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# Inhibition of TCDD-Induced Lipid Peroxidation, Glutathione Peroxidase Activity and Toxicity by BHA and Glutathione

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The mechanism of toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been extensively investigated in recent years, but the cause of liver injury and lethality is still unknown (Poland and Knutson, 1982). The toxicity of TCDD may involve in part enhanced lipid peroxidation (Stohs et al., 1983). TCDD administration to rats increases hepatic lipid peroxidation as shown by the increased production of such indicators of lipid peroxidation as malondialdehyde and conjugated dienes. In addition, lipofuscin pigments, which are considered to be by-products of lipid peroxidation, accumulate in the hearts of TCDD-treated rats (Albro et al., 1978). Hassan et al. (1983) have recently shown that TCDD has no effect on hepatic lipid peroxidation or glutathione peroxidase activity in hamsters which are highly resistant to this compound. Furthermore, TCDD induced hepatic lipid peroxidation in guinea pigs which are very sensitive to TCDD. Lipid peroxidation is regarded as one of the primary and key events in cellular damage.

Antioxidants are capable of inhibiting the toxicity of various chemicals when the antioxidants are administered either prior to or at the same time as the xenobiotic (Wattenberg et. al., 1980). The phenolic antioxidant 2(3)-t-butyl-4-hydroxyanisole (BHA) in the diet of mice prevents onset of TCDD-induced porphyria (Sweeney, 1982). BHA administration to mice also increases by several fold the specific activities of the phase II detoxication enzymes, glutathione S-transferases and epoxide hydratase, in the liver and other tissues (Benson et al., 1978), and its administration is associated with an increase in hepatic glutathione content (Baltzinger et al., 1978).

Glutathione (GSH), the major nonprotein sulfhydryl compound in most cells, plays an important role in the protection of cellular constituents against oxidative damage and in the detoxification of many electrophiles. Lipid peroxidation is known to occur as a consequence of GSH depletion (Younes and Siegers, 1981).

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The effects of the water soluble antioxidants, BHA and GSH, on hepatic lipid peroxidation and other biochemical parameters in rats after TCDD administration were studied.

## MATERIALS AND METHODS

Female Sprague-Dawley rats weighing 140-160 gm (Sasco Inc., Omaha, NE) were housed in stainless steel hanging cages and provided free access to feed and water. BHA was obtained from Sigma Chemical Co. BHA (500 mg/kg/day) in corn oil was administered orally for 3 days to one group of rats followed by 200 mg/kg/day for 9 additional days. Control animals received the corn oil vehicle only. Reduced glutathione (GSH) (300 mg/kg/day) in distilled water was administered orally for 12 days to a third group of animals. Half of each of the 3 groups were given TCDD (40 µg/kg) in corn oil orally on days 4, 5 and 6.

All the animals were killed on day 13. Livers were homogenized and the microsomal and cytosolic fractions were isolated by differential centrifugation. Protein concentrations were determined according to the method of Lowry et al. (1951). Liver content of reduced GSH (sulfhydryl) was assayed by the method of Hissin and Hilf (1976). For determination of lipid peroxidation, isolated microsomes were suspended in 0.10 M phosphate buffer, pH 7.4, and incubated with 0.40 mM NADPH for 10 min. The malondialdehyde which was formed as a result of lipid peroxidation was measured by the thiobarbituric acid method as described by Miles et al. (1980). Glutathione peroxidase (GSHPx) was estimated using the procedure of Paglia and Valentine (1967). Glutathione S-transferase (GST) and glutathione reductase (GR) activities of cytosolic fractions were measured by the methods of Habig et al. (1974) and Carlberg and Mannervik (1975), respectively. Aryl hydrocarbon hydroxylase (AHH) activity was determined by the method of Dehnen et al. (1973). Data were analyzed by a one-way analysis of variance with mean values compared by Student's t test.

For the survival studies, 10 rats were given 500 mg BHA/kg/day for 3 days orally in corn oil followed by 200 mg BHA/kg/day for an additional 23 days. A second group of 10 animals received only the corn oil vehicle. All animals received 40 µg TCDD/kg/day orally in corn oil on days 4, 5 and 6. Body weights and per cent of animals surviving with time in each group were determined.

