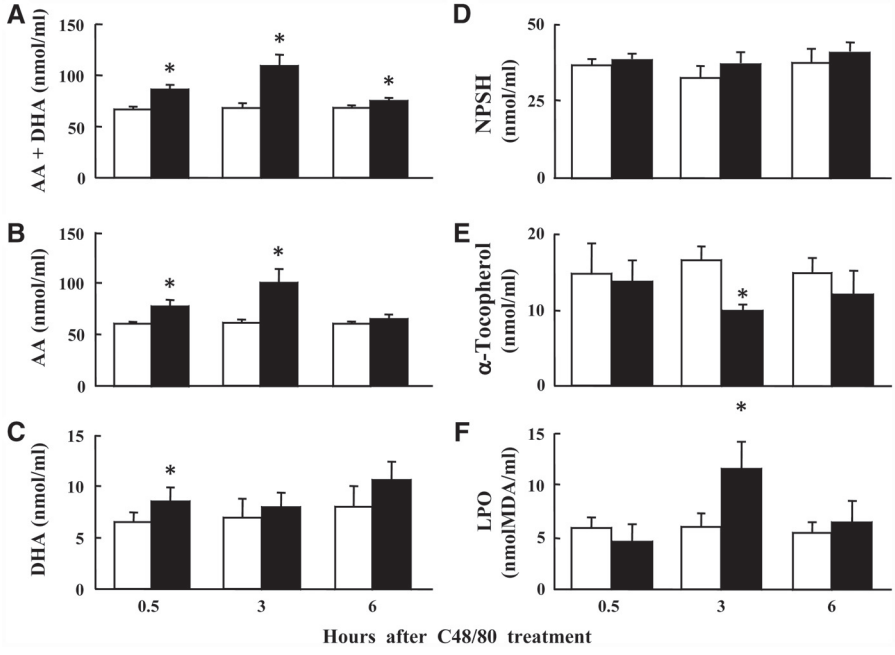

Figure 2. Effect of a single C48/80 treatment on serum AA + DHA (A), AA (B), DHA (C), NPSH (D), α-tocopherol (E) and LPO (F) concentrations in rats. C48/80 (0.75 mg/kg) was intraperitoneally injected to fasted rats. C48/80-treated and untreated rats were killed 0.5, 3 or 6 h after treatment. Serum AA + DHA, AA, DHA, NPSH, α-tocopherol and LPO were assayed as described in Materials and methods. Open bar, untreated control rats; closed bar, C48/80-treated rats. Each value is a mean ± SD (n = 8 per group). $^{*}p < 0.05$ (vs untreated control rats).

