Reduction of the Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Mice Using an Antiulcer Drug, Geranylgeranylacetone

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The protective effect of geranylgeranylacetone (GGA), an antiulcer drug, against the acute toxicity and teratogenicity produced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was examined in C57BL/6J mice. When mice were co-treated, GGA reduced the loss of body weight gain and lethality produced by TCDD but hepatomegaly and thymic atrophy were not improved. Additionally, no protective effect of GGA was observed in the formation of cleft palate and hydrenephrosis in mouse fetuses caused by maternal exposure to TCDD. To clarify the reducing mechanism by GGA, the Hsp70.1 mRNA levels in liver and intestine were analyzed. However, it was difficult to explain the effect of GGA from the induction of Hsp70.1. GGA had also no effect on the induction of hepatic ethoxyresorufin 0-deethylase activity by TCDD. These data suggest that GGA exhibits a protective effect against some forms of dioxin toxicity by a mechanism without involving inhibition of arylhydrocarbon receptor activation.

Key words 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD); geranylgeranylacetone (GGA); reduction; toxicity; wasting syndrome

Dioxins are one of the most widely distributed environmental pollutants. Because of their lipophilicity, stability and resistance to biodegradation, dioxins bioaccumulate in the food chain and are ubiquitously present in human adipose tissue. They are extremely potent in producing a variety of adverse effects in experimental animals and humans based on traditional toxicology studies. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic congener of a large group of dioxins that cause a wide variety of apparently unrelated adverse effects. The adverse effects of TCDD include a number of phenomena such as lethality, wasting syndrome, hepatomegaly, atrophy of the thymus and spleen, tumor promotion, immunosuppression, teratogenicity, and endocrine changes. In addition, it is also known that exposure to TCDD induces transcriptional activation of genes encoding xenobiotic metabolizing enzymes such as cytochrome P450. Dioxins are believed to exert their adverse effects through interaction with the arylhydrocarbon receptor (AhR). In agreement with this, it has been reported that AhR-deficient mice are resistant to TCDD toxicity including hepatomegaly, thymic atrophy and teratogenic response. However, some effects of TCDD have been shown not to require AhR; i.e., immunosuppression, induction of protein kinases and phospholipase C, and effects on plasma membranes and low-density lipoprotein receptor. Therefore, the exact mechanisms governing dioxin toxicity remain to be fully elucidated.

In our previous study, we found that the heat shock protein (HSP) 70 is induced in hepatic cytostol of rats treated with a dioxin, 3,3′,4,4′,5-pentachlorobiphenyl (IUPAC No. PCB 126). The HSPs are known to be one of the chaperon proteins, and they are an important family of endogenous protective proteins that are increased in response to a variety of stresses such as heat shock, hypoxia, hydrogen peroxide, inflammation, and ischemia. Among the various HSP isoforms, a critical role in the cellular response to acute stress situations has been assigned to the HSP70 family, which is an abundant and highly conserved group of proteins in eukaryotic cells. Therefore, the induction of HSP70 by PCB126 was postulated to be a defensive response to rescue cells from dioxin damage. These studies led us to the hypothesis that the induction of HSP70 in advance is connected with the reduction in toxicity following reinforcement of the defense system against dioxins. However, little is known about the protective role of HSP70 against the adverse effects produced by dioxins.

Geranylgeranylacetone (GGA, see Fig. 1) is a known nontoxic antiulcer drug widely used in clinical situations. This drug has a wide margin of safety and there is little information about any adverse drug actions. The antiulceric mechanism of GGA is believed to be due to the fact that this drug stimulates the synthesis and secretion of mucin, thereby preventing degradation of gastric mucus exposed to several types of stress insults. Also, the gastric mucosa, liver, small intestine and heart have been shown to be target organs for GGA cytoprotection. However, the mechanism of the cytoprotective effect against the variety of insults by endogenous and exogenous agents is a complex one and appears to be multicomponential. Recently, GGA was shown to activate heat shock factor and induce the expression of HSPs in cultured rat gastric mucosa and it has been argued that this may partly explain the molecular mechanism governing the cytoprotective effect of GGA. Furthermore, it has been reported that the main HSPs induced by a single oral dose of GGA is the HSP70 family but not other HSPs such as HSP60.

Fig. 1. Structure of GGA
and HSP27.\textsuperscript{16} In this study, we examined the effect of GGA on the toxic manifestations of TCDD in order to clarify whether HSP70 plays a defensive role against dioxin toxicity. Though we failed to elucidate this issue, our results show that GGA is able to reduce components of TCDD toxicity such as wasting syndrome and lethality.

MATERIALS AND METHODS

Reagents TCDD (purity $\geq$ 99\% as determined by GC/MS) was obtained from AccuStandard, Inc. (New Haven, CT, U.S.A.). A stock solution of 40 $\mu$g/ml was prepared by dissolving TCDD in acetone and stored at $-20$ \textdegree C until use. GGA was donated by Eisai Co. Ltd. (Tokyo, Japan). The drug was stored at 4 \textdegree C in the dark. Ex Taq\textsuperscript{TM} DNA polymerase was purchased from Takara Bio Inc. (Ohtsu, Japan). All other chemicals were of analytical grade and commercially available.

Animals and Treatments A TCDD stock solution dissolved in acetone was diluted with corn oil. The acetone was then evaporated under nitrogen. As a control, the same volume of acetone was added to corn oil and then the acetone was removed in a similar fashion. GGA was emulsified briefly with 5\% gum arabic and 0.6\% Tween 80. As a control of GGA, the same component without GGA was prepared and emulsified. All prepared solutions were stored in the dark until administration.

Male C57BL/6J mice (4 weeks old) were purchased from CLEA Japan (Tokyo, Japan), and acclimatized for one week prior to treatment. Throughout the experiment, mice were allowed access to food and water \textit{ad libitum}. Mice were randomly assigned to treatment groups by body weight. Ten (Experiment 1) or five (Experiment 2) mice per group were housed in cages. The schedule of administration is shown in Fig. 2a. On day 0, GGA was administrated by gavage at a dose of 200 mg/kg body weight/4 ml emulsified solution. The dose of GGA was expected to be sufficient for HSP70 induction, based on the studies of Hirakawa \textit{et al.}\textsuperscript{17} and Ooie \textit{et al.}\textsuperscript{16} Then, 4 h after GGA treatment, TCDD was given by gavage once at a dose of 100 $\mu$g/kg body weight/6 ml corn oil (TCDD low dose, TL; Experiment 1) or 200 $\mu$g/kg body weight/6 ml corn oil (TCDD high dose, TH; Experiment 2). After the initial treatment on day 0, GGA was administrated once a day at the same dose for 30 d. The same volume of each vehicle (emulsified solution and/or corn oil) was given to mice not receiving TCDD/GGA. During the study, the body weights of all mice were measured. One day after the last administration of GGA, the organ weights of all mice were measured.

In the teratogenicity study, plug-positive female C57BL/6J mice (11 weeks old) were purchased from CLEA Japan, Inc. (Tokyo, Japan). The date on which a vaginal plug was found was defined as Gestation Day (GD) 0. Each dam was housed in different cages, and given food and water \textit{ad libitum}. As schematically shown in Fig. 2b, dams were treated by gavage once a day, following the initial treatment with GGA and TCDD. Fetuses were removed from dams on GD18, and dehydrated in 4\% paraformaldehyde–0.1 m potassium phosphate buffer (pH 7.4). After dehydration, they were fixed using the paraffin method. The palatal structure was evaluated by cutting between the upper and lower jaws. Each kidney was sliced longitudinally and examined under a dissecting microscope after staining with hematoxylin/eosin. To estimate any kidney malformation (expansion of renal pelvis), the area ratio (pelvis/whole kidney) was calculated. This involves measuring the longest horizontal and vertical lengths of the renal pelvis and whole kidney and estimating the areas by multiplying those lengths.

Analysis of Hsp70.1 mRNA Using Semi-quantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Total RNA from each tissue was extracted using a commercial kit, RNeasy\textsuperscript{TM} Midi Kit (QIAGEN, GmbH, Hilden, Germany), according to the manufacturer’s instructions. Reverse transcription was performed using the SUPERSCRIPT\textsuperscript{TM} First-Strand Synthesis System for RT-PCR (GIBCO\textsuperscript{TM} Invitrogen Corp., Carlsbad, CA, U.S.A.) with Oligo (dT)\textsubscript{12–18} primer.

