Effects of Peroxisome Proliferators on Antioxidant Enzymes and Antioxidant Vitamins in Rats and Hamsters

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Received August 9, 2000; accepted December 4, 2000

Peroxisome proliferators (PPs) cause hepatomegaly, peroxisome proliferation, and hepatocarcinogenesis in rats and mice, whereas hamsters are less responsive to PPs. PPs increase the activities of enzymes involved in peroxisomal β-oxidation and ω-hydroxylation of fatty acids, which has been hypothesized to result in oxidative stress. The hypothesis of this study was that differential modulation of antioxidant enzymes and vitamins might account for differences in species susceptibility to PPs. Accordingly, we measured the activities of DT-diaphorase and superoxide dismutase (SOD) and the hepatic content of ascorbic acid and α-tocopherol in male Sprague-Dawley rats and Syrian hamsters fed 2 doses of 3 known peroxisome proliferators (dibutyl phthalate [DBP], gemfibrozil, and [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid (Wy-14,643) for 6, 34, or 90 days. In untreated animals, the activity of DT-diaphorase was much higher in hamsters than in rats, but the control levels of SOD, ascorbic acid, and α-tocopherol were similar. In rats and hamsters treated with Wy-14,643, we observed decreases in α-tocopherol content and total SOD activity. DT-diaphorase activity was decreased in activity following Wy-14,643 treatment in rats at all time points and doses, but only sporadically affected in hamsters. Rats and hamsters treated with DBP demonstrated increased SOD activity at 6 days; however, in the rat, DBP decreased SOD activity at 90 days and α-tocopherol content was decreased throughout. In gemfibrozil treated rats and hamsters, a decrease in α-tocopherol content and an increase in DT-diaphorase activity were observed. In either species, no consistent trend was observed in total ascorbic acid content after treatment with any of the PPs. In conclusion, these data suggest that both rats and hamsters are compromised in antioxidant capabilities following PP treatment and additional hypotheses for species susceptibility should be considered.

Key Words: Wy-14,643, dibutyl phthalate, gemfibrozil, superoxide dismutase, DT-diaphorase, α-tocopherol, ascorbic acid, peroxisome proliferator (PP), Sprague-Dawley rat, Syrian hamster.

Dibutyl phthalate (DBP), [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid (Wy-14,643), and gemfibrozil are unrelated compounds known to cause an increase in the size and number of peroxisomes, termed peroxisome proliferation (PP), in the livers of mice and rats (Lake, 1995; Reddy and Lalwai, 1983). Accordingly, this induced proliferation of peroxisomes is considered central to the yet undefined mechanism whereby treatment with these compounds results in hepatomegaly and hepatocarcinogenesis in these same rodents (Lake, 1995; Rao and Reddy, 1991). Contrary to the effects observed in rats and mice, nonhuman primates, guinea pigs, and dogs are nonresponsive, whereas hamsters are less responsive to these compounds (Isenberg et al., 2000; James and Roberts, 1996; Lake et al., 1993, 2000; Reddy and Lalwai, 1983; Styles et al., 1988). Furthermore, these compounds are negative in genotoxicity assays (Ashby et al., 1994; Budroe and Williams, 1993), instead facilitating their effects through activation of the peroxisome proliferator activated receptor (PPARα in the liver) ultimately influencing the transcription of PP-responsive genes (Desvergne and Wahli, 1999). Consequently, reactive oxygen species (ROS) resulting from the increased activity of peroxisomal fatty acyl-CoA oxidase (AOX) and cytochrome P4504A1/6 have been hypothesized to mediate the resulting hepatocarcinogenesis (Lake, 1995; Lake et al., 1990; Rao and Reddy, 1991).

The involvement of enzymes facilitating the removal of ROS in the mechanism of PP-mediated carcinogenesis has been examined in various rat and mouse strains following treatment with a wide array of PPs (Ashby et al., 1994). Superoxide dismutases (SOD) are enzymes that catalyze the conversion of O2·− to H2O2 and O2. In eukaryotes, 3 genes encode 3 forms of SOD, which are highly compartmentalized. Homodimeric Cu,Zn-SOD is partitioned in the cytosol, homotetrameric Mn-
SOD is in the mitochondria, and homotetrameric extracellular (EC) SOD, also containing Cu/Zn, is in the extracellular space (Mates et al., 1999). Converting O$_2^-$ to H$_2$O$_2$ in the absence of the equivalent activity of both catalase and selenium-dependent glutathione peroxidase (Se-GPx), both of which are able to convert H$_2$O, to H$_2$O, can be more detrimental to the cell than the original ROS (de Haan et al., 1996). Indeed, increased SOD activity leading to increased H$_2$O$_2$ concentrations in the presence of transition metals can result in the formation of HO$^·$ through increased peroxisomal glutathione peroxidase (Se-GPx), both of which are able to convert H$_2$O$_2$ to H$_2$O, can be more detrimental to the cell than glutathione peroxidase (Se-GPx), both of which are able to convert H$_2$O$_2$ to H$_2$O, can be more detrimental to the cell than glutathione peroxidase (Se-GPx), both of which are able to convert H$_2$O$_2$ to H$_2$O. Iglesia, 1984; Lake et al, 1999). More reactive than O$_2^-$ or H$_2$O$_2$, HO$^·$ immediately reacts with nucleophilic macromolecules resulting in cellular damage, including lipid hydroperoxides (LOO$^·$). Within cells, the lipid-soluble, antioxidant α-tocopherol can terminate LOO$^·$ chain reactions (Tappel, 1980).

Tocopherols exist as 4 forms depending on the substitution on the chromanol ring (α, β, γ, δ) (Brigelius-Flohe and Traber, 1999). Additionally, the tocotrienols (α, β, γ, δ) also contribute to vitamin-E activity. Of the 8 molecules collectively referred to as vitamin E, α-tocopherol is the most active antioxidant, facilitating the protection of cells from ROS-mediated lipid peroxidation and breaking the chain of radical formation (Brigelius-Flohe and Traber, 1999; Serbinova and Packer, 1994). The resultant α-tocopheroxy radical can be reduced by ascorbic acid, thus regenerating α-tocopherol (Halliwell, 1989). Furthermore, ascorbic acid has been demonstrated to “spare” α-tocopherol, leading to the conclusion that together these two molecules act synergistically in protecting cells from ROS (Halpern et al., 1998; Webers and Sies, 1988). Additionally, the α-tocopherol quinone formed by chain-terminating lipid reactions can be reduced by the cytosolic flavoprotein, DT-diaphorase, resulting in the formation of the antioxidant α-tocopherol hydroquinone (Nakamura and Hayashi, 1994; Siegel et al., 1997). Collectively, all 3 of these antioxidant entities have been hypothesized to inhibit the carcinogenic process (Birt, 1986; Chen et al., 1988; Joseph et al., 1994; McCullum and Frei, 1999; Rauth et al., 1997).

As described earlier, PP treatment results in species-specific hepatocarcinogenesis. Although hamsters treated with PPs do not develop hepatocellular carcinomas or undergo increased cell proliferation, increased production of H$_2$O$_2$ does occur through increased peroxisomal β-oxidation (Gray and de la Iglesia, 1984; Lake et al., 1993, 2000). Accordingly, we hypothesized that antioxidant enzymes such as SOD or DT-diaphorase, and/or the antioxidant vitamins C and E may be modulated differently in hamsters and rats following PP treatment.

**MATERIALS AND METHODS**

**Materials.** Wy-14,643 was obtained from Chemsyn Science Laboratories (Lenexa, KS). DBP was obtained from Research Triangle Institute (Research Triangle Park, NC). Gemfibrozil and all other reagents, unless specifically stated otherwise, were purchased from Sigma Chemical Company (St. Louis, MO). All enzyme assays were analyzed using a Shimadzu UV-Vis spectrophotometer, Model UV-1601.

**Experimental design and animal treatments.** The National Toxicology Program, Research Triangle Park, NC, performed all animal treatments. Male, Sprague-Dawley rats (4–6 weeks old, Harlan Sprague-Dawley Inc., Indianapolis, IN) and male, Syrian hamsters (4–6 weeks old, Frederick Cancer Research and Development Center, Frederick, MD) were treated with PPs in an NTP-2000 unreinforced diet (Ziegler Brothers, Inc., Gardners, PA). Animals (n = 5) were administered a control diet or the following amounts of PPs: Wy-14,643 at 50 and 500 ppm, DBP at 5000 and 20,000 ppm in both rats and hamsters, and gemfibrozil at 1000 and 16,000 ppm in rats, 6000 and 24,000 ppm in hamsters. Gemfibrozil was used at higher concentrations in hamsters, due to its predominant urinary excretion in this species; thus using the same dose in both animals may result in hamsters receiving a lesser biological dose (Drengsten et al., 1999). Animals were fasted for 6, 34, or 90 days and euthanized by carbon dioxide overexposure. Livers were then frozen in liquid nitrogen with subsequent storage at –80°C.