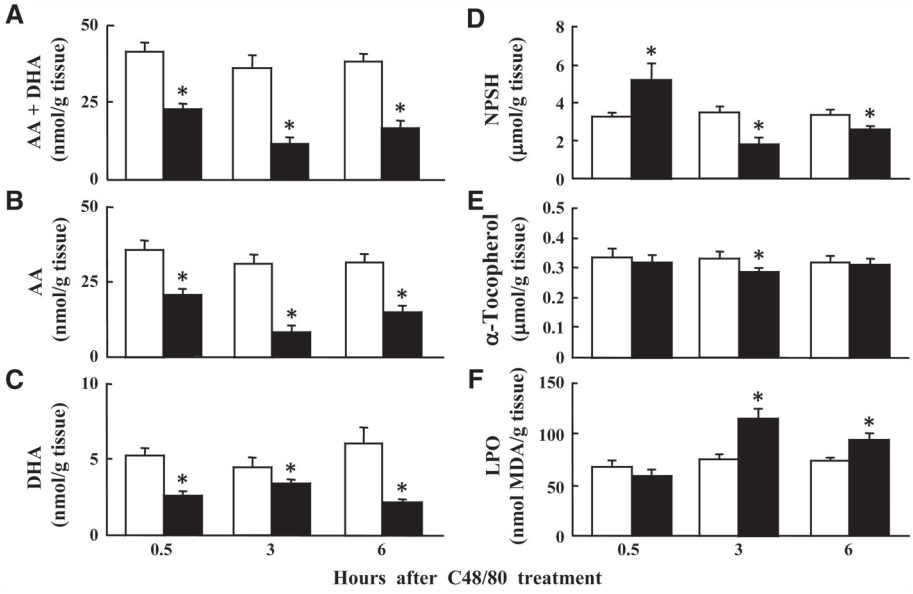

Figure 3. Effect of a single C48/80 treatment on adrenal AA + DHA (A), AA (B), DHA (C), NPSH (D), *α*-tocopherol (E) and LPO (F) contents in rats. C48/80 (0.75 mg/kg) was intraperitoneally injected to fasted rats. C48/80-treated and untreated rats were killed 0.5, 3 or 6 h after treatment. Adrenal AA + DHA, AA, DHA, NPSH, *α*-tocopherol and LPO were assayed as described in Materials and methods. Open bar, untreated control rats; closed bar, C48/80-treated rats. Each value is a mean ± SD (n = 8 per group). $^{*}p < 0.05$ (vs untreated control rats).

content was slightly less in the C48/80-treated group than in the control group at 3 h, but there was no significant difference in adrenal *α*-tocopherol content between the C48/80-treated and control groups at 0.5 or 6 h (Figure 3E). Adrenal LPO content was significantly higher in the C48/80-treated group than in the control group at 3 or 6 h, but not 0.5 h, although the extent of increase in adrenal LPO content in the C48/80-treated group was larger at 3 h than at 6 h (Figure 3F).

When ketotifen and *α*-tocopherol were administered to C-48/80-treated rats at 0.5 h before and after treatment, respectively, the administered ketotifen significantly attenuated the increased serum histamine, serotonin and corticosterone concentrations found at 3 h after C48/80 treatment and maintained the serum histamine, serotonin and corticosterone concentrations near the levels of the control group, while the administered *α*-tocopherol did not affect the increased levels of these components at all (Figure 4).

The administration of ketotifen significantly attenuated the increased serum AA + DHA and AA concentrations found at 3 h after C48/80 treatment and maintained the serum AA + DHA and AA concentrations at the levels of the control group, although the administered ketotifen did not affect the serum DHA concentration at the same time point (Figures 5A–C). The administration of ketotifen significantly attenuated the decreased serum *α*-tocopherol concentration and the increased serum LPO concentration found at 3 h after treatment and maintained the serum *α*-tocopherol and LPO concentrations at the levels of the control group, although the administered ketotifen did not affect the serum NPSH concentration at the same time point (Figures 5D–F).

The administration of *α*-tocopherol significantly attenuated the increased serum AA + DHA and AA concentrations found at 3 h after treatment and maintained the serum AA + DHA and AA concentrations near the levels of the control group, although the administered *α*-tocopherol did not affect the serum DHA concentration at the same time point (Figures 5A–C). The administration of *α*-tocopherol significantly attenuated the decreased serum *α*-tocopherol concentration and the increased serum LPO concentration found at 3 h after treatment and maintained the serum *α*-tocopherol concentration at 1.7-fold higher than the level of the control group and the serum LPO concentration at the level of the control group, although the administered *α*-tocopherol did not affect the serum NPSH concentration at the same time point (Figures 5D–F).

The administration of ketotifen significantly attenuated the decreased adrenal AA + DHA and AA contents found at 3 h after C48/80 treatment and maintained the adrenal AA + DHA, AA and DHA contents at the levels of the control group (Figures 6A–C). The administration of ketotifen significantly attenuated the decreased adrenal NPSH and *α*-tocopherol contents and the increased adrenal LPO contents found at 3 h after treatment and maintained the adrenal *α*-tocopherol, NPSH and LPO contents at the levels of the control group (Figures 6D–F).

The administration of *α*-tocopherol significantly attenuated the decreased adrenal AA + DHA and AA contents found at 3 h after C48/80 treatment

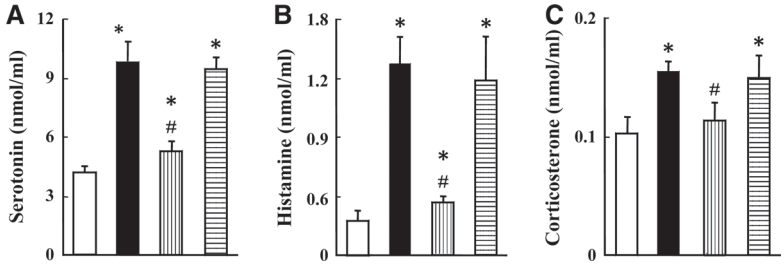

Figure 4. Effects of ketotifen and α-tocopherol administrations on serum serotonin (A), histamine (B) and corticosterone (C) concentrations in rats treated with C48/80. Ketotifen (1 mg/kg) was intraperitonelly administered to C48/80-treated rats at 0.5 before treatment and α-tocopherol (250 mg/kg) was orally administered to the treated rats 0.5 h after treatment. All rats were killed 3 h after C48/80 treatment. Serum serotonin, histamine and corticosterone were assayed as described in Materials and methods. Open bar, untreated control rats; closed bar, C48/80-treated rats; vertically striped bar, C48/80-treated rats pre-administered with ketotifen; horizontally striped bar, C48/80-treated rats post-administered with α-tocopherol. Each value is a mean ± SD (n = 8 per group). $^{*}p < 0.05$ (vs untreated control rats); $^{\#}p < 0.05$ (vs rats treated with C48/80 alone).

and maintained the adrenal AA + DHA and AA contents at ~ 50% of the levels of the control group, although the administered α-tocopherol further reduced the adrenal DHA content at the same time point (Figures 6A–C). The adrenal AA + DHA, AA and DHA contents in the C48/80-treated rats administered with α-tocopherol were almost equal to those in the C48/80-treated rats found at 0.5 h after treatment (Figures 6A–C and 3A–C). The administration of α-tocopherol significantly attenuated the decreased NPSH and α-tocopherol concentrations and the increased serum LPO concentration found at 3 h after C48/80 treatment and maintained the adrenal α-tocopherol content at 1.5-fold higher than the level of the control group and the adrenal NPSH and LPO contents at the level of the control group (Figures 6D–F).

Discussion

In the present study, a single intraperitoneal injection of C48/80 (0.75 mg/kg) to Wistar rats fasted for 24 h caused marked increases in serum serotonin and histamine concentrations at 0.5 h after injection with a gradual reduction of serum serotonin concentration and a rapid reduction of serum histamine concentration at 3 and 6 h, as reported previously [32,33]. Bousquet et al. [8] reported that, in fed rats with a single intraperitoneal injection of C48/80 (200 μg/animal), an increase in plasma corticosterone level occurred with a decrease in adrenal AA concentration at 0.5 h after injection. Földes et al. [34] reported that the hypothalamic-pituitary-adrenal axis was activated in fed rats with a single intravenous injection of C48/80 (50 μg/kg), resulting in an increase in plasma

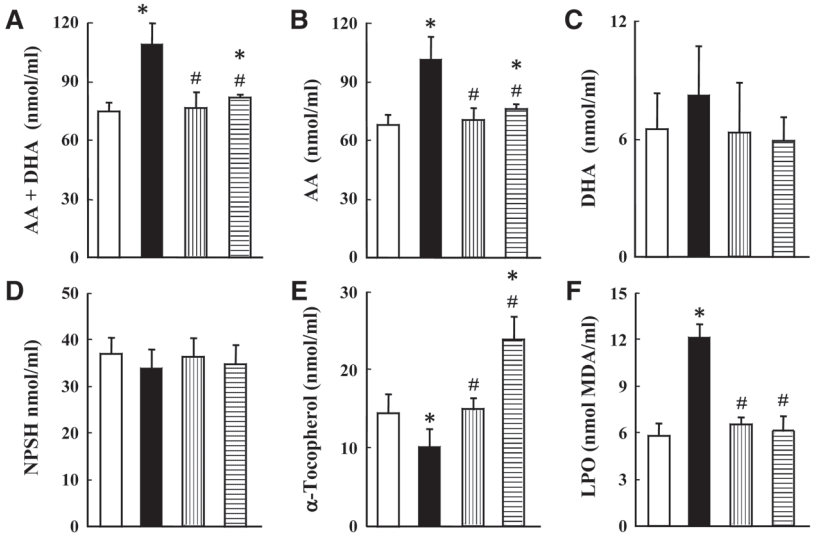

Figure 5. Effects of ketotifen and α-tocopherol administrations on serum AA + DHA (A), AA (B), DHA (C), NPSH (D), α-tocopherol (E) and LPO (F) concentrations in rats treated with C48/80. Ketotifen (1 mg/kg) was intraperitonelly administered to C48/80-trearted rats at 0.5 h before treatment and α-tocopherol (250 mg/kg) was orally administered to the treated rats 0.5 h after treatment. All rats were killed 3 h after C48/80 treatment. Serum AA + DHA, AA, DHA, NPSH, α-tocopherol and LPO were assayed as described in Materials and methods. Open bar, untreated control rats; closed bar, C48/80-treated rats; vertically striped bar, C48/80-treated rats pre-administered with ketotifen; horizontally striped bar, C48/80-treated rats post-administered with α-tocopherol. Each value is a mean ± SD (n = 8 per group). $^{*}p < 0.05$ (vs untreated control rats); $^{\#}p < 0.05$ (vs rats treated with C48/80 alone).

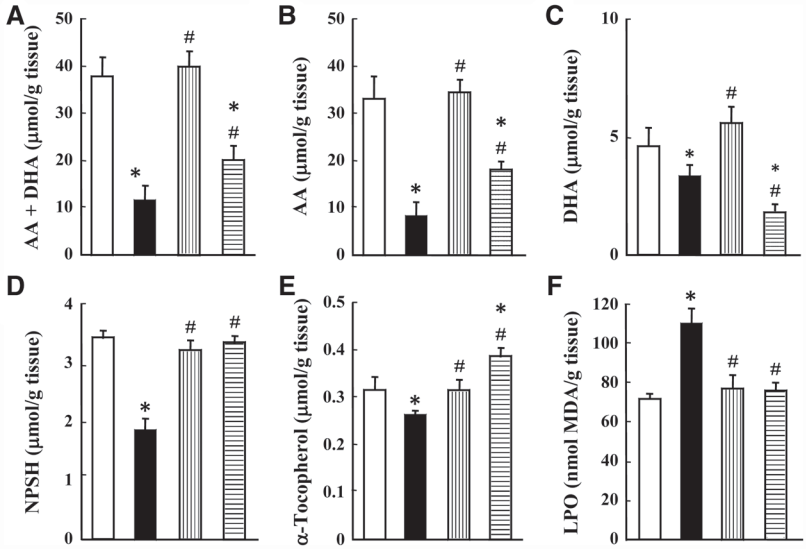

Figure 6. Effects of ketotifen and α-tocopherol administrations on adrenal AA + DHA (A), AA (B), DHA (C), NPSH (D), α-tocopherol (E) and LPO (F) contents in rats treated with C48/80. Ketotifen (1 mg/kg) was intraperitonelly administered to C48/80-treated rats at 0.5 h before treatment and α-tocopherol (250 mg/kg) was orally administered to the treated rats 0.5 h after treatment. All rats were killed 3 h after C48/80 treatment. Adrenal AA + DHA, AA, DHA, NPSH, α-tocopherol and LPO were assayed as described in Materials and methods. Open bar, untreated control rats; closed bar, C48/80-treated rats; vertically striped bar, C48/80-treated rats pre-administered with ketotifen; horizontally striped bar, C48/80–treated rats post-administered with α-tocopherol. Each value is a mean ± SD (n = 8 per group). $^{*}p < 0.05$ (vs untreated control rats); $^{\#}p < 0.05$ (vs rats treated with C48/80 alone).

adrenocorticotropic hormone (ACTH) level with its peak around 0.5 h after injection. Hinson et al. [35] have shown that C48/80 enhances corticosterone and aldosterone secretion by the isolated perfused rat adrenal gland *in situ* and have suggested that C48/80 exerts its action on adrenocortical steroid secretion by provoking the release of histamine and serotonin from the capsular mast cells. In the present study, fasted rats with a single C48/80 treatment showed an increase in serum corticosterone concentration at 0.5 h after treatment with its reduction to the level of untreated rats at 6 h. The increases in serum serotonin, histamine and corticosterone concentrations found at 3 h after C48/80 treatment were diminished almost completely by pre-administration of ketotifen, a mast cell stabilizer [19]. As it is known that vitamin E inhibits mast cell degranulation in rat peritoneal mast cells [18], α-tocopherol was orally administered to C-48/80-treated rats at 0.5 h after treatment. This post-administration of α-tocopherol did not change the increased serum serotonin, histamine and corticosterone concentrations at 3 h after treatment, indicating that the administered α-tocopherol had no effect on mast cell degranulation in rats treated once with C48/80.

In rats treated once with C48/80, increases in serum AA + DHA, AA and DHA concentrations occurred at 0.5 h after treatment, further increases in serum AA + DHA and AA concentrations and the reduction of increased serum DHA concentration to the level of untreated rats were found at 3 h and the reduction of the increased serum AA + DHA and AA concentrations near the levels of untreated rats was observed at 6 h, as shown in our previous report [9]. In C48/80-treated rats, a decrease in serum α-tocopherol concentration and an increase in serum LPO concentration were found 3 h after treatment, although serum NPSH concentration was unchanged at 0.5, 3 or 6 h. These results suggest that oxidative stress could occur systemically in rats with a single C48/80 treatment.

The pre-administration of ketotifen prevented the increases in serum AA + DHA, AA and LPO concentrations and the decrease in serum α-tocopherol concentration at 3 h after C48/80 treatment and maintained the serum AA + DHA, AA, LPO and α-tocopherol concentrations at the levels of untreated control rats. These results indicate that mast cell degranulation evoked by a single C48/80 treatment causes increases in serum AA + DHA, AA and LPO concentrations and a decrease in α-tocopherol concentration in rats. The post-administration of serum α-tocopherol restored the increased serum AA + DHA and AA concentrations near the levels of untreated control rats and the increased serum LPO concentration at the level of untreated control rats by increasing the decreased serum α-tocopherol concentration above the level of untreated control rats at 3 h after C48/80 treatment. These results indicate that a single C48/80 treatment causes oxidative stress systemically in rats through mast cell degranulation.

In the present study, rats treated once with C48/80 showed a marked decrease in adrenal AA content, as shown in previous reports [7,8]. The adrenal

AA + DHA and AA contents in C48/80-treated rats were ~ 50% of those in untreated control rats at 0.5 h after treatment and about one-third of those in untreated control rats at 3 h. The reduction of adrenal AA + DHA or AA content found at 3 h after C48/80 treatment was partially recovered at 6 h. We have suggested that, in rats treated once with C48/80, the increase in serum AA concentration found at 3 h after treatment could be due to the release of AA increasing in the liver into the bloodstream [9]. Therefore, it seems likely that the release of AA from the adrenal tissue of rats treated with C48/80 contributes to the above-described increase in serum AA concentration at 0.5 h after treatment. DHA content in the adrenal of untreated control rats was ~ 10% of total ascorbic acid (AA + DHA) content in the tissue, as reported by Harding and Nelson [36]. Adrenal DHA content in C48/80-treated rats was below half of that in untreated rats at 0.5 or 6 h after treatment, but the adrenal DHA content was ~ 80% of that in untreated rats at 3 h. These results indicate that although about two-third of AA + DHA present in the adrenal of rats is lost 3 h after a single C48/80 treatment, a part of AA remaining in the tissue is converted to DHA in the tissue. Most of NPSH present in the adrenal tissue of rats is GSH [37]. It is known that GSH regenerates AA from DHA by chemical and enzymatic reactions [16]. GSH-dependent dehydroascorbate reductase exists in the adrenal tissue of rats [38]. In the present study, adrenal NPSH content in C48/80-treated rats increased 0.5 h after treatment, but the increased adrenal NPSH content was reduced below the level of untreated control rats at 3 h, although the reduction of adrenal NPSH content was partially recovered at 6 h. This time course of adrenal NPSH content suggests that the reduction of NPSH content in the adrenal of C48/80-treated rats may contribute to the oxidation of AA to DHA in the tissue. It has been reported that adrenal NPSH level is increased in rats administered with a small dose of ACTH [38]. As described above, it is known that a rapid increase in plasma ACTH level occurs in rats treated once with C48/80 [34] and a rapid increase in serum corticosterone concentration was found in rats with a single C48/80 treatment. Accordingly, the increase in adrenal NPSH content at 0.5 h after C48/80 treatment may occur via C48/80-mediated ACTH secretion. Adrenal α-tocopherol content was reduced 3 h after C48/80 treatment. As described above, serum α-tocopherol concentration was reduced 3 h after C48/80 treatment. Accordingly, it seems likely that the reduction of adrenal α-tocopherol content in C48/80-treated rats is, at least in part, due to the reduction of serum α-tocopherol concentration. AA is known to support the chain-breaking antioxidant action of α-tocopherol by regenerating α-tocopherol from its radical form at the lipid/aqueous interface [17]. Therefore, it seems likely that the reduction of AA content in the adrenal tissue of C48/80-treated rats contributes to the reduction of α-tocopherol content in the tissue. Adrenal LPO content was increased 3 h after C48/80 treatment, although the increase in adrenal LPO content was partially recovered at 6 h. Thus, LPO content increased with concomitant decreases in AA, NPSH and α-tocopherol contents in the adrenal tissue of rats with a single C48/80 treatment. Taken together, it is suggested that, in the adrenal gland of rats treated once with C48/80, oxidative stress could occur a little late after the appearance of C48/80-evoked mast cell degranulation.

