Attenuation of 2,3,7,8-Tetrachlorodibenzo-p-dioxin Toxicity by Resveratrol: A Comparative Study with Different Routes of Administration

Takumi Ishida, a Tomoki Takeda, a Takayuki Koga, a Masahiro Yahata, a Ayako Ike, a Chihiro Kuramoto, a Junko Takeo, a Isamu Hashiguchi, b Akifumi Akamine, b Yuji Ishii, * a and Hideyuki Yamada a

a Graduate School of Pharmaceutical Sciences, Kyushu University; and b Graduate School of Dental Science, Kyushu University; 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan.

Received January 5, 2009; accepted March 2, 2009; published online March 5, 2009

The activation of aryl hydrocarbon receptor with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is known to be antagonized by co-treatment with resveratrol. However, such a protective effect has been suggested from studies using subcutaneous injection of this polyphenol. To evaluate the practical usefulness of resveratrol, this study examined the protective effect of oral resveratrol on the sub-acute toxic effects of TCDD in C57BL/6J mice. A TCDD-induced wasting syndrome was not alleviated by treating mice for 28 d with oral resveratrol. However, subcutaneous injection of resveratrol for 5 d significantly improved the symptoms. Neither oral nor subcutaneous administration of resveratrol alleviated TCDD-induced hepatomegaly and thymic atrophy. Steatosis produced by TCDD was markedly counteracted by co-treatment with oral resveratrol, whereas resveratrol injected subcutaneously had no effect. The reason for the lack of protective effect via the latter dosing route was assumed to be due to the minor accumulation of hepatic lipids 5 d after TCDD treatment. To clarify the mechanisms, the activity of ethoxyresorufin O-deethylase and the content of thiobarbituric acid-reactive substances in the liver were measured. Both indices increased by TCDD treatment were significantly suppressed by subcutaneous injection of resveratrol. In contrast, oral resveratrol failed to rescue them. In agreement with the greater protective effects of subcutaneously-injected resveratrol, pharmacokinetic studies indicated that the area under the curve extrapolated to infinity (AUC ∞) was 8.2-times greater following subcutaneous injection compared with oral administration. These data suggest that 1) oral resveratrol is an attractive candidate as an agent capable of combating dioxin toxicity and 2) increasing the bioavailability of this polyphenol enhances its protective effect.

Key words 2,3,7,8-tetrachlorodibenzo-p-dioxin; resveratrol; protective effect; wasting syndrome; hepatic steatosis; bioavailability

Dioxins and related halogenated aryl hydrocarbons are widespread, persistent and highly toxic environmental pollutants. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic congener and has been widely investigated as a prototype of this class of chemicals. It is widely accepted that dioxin exerts its toxic effects by activating aryl hydrocarbon receptor (AhR) 1—3. In addition, it has also been suggested that oxidative stress produced by dioxins plays a role in the manifestation of toxic symptoms (see review of Ishida et al. 4). In spite of these findings, the mechanisms of dioxin toxicity following AhR activation and/or the generation of oxidative stress have not been fully elucidated, mainly because of their complexity: for example, dioxins cause different sorts of toxic effects in tissue-, sex-, age-, and species-specific manners. 5—8

Resveratrol (trans-3,4’,5-trihydroxystilbene) is a phenolic ingredient of red wine. An epidemiological study has suggested that the consumption of red wine reduces the incidence of mortality and morbidity due to coronary heart diseases. 9 Resveratrol is assumed to be one of the key ingredients having a protective effect mentioned above. Since the above finding, this polyphenol has acquired considerable attention because of its beneficial roles on health as a functional component of foods. For example, resveratrol is found in grapes, peanuts, and cranberries, 10 and it has been reported to have cancer chemopreventive, 11 antioxidant, 12,13 antiplatelet, 14 antifungal, 15 and phytoestrogenic 16 effects. In addition, it has been demonstrated that the activation of AhR with TCDD is antagonized by co-treatment with resveratrol in an in vitro study. 17,18 Regarding the in vivo effect, Casper et al. 17 have demonstrated that resveratrol has the ability to attenuate the induction of cytochrome P450 (CYP) 1A1 in the lung and kidney of rats treated with a mixture of benzo[a]pyrene/7,12-dimethylbenz[a]anthracene. Therefore, it is reasonable to expect that resveratrol has a prophylactic effect against TCDD-induced toxicity. However, the effect of resveratrol reported so far was focused only on the effect on the induction of CYP1A1 produced by TCDD, and this was examined after subcutaneous injection of the polyphenol. Thus, it remains to be clarified whether the adverse effects of dioxins are actually attenuated by resveratrol given via more practical route of administration. To address this issue, we examined the effects of oral resveratrol on the toxic effects of TCDD in male C57BL/6J mice. In addition, we compared the effects of subcutaneously-injected resveratrol with those given orally to assess the relationship between protective potency and bioavailability.