**Preparation of whole liver homogenates (WLH).** WLH for enzyme assays were prepared by homogenizing samples, using a Tekmar TR-10 tissue homogenizer (Tekmar, Cincinnati, OH) in 1.15% KCl, 0.1 mM EDTA, pH 7.4. Protein concentrations (mg/ml) of WLH were determined using the Bicinchoninic Acid (BCA) method (Pierce Chemical Co., Rockford, IL), using IgG as the standard.

**SOD activity gels.** SOD activity gels were conducted using the method of Beauchamp and Fridovich (1971) as modified by St. Clair et al. (1991). Sonicated samples used in the cytochrome C assay were pooled (n = 5, 20 μg each) to a total of 100 μg and diluted in sample buffer with a final concentration of 10% glycerol, 62.4 mM Tris-Cl, pH 6.8, and 0.01% bromophenol blue. Samples and standards (1 unit Mn-SOD, E. coli-derived, and 1 unit CuZn-SOD, Bovine erythrocyte derived) were kept on ice at all times and loaded onto a nondenaturing, 5% polyacrylamide stacking gel (0.125 M Tris-Cl, pH 6.8, 0.01% bromophenol blue). Protein concentrations (mg/ml) of WLH were determined using the Bicinchoninic Acid (BCA) method (Pierce Chemical Co., Rockford, IL), using IgG as the standard.

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**DT-diaphorase assay.** Dicumarol-sensitive DT-diaphorase was measured using 2,6-dichlorophenolindophenol (DCPIP) as substrate, according to the method of Lind et al. (1990) with consideration of the findings of Hodnick and Sartorelli (1997). Briefly, WLH was added to a reaction containing 50 mM Tris-Cl, pH 7.5, 0.08% Triton X-100, 40 μM DCPIP, and 0.05 mM NADH. The rate of DCPIP reduction was measured at 600 nm. Dicumarol was added to the reaction at a final concentration of 10 μM and the rate of DCPIP reduction was measured at 600 nm. DT-diaphorase activity was calculated by
RESULTS

To determine if hamsters and rats differ in their ability to detoxify oxidative stress resulting from PP treatment, separating the two mechanistically in their responsiveness to these compounds, we examined enzymes and vitamins that detoxify ROS. First, to determine if either of these species differ in their ability to catalyze the dismutation of superoxide, we examined the total activity of SOD in whole liver homogenates following treatment with DBP, gemfibrozil, or Wy-14,643 for 6, 34, and 90 days. With the exception of hamsters administered WY-14,643 at 50 ppm for 6 days, WY-14,643 treatment conclusively decreased SOD activity in both rats and hamsters at all other time points and doses examined using cytochrome C as the final electron acceptor (Fig. 1). Total SOD activity was increased in both rats and hamsters treated with DBP for 6 days. However, 90-day DBP treatment of rats decreased total SOD activity. Gemfibrozil treatment was the least effective at modifying SOD activity; minor increases in SOD activity were observed, but only the 34-day, 6000-ppm doses in hamsters was significantly altered. We next examined SOD using qualitative activity gels, which, following size and charge separation using polyacrylamide gel electrophoresis, demonstrate the presence of SOD via inhibition of NBT reduction by O$_2^-$, producing a darkened area on the inverted gel (Fig. 2). Through the use of these gels, we hoped to be able to distinguish between Mn-SOD and CuZn-SOD, therefore allowing for the determination of which of the enzymes is altered by PP treatment. However, due to technical limitations, we could not determine if changes in Mn-SOD activity account for the changes in total SOD activity in the rat. Examination of the gels clearly revealed that the decreased SOD activity from Wy-14,643 treatment is due in part to decreases in Cu,Zn-SOD based on the migration of purified Cu,Zn-SOD. The analysis of

gemfibrozil and DBP-treated animals using the activity gels (not shown) did not demonstrate observable differences in total SOD activity as assayed by cytochrome C assay (Fig. 1).

