Suppressive effects of dietary curcumin on the increased activity of renal ornithine decarboxylase in mice treated with a renal carcinogen, ferric nitrilotriacetate

Yasumasa Okazaki, Mohammad Iqbal, Shigeru Okada*

Department of Pathological Research, Faculty of Medicine, Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-Cho, Okayama 700-8558, Japan

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Abstract

Curcumin, a natural, biologically active compound extracted from rhizomes of Curcuma species, has been shown to act as a biological response modifier in various disorders. We have reported previously that the dietary supplementation of curcumin enhances the activities of antioxidant and phase II metabolizing enzymes in mice (M. Iqbal, S.D. Sharma, Y. Okazaki, M. Fujisawa, S. Okada, Dietary supplementation of curcumin enhances antioxidant and phase II metabolizing enzymes in ddY mice: possible role in protection against chemical carcinogenesis and toxicity, Pharmacol and Toxicol. 92 (2003) 33-38.) and inhibits ferric nitrilotriacetate (Fe-NTA) induced oxidative injury of lipids and DNA in vitro (M. Iqbal, Y. Okazaki, S. Okada, In vitro curcumin modulates Ferric Nitrilotriacetate (Fe-NTA) and hydrogen peroxide (H₂O₂)-induced peroxidation of microsomal membrane lipids and DNA damage, Teratogenesis Carcinogenesis and Mutagenesis Supplement 23 (2003) 151-160.). In our present study, Fe-NTA, a known complete renal carcinogen, which generate ROS in vivo, was given intraperitoneally to mice and curcumin was tested for its ability to inhibits oxidative stress and the activity of ornithine decarboxylase (ODC) as well as histopathological changes in the kidney. Substantial changes in glutathione, antioxidant enzymes as well as changes in phase II metabolizing enzymes were observed in the kidney at 12 h after treatment with Fe-NTA (9.0 mg Fe/kg body weight). Effect of oxidative stress induced by Fe-NTA were also demonstrated by the increase in lipid peroxidation as monitored by formation of thiobarbituric acid-reactive substances and 4-hydroxy-2-nonenal (HNE)-modified proteins in kidney. Likewise, the level of protein carbonyl contents, an indicator of protein oxidation was also increased after Fe-NTA administration. However, the changes in these parameters were restored to normal in curcumin-pretreated mice. The ODC activity in the kidney was significantly increased by Fe-NTA, while the increased ODC activity induced by Fe-NTA was normalized in curcumin-pretreated mice. In addition, curcumin pretreatment almost completely prevented kidney biomolecules from oxidative damage and protected the tissue against observed histopathological alterations.

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Keywords: Ferric nitrilotriacetate; Antioxidant enzyme; Ornithine decarboxylase; Oxidative stress; Renal tubular necrosis; Lipid peroxidation; Protein carbonyl; Curcumin; 4-hydroxy-2-nonenal-modified protein

1. Introduction

Curcumin (diferuloylmethane), a major yellow pigment of turmeric obtained from the powdered rhizomes of the plant Curcuma longa Linn.; is commonly used as a coloring agent in foods, drugs and cosmetics [1] possess anti-inflammatory and antioxidant properties [2,3]. Curcumin blocks tumor initiation induced by benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene [4], and suppresses phorbol ester-induced tumor promotion [5]. In vivo, curcumin suppresses carcinogenesis of skin [4,5], the stomach [6], the colon [7], the breast [8], and the liver [9] in mice. Additionally, curcumin exhibits anti-metastatic activity [10]. A wide range of biological, pharmacological and clinical potentials of curcumin have been investigated so far. How curcumin exhibits its anticarcinogenic and anti-metastatic effects is not fully understood, but its ability to inhibit the
growth of endothelial cells [11], suppress angiogenesis in vivo [12], abrogate FGF2-induced angiogenic response and matrix metalloprotease-9 expression [13], block expression of adhesion molecule [14], and cyclooxygenase-2 [15] may play important roles. In vitro curcumin was found to induce apoptosis of a wide variety of tumor cells [16] and inhibits ferric nitritotriacetate (Fe-NTA)-induced oxidative injury of lipids and DNA [17].

Most of the toxicants [18,19] including Fe-NTA, which behave as tumor promoters acts through the generation of free radicals, induction of ornithine decarboxylase (ODC) activity, a rate-limiting enzyme in polyamine biosynthesis, and by enhancing the rate of DNA synthesis with simultaneous decrease in antioxidant defenses [20–24]. Therefore, oxidative stress and ODC are widely used as biomarkers of tumor promotion [20–24]. Since curcumin acts as a promising biological response modifier, antioxidant, antimutagenic and/or anticarcinogenic agent [1–17]. However, the precise mechanisms by which curcumin exerts its beneficial effects remain to be elucidated and supports the need for further study. In the view of above observations, it was thought to study the mechanism by which curcumin suppresses early events of tumorigenesis in a mice model induced by Fe-NTA. Our results suggest that dietary curcumin suppresses Fe-NTA induced renal ODC activity in mice.

2. Materials and methods

2.1. Chemicals

Tris–HCl, thiobarbituric acid, 2,4-dinitrophenylhydrazine, guanidine-HCl, oxidized and reduced glutathione, nicotinamide adenine dinucleotide phosphate reduced, 2-mercaptopethanol, dithiothreitol, phenyl methylsulfonyl fluoride, pyridoxal-5-phosphate, glucose-6-phosphate, 1-chloro-2,4-dinitrobenzene, glutathione reductase, 5,5'-dithio-bis-2-nitrobenzoic acid, bovine serum albumin, hydrogen peroxide, tween 80, brig 35, methoxyethanol, ethanolamine, ethyl acetate, sodium bicarbonate, trichloroacetic acid, curcumin, and nitritotriacetic acid disodium salt were purchased from either Sigma Chemical Company, St. Louis, MO, USA or Aldrich, USA. [14C]Ornithine (specific activity 56 m Ci/mmol) was purchased from Amersham Corp., UK. All other chemicals/reagents were of highest quality available from Wako Pure Chemical Industries Ltd., Osaka, Japan.

2.2. Antibody

Monoclonal antibody, HNEJ-2, specific for 4-hydroxy-2-nonenal (HNE)-modified protein adducts [25] was used for immunohistochemical studies and kindly provided by Dr. S. Toyokuni (Department of Pathology and Biology of Diseases, Graduate School of Medicine, Kyoto University, Japan).

2.3. Preparation and injection of Fe-NTA solution

A solution of Fe-NTA was prepared by the method of Awai et al. [26]. Briefly, ferric nitrate and NTA was dissolved in double-distilled water. The respective solutions were mixed to achieve a molar ratio of 1:3 of Fe-NTA. The pH was adjusted to 7.4 with sodium bicarbonate with constant stirring. All solutions were prepared fresh immediately before its use. Fe-NTA was intraperitoneally injected into the animals.

2.4. Animals and treatment

Animal experiments were approved by the animal care committee of Okayama University Medical School, care and handling of the animals were in accordance with National Institutes of Health guidelines. Male ddY mice (4–6 weeks old) weighing 20–30 g obtained from Shizuoka Laboratory Centre (Shizuoka, Japan) were used. They were housed in a temperature-controlled (25 °C) room with alternating 12-h/12-h light/dark cycles, and were allowed to acclimatize for 1 week before study and had free access to standard laboratory chow and water ad libitum. The basic diet without curcumin supplement is referred to as the control diet and that supplemented with curcumin is referred to as the curcumin diet.

For studying, the effect of dietary curcumin on Fe-NTA-mediated renal ODC activity, oxidative stress, and histopathological studies, 20 male mice were divided randomly into five groups consisting of four animals in each group. Group I animals received normal diet and served as negative control. Group II animals received curcumin (1.0% curcumin diet, higher dose for 4 weeks) and served as control. Groups IV and V animals received two different concentrations of curcumin (0.5% and 1.0% curcumin diet for 4 weeks). Group III animals received normal diet. After 4 weeks of the dietary supplementation of curcumin, animals of groups III, IV and V received an i.p. injection of Fe-NTA (9.0 mg Fe/kg body weight). All the animals were killed at 12 h after the treatment with Fe-NTA or saline by decapitation and both kidneys of each animal were removed immediately. One kidney of each animal was homogenized in 0.1 M phosphate buffer, pH 7.4 containing 1.15% KCl for the preparation of post-mitochondrial supernatant (PMS) and cytosol as described previously [20,21,24,27]. The other kidney of each animal was fixed in 10% buffered formalin for 24 h and then embedded in paraffin, sectioned at 3.5 µm, mounted on glass slide either for hematoxylin and eosin staining/or for immunohistochemical studies. The selection of dose regime of curcumin and Fe-NTA were based on previous published data [4–9,20–24].

2.5. Measurement of GSH (reduced)

Reduced glutathione in kidney was measured by the method of Jollow et al. [28], as described previously by Iqbal et al. [29]. An aliquot of 2.0 ml of renal PMS (10% w/v) was
precipitated with 2.0 ml of sulfosalicylic acid (4% w/v). The samples were kept at 4 °C for at least 1 h and then subjected to centrifugation at 2000×g for 20 min. The assay mixture contained 0.1 ml filtered aliquot, 2.7 ml phosphate buffer (0.1 M, pH 7.4) and 0.2 ml DTNB (4 mg/ml of phosphate buffer, 0.1 M, pH 7.4) in a total volume of 3.0 ml. The yellow color developed was read immediately at 412 nm on a spectrophotometer model U-2001.

2.6. Measurement of lipid peroxidation

The assay for lipid peroxidation in renal PMS was done following the method of Wright et al. [30], as described by Iqbal et al. [31], by measuring the rate of production of TBARS (expressed as malondialdehyde equivalents). The reaction was started by addition of 1.0 ml trichloroacetic acid (10% w/v). Following addition of 1.0 ml thiobarbituric acid (0.67% w/v), all the tubes were placed in a boiling water bath for a period of 20 min. At the end, the tubes were shifted to an ice bath and centrifuged at 10,000×g for 20 min at 4 °C using Hitachi cold centrifuge model CR 15B. The amount of malonaldehyde formed in each of the samples was assessed by measuring the optical density of the supernatant at 535 nm using spectrophotometer model U-2001 at 37 °C against a reagent blank. The results were expressed as nmol MDA/g of tissue using a molar extinction coefficient of 1.56×10⁵ M/cm.

2.7. Measurement of ODC activity

ODC activity was measured by utilizing 0.4 ml (1.2 mg protein) of renal 105,000×g supernatant fraction (20% w/v) per assay tube and measuring the release of 14CO₂ from DL-[1-14C]ornithine by the method of O’Brien et al. [32], as described by Iqbal et al. [31]. The kidneys were homogenized in Tris–HCl buffer (pH 7.5, 50 mM) which contained EDTA (0.1 mM), pyridoxal phosphate (0.1 mM), PMSF (0.1 mM), 2-mercaptoethanol (0.1 mM), dithiothreitol (0.1 mM) and tween 80 (0.1% w/v), at 4 °C using polytron homogenizer. In brief, the reaction mixture contained 400 μl enzyme and 0.095 ml co-factor mixture that contained pyridoxal phosphate (0.32 mM), EDTA (0.4 mM), dithiothreitol (0.1 mM), ornithine (0.4 mM), briz 35 (0.02% w/v) and [1-14C]ornithine (0.05 μCi) in a total volume of 0.495 ml. After adding buffer and the co-factor mixture to the blank and another test tube, the tubes were covered immediately with a rubber cork containing 0.2 ml ethanolamine and methoxyethanol mixture in the center well, and were kept in a water bath at 37 °C. After 1 h of incubation, the enzyme activity was arrested by injecting 1.0 ml of citric acid solution (2.0 M) along the sides of glass tubes and incubation was continued for 1 h to ensure complete absorption of 14CO₂. Finally, the central well was transferred to a vial containing 2.0 ml of ethanol and 10 ml toluene-based scintillation fluid was added to it, followed by counting the radioactivity in a liquid scintillation counter. ODC activity was expressed as pmol 14CO₂ released/h/mg protein.

2.8. Measurement of antioxidant and phase II enzyme activity

Glutathione peroxidase activity was measured according to the procedure of Mohandas et al. [27], as described by Iqbal and Athar [33]. Glucose-6-phosphate dehydrogenase activity was measured by the method of Zaheer et al. [34], as described by Ansar et al. [35]. Glutathione reductase activity was measured by the method of Carlbeg and Mannervik [36], as modified by Athar and Iqbal [21]. Catalase activity was determined by the method of Claihorne [37], as described by Iqbal and Athar [33]. Glutathione S-transferase activity was determined by the method of Benson et al. [39], as described by Iqbal et al. [40], using 2,6-dichloroindophenol as an electron acceptor.

2.9. Immunohistochemistry

Immunohistochemical studies were conducted using avidin–biotin complex peroxidase method of Hsu et al. [41] as described by Toyokuni et al. [42]. Samples were fixed with 10% phosphate-buffered formalin for overnight and embed in paraffin. After deparaffinization and dehydration through a graded xylene/ethanol series, incubation in 3.0% hydrogen peroxide in 10 mM phosphate-buffered saline (PBS) was applied for the inhibition of endogenous peroxidase. After these procedures, normal rabbit serum (Dako, Glostrup, Denmark; diluted to 1:10) for the inhibition of non-specific binding of secondary antibody, partially purified mouse monoclonal antibody, HNEJ-2 against HNE-modified protein adducts (10 μg/ml) followed by biotinylated rabbit anti-mouse IgG antiserum (Dako; diluted to 1:100) and ABC (Vectastain ABC Kit, Vector Laboratories, Burlingame, CA; diluted to 1:100) were sequentially applied to the sections. Finally, the sections were incubated with liquid 3,3-diaminobenzidine for 3 min (controls using pre-immune mouse serum and PBS instead of antibody against HNE-modified protein adducts showed no or negligible positivity).

2.10. Measurement of protein carbonyl contents

Protein carbonyl contents in kidney and liver were assayed by the method of Levine et al. [43] as described by Iqbal et al. [40]. An aliquot 0.5 ml (10%, w/v) of renal and hepatic 105,000×g cytosolic fractions was treated with an equal volume of 2,4-DNPH (0.1%) in 2N HCl and incubated for 1 h at room temperature. This mixture was treated with 0.5 ml TCA (10%, w/v) and after centrifugation, the precipitate was extracted three times with ethanol/ethyl acetate (1:1, v/v). The protein sample was then dissolved with 2.0 ml of solution containing guanidine hydrochloride (8 M)/EDTA (13 mM)/tris (133 mM) (pH 7.2) and UV absorbance was measured at 365 nm. The results were expressed as nmol of 2,4-DNPH-incorporated/mg protein based on the molar extinction
coefficient of 21.0 mM\(^{-1}\) cm\(^{-1}\). Protein concentration in all samples was determined using a bicinchonic acid protein assay kit (Pierce, Rockford, IL).

2.11. Statistical analysis

The significance between the group was based on Student’s t-test followed by analysis of variance. A P value less than 0.05 was considered as significant difference. All data were expressed as mean±S.E. of four animals.

3. Results

3.1. Suppressive effects of curcumin pretreatment on Fe-NTA-induced renal ODC activity

ODC activity is generally considered to be a biomarker of tumor promotion [44–46]. It was shown that Fe-NTA increased the activity of renal ODC maximally at 12 h after its administration in rat and mice [20,21]. In this study, treatment with Fe-NTA to animals alone, however, resulted in an about 4.4-fold increase in renal ODC activity at 12 h (P<0.05) as shown in Fig. 1. Curcumin pretreatment strongly inhibits Fe-NTA-induced increased renal ODC activity in a dose-dependent manner. At the maximum dose of curcumin (1.0% diet) used in this study, 63% inhibition (P<0.05) in ODC activity was observed (Fig. 1). Similarly, a lower dose of curcumin (0.5% diet), also resulted in a significant inhibition of about 31% (P<0.05) in the ODC activity. Curcumin treatment alone at a higher dose (1.0% diet) had no effect on basal enzyme activity and did not cause any induction of renal ODC activity.

3.2. Suppressive effects of curcumin pretreatment on Fe-NTA-induced renal GSH (reduced)

GSH (reduced) is an important endogenous antioxidant whose nucleophilic and reducing properties play a central role in metabolic pathways, as well as in the antioxidant system of aerobic cells. Enhanced generations of ROS are expected to consume the endogenous tissue antioxidants such as GSH (reduced), thereby depleting its concentration within the tissue. As shown in Fig. 2, compared with saline-treated control, Fe-NTA treatment in animals resulted in a strong depletion of about 63% (P<0.05) of observed renal GSH (reduced). However, curcumin pretreatment resulted in a significant protection of Fe-NTA-induced depletion of GSH (reduced) in a dose-dependent manner. At a dose of 0.5% curcumin diet pretreatment, a 24% inhibition (P<0.05) in GSH (reduced) levels was evident. Similarly, at a higher dose of 1.0% curcumin diet pretreatment, about 56% inhibition in renal GSH (reduced) was observed (P<0.05). There were no obvious differences in renal GSH (reduced) between the mice fed with normal diet and 1.0% curcumin diet (data not shown in Fig. 2).

3.3. Suppressive effects of curcumin pretreatment on Fe-NTA-induced renal lipid peroxidation

One of the hallmarks of oxidative stress is the formation of oxidized macromolecules such as lipid peroxidation.
Therefore, we used lipid peroxidation as a marker of oxidative stress. In our present study, we found that the treatment of animals with Fe-NTA increases the level of renal lipid peroxidation to about 1.8-fold at 12 h when measured in the form of TBARS production ($P<0.05$).

Curcumin diet treatment prior to Fe-NTA administration was found to decrease the level of renal TBARS formation and thus inhibited Fe-NTA-induced renal lipid peroxidation by 1.3- and 1.1-fold at 12 h after Fe-NTA treatment at a dose of 0.5% and 1.0% curcumin diet, respectively, ($P<0.05$) as shown in Table 1. Curcumin was thus able to normalize Fe-NTA-induced renal lipid peroxidation, as monitored by TBARS formation.

### 3.4. Suppressive effects of curcumin pretreatment on Fe-NTA-induced renal histopathological alterations

The presence of renal injuries in the kidney of mice treated with Fe-NTA was revealed by marked histopathological studies as shown in Fig. 3b. The treatment of animals with Fe-NTA induces kidney swelling (kidney \((g)\) relative to body weight \((g)\) for saline control, \(0.39\pm 0.01;\) Fe-NTA, \(0.47\pm 0.01;\) curcumin+Fe-NTA, \(0.40\pm 0.01,\) were, respectively) with obliteration of space in Bowman’s capsule, nuclear pycnosis, presence of necrotic debris and proteinaceous casts in tubular lumens, flattened epithelia, congested blood vessels, prominent nucleoli and ample cytoplasm with increased homogenous eosinophilia were observed. Some degeneration of renal proximal tubules with sloughing of cells into the tubular epithelium was also noted, as shown in Fig. 3b. As indicated by the staining method, curcumin diet pretreat-

![Table 1](#)

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>nmol MDA/g of tissue</th>
<th>Control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline alone</td>
<td>16.0±0.74</td>
<td>100</td>
</tr>
<tr>
<td>Curcumin alone (1.0%)</td>
<td>15.0±0.87</td>
<td>97</td>
</tr>
<tr>
<td>Fe-NTA alone</td>
<td>28.0±0.44*</td>
<td>175</td>
</tr>
<tr>
<td>Curcumin (0.5%)+Fe-NTA</td>
<td>20.0±0.60**</td>
<td>128</td>
</tr>
<tr>
<td>Curcumin (1.0%)+Fe-NTA</td>
<td>17.0±0.47**</td>
<td>109</td>
</tr>
</tbody>
</table>

Each value represents mean±S.E. of four animals. Dose regimen, treatment protocols and other details are described in the text.

* $P<0.05$ vs. saline treatment control group.

** $P<0.05$ vs. Fe-NTA treatment group.

Fig. 3. Suppressive effects of curcumin pretreatment on Fe-NTA-induced renal histopathological changes. Dose regimen, treatment protocols and other details are described in the text. (a) Saline-treated control animals, (b) Fe-NTA-treated animals, (c) 4 weeks of 1.0% curcumin diet pretreatment and Fe-NTA-treated animals. Specimens stained with hematoxylin and eosin, (a, b and c)×40.
ment showed prominent inhibitory effects on kidney swelling, necrotic changes, normal cellular lining and space in Bowman’s capsule (Fig. 3c).

3.5. Suppressive effects of curcumin pretreatment on Fe-NTA-induced alterations in renal antioxidant enzymes

Fe-NTA treatment of animals for 12 h diminished the activities of antioxidant enzymes as shown in Table 2. The observed decrease in the activities for glutathione reductase, glucose-6-phosphate dehydrogenase, catalase and glutathione peroxidase were 49%, 36%, 38% and 50% as compared to saline treated control, respectively ($P<0.05$). However, curcumin pretreatment of mice resulted in the recovery of diminished activities of antioxidant enzymes. The protection provided by curcumin was dose-dependent. At a lower dose of 0.5% curcumin diet treatment, the protection provided by curcumin was dose-dependent. At a lower dose of 0.5% curcumin diet treatment, the protection showed prominent inhibitory effects on kidney swelling, necrotic changes, normal cellular lining and space in Bowman’s capsule (Fig. 3c).

3.6. Suppressive effects of curcumin pretreatment on Fe-NTA-induced alterations in renal phase II-metabolizing enzymes

Treatment of animals with Fe-NTA for 12 h resulted in a strong depletion of phase II-metabolizing enzymes in kidney as shown in Table 3. The observed reduction in enzyme activities for glutathione S-transferase and quinone reductase in kidney following Fe-NTA treatment were 44% and 38% of the saline treated control, respectively ($P<0.05$). In the studies assessing the effect of curcumin pretreatment on Fe-NTA-induced depletion of phase II-metabolizing enzymes, it showed a dose-dependent significant protection (Table 3). In case of glutathione S-transferase, pretreatment of animals with curcumin diet at a concentration of 0.5% and 1.0% showed 32% ($P<0.05$) and 54% ($P<0.05$) protection against Fe-NTA-induced depletion of GST activity in mouse kidney, respectively (Table 3). Similarly, Fe-NTA-induced depletion of renal quinone reductase activity was also recovered by 47% ($P<0.05$) and 86% ($P<0.05$) following pretreatment with 0.5% and 1.0% of curcumin diet, respectively. Similarly, curcumin alone treatment at higher dose (1.0% diet) was without any significant effect on basal renal phase II-metabolizing enzymes activities (data not shown in Table 3).

3.7. Suppressive effects of curcumin pretreatment on Fe-NTA-induced formation of HNE-modified protein adducts in kidney

Since HNE, a major aldehydic product of lipid peroxidation, is believed to be largely responsible for cytopathological effects observed during oxidative stress [42,47–49]. Therefore, we evaluated the effects of curcumin against Fe-NTA-induced formation of HNE-modified protein adducts in kidney. Immunohistochemical examination revealed that after the administration of Fe-NTA, there was an increased formation of HNE-modified protein adducts in the cytoplasm of the renal proximal tubules (Fig. 4b). In contrast, prophylactic treatment of rats with 1.0% curcumin in diet almost completely suppressed the formation of HNE-modified protein adducts in kidney.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Glutathione reductase (nmol NADPH oxidized/min/mg protein)</th>
<th>Glucose-6-phosphate dehydrogenase (nmol NADPH formed/min/mg protein)</th>
<th>Catalase (nmol H$_2$O$_2$ consumed/min/mg protein)</th>
<th>Glutathione peroxidase (nmol NADPH oxidized/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline alone</td>
<td>406.9±8.0</td>
<td>38.9±3.5</td>
<td>839.0±24.6</td>
<td>641.4±29.4</td>
</tr>
<tr>
<td>Fe-NTA alone</td>
<td>210.2±10.6*$b$</td>
<td>25.0±3.4*</td>
<td>526.9±89.7*$b$</td>
<td>324.4±48.8*$b$</td>
</tr>
<tr>
<td>Curcumin (0.5%)+Fe-NTA</td>
<td>251.7±17.8**</td>
<td>30.5±3.7***</td>
<td>656.2±29.7**</td>
<td>487.1±70.5**</td>
</tr>
<tr>
<td>Curcumin (1.0%)+Fe-NTA</td>
<td>340.8±13.3**</td>
<td>37.3±1.4**</td>
<td>793.1±24.2**</td>
<td>570.7±30.7**</td>
</tr>
</tbody>
</table>

Each value represents mean±S.E. of four animals.

Dose regimen, treatment protocols and other details are described in the text.

* $P<0.05$ vs. saline treatment control group.

** $P<0.05$ vs. Fe-NTA treatment group.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Glutathione S-transferase activity (nmol CDNB conjugate formed/min/mg protein)</th>
<th>Quinone reductase activity (nmol 2,6 DCIP reduced/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline alone</td>
<td>457.2±2.0</td>
<td>491.0±51.9</td>
</tr>
<tr>
<td>Fe-NTA alone</td>
<td>257.5±27.4*$*$</td>
<td>305.2±34.7*$*$</td>
</tr>
<tr>
<td>Curcumin (0.5%)+Fe-NTA</td>
<td>320.4±24.2**</td>
<td>396.3±18.0**</td>
</tr>
<tr>
<td>Curcumin (1.0%)+Fe-NTA</td>
<td>368.7±23.9**</td>
<td>469.5±19.58**</td>
</tr>
</tbody>
</table>

Each value represents mean±S.E. of four animals.

Dose regimen, treatment protocols and other details are described in the text.

* $P<0.05$ vs. saline treatment control group.

** $P<0.05$ vs. Fe-NTA treatment group.
Fe-NTA-induced formation of HNE-modified protein adducts in kidney (Fig. 4c).

3.8. Suppressive effects of curcumin pretreatment on Fe-NTA-induced formation of protein carbonyl contents in kidney and liver

Because studies have shown that oxidation of some amino acid residues of proteins such as lysine, arginine and proline leads to the formation of carbonyl derivatives that affect the nature and function of proteins [50]. The presence of carbonyl groups has become a widely accepted measure of oxidative damage of proteins under the conditions of oxidative stress, which react with 2,4-DNPH to form stable hydrazone derivatives [51]. Therefore, we analyzed the effects of curcumin on Fe-NTA-induced formation of protein carbonyl contents in kidney and liver as a measure of oxidative damage. As shown in Table 4, Fe-NTA treatment alone resulted in ~125% induction of protein carbonyl contents in kidney and ~107% in liver as compared with their respective

Table 4

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Renal protein carbonyl contents (nmol of 2,4-DNPH incorporated/mg protein)</th>
<th>Control (%)</th>
<th>Hepatic protein carbonyl contents (nmol of 2,4-DNPH incorporated/mg protein)</th>
<th>Control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline alone</td>
<td>0.36±0.01</td>
<td>100</td>
<td>0.42±0.01</td>
<td>100</td>
</tr>
<tr>
<td>Curcumin alone (1.0%)</td>
<td>0.35±0.02</td>
<td>97</td>
<td>0.42±0.01</td>
<td>100</td>
</tr>
<tr>
<td>Fe-NTA alone</td>
<td>0.45±0.01*</td>
<td>125</td>
<td>0.45±0.02</td>
<td>107</td>
</tr>
<tr>
<td>Curcumin (0.5%)+Fe-NTA</td>
<td>0.40±0.02**</td>
<td>111</td>
<td>0.44±0.01**</td>
<td>104</td>
</tr>
<tr>
<td>Curcumin (1.0%)+Fe-NTA</td>
<td>0.37±0.01**</td>
<td>102</td>
<td>0.42±0.02**</td>
<td>100</td>
</tr>
</tbody>
</table>

Each value represents mean±S.E. of four animals.
Dose regimen, treatment protocols and other details are described in the text.
* P<0.05 vs. saline treatment control group.
** P<0.05 vs. Fe-NTA treatment group.

Fig. 4. Suppressive effects of curcumin pretreatment on Fe-NTA-induced formation of HNE-modified protein adducts in kidney. Dose regimen, treatment protocols and other details are described in the text. (a) Saline-treated control animals, (b) Fe-NTA-treated animals (increased formation of HNE-modified protein adducts), (c) 4 weeks of 1.0% curcumin diet pretreatment and Fe-NTA-treated animals (decreased formation of HNE-modified protein adducts), (a, b and c)×40. a–c: avidin–biotin complex peroxidase method.
saline-treated controls. However, pretreatment of mice with curcumin reduced induction of protein carbonyl contents in both organs as shown in Table 4. Treatment with curcumin alone (1.0% diet) was without any effect on protein carbonyl contents in kidney and liver.

4. Discussion

We have previously reported that treatment with Fe-NTA induced a variety of changes in vivo, i.e. enhanced lipid peroxidation, depletion of enzymatic and non-enzymatic antioxidant molecules, induction of carbonyls protein content, increased prostaglandins F2α, increased expression of HNE-modified proteins, increased ODC activity and DNA synthesis in kidney [20–24,31,40,52]. The results of the present study have demonstrated that curcumin diet pretreatment suppresses the Fe-NTA-mediated cellular oxidative stress, renal ODC induction and alleviates nephrotoxicity. Cell proliferation plays an important role in multistage carcinogenesis and involves multiple genetic alterations [53]. Several lines of evidence have shown that certain naturally occurring substances possess significant antimutagenic properties in the tumor promotion animal model [29,35]. Fe-NTA is a potent renal tumor promoter and acts through the generation of oxidative stress [20,21,24]. Polyamines and polyamine synthetic enzyme activities are associated with cell proliferation [32]. ODC is the rate-limiting enzyme in the biosynthesis of polyamines, which appears to be a prerequisite for cell proliferation, differentiation, and neoplastic transformation [44–46,53]. The induction of ODC has been suggested to play a significant role in tumor promotion [44]. ODC inhibition was shown to be a promising tool for screening inhibitors of tumorigenesis [46,54]. Based on this information, in the present study, we assessed the effect of curcumin pretreatment on Fe-NTA-mediated induction of biochemical response of tumor promotion. The observed suppression in the Fe-NTA-mediated enhancement of ODC activity in kidney with curcumin pretreatment in mice in the present study suggests its ability to act as potent antitumor promoter. Therefore, it is reasonable to believe that curcumin pretreatment in mice inhibited the action of the tumor promoter and/or the enzymatic pathway(s) that regulates the ODC induction. The suppression of Fe-NTA-induced renal ODC activity by curcumin pretreatment is in accordance with previous data, which shows that curcumin pretreatment inhibit TPA-induced cutaneous ODC activity due to their antioxidant activity [5].

Although the sequence of events is not established, it is largely agreed that oxidative stress is an important contributor [20–24,31,33,40,52] in Fe-NTA-induced renal ODC activity. Glutathione as the main component of endogenous non-protein sulphydryl pool participates in scavenging free radicals, reducing peroxide or being conjugated with electrophilic compounds, thus providing the cell with multiple defenses not only against ROS but also against their toxic products [55]. In the present study, administration of a single dose of Fe-NTA decreases the enzymatic and non-enzymatic antioxidant molecules, supporting the notion that depletion of these enzymes is one of the major factors leading to lipid peroxidation and subsequent tissue damage after Fe-NTA treatment. Administration of curcumin ameliorated Fe-NTA-mediated oxidative stress in the kidney of animals, which is manifested by decrease in lipid peroxidation (both in the formation of TBARS and HNE-modified proteins), and reversal of enzymatic and non-enzymatic antioxidant molecules. The protective effect of curcumin was also observed against Fe-NTA-induced protein oxidation as monitored by measuring protein carbonyl contents in kidney and liver and is a clear further indication that this agent imparts its preventive action, at least in part, via its antioxidant property. These findings are consistent with the previous reports [56] and with our earlier observations that several antioxidants prevent Fe-NTA-mediated oxidative stress [17,29,35,57]. Our results also demonstrated that curcumin pretreatment could restore and/or activate quinone reductase activity in Fe-NTA-treated mice and are in good accordance with our previous report [58]. These data not only indicate that curcumin intercepts the Fe-NTA-mediated generation of free radicals but also indicates that these radicals are responsible for stimulating physiological response which enhances antioxidant generation and dwindle antioxidant defenses in the kidney of Fe-NTA-treated animals. In addition, curcumin pretreatment markedly reduced the histopathological changes in mice kidney as evidenced by necrotic changes, normal cellular lining and space in Bowman’s capsule.

Our results, which show inhibition of Fe-NTA-induced renal oxidative stress by curcumin, may have been caused by the antioxidant and free radical scavenging action of curcumin [1,3], and may play a critical role in protecting the cells/tissues against the cytotoxic/genotoxic effects of peroxides and OH generated by Fe-NTA. By scavenging free radicals and inhibiting ODC induction, curcumin may intercept the growth-promoting and mutagenic function of polyamines and arachidonic acid metabolites. Another protective mechanism of curcumin may be due to its antiapoptotic effect on Fe-NTA-induced apoptosis [59]. However, this possibility remained to be confirmed. Further experiments are desired to confirm or reject this hypothesis.

In summary, our data indicate that curcumin suppresses renal ODC induction and alleviate tissue damages induced by Fe-NTA. It is likely that curcumin has the potential to be used as a chemopreventive agent in free radical-mediated disorders.

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