INDUCTION OF HEPATIC MICROSOMAL MONOOXYGENASES IN FEMALE RATS GIVEN VARIOUS SUBSTITUTED PHENOLS AND HYDROQUINONES

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Accepted February 14, 1981

Butylated hydroxytoluene (BHT), a commonly used food antioxidant, induces hypoprothrombinemia and a resultant hemorrhagic death in male rats (1). These adverse effects of BHT are prevented by the administration of vitamin K (2, 3). Takahashi and Hiraga indicated that the structural characteristics of BHT were to a greater extent responsible for the hemorrhagic effect than were the antioxidant properties, by comparing the hemorrhage-inducing ability of various compounds (4, 5). Considering the species, strain and sex differences in the hemorrhage-inducing effect of BHT, this phenomenon may be related to the metabolism of this compound (6). In the present investigation we examined the ability of various substituted phenols and hydroquinones to induce hepatic microsomal monooxygenases, and discussed the relationship to hemorrhagic phenomenon.

Butylated hydroxytoluene (2,6-di-tert-butyl-4-methylphenol, BHT), ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinone, EQ), tert-butylhydroquinone (BHQ), 2,4-di-tert-butylphenol (2,4-DBP), 2-tert-butyl-4-sec-butylphenol (BBP) and 4-tert-butyl-2,6-diisopropylphenol (BDPP) were purchased from Tokyo Kasei Kogyo Co., 2,4,6-tri-tert-butylphenol (TBP) and 3,5-di-tert-butylphenol (3,5-DBP) were from Aldrich Chemical Co., and 2,5-di-tert-butylhydroquinone (DBHQ) from Wako Pure Chemical Industries Ltd.

Five week-old, Wistar-JCL female rats (obtained from CLEA Japan Inc.) were fed either commercial stock ration (powdered CLEA CE-2) alone or the same ration containing the above-described chemicals, at a concentration of 0.5% for 6 days. After maintenance on the stock ration for the following 24 hr, the animals were decapitated. All other materials and techniques were the same as previously described (7, 8).

Liver weight and microsomal protein content, cytochrome P-450 content and two monooxygenase activities in liver of rats given eight kinds of substituted phenols and hydroquinones and an antioxidant ethoxyquin (EQ) are summarized in Table 1. Data are expressed on a 100 g body weight basis. All the test compounds except for DBHQ produced a significant enlargement of the liver. TBP and BDPP administration brought about a doubling of liver weight, whereas BHT, EQ and BBP produced only a moderate increase (more than 30%). All the compounds showing a marked (TBP and BDPP) or a moderate (BHT, EQ and BBP) hepatic enlargement caused an extreme augmentation in cytochrome P-450 content: both groups of compounds elicited 7- and 2-fold increases, respectively. The effects of these compounds on the activities of microsomal monooxygenases: i.e. p-nitroanisole demethylase, aminopyrine demethylase and aniline
hydroxylase were more potent (the data concerning the last compound are not shown). TBP, BDPP, BHT and BBP administration caused 10-, 8-, 4-, and 3-fold increases, respectively, in those three activities. DBHQ produced neither hepatic enlargement nor augmentation of cytochrome P-450 content, but did cause a moderate increase in monooxygenase activities. The other three compounds showed a slight (2,4-DBP) or no induction of monooxygenases (3,5-DBP and BHQ). Phenobarbital (PB), which was given to rats at a concentration of 0.1% in drinking water, caused a 4-fold increase in cytochrome P-450 content and a 6-fold increase in the enzymatic activities (data not shown). Thus, we estimate that the inducibility of TBP and BDPP is not at all inferior to that of PB.

All the compounds examined changed neither the Soret peak wavelength of the CO-binding difference spectrum (499 nm) nor the 455 nm/430 nm peak height ratio of the ethyl-isocyanide difference spectrum (0.45–0.57).

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoretic patterns of microsomes prepared from female rats given various test compounds are shown in Fig. 1. Four (or five) polypeptide bands were visualized in the range between 45,000 and 55,000 molecular weight. PB administration (well 2) enlarged three polypeptide bands among them, the molecular weight of which was tentatively calculated to be 53,000, 50,000 and 47,000, according to the method of Shapiro et al. (9). Polychlorinated dibenzofuran (PCDF, a 3-methylcholanthrene

<table>
<thead>
<tr>
<th>Drug</th>
<th>Liver weight (g/100 g BW)</th>
<th>Microsomal protein (mg/100 g BW)</th>
<th>Cytochrome P-450 (pmoles/100 g BW)</th>
<th>p-Nitroanisole demethylase (nmol/min/100 g BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.85 ± 0.29</td>
<td>80.9 ± 8.0</td>
<td>76.2 ± 9.4</td>
<td>0.97 ± 0.13</td>
</tr>
<tr>
<td>TBP</td>
<td>11.32 ± 0.94**</td>
<td>257.8 ± 24.9***</td>
<td>486.0 ± 66.3***</td>
<td>9.09 ± 0.26***</td>
</tr>
<tr>
<td>DBHQ</td>
<td>5.25 ± 0.41</td>
<td>59.4 ± 5.1**</td>
<td>55.5 ± 8.2</td>
<td>1.92 ± 0.17***</td>
</tr>
<tr>
<td>BHT</td>
<td>6.18 ± 0.84*</td>
<td>122.5 ± 34.4</td>
<td>145.1 ± 59.6*</td>
<td>3.88 ± 1.84*</td>
</tr>
<tr>
<td>EO</td>
<td>6.50 ± 0.53**</td>
<td>129.4 ± 10.4**</td>
<td>152.0 ± 14.9***</td>
<td>3.27 ± 0.29***</td>
</tr>
</tbody>
</table>

Experiment 1.

Control | 4.79 ± 0.13           | 71.9 ± 14.3                      | 50.9 ± 11.7                      | 1.20 ± 0.17                                    |
| DBHQ   | 5.07 ± 0.24           | 66.9 ± 15.1                      | 64.8 ± 19.5                      | 2.24 ± 0.47***                                 |
| BHQ    | 5.19 ± 0.19*          | 72.2 ± 10.8                      | 54.2 ± 16.4                      | 1.27 ± 0.14                                    |
| 3,5-DBP | 5.50 ± 0.23**        | 72.7 ± 9.4                       | 60.5 ± 7.0                       | 1.61 ± 0.17*                                   |
| 2,4-DBP | 6.08 ± 0.18**        | 92.5 ± 17.8                      | 80.6 ± 17.2*                     | 2.12 ± 0.30**                                  |
| BBP    | 6.65 ± 0.67**         | 98.9 ± 22.2                      | 106.4 ± 31.7*                    | 23.37 ± 0.53***                                |
| BDPP   | 9.75 ± 0.78**         | 177.6 ± 39.7*                    | 360.1 ± 94.2*                    | 7.77 ± 0.07**                                  |

Experiment 2.

Control | 4.64 ± 0.29           | 88.0 ± 9.2                       | 64.5 ± 7.7                       | 1.32 ± 0.11                                    |
| BHT    | 6.39 ± 0.39**         | 155.1 ± 8.2**                    | 168.6 ± 17.4**                   | 5.17 ± 0.51*                                   |

Experiment 3.

Table 1. Liver weight and microsomal protein content, cytochrome P-450 content and two microsomal monooxygenase activities in liver of female rats given various substituted phenols and hydroquinones at a level of 0.5% in diets.

Each value represents the mean ± SD. Significantly different from each control group: *P<0.05; **P<0.01; ***P<0.001. *Number of animals examined. **In this group, the drug was added to a diet at a concentration of 0.2%. Mean body weights of these groups were 137, 126, 95, 127 and 131 g in experiment 1, 144, 125, 132, 128 and 128 g in experiment 2, and 142 and 137 g in experiment 3, respectively.
Type inducer (8)) administration (well 1) enlarged the 53,000 molecular weight peptide band and elicited the appearance of a 56,000 molecular weight peptide band. The above-mentioned 47,000, 53,000 and 56,000 molecular weight polypeptide bands seem to correspond to cytochromes P-450, P-450b and P-450, respectively, which were purified by Ryan et al. (10), as assessed from the position on the electrophrogram.

TBP and BDPP administration remarkably enlarged PB-inducible three polypeptide bands (wells 4 and 12). A similar change was produced by DBHQ, but to a lesser extent (well 9). BHT and EQ conspicuously enlarged 47,000 molecular weight peptide band, and to a lesser extent 53,000 and 50,000 molecular weight bands (wells 5 and 6). A similar but a less marked change was produced by 2,4-DBP and BBP (wells 10 and 11). TBP, which was the most potent inducer of cytochrome P-450, also increased several other microsomal polypeptides in the range between 58,000 and 62,000 molecular weight. None of the test compounds increased PCDF-inducible 56,000 molecular weight polypeptide. Using two dimensional electrophoresis of microsomes from BHT-given rats (first dimension: Thomas et al. (11); second dimension: Laemmli (12)), we have confirmed that heme-containing polypeptides exist in a broad area between molecular weight 47,000 and 53,000, particularly in the neighborhood of 47,000 (unpublished data).

Kahl and Wulff (13) observed a marked increase in a band with the molecular weight between 42,000 and 45,000 in BHT-given rats, and assigned it to epoxide hydratase. This molecular weight is considerably smaller than weights reported by other investigators (14-16) (about 50,000). This band seems to be identical with our 47,000 polypeptide band. Considering that heme-containing peptide(s) exists in this position in a considerable amount as above described, the existence of cytochrome P-450 seems to be undeniable, even if this band also contains epoxide hydratase.

Takahashi and Hiraga (4, 5) compared the hemorrhage-inducing ability of various substituted phenols, and found that among them only TBP, BDPP and to a lesser extent BHT caused a hemorrhagic death in male rats. In the present experiment, we have shown that these compounds also induce hepatic microsomal monooxygenases extremely (TBP and BDPP) or moderately (BHT). Thus, through an unknown mechanism, the hemorrhage-inducing process may be related to the monooxygenase-inducing process.
However, as shown in our previous report (7), the 47,000 molecular weight polypeptide (perhaps cytochrome P-450α, named by Ryan et al. (10)) is only induced in the rat, i.e. a hemorrhage-inducible species (6), but not in the mouse, i.e. a hemorrhage-non-inducible species (6). Thus, in the case of BHT, it may be inferred that the metabolite(s) formed by a particular type of monoxygenase (cytochrome P-450α) cause(s) a hemorrhage.

In conclusion, two kinds of substituted phenols, TBP and BDPP, the structures of which markedly differ from PB, were potent PB type inducers of microsomal monoxygenases in spectral, catalytic and electrophoretic properties. However, in spite of the similarity in the structure, BHT showed some differences in the electrophorogram from these compounds.

Acknowledgement: We are grateful to Dr. O. Takahashi in our laboratory for pertinent suggestions.

REFERENCES