MATERIALS AND METHODS

Reagents TCDD (purity ≥99%) was obtained from AccuStandard, Inc. (New Heaven, CT, U.S.A.). Resveratrol (purity ≥99%), nicotinamide adenine dinucleotide phosphate (NADP), 7-ethoxyresorufin and thiobarbituric acid were purchased from Sigma (St. Louis, MO, U.S.A.). All other reagents were of analytical grade and commercially avail-

* To whom correspondence should be addressed. e-mail: ishii@phar.kyushu-u.ac.jp © 2009 Pharmaceutical Society of Japan
able.

**Animals and Treatments** All experiments were pre-approved by the Institutional Animal Care and Experiment Committee of Kyushu University. TCDD stock solution was prepared by dissolving TCDD in acetone (40 \( \mu \text{g/ml} \)) and this was stored at \(-20^\circ\text{C}\) until use. A stock solution was diluted with corn oil to give a concentration of 20 \( \mu\text{g/ml} \), and the acetone was evaporated under nitrogen. All animals were maintained under controlled temperature (22±5\(^\circ\text{C}\)), lighting (12 h light and night) and humidity (50±15\%) conditions, and they were allowed free access to water and CELA radiation-sterilized rodent diet (CE-2; CLEA Japan, Inc., Tokyo, Japan), during the course of the study. In the oral administration study, resveratrol was suspended in 0.5% methylcellulose/0.2% Tween 80 (4 mg/ml). The solution was kept in the dark until use, and then vortex-mixed immediately before administration. Male C57BL/6J mice (4 weeks old, CLEA Japan, Inc., Tokyo, Japan) were randomly divided into 4 groups, and given resveratrol by gavage (20 mg/kg body weight/5 ml) or vehicle. The dose of resveratrol was chosen so that it had no effect on the hepatic activity of ethoxyre sorufin O-deethylase (EROD) and the content of thiobarbituric acid-reactive substances (TBARS), on the basis of our preliminary experiment (data not shown). Mice were then given TCDD orally (100 \( \mu\text{g/kg body weight} \)) or vehicle 90 min after resveratrol treatment. After the treatment described above on day 0, the same dose of resveratrol was administrated once a day for the next 28 d. During the experiment, the body weights of mice were measured before administration. Thirty minutes after the last treatment, organs from all mice were removed and weighed. Hepatic homogenates (10%) were prepared with 1.15% KCl, and aliquots were centrifuged at 9000 \( \times g \) for 20 min to prepare supernatants as a source of CYP. All prepared samples were stored at \(-80^\circ\text{C}\) until use.

In a study involving subcutaneous injection, resveratrol was dissolved in propylene glycol to give a concentration of 45 mg/ml. Male C57BL/6J mice (4 weeks old) received injections of resveratrol (225 mg/kg body weight/5 ml) or vehicle into their backs. Mice were then given TCDD orally (100 \( \mu\text{g/kg body weight} \)) or vehicle 90 min after resveratrol treatment. After the treatment described above on day 0, resveratrol was administrated once a day at the same dose for the next 5 d. All other conditions were the same as those described in the oral administration study.

**Determination of Resveratrol in Plasma by HPLC** The determination of plasma concentrations of resveratrol was carried out by the method of He et al.\(^{19}\) with minor modifications. The blood from a resveratrol-treated mouse was collected in a tube containing 0.1% (w/v) di-sodium ethylenediaminetetraacetic acid (EDTA) dihydrate. After centrifugation at 2000 \( \times g \) and 4\(^\circ\text{C}\) for 15 min, 50 \( \mu\text{l} \) of the plasma was transferred to a new tube, and then 10 \( \mu\text{l} \) 7-ethoxycoumarin (10 \( \mu\text{g/ml} \)) was added as the internal standard. Resveratrol was extracted three times with 100 \( \mu\text{l} \) ethyl acetate, by vortex-mixing and centrifugation at 15000 \( \times g \) and 4\(^\circ\text{C}\) for 1 min. The organic solvent of the pooled extract was evaporated under nitrogen, the residue was dissolved in 40 \( \mu\text{l} \) methanol, and a portion (20 \( \mu\text{l} \)) was subjected to HPLC analysis. The HPLC conditions used were as follows: instruments, a Hitachi L-7100 intelligence pump (Hitachi, Ltd., Tokyo, Japan) equipped with a UV detector (Hitachi model L-2400), an autosampler (Hitachi model L-7200), and a data integrator (Hitachi model D-2500); column, YMC Pak C8 (6 mm×100 mm i.d., 10 \( \mu\text{m} \)) (YMC Co., Ltd., Kyoto, Japan) attached to a Guard-PakTM precolumn module containing Nova-Pack C18 as the Guard-PakTM insert (Waters Corp., Milford, MA, U.S.A.); mobile phase, 50% methanol containing 1% acetic acid; flow rate, 2 ml/min; detection wavelength, 303 nm. Under these conditions, resveratrol and 7-ethoxycoumarin were detected at the retention times of 3.40 and 8.20 min, respectively.

**Histopathological Examination** The lipid accumulation in hepatocytes was assessed by Oil red-O staining.\(^{20}\) Briefly, mouse liver was fixed in 4% paraformaldehyde, embedded in Tissue-Tek® OCT Compound (Sakura Finetechanical Co., Ltd., Tokyo, Japan), and frozen in dry ice–ethanol. The same lobe of each frozen liver was sliced into sections with a thickness of 6 \( \mu\text{m} \), and then the prepared cryosections were mounted on glass slides. The cellular lipid droplets were stained with Oil red-O (0.3% in 60% isopropanol), a dye permeable to lipid droplets, for 15 min at room temperature, and then excess dye was removed by washing with distilled water. Then, the cryosections stained with Oil red-O were counterstained with methyl green, followed by microscopic examination (×10).

**Other Methods** The hepatic EROD activity and TBARS concentration were measured by the methods of Burke and Mayer\(^{21}\) and Ohkawa et al.,\(^{22}\) respectively. The protein concentrations were determined by the method of Lowry et al.,\(^{23}\) with bovine serum albumin as a standard. Statistical significance was calculated by ANOVA with a post hoc test using Fischer’s Protect Least Significant Difference procedure.

**RESULTS**

**Effects of Resveratrol on TCDD-Induced Wasting Syndrome** In an experiment examining the effect of oral resveratrol, TCDD-treated mice showed a loss of body weight gain from day 1 compared with those of control and resveratrol-treated animals (Fig. 1A). Although co-treatment with resveratrol tended to produce recovery from the symptom from day 18, no significant differences were detected. The effect of resveratrol given by subcutaneous injection was evaluated over a period (5 d) shorter than that for oral administration, because further injections were not performed to avoid excess stress to the animals. Also in this trial, a loss of body weight gain was observed in mice treated with TCDD alone (Fig. 1B). Co-treatment with resveratrol injected subcutaneously alleviated the symptom to the control level (Fig. 1B). Table 1 shows the effect of resveratrol on changes in organ weights produced by TCDD. As has been well-established, TCDD caused hepatomegaly and thymic atrophy. Neither oral nor subcutaneous administration of resveratrol improved these effects. These data demonstrate that resveratrol has the ability to reduce TCDD-produced wasting syndrome, although it failed to improve changes in organ weights. The data obtained also suggest that increasing the bioavailability of resveratrol (subcutaneous>oral) enhances the protective effect. The difference in resveratrol bioavailability between oral and subcutaneous administration is described later.