## RESULTS AND DISCUSSION

TCDD administration increased hepatic microsomal lipid peroxidation by approximately 7.3-fold (Table 1). When rats were treated with 500 mg BHA/kg/day for 3 days followed by 200 mg/BHA/kg/day for an additional 9 days, a 31% decrease in hepatic lipid peroxidation occurred when compared to the corn oil treated control group (Table 1). BHA treated rats administered TCDD demonstrated a 2.2-fold increase in lipid peroxidation relative to control animals. This represented a 70% decrease in lipid peroxidation as compared to the group which received TCDD without BHA. Oral administration of GSH

(300 mg/kg/day) for 12 days produced a 33% decrease in lipid peroxidation. TCDD treatment of animals receiving GSH resulted in only a 1.8-fold increase in lipid peroxidation or a 75% decrease in lipid peroxidation as compared to the TCDD only group (Table 1).

Table 1. Effects of BHA and Glutathione on TCDD-Induced Changes in Hepatic Glutathione Peroxidase Activities and Lipid Peroxidation in Female Rats

| Treatment | Total glutathione peroxidase (μmol/min/mg protein) | Se-dependent glutathione peroxidase (μmol/min/mg protein) | Lipid peroxidation (nmol/min/mg protein) |
|---|---|---|---|
| Control (corn oil) | 0.43 ± 0.02 | 0.26 ± 0.02 | 1.33 ± 0.21 |
| TCDD | 0.25 ± 0.03[a] | 0.09 ± 0.02[a] | 9.69 ± 1.58[a] |
| BHA + corn oil | 0.54 ± 0.07[a] | 0.39 ± 0.07[a] | 0.92 ± 0.25 |
| BHA + TCDD | 0.43 ± 0.06 | 0.16 ± 0.04[a,b] | 2.90 ± 1.03[a,b] |
| Glutathione + corn oil | 0.38 ± 0.03 | 0.24 ± 0.01 | 0.89 ± 0.25 |
| Glutathione + TCDD | 0.34 ± 0.05[a,b] | 0.13 ± 0.04[a] | 2.43 ± 0.59[a,b] |

Rats were given 500 mg BHA/kg/day for 3 days followed by 200 mg BHA/kg/day for 9 days, 300 mg GSH/kg/day for 12 days or the corn oil vehicle P.O. Rat receiving TCDD were given 40 μg/kg/day P.O. in corn oil on days 4, 5 and 6. All animals were killed on day 13. [a]$P < 0.05$ with respect to corn oil control group. [b]$P < 0.05$ with respect to the group which received TCDD alone. Each value represents the mean ± S.D. from 4-6 animals.

TCDD administration produced a significant decrease in both total and selenium dependent glutathione peroxidase (GSHPx) activities (Table 1). A 65% inhibition of the selenium dependent enzyme was observed. BHA treatment alone produced a small but significant increase in the activities of both peroxidases. When TCDD was administered to BHA treated rats, total GSHPx activity was unchanged as compared to the control group, while selenium dependent GSHPx activity was less than the control group but greater than the activity from rats receiving only TCDD (Table 1). GSH administration to rats had no effect on total or selenium dependent glutathione peroxidase. Administration of TCDD to GSH

Table 2. Effects of BHA and Glutathione on TCDD-Induced Hepatic Changes in Female Rats

| Treatment | Glutathione reductase (μmol/min/mg protein) | Glutathione S-transferase (μmol/min/mg protein) | Aryl hydrocarbon hydroxylase (nmol/min/mg protein) | Reduced glutathione (μg/mg protein) |
|---|---|---|---|---|
| Control (corn oil) | 233.6 ± 53.3 | 21.8 ± 1.0 | 0.16 ± 0.03 | 9.13 ± 1.52 |
| TCDD | 381.9 ± 41.0[a] | 64.3 ± 8.9[a] | 1.29 ± 0.15[a] | 6.72 ± 1.23[a] |
| BHA + corn oil | 609.6 ± 40.1[a] | 33.8 ± 3.9[a] | 0.23 ± 0.04 | 13.87 ± 1.47[a] |
| BHA + TCDD | 595.2 ±136.5[a,b] | 50.5 ± 7.1[a] | 0.88 ± 0.17[a,b] | 14.24 ± 1.35[a,b] |
| Glutathione + corn oil | 272.0 ± 44.2 | 31.0 ± 3.7[a] | 0.73 ± 0.17[a] | 7.57 ± 1.43 |
| Glutathione + TCDD | 451.8 ± 30.4[a] | 76.2 ±12.0[a] | 1.89 ± 0.35[a,b] | 6.38 ± 0.76[a] |

Female rats were treated with 500 mg BHA/kg/day for three days followed by a maintenance dose of 200 mg/kg/day for nine additional days, 300 mg glutathione/kg/day for 12 days, or the corn oil vehicle P.O. Rats receiving TCDD were given 40 μg/kg/day P.O. in corn oil on days 4, 5 and 6. All animals were killed on day 13. [a]$P < 0.05$ with respect to corn oil control. [b]$P < 0.05$ with respect to TCDD alone. Each value represents the mean ± S.D. from animals.

treated rats resulted in a significant decrease in selenium dependent GSHPx activity with respect to the group receiving only GSH (Table 1).

TCDD treatment produced a 26% decrease in hepatic GSH (sulfhydryl) content as compared to the control group (Table 2). BHA treatment alone and BHA treatment in combination with TCDD administration both resulted in over a 50% increase in hepatic GSH content. Oral administration of GSH for 12 days had no effect on GSH content, while the TCDD treatment of rats which had been administered GSH orally resulted in a 30% decrease in hepatic GSH content.

TCDD resulted in an 8-fold increase in the activity of AHH (Table 2). Treatment of rats with BHA for 12 days produced a small but insignificant increase in AHH activity. In the presence of BHA, TCDD elicited only a 5.5-fold increase in hepatic AHH activity. In GSH treated animals, liver AHH activity was 4.5-fold greater than in the corn oil control rats. The TCDD and GSH treated animals showed a 2.6-fold higher AHH activity than animals given only GSH for 12 days (Table 2), an 11.8-fold increase as compared to the corn oil control group, and 46.5% greater activity than animals given TCDD alone.

TCDD was shown to be an inducer of both glutathione reductase (GR) and glutathione-S-transferase (GST), producing 1.6 and 2.9-fold increases, respectively, in these two enzymes (Table 2). BHA treatment for 12 days resulted in 2.6 and 1.6-fold increases in GR and GST, respectively. TCDD treatment in combination with BHA resulted in a hepatic GST activity which was similar to the activity for TCDD, treatment alone while GR activity for BHA plus TCDD was higher than for TCDD treatment alone. The administration of GSH for 12 days had no effect on hepatic GR, but a 1.4-fold increase in GST was observed (Table 2). TCDD treatment produced 1.9 and 3.5-fold increases in the activities of GR and GST, respectively, in animals which had been given GSH as compared to the corn oil control results (Table 2).

Progressive body and liver weight losses are characteristics of TCDD toxicity. The effect of TCDD on these parameters in animals treated with BHA and GSH is presented in Table 3. Six days after TCDD administration, a 20% decrease in body weight of the animals was observed. BHA and GSH without TCDD produced small but insignificant increases in body weight. BHA and GSH provided partial protection against TCDD-induced weight loss. Both BHA and GSH also protected against TCDD-induced liver weight loss, with BHA treated animals actually realizing an increase in liver weight (Table 3).

The effect of BHA treatment on survival of rats following a lethal dose of TCDD was determined (Fig. 1). The animals were treated with a dose of 500 mg BHA/kg/day for 3 days followed by a maintenance dose of 200 mg/kg/day for 23 days or the corn oil vehicle. Each group of animals was treated with 40 $\mu$g TCDD/kg/day on days 4,5 and 6. By day 26 of the study 80% of the rats

Table 3. Effects of BHA and Glutathione on TCDD-Induced Changes in Body Weights and Liver Weights of Female Rats

| Treatment | Body Weight (gm) Initial | Final | Liver weight gm | gm/gm body wt. |
|---|---|---|---|---|
| Control (corn oil) | 154.3 ± 5.8 | 169.7 ± 6.9[a] | 6.98 ± 0.37 | 0.041 ± 0.003 |
| TCDD | 158.2 ± 8.7 | 139.3 ± 9.8[a] | 5.60 ± 1.04 | 0.042 ± 0.002 |
| BHA + corn oil | 144.2 ± 6.4 | 174.2 ±11.2[a] | 8.11 ± 0.79 | 0.047 ± 0.005 |
| BHA + TCDD | 144.5 ± 4.1 | 149.6 ±12.8 | 7.56 ± 2.08 | 0.050 ± 0.013 |
| Glutathione + corn oil | 164.8 ± 6.6 | 181.8 ± 8.2[a] | 6.93 ± 0.99 | 0.038 ± 0.004 |
| Glutathione + TCDD | 170.2 ±12.3 | 162.2 ±13.6 | 6.49 ± 1.41 | 0.039 ± 0.007 |

Female rats were given 500 mg BHA/kg/day P.O. in corn oil for three days followed by 200 mg/kg/day for nine days, 300 mg glutathione/kg/day for 12 days, or the vehicle. Rats receiving TCDD were given 40 μg/kg/day on days 4, 5, and 6. All animals were killed on day 13. [a]$P < 0.05$ with respect to body weight before treatment began. Each value represents the mean ± S.D. from 4-6 rats.

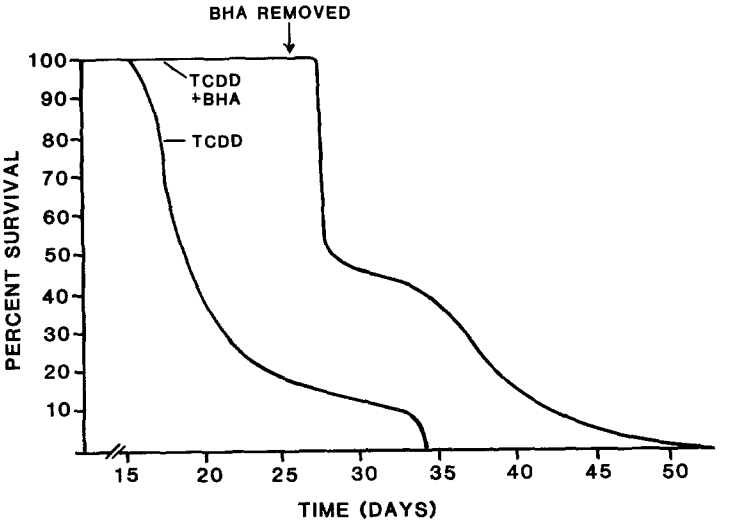


Fig. 1. Percent survival as a function of time following administration of 40 µg TCDD/kg/day for three days. Ten rats received 500 mg BHA/kg/day for three days orally in corn oil, followed by 200 mg BHA/kg/day for 23 days. An equal number of animals received only the corn oil. TCDD was given on days 4, 5 and 6.

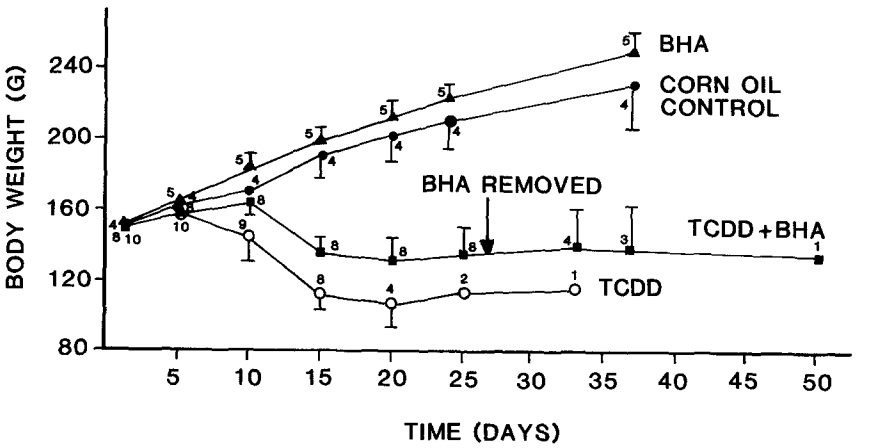


Fig. 2. Changes in body weight with time following BHA and TCDD administration. Rats were given corn oil daily with some of the animals receiving 40 µg TCDD/kg on days 4,5 and 6 of the study. A group of rats received 500 mg BHA/kg/day for three days orally in corn oil, followed by 200 mg BHA/kg/day for 23 days. Eight of the BHA treated rats were given 40 µg TCDD/kg on days 4,5 and 6.

receiving TCDD only had died while none of the rats receiving BHA daily for the 26 days in addition to the TCDD had died. Therefore, BHA administration was able to protect these animals from the lethality of TCDD. After day 26 the BHA administration was discontinued. Subsequently, all animals that had been receiving the BHA died.

The effect of TCDD and daily BHA treatment on body weights of rats is presented in Fig. 2. The body weights of animals receiving daily BHA were not significantly different than body weights of rats receiving only the corn oil vehicle. Body weights of rats receiving TCDD on days 4,5 and 6 were significantly lower than the respective control groups by day 10. However, the body weights of TCDD treated rats receiving BHA were significantly higher at all time points after day 10 as compared to animals receiving only the TCDD.

Daily BHA administration delayed TCDD lethality (Fig. 1), but did not prevent the lack of gain in body weight caused by TCDD (Fig. 2). Cessation of daily BHA treatment resulted in the deaths of these animals. The elimination half lives of TCDD and BHA are 31 days (Rose et al., 1976) and 14 hours (Golder et al., 1962), respectively. Within 2 days, most of the BHA will have been eliminated while over 50% of a triple $LD_{50}$ dose of TCDD will still be present in the animals.

The ability of TCDD to inhibit GSHPx activity is shown in Table 1. The results suggest that it is the selenium dependent form of the enzyme which is inhibited to the greatest extent and that BHA but not GSH was able to provide partial protection. Protection from TCDD toxicity by BHA may be partially due to the enhanced peroxidase activity. Inhibition of GSHPx by TCDD alone may result in the accumulation of $H_2O_2$, and $H_2O_2$ with superoxide or iron can generate hydroxyl radicals and singlet oxygen which are effective initiators of lipid peroxidation (Dixit et al., 1982). As such, a portion of the TCDD-induced lipid peroxidation may be due to the inhibition of GSHPx.

Sweeney (1982) has shown that feeding 0.75% BHA in the diet of mice prevents the onset of TCDD-induced porphyria, supporting the hypothesis that lipid peroxidation or a related free radical mechanism is involved in the toxicity of TCDD. Robertson et al. (1983) reported that the antioxidants vitamin E, BHA and butylated hydroxy toluene were unable to prevent in rats the toxic effects of tetrabromobiphenyls, compounds closely related to TCDD. The dose of BHA may have been insufficient to reverse the toxic manifestations of the brominated biphenyls.

GSH is a major water soluble antioxidant in cells. BHA administration can increase hepatic GSH content (Table 2). However, oral administration of GSH did not increase hepatic GSH content. Yoshimura et al. (1982) also did not observe an increase in GSH content of the liver after giving GSH orally to rats. These results are presumably due to the poor absorption of intact GSH.

The observed effect of oral GSH may be due to absorbed cysteine. The role of GSH in preventing lipid peroxidation is well known. Depletion of GSH results in an increase in hepatic lipid peroxidation (Younes and Siegers, 1981), and the data in Table 2 indicate that TCDD does produce a decrease in hepatic GSH (sulfhydryl) content.

The roles of GST and GR in TCDD toxicity are not known. The data in Table 2 demonstrate that TCDD is an inducer of these enzymes. BHA administration induced both GST and GR, and the activities did not change significantly when TCDD was given to these animals.

The results demonstrate that a significant increase in lipid peroxidation occurs at acutely toxic doses of TCDD, and this may contribute to the characteristic symptoms associated with this xenobiotic. A combination of factors may be involved in TCDD induced lipid peroxidation including enhanced AHH activity, inhibited glutathione peroxidase activity and possibly the decrease in hepatic reduced GSH content. Oral BHA and GSH have been shown to modulate the effects of TCDD on lipid peroxidation.

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