The pre-administration of ketotifen to rats treated with C48/80 restored the decreased adrenal AA + DHA, AA and DHA contents to the levels of untreated control rats at 3 h after treatment, indicating that a single C48/80 treatment causes decreases in adrenal AA + DHA, AA and DHA contents in rats through mast cell degranuation. The pre-administration of ketotifen restored the decreased adrenal NPSH and α-tocopherol contents and the increased LPO content to the levels of untreated control rats at 3 h after C48/80 treatment. These results indicate that a single C48/80 treatment causes oxidative stress in the adrenal of rats through mast cell degranulation. The decreases in adrenal AA + DHA and AA contents at 3 h after C48/80 treatment was partially recovered by the post-administration of α-tocopherol, while the adrenal DHA content was further reduced by this administration. In addition, the adrenal AA + DHA, AA and DHA contents in C48/80-treated rats administered with α-tocopherol were almost equal to those in C48/80-treated rats at the time when α-tocopherol was administered, i.e. at 0.5 h after treatment. Accordingly, these results indicate that AA remaining in the adrenal tissue of C48/80-treated rats at 0.5 h after treatment is oxidized to DHA in the tissue at 3 h. The post-administration of α-tocopherol restored the decreased adrenal NPSH content to the level of untreated control rats and increased the decreased adrenal α-tocopherol content above the levels of C48/80-treated and untreated control rats at 3 h after treatment. In addition, the post-administration of α-tocopherol restored the increased adrenal LPO content to the level of untreated control rats at 3 h after C48/80 treatment. These results indicate that oxidative stress in the adrenal gland of rats treated with C48/80 is caused through an enhancement of lipid peroxidation associated with the reductions of AA, NPSH and α-tocopherol contents in the tissue under mast cell degranulation.

It has been reported that histamine administration reduces adrenal AA level with concomitant increases in adrenal corticosterone level and serum AA level in rats [39]. It is known that a single treatment of rats with ACTH causes a marked reduction of adrenal AA level [40]. It is also known that ACTH treatment reduces AA level by inhibiting the uptake of AA in

isolated rat adrenal cells [41]. It has been shown that AA is converted to DHA due to the action of ACTH in the adrenal cortex of rats [42]. Therefore, there is a possibility that histamine released from degranulated mast cells and ACTH secreted via activation of the hypothalamic-pituitary-adrenal axis contribute to the occurrence of oxidative stress in the adrenal of C48/80-treated rats.

We have reported that oxidative stress occurs in the gastric mucosa of rats treated once with C48/80 (0.75 mg/kg) through an ischemia-reperfusion-like change in gastric mucosal blood flow due to degranulation of connective tissue mast cells followed by decreases in AA and α-tocopherol contents and the activity of Se-glutathione peroxidase, an enzyme to metabolize hydrogen peroxide and lipid hydroperoxides and increases in infiltrated neutrophils and the activity of xanthine oxidase, an enzyme to generate ROS, in the tissue [20,29,33,43]. It is known that C48/80 treatment causes a significant increase in perfusion medium flow rate through mast cell degranulation in the isolated rat adrenal gland [44]. Therefore, such a change in adrenal blood flow, in addition to a rapid depletion of adrenal AA, due to mast cell degranulation may contribute to the occurrence of oxidative stress in the adrenal gland of rats treated once with C48/80.

In conclusion, the results obtained in the present study indicate that a single C48/80 treatment causes oxidative stress through an enhancement of lipid peroxidation associated with the decreases in AA, NPSH and α-tocopherol contents in the adrenal gland of rats through mast cell degranulation. However, further investigation is needed to clarify the exact mechanism by which oxidative stress is caused in the adrenal gland of rats treated once with C48/80.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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This paper was first published online on Early Online on 29 October 2009.

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