**Improvement of TCDD-Induced Lipid Accumulation**
The accumulation of hepatic triglycerides and cholesterol, which is considered to be the initial manifestation of steatosis, is one of the typical biological responses produced after exposing mammals to dioxins. We then examined the effect of resveratrol on the TCDD-induced lipid accumulation in liver. Similarly to the above reports, marked accumulation of lipid droplets in hepatocytes was observed 28 d after TCDD treatment (Fig. 2C; reddish stain means the accumulation of lipid). The co-treatment with oral resveratrol markedly reduced the lipid droplets (Fig. 2D). Lipid accumulation in hepatocytes was also observed 5 d after TCDD administration (Fig. 2G), although the magnitude of this effect was less than that after 28 d (Fig. 2C). In contrast to oral resveratrol, subcutaneously-administered resveratrol had no apparent effect on lipid accumulation by TCDD (Fig. 2H).

Effects of Resveratrol on AhR Activation and Oxidative Stress Induced by TCDD

To investigate whether resveratrol modifies AhR activation or the occurrence of oxidative stress by TCDD, the effects of resveratrol on TCDD-produced changes in hepatic EROD activity and TBARS concentration were measured. The hepatic EROD activity and TBARS concentration were significantly increased by treating mice with TCDD (Figs. 3, 4). Co-treatment with resveratrol by the subcutaneous route significantly reduced the TCDD effects on both indices, although the magnitude of the decrease was not marked. In contrast, oral administration

Table 1. Effect of Resveratrol Following Oral and Subcutaneous Administration on the Organ Weights of Mice Treated with TCDD

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1 (oral resveratrol)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (5)</td>
<td>5.18±0.31</td>
<td>0.27±0.03</td>
<td>0.18±0.02</td>
</tr>
<tr>
<td>Resveratrol (5)</td>
<td>5.32±0.23</td>
<td>0.24±0.02</td>
<td>0.15±0.02</td>
</tr>
<tr>
<td>TCDD (4)</td>
<td>8.59±0.30***</td>
<td>0.19±0.10*</td>
<td>0.07±0.06***</td>
</tr>
<tr>
<td>Resveratrol+TCDD (5)</td>
<td>8.57±0.88***</td>
<td>0.23±0.04</td>
<td>0.04±0.02***</td>
</tr>
<tr>
<td>Experiment 2 (subcutaneous resveratrol)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (5)</td>
<td>5.90±0.23</td>
<td>0.32±0.02</td>
<td>0.23±0.03</td>
</tr>
<tr>
<td>Resveratrol (5)</td>
<td>6.20±0.09</td>
<td>0.37±0.02</td>
<td>0.21±0.02</td>
</tr>
<tr>
<td>TCDD (4)</td>
<td>7.11±0.13***</td>
<td>0.26±0.03*</td>
<td>0.09±0.02***</td>
</tr>
<tr>
<td>Resveratrol+TCDD (5)</td>
<td>6.78±0.71**</td>
<td>0.29±0.07</td>
<td>0.08±0.02***</td>
</tr>
</tbody>
</table>

Tissue weights were measured after 28 (Experiment 1) and 5 (Experiment 2) d, respectively, following a single administration of TCDD. Resveratrol was administered to mice every day during both experiments (see Materials and Methods for the details). The values represent the mean±S.D. The number of animals is shown in parenthesis, except for the thymus (n=3) in the TCDD-treated mice of Experiment 1. Significantly different from the control: *p<0.05; **p<0.01; ***p<0.001.
failure to attenuate a TCDD-produced increase in EROD activity and TBARS content (Figs. 3, 4). Thus, the results obtained demonstrate that resveratrol injected subcutaneously partially antagonizes AhR activation and oxidative stress by TCDD.

Pharmacokinetic Profiles of Resveratrol Following Oral and Subcutaneous Administration

To clarify the factors contributing to the difference in the effect of oral and subcutaneous resveratrol, we compared the pharmacokinetics profiles of resveratrol between the two routes. The plasma concentration of resveratrol given orally reached a maximum at 5 min after administration, and then it declined rapidly over 2 h (Fig. 5A). In contrast, when resveratrol was injected subcutaneously into mice, the maximum plasma concentration was observed 20 min after treatment (Fig. 5B). Furthermore, the concentration of approximately a tenth of the maximum was maintained until 24 h (Fig. 5B). In agreement with these results, the area under the curve, mean residence time and elimination half-life of resveratrol injected subcutaneously was 93-, 522- and 23-times greater, respectively, compared with the values for oral resveratrol (Table 2). It should be noted that the dose of resveratrol differed between oral and subcutaneous administration. However, since the relative bioavailability (\(\frac{AUC_{\text{subcutaneous}}}{AUC_{\text{oral}}\)} normalized by the difference in dose was 8.2, it is clear that the bioavailability of resveratrol failed to attenuate a TCDD-produced increase in EROD activity and TBARS content (Figs. 3, 4). Thus, the results obtained demonstrate that resveratrol injected subcutaneously partially antagonizes AhR activation and oxidative stress by TCDD.