To ensure quantitative amplification in PCR, the amplifications were carried out under different conditions of template content and cycle number. Based on this experiment, PCR conditions that guarantee a dose (template amount)–response relationship were set as follows. The reaction mixture (50 $\mu$l) consisted of 0.5 $\mu$l 40-fold diluted cDNA solution, 1.25 units Takara Ex Taq\textsuperscript{TM} DNA polymerase, 0.2 $\mu$m specific primers for mouse Hsp70.1 (Table 1) and 5 $\mu$l attached 10$\times$ Ex Taq\textsuperscript{TM} buffer. The reaction was carried out under the following conditions: 3 min at 95 \textdegree C–(1 min at 95 \textdegree C–1 min at 64 \textdegree C–1 min at 72 \textdegree C)$\times$30 cycles – 20 min at 72 \textdegree C–hold at 4 \textdegree C. $\beta$-Actin cDNA, a standard for normalization, was also amplified using the specific primers (Table 1).

PCR products were separated by agarose gel (2\%) electrophoresis and stained with ethidium bromide. The band intensity of PCR products was calculated using NIH image software (version 1.52, Wayne Rasband, Bethesda, MD, U.S.A.).

Other Methods The protein content of microsomes was determined by the method of Lowry \textit{et al.}\textsuperscript{19} with BSA as a standard. The ethoxyresorufin O-deethylase (EROD) activity was measured by the method of Burke and Mayer.\textsuperscript{20} Statisti-
mental significance was calculated by Scheffe’s F-test.

RESULTS

The Effect of GGA on the Acute Toxicity Produced by TCDD To study whether GGA reduces wasting syndrome by TCDD, mice were treated with GGA and a low (100 μg/kg, TL) or high (200 μg/kg, TH) dose of TCDD. The changes in body weight gain of the TL- and GGA+TL-treated groups are shown in Fig. 3 (Experiment 1). Significant loss of body weight gain was found to occur in the TL group at day 8, and thereafter a marked suppression of body weight gain was seen in the mice and co-treatment with GGA apparently prevented TCDD-induced body weight loss, although the effect was incomplete. During experimental days, no reduced feed intake was observed in the TL- or GGA+TL-treated group in comparison with the control. The diet consumption in the control, TL, GGA and GGA+TL groups was 2.71±0.14, 2.87±0.16, 2.53±0.31 and 2.81±0.30 g/d/mice, respectively. In Experiment 1, 1 out of 10 mice died at day 29 of the experiment in the TL group, while no mice died in GGA+TL group. The mortality following TH treatment was also reduced by co-treatment with GGA (Fig. 3, Experiment 2). While all five mice died during the 27 d of the experiment in the TH group, 2 out of 5 mice survived in the GGA+TH group. In Experiment 2, GGA showed no significant effect on the TCDD-induced reduction in body weight.

The effect of GGA co-treatment on organ weight change induced by TCDD is shown in Table 2. Although GGA rescued mice from TCDD-induced wasting syndrome as described above, both hepatomegaly and thymic atrophy were observed not only in TL-treated mice but also in TL+GGA-treated mice. The magnitude of change in organ weights was almost similar in the two groups. In Experiment 2, the GGA+TH group also showed a marked increase in liver weight and atrophy of the thymus. Mice in TH group exhibited the same trend including a marked hepatomegaly and atrophy of the thymus when they died (data not shown in Table 2).

The Effect of GGA on Teratogenicity Produced by TCDD We next studied the effect of GGA on the teratogenicity of TCDD. The results are shown in Table 3. In all fetuses from control and GGA-treated dams, no notable teratogenic index like a cleft palate was detected. In contrast, the incidence of hyperplasia of the renal pelvis was more than 40% despite the control and GGA-treated mice. However, the symptoms seemed to be minor because the affected area of renal pelvis in both groups was approximately 1% of the whole kidney. On the other hand, these teratogenic indexes were detected in all fetuses from TCDD- and GGA+TCDD-treated dams. No significant differences were observed in the frequency and severity of cleft palate and

Table 1. Sequences of Primers

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsp70.1</td>
<td>5'-TAATGTTGGGAGCAGCACTGT-3'</td>
<td>325</td>
</tr>
<tr>
<td>Sense</td>
<td>5'-AGGGTGCCAGTGTAGACATGTA-3'</td>
<td></td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-CACCATGTACCCAGGCATCGC-3'</td>
<td>194</td>
</tr>
<tr>
<td>β-Actin</td>
<td>5'-AGGGGCCGAGCTCATGATC-3'</td>
<td></td>
</tr>
<tr>
<td>Sense</td>
<td>5'-GAGGGGCAGCGTCTCATGATC-3'</td>
<td></td>
</tr>
</tbody>
</table>

The values represent the mean±S.E. except for the GGA+TH treatment group in Experiment 2. Although the number of samples is shown in parentheses, the value of the thymus of the TL-treated mice in experiment 1 was calculated from 6 samples because three thymuses weighed less than 0.001 g. Significantly different from control; *p<0.05; **p<0.01; ***p<0.001. N.D., no data.

Table 2. Changes in Organ Weights of C57BL/6J Mice Following Treatment with TCDD and GGA

|------------|--------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Experiment 1
| Control (10) | 5.00±0.05                | 0.19±0.01                 | 0.27±0.01                 | 0.79±0.01                 | 1.21±0.01                 |
| GGA (10)    | 4.93±0.07                | 0.18±0.01                 | 0.25±0.01                 | 0.77±0.02                 | 1.18±0.02                 |
| TL (6 or 9) | 7.73±0.30***             | 0.05±0.01***              | 0.24±0.02                 | 0.88±0.03*                | 1.31±0.01                 |
| GGA+TL (10) | 7.55±0.18***             | 0.06±0.01***              | 0.30±0.03                 | 0.75±0.02                 | 1.38±0.05**               |
| Experiment 2
| Control (5)  | 5.18±0.10                | 0.17±0.01                 | 0.27±0.01                 | 0.82±0.03                 | 1.34±0.03                 |
| GGA (5)     | 5.26±0.16                | 0.15±0.01                 | 0.27±0.01                 | 0.81±0.02                 | 1.34±0.03                 |
| TH (0)      | N.D.                     | N.D.                      | N.D.                      | N.D.                      | N.D.                      |
| GGA+TH (2)  | 9.25, 6.34               | 0.03, 0.04                | 0.17, 0.26                | 0.85, 0.77                | 1.50, 1.30                |

Number of fetuses examined is shown in parentheses. The areas of renal pelvis were calculated by dividing the area of pelvis by the area of whole kidney. Significantly different from control; *p<0.05; **p<0.01; ***p<0.001. N.D., no data.

Table 3. Incidence of Cleft Palate and Hydronephrosis of Fetuses from Dams Following Treatment with GGA and TCDD

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cleft palate (%)</th>
<th>Hydroplasia (%)</th>
<th>Mean area of renal pelvis (% of the area of kidney±S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (28)</td>
<td>0</td>
<td>46</td>
<td>0.9±0.3</td>
</tr>
<tr>
<td>GGA (30)</td>
<td>0</td>
<td>43</td>
<td>1.1±0.3</td>
</tr>
<tr>
<td>TCDD (33)</td>
<td>100</td>
<td>100</td>
<td>16.0±0.8***</td>
</tr>
<tr>
<td>GGA+TCDD (37)</td>
<td>100</td>
<td>100</td>
<td>18.0±0.7***</td>
</tr>
</tbody>
</table>

Number of fetuses examined is shown in parentheses. The areas of renal pelvis were calculated by dividing the area of pelvis by the area of whole kidney. Significantly different from TCDD treatment group; †p<0.05.
hyperplasia of the renal pelvis between TCDD- and GGA+TCDD-treated groups.

Effects of GGA on Cellular Level of Hsp70 and AhR-Mediated Gene Expression

To obtain information about the mechanism for the action of GGA on TCDD toxicity, we compared the Hsp70 mRNA level between the groups in Experiment 1 (Fig. 3), using semi-quantitative RT-PCR. Among Hsp70 isoforms, we focused on the Hsp70.1 isoform because of its higher responsiveness toward acute stimuli compared with the other isoforms. The hepatic and intestinal levels of Hsp70.1 mRNA are shown in Figs. 4 and 5, respectively. In liver, TCDD (TL) increased significantly Hsp70.1 mRNA expression (Fig. 4). While GGA alone failed to elevate Hsp70.1, this drug tended to enhance TCDD-induced expression of Hsp70.1 although it was not statistically significant (TCDD vs. TCDD+GGA groups). The increase in the mRNA level in the TL and GGA+TL treatment groups was approximately 1.6- and 1.8-fold greater than the control, respectively (Fig. 4b). No significant differences in the Hsp70.1 mRNA level in the intestine were detected in any of the treatment groups (Fig. 5). The level of Hsp70.1 protein in hepatic and intestinal cytosol was confirmed to have the same pattern as the mRNA expression obtained by immunoblotting (data not shown).

It is conceivable that GGA plays a role by affecting TCDD-mediated activation of AhR-containing pathways. To address this issue, we next measured the hepatic microsomal activity of EROD, which is mediated by Cyp1a1, one of the proteins the expression of which is governed by AhR. The EROD activities were significantly increased by GGA+TL- as well as TL-treatment (Table 4). No significant difference was found between the TL and GGA+TL groups. This observation suggests that GGA does not have any effect on AhR pathways.

Table 4. EROD Activity of Liver Microsomes from C57BL/6J Mice Following Treatment with TCDD and GGA

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Activity (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>100±12</td>
</tr>
<tr>
<td>GGA</td>
<td>5</td>
<td>119±6</td>
</tr>
<tr>
<td>TL</td>
<td>5</td>
<td>6970±387***</td>
</tr>
<tr>
<td>GGA+TL</td>
<td>5</td>
<td>7950±209***</td>
</tr>
</tbody>
</table>

The doses of TCDD and GGA are referred to Experiment 1. The values represent the mean±S.E. of 5 randomly selected mice. The EROD activity of the control was 0.016±0.002 nmol resorufin formed/min/mg protein. Significantly different from control; ***p<0.001.
DISCUSSION

Our results show that the acute toxic effects of TCDD are reduced by GGA. It is widely accepted that dioxins exert their toxicity through the interaction with AhR. However, our results strongly suggest that GGA reduces the toxic effects produced by TCDD without any change in the function of AhR (Table 4). Many studies describing the TCDD effects caused by the AhR-independent pathway (see introduction for examples) together with the data presented here support this view. Our results may support a view that the wasting syndrome produced by dioxin involves a mechanism(s) independent of the AhR-mediated pathways. However, it should be noted that the loss of body weight and lethality produced by dioxins correlate with the affinity of dioxins for AhR.23) As for the mechanism of wasting syndrome produced by TCDD, recent studies showed that the wasting syndrome including hyperlipidemia and body weight loss depend on the activation of protein tyrosine kinase pp60 c-Src because those manifestations by TCDD were reduced in c-Src knockout mice as compared to wild type C57BL/6j mice.25) Furthermore, Vogel et al.23) suggested that the reduction of wasting syndrome in c-Src knockout mice can be attributable to the decreased response in the hepatic expression of CCAAT/enhancer binding protein (C/EBP) α and C/EBPβ mRNAs, both which contribute to the regulation of phosphoenolpyruvate carboxykinase, one of the key candidates participating in wasting syndrome. On the other hand, it has been reported that TCDD-induced anorexia and body weight loss may result from the alteration of pituitary function—which is regulated by AhR. In support of this, AhR ligand increases the levels of gonadotropine II and adrenocorticotropic hormone produced in pituitary.24–26) From reports above mentioned, one possibility with respect to the reducing mechanism of GGA is raised that GGA exerts its protective effects by affecting a target(s) located downstream of the AhR pathways.

As has been reported already, the minimum dose of TCDD required to produce toxicity differs depending on the particular toxic effect; for example, in C57BL mice, loss of body weight gain, over 95 µg/kg; LD50, 114 µg/kg; hepatitis and thymic atrophy, 1 µg/kg; and cleft palate and kidney malformation, 3 µg/kg.27) Therefore, each toxic sign has a different threshold. In our study, the two doses of TCDD (TL, 100 µg/kg; TH, 200 µg/kg) were used to attain the reproductive loss of body weight gain and lethality by TCDD in C57BL/6j mice. It is noteworthy that GGA showed the reducible effect against toxicities produced by the doses of TCDD (Fig. 3). Unfortunately, we were unable to clarify the mechanisms governing the effect of GGA. However, the data mentioned above suggest that GGA elevates the threshold determining the occurrence of body weight loss and lethality produced by TCDD. Since the dose of TCDD (>100 µg/kg) employed in this study was much greater than the threshold for organ weight change, GGA was assumed to be unable to enhance the threshold to the level greater than the dose. The same may be true for fetal hydrenephrosis; the TCDD dose (10 µg/kg) employed in this study was too high and GGA might not elevate the threshold to the level over the doses. The protective effect of GGA on body weight/lethality may be due to increased elimination of TCDD from the body. However, this possibility is unlikely, because the hepatic content of TCDD in mice treated with TCDD alone and with TCDD + GGA was comparable (data not shown). Placental transfer of GGA to the fetus has been confirmed by other workers.29) This observation does not support the possibility that any absence of a GGA effect on teratogenicity is due to limited accumulation of this drug in the fetus.

In this study, we proposed the hypothesis that the induction of Hsp70 may cause a reduction in dioxin toxicity. The Hsp70 family plays a defensive role against acute stimulation in eukaryotic cells and, in the mouse, this family contains at least seven proteins, including heat shock cognate protein (Hsc70), glucose-regulated proteins (Grp) 75 and Grp78, spermatocyte-specific Hsp70.2 and testis specific Hsc70. In addition, the exposure of cells to stress activates a survival response via induction of the intronless Hsp70.1 and Hsp70.3 genes.30) Among these, since Hsp70.1/70.3 are rapidly expressed in cells in response to acute stress stimuli, these chaperons are considered to be important as quenchers of acute damage to cells. Our previous study also identified that the Hsp70.1 mRNA was induced preferentially in the liver of rat treated with PCB126 (data not shown). From these reasons, we focused on the alteration of Hsp70.1 and examined whether this protein is able to modify TCDD toxicity. Since GGA failed to significantly elevate Hsp70.1 over the level attained with TCDD alone, a protective effect of GGA on an acute toxic effect would not be attributable to altered hepatic and intestinal Hsp70.1 expression. If GGA exerts a protective effect through Hsp70 induction, it would occur in organs other than liver and intestine. In this study, the level of Hsp70.1 in liver and intestine was analyzed after 30 d of TCDD treatment. One possibility is that GGA causes a change in the Hsp70.1 level at an earlier period following co-treatment with TCDD. It is generally believed that the ability to resist stress is increased in proportion to the expression level of HSPs.31) However, excessive expression of Hsp70 does not seem to always result in a defensive effect against stress. For example, Liu and Stocco32) reported that excess Hsp70 decreases steroid bio-synthesis in mouse Leydig tumor MA-10 cells by reducing the expression of steroidogenic acute regulatory protein, an important mechanism for cholesterol transportation. It is, therefore, assumed that an appropriate expression level is required for the protective effect of HSP70. Thus, the possibility that an enhanced increase in Hsp70.1 by GGA over the TCDD-mediated increase contributes to the improvement in acute toxicity could be raised, although the effect was minor. GGA is also reported to induce thioredoxin in primarily cultured rat hepatocytes, and to enhance transcriptional activity partly via an increase in thioredoxin.33) Since thioredoxin has the ability to reduce oxidized thiol and is one of the oxidative stress quenchers,34) another possibility is that GGA combats TCDD toxicity by its effect as an oxidative stress quencher inducer. This possibility remains to be clarified.

In conclusion, we have shown that GGA reduces adverse effects such as the loss of body weight gain and lethality produced by TCDD. Although the mechanism is not yet fully understood, GGA seems to exhibit the above effect without affecting AhR activation by TCDD. Though this study could not detect any target explaining to the protective effect by GGA, our results will provide interesting new sights into the development of therapeutic and preventive approach for
dioxin toxicity.

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