To determine if DT-diaphorase is altered following PP treatment, we analyzed whole-liver homogenates for dicumarol-sensitive enzyme activity involving the 2-electron reduction of
ability to mediate species-specific hepatocarcinogenesis by a significant decrease only in rats. or Wy-14,643 treatment, whereas DBP treatment resulted in copherol levels were observed in both species with gemfibrozil activity would result in either an increased or decreased capac- DT-diaphorase activity seen with low-dose treatment of gemfibrozil was not apparent in the high dose, which confounds the biological significance. Treatment of rats with DBP resulted in nonsignificant decreases in activity at 90 days. Alternatively, hamsters were not affected by DBP and only sporadic decreases in activity were observed following Wy-14,643 treatment. In addition, higher levels of DT-diaphorase activity were observed in the hamster at all time points examined. This increased DT-diaphorase activity seen with low-dose treatment of gemfibrozil was not apparent in the high dose, which confounds the biological significance. Treatment of rats with DBP resulted in nonsignificant decreases in activity at 90 days. Alternatively, hamsters were not affected by DBP and only sporadic decreases in activity were observed following Wy-14,643 treatment. In addition, higher levels of DT-diaphorase activity were observed in the hamster (mean control DT-diaphorase activity was 16.81 and 40.33 nmol/min/mg protein for rats and hamsters, respectively).

DISCUSSION

Hepatic ascorbic acid (Table 1) and α-tocopherol (Table 2) concentrations were analyzed to determine the status of small molecular weight antioxidants in both rats and hamsters treated with any of the 3 PPs. Few significant changes were observed in total hepatic ascorbic acid content in rats, although DBP generally increased ascorbic acid in hamsters at all time points and Wy-14,643 generally increased ascorbic acid at 6 and 34 days; only Wy-14,643 treatment for 34 days resulted in a significant increase (~2.5-fold). Significant decreases in α-tocopherol levels were observed in both species with gemfibrozil or Wy-14,643 treatment, whereas DBP treatment resulted in significant decreases only in rats.

PPs have been studied over the past several decades for their ability to mediate species-specific hepatocarcinogenesis by a nongenotoxic mechanism (Lake, 1995; Reddy and Lalwai, 1983). Accordingly, although neither they nor their metabolites bind to DNA, their ability to increase peroxisomal enzyme activity with concomitant increased H2O2 production has been hypothesized to mediate their carcinogenic effect (Lake, 1995; Lake et al., 1990; Rao and Reddy, 1991). Differences in detoxifying ROS may explain PPs’ species-specific effects, as ROS not only damage cells but are potent second messengers affecting numerous transcription factors and ultimately the transcription of their target genes (Dalton et al., 1999). The objective of this study was to address whether antioxidant vitamins or enzymes, which facilitate the removal of ROS, are modulated differently in hamsters, a less responsive species, than in rats, a responsive species, following PP administration.

PPs can induce the activity of cytochrome P450s 4A1 and 4A6 and increased P450 activity has been hypothesized to increase intracellular O2− and H2O2 (Bondy and Naderi, 1994; Kappus, 1987; Makowska et al., 1992; Starke et al., 1997). Increased O2− not removed by the action of SOD may result in damage to cellular macromolecules directly, or can interact with H2O2 in the Haber-Weiss reaction, producing the most reactive ROS, HO' (Kappus, 1987; Miichels et al., 1994). In this study, short-term (6 days) treatment with DBP increased SOD activity in both species. On the other hand, SOD was decreased in both species by Wy-14,643 at all time points and in rats treated with DBP for 90 days. Decrease hepatic or plasma SOD activity following treatment with other PPs has been previously reported in both rats and mice (Cai et al., 1995; Dhaunsi et al., 1994; Rivero et al., 1994). Opposing these results, increased SOD following PP treatment has also been reported (Arnaiz et al., 1995; Becuwe et al., 1999; Glaueur et al., 1992). Combining both the cytochrome C assays with NBT activity gels, it can be concluded that the decreased activity observed following PP treatment herein was due in part to decreases in Cu,Zn-SOD activity. This decreased SOD ac-

FIG. 2. SOD activity gels. Pooled (n = 5) 100 µg samples of sonicated homogenates were electrophoresed on a native polyacrylamide gel followed by analysis of SOD activity as described in Materials and Methods. After gels were exposed to light, cleared areas inhibiting the reduction of NBT were considered to contain SOD. The color in the gel was inverted for ease of reading. Time of treatment is listed in days and Species:Compound are abbreviated as follows: rat (R), hamster (H), and Wy-14,643 (WY). The numbers 1, 2, and 3 indicate no dose, low dose, and high dose, respectively. Arrows (top and bottom) indicate the migration of Mn-SOD (Mn) and Cu,Zn-SOD (Cu) in rats (r) and hamsters (h). *Purified Cu,Zn-SOD (derived from Bovine erythrocytes); **purified MnSOD (E.coli) as described in Materials and Methods.
activity may be due to decreased protein as has been reported following ciprofibrate treatment (Dhaunsi et al., 1994). The role of PPARα in mediating these events is unknown, but it should be mentioned that a PPRE has been identified in the rat Cu,Zn-SOD gene and transient transfection experiments have demonstrated induction of this element with arachidonic acid (Yoo et al., 1999). Additionally, a positive correlation between Cu,Zn-SOD and PPARα mRNA has been observed (Inoue et al., 1998). Furthermore, there is evidence that Cu²⁺ metabolism is altered in PP-treated rats, affecting ceruloplasmin oxidase activity and the mRNA for ceruloplasmin (Eagon et al., 1999). How these observations correlate with the decreased SOD activity observed herein is unknown.

Numerous recent studies have speculated about the involvement of DT-diaphorase in carcinogenesis and oxidative stress (Rauth et al., 1997). The endogenous role of this mainly cytosolic enzyme may involve vitamin-K metabolism, although recent data have clearly demonstrated its role in reducing α-tocopherol quinone to α-tocopherol hydroquinone (Nakamura and Hayashi, 1994; Siegel et al., 1997) and maintaining reduced coenzyme Q (Beyer et al., 1998). Furthermore, there is evidence that Cu²⁺-induced superoxide anion is crucial in mediating these events is unknown, but it should be mentioned that a PPRE has been identified in the rat Cu,Zn-SOD gene and transient transfection experiments have demonstrated induction of this element with arachidonic acid (Yoo et al., 1999). Additionally, a positive correlation between Cu,Zn-SOD and PPARα mRNA has been observed (Inoue et al., 1998). Furthermore, there is evidence that Cu²⁺ metabolism is altered in PP-treated rats, affecting ceruloplasmin oxidase activity and the mRNA for ceruloplasmin (Eagon et al., 1999). How these observations correlate with the decreased SOD activity observed herein is unknown.

TABLE 1
Effects of Peroxisome Proliferators on Hepatic Ascorbic Acid Concentration in Male Sprague-Dawley Rats and Male Syrian Hamsters

<table>
<thead>
<tr>
<th>Compound</th>
<th>Days</th>
<th>ppm</th>
<th>Rat ppm</th>
<th>Hamster ppm</th>
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</thead>
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<tr>
<td>DBP</td>
<td>6</td>
<td>0</td>
<td>0.24 ± 0.03</td>
<td>0.32 ± 0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5000</td>
<td>0.20 ± 0.03</td>
<td>0.54 ± 0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20,000</td>
<td>0.25 ± 0.03</td>
<td>0.66 ± 0.14</td>
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<tr>
<td></td>
<td>34</td>
<td>0</td>
<td>0.21 ± 0.02</td>
<td>0.16 ± 0.02</td>
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<td>5000</td>
<td>0.24 ± 0.03</td>
<td>0.19 ± 0.05</td>
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<td>20,000</td>
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<td>90</td>
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<td>0.30 ± 0.04</td>
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<td>0.18 ± 0.02</td>
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<td>0.15 ± 0.04</td>
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Note. Results are expressed as mean (mg/g liver) ± SEM; n = 4–5.
*Significantly different from controls, p < 0.05 as determined using Bonferroni analysis.
Effects of Peroxisome Proliferators on Hepatic \( \alpha \)-Tocopherol Concentrations in Male Sprague-Dawley Rats and Male Syrian Hamsters

<table>
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<th>Rat ppm</th>
<th>Hamster ppm</th>
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<td>DBP</td>
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<td>5000 3.13 ± 0.13</td>
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<td>Gemfibrozil</td>
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<td>5000 3.14 ± 0.25</td>
<td>20000 1.94 ± 0.15</td>
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</table>

Note. Results are mean (\( \mu \text{g/mg protein} \)) ± SEM; \( n = 4-5 \).

*Significantly different from controls, \( p < 0.05 \) as determined using Bonferroni analysis.

published data that DT-diaphorase activity varies with sex, strain, and species (Horie, 1990). Rats had decreased DT-diaphorase activity following Wy-14,643 treatment across all time points examined, indicating that they may have a decreased ability to defend against lipid peroxidation or quinone redox cycling. Only short-term (6-day) treatment of hamsters with Wy-14,643 resulted in significant decreases in DT-diaphorase activity. Decreased DT-diaphorase activity has been reported previously in PP-treated mice and opposing effects (increased DT-diaphorase) have been reported in PP-treated mice (Cai et al., 1995; Glaubert et al., 1992; Sohlenius et al., 1992, 1993). Interestingly, gemfibrozil treatment increased DT-diaphorase activity in rats at the 2 earlier time points, indicating the effect on this enzyme may be compound-specific. Although the role of DT-diaphorase in vitamin K metabolism is still underdetermined, it is interesting that survival of Wy-14,643-treated rats was increased to control levels by supplementing the diet with vitamin K (Hurt et al., 1997). These data suggest more experimentation is needed to determine if these 2 observations are related, and how important a role DT-diaphorase has in both survival and carcinogenesis with regard to PPs.

\( \alpha \)-Tocopherol and ascorbic acid are known to combat ROS in a synergistic manner (Halpner et al., 1998). Although no conclusive significant changes were observed with ascorbic acid, depletion of \( \alpha \)-tocopherol was apparent following PP treatment. Moreover, this depletion was neither compound-specific nor species-specific. The data herein agree with previous reports concerning the depletion of \( \alpha \)-tocopherol by PPs (Conway et al., 1989; Glaubert et al., 1992; Lake et al., 1989). The underlying mechanism may be related to one or more circumstances. As has been proposed by Arnaiz et al. (Lores Arnaiz et al., 1997), hypolipidemic drugs obviously affect lipoprotein transport and this would affect the uptake of tocopherols that are transported the same as fatty acids (Drevon, 1991). Although \( \alpha \)-tocopherol is clearly reduced whereas ascorbic acid content is not, in these animals, this may not be indicative of the situation that occurs in humans since they, unlike these rodents, are unable to synthesize ascorbic acid (Johnson, 1979). Nonetheless, ascorbic acid inhibits the loss of \( \alpha \)-tocopherol by restoring it to its reduced form following interaction with peroxide radicals (Halpner et al., 1998). In addition, ascorbic acid serves to spare \( \alpha \)-tocopherol by interacting with peroxyl radicals as well as its ability to inhibit DNA adduction due to ROS (Barja et al., 1994; Halpner et al., 1998). However, in the rodent model, increased ascorbic acid with decreased \( \alpha \)-tocopherol may render cells susceptible to the "pro-oxidant" effects of ascorbic acid (Bogaards et al., 1992; Foliot and Beaune, 1994).

In summary, these results only partially support the hypothesis that these antioxidants or antioxidant enzymes are differentially modified in rats and hamsters. DT-diaphorase is clearly altered differentially: hamsters have a much higher basal level of this enzyme, and it is lowered in rats but not in hamsters by Wy-14,643, the most efficacious of these peroxisome proliferators at inducing hepatocellular carcinomas in rats and mice. For SOD, ascorbic acid, and \( \alpha \)-tocopherol, there are minor species differences after peroxisome-proliferator administration, but the overall effects appear to be similar. Accordingly, although DT-diaphorase is modified differentially, other mechanisms additionally must be considered, such as modification of genes containing peroxisome-proliferator response elements (PPREs) (Corton et al., 2000), changes in DNA synthesis and apoptosis (Roberts et al., 2000), and changes in cell to cell communication (Isenberg et al., 2000), among others.

ACKNOWLEDGMENTS

We are grateful to Drs. Bernhard Hennig and Larry Robertson, University of Kentucky, for use of their spectrophotometer and HPLC, respectively. This research was funded by NIH grant ES09771 and by the Kentucky Agricultural Experiment Station. M.L.O. and T.P.T. were supported by NIEHS Training Grant T32 ES07266. M.L.O. was also supported by the Dissertation Year Fellowship from The Graduate School, University of Kentucky.
REFERENCES