**Pharmacokinetic Profiles of Resveratrol Following Oral and Subcutaneous Administration**

To clarify the factors contributing to the difference in the effect of oral and subcutaneous resveratrol, we compared the pharmacokinetics profiles of resveratrol between the two routes. The plasma concentration of resveratrol given orally reached a maximum at 5 min after administration, and then it declined rapidly over 2 h (Fig. 5A). In contrast, when resveratrol was injected subcutaneously into mice, the maximum plasma concentration was observed 20 min after treatment (Fig. 5B). Furthermore, the concentration of approximately a tenth of the maximum was maintained until 24 h (Fig. 5B). In agreement with these results, the area under the curve, mean residence time and elimination half-life of resveratrol injected subcutaneously was 93-, 522- and 23-times greater, respectively, compared with the values for oral resveratrol (Table 2). It should be noted that the dose of resveratrol differed between oral and subcutaneous administration. However, since the relative bioavailability \(\frac{AUC_{\text{subcutaneous}}}{AUC_{\text{oral}}\)} normalized by the difference in dose was 8.2, it is clear that the bioavailability of

Table 2. Pharmacokinetic Parameters of Resveratrol Given by Oral Administration and Subcutaneous Injection Obtained with Non-compartmental Analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>p.o.</th>
<th>s.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>20</td>
<td>225</td>
</tr>
<tr>
<td>(AUC_{\text{area}}) (µg·min·mL(^{-1}))</td>
<td>63</td>
<td>5870</td>
</tr>
<tr>
<td>(MRT) (min)</td>
<td>32</td>
<td>16700</td>
</tr>
<tr>
<td>(t_{1/2}) (min)</td>
<td>20</td>
<td>451</td>
</tr>
</tbody>
</table>

\(AUC_{\text{area}}\), area under the curve extrapolated to infinity; \(MRT\), mean residence time; \(t_{1/2}\), elimination half-life. Each represents the optimized value.
resveratrol is improved by dosing \textit{via} the subcutaneous route.

**DISCUSSION**

Although previous studies have demonstrated the antagonistic effect of resveratrol on AhR activation occurred in the \textit{in vivo} as well as \textit{in vitro} conditions,\textsuperscript{17,18} it has been unclear whether this polyphenol by oral administration actually reduces the \textit{in vivo} toxicity of dioxin. Regarding this, Jang \textit{et al.}\textsuperscript{26} have reported recently that the pretreatment of pregnant mice with oral resveratrol significantly reduces the incidence of cleft palate and the severity of renal malformations in the pups caused by \textit{in utero} exposure to TCDD. In addition, above study has showed that oral resveratrol attenuates the wasting syndrome and lipid accumulation in the liver produced by TCDD. However, present study indicated that the protective effect of oral resveratrol is not marked, probably due to its insufficient serum concentration and short persistency. Indeed, it has been reported that resveratrol given orally shows a poor bioavailability due to its high rate of degradation by metabolism.\textsuperscript{27,28} Therefore, changing the administration pathway to the parenteral route is expected to improve the bioavailability. In the present study, we used subcutaneous injection to achieve this. As expected, the results obtained showed that subcutaneously-injected resveratrol has a greater bioavailability and more markedly reduces TCDD toxicity than oral resveratrol. Therefore, it is highly likely that increasing the bioavailability of resveratrol enhances its protective effect against TCDD toxicity.

Wasting syndrome, including a loss in body weight gain, is one of the typical toxic effects observed following exposure of rodents to dioxins. Although the mechanism is not yet fully understood, it is believed that AhR activation by dioxins is a key factor.\textsuperscript{29} Regarding the mechanism of the protective effect of resveratrol, since this substance has been reported to act as an antagonist of AhR activation \textit{in vitro} and \textit{in vivo},\textsuperscript{17,18} it is reasonable to postulate that a reduction in wasting syndrome is attributable to this antagonism. In fact, α-naphthoflavone, an antagonist against AhR activation by TCDD, attenuates cleft palate, and renal pelvic and ureteric dilatations caused by TCDD.\textsuperscript{30} The antagonism of resveratrol on AhR signaling is partially supported by our observation that the effect of resveratrol on EROD activity, an index of AhR activation, was seen only after its subcutaneous injection. In addition, resveratrol is also known to have an inhibitory effect on EROD reduction in the experiment involving subcutaneous injection. 

Inflammatory reactions, including the production of oxidative stress, appear to contribute to the toxic effects of TCDD. For example, TCDD-produced wasting syndrome is attenuated by co-treatment with either anti-tumor necrosis factor-α antibody or dexamethasone in mice.\textsuperscript{32} Recently, it has been reported that resveratrol inhibits the interleukin-1β-induced release of reactive oxygen species in chondrocytes.\textsuperscript{33} Therefore, another possibility is that repeated administration of resveratrol reduces TCDD-produced wasting syndrome by its anti-inflammatory and/or anti-oxidative stress effects. This possibility seems to be likely because co-treatment with resveratrol injected subcutaneously significantly reduced the hepatic TBARS content increased by TCDD (Fig. 4B). In contrast, oral resveratrol failed to compete with the TCDD-produced enhancement of hepatic TBARS, although resveratrol concentration increases to a high level at the endpoint of tissue preparation for TBARS assay (30 min after resveratrol administration). Although we are unable to explain this discrepancy, it may again be due to a difference in pharmacokinetics between the two dosing routes. For example, maintaining the serum concentration of resveratrol rather than its transient increase would be more important to cause a reduction in TBARS.

The accumulation of lipids involving triglycerides and cholesterol in the liver is one of the typical biological manifestations produced by dioxins, and it is considered as an initial stage of steatosis.\textsuperscript{24,25} The lipid accumulation in the hepatocytes produced by TCDD was markedly improved by co-treatment with oral resveratrol. Although the mechanism of TCDD-induced steatosis is still unclear, it is suggested that this symptom is caused by an increase in lipid uptake by hepatocytes and/or a reduction in lipid metabolism by AhR activation.\textsuperscript{34–37} On the other hand, since a putative link between oxidative stress and steatosis has been demonstrated (see review of Mantena \textit{et al.}\textsuperscript{38}), an alternative mechanism may involve oxidative stress that contributes to the production and progression of TCDD-induced steatosis. However, the observation that oral resveratrol had no effect on TBARS elevation by TCDD, even although it is protective against steatosis, does not support the above view. Thus, as far as TCDD-produced hepatic lipoidosis is concerned, a mechanism other than enhanced oxidative stress seems to play a more important role in causing the disorder. In contrast with oral administration, subcutaneously-injected resveratrol exhibited no notable effect on lipid accumulation in hepatocytes, in spite of its high bioavailability. The reason for this inconsistency remains unknown. The livers were removed for histological examination 28 (oral resveratrol) and 5 d (subcutaneous resveratrol) after TCDD treatment. Owing to this difference, in the period of TCDD exposure, lipid accumulation in hepatocytes is assumed to be lower in the subcutaneous injection study than in the oral administration study. In fact, in the former study, stained lipid drops in the TCDD-treated liver were apparently fewer compared with those in the latter study (Figs. 2C, G). Thus, the reason for the lack of a protective effect by subcutaneous resveratrol on lipoidosis is considered to be, at least in part, due to the low accumulation of lipids.

This study shows that resveratrol administrated orally partially restores the sub-acute toxicity of TCDD. In addition, it is suggested that such an effect of resveratrol can be en-
hanced by improving its bioavailability. Although the mechanisms governing the protective effect remain to be clarified, it is suggested that resveratrol as a food ingredient is an effective candidate agent to combat dioxin toxicity.

Acknowledgements  This work was supported by a Research Grant 15190101 from the Ministry of Health, Labour, and Welfare in Japan.

REFERENCES