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THE EFFECTS OF α-TOCOPHEROL AND WATER-SOLUBLE ANALOGUES ON LIVER MICROSOMAL LIPID PEROXIDATION

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Lipid peroxidation has been suggested to play a key role in many cases of drug-induced tissue injury (1). Antioxidants are often used to evaluate whether a drug exerts its effects via lipid peroxidation "in vivo" and "in vitro". α-Tocopherol is thought to be the most important chain breaking lipophilic antioxidant (2), but because of its hydrophobic nature it is a weak inhibitor of lipid peroxidation in some systems "in vitro" due to its slow rate of penetration into membrane dispersed in aqueous suspensions. In consequence, we have used water-soluble analogues of Vitamin E, Trolox C (Hoffman la Roche) and TPGS (Eastman Kodak; α-tocopherol polyethylen glycol 1000 succinate) to clarify the low apparent antioxidant activity of α-tocopherol in aqueous suspensions of liver microsomes "in vitro".

METHODS

Washed liver microsomes were prepared as described previously (3) from adult male Wistar rats. The incubations were carried out in a shaking water bath at 37°C. Incubations were as follows:

1) NADPH/CCl_4 system: Microsomes (2 mg/ml) were usually incubated for 15 min in an NADPH-generating buffered (pH 7.4) system. CCl_4 was added at a final concentration of 6.9 mM (3). In some cases a post-mitochondrial supernatant fraction was used and supplemented with glucose-6- phosphate and $NADP^+$.

2) Cumene hydroperoxide system: Microsomes (1.5 mg/ml) were incubated for 15 min with cumene hydroperoxide (100 μM) (4).

3) Iron-cysteine system: Microsomes (1.5 mg/ml) were incubated for 15 min with $FeSO_4$ (5μM) and cysteine (500 μM) (5).

After incubations of the above systems, TBA-reactive materials were assayed as described in ref.3. Covalent binding was determined as per (6). Cytochrome P-450 was determined by its reduced CO-binding spectrum. The pulse radiolysis technique was performed as in (7).

RESULTS

Table 1 shows the effects of α-tocopherol and Trolox C on the NADPH-CCl_4 and iron-cysteine systems. Similar results have been obtained in the cumene hydroperoxide system (data not shown). Trolox C was not efficient in protecting cytochrome P-450 from CCl_4-dependent damage (data not included); this suggests that covalent binding of CCl_4-metabolites may play a role in the destruction of cytochrome P-450. Pulse radiolysis studies have shown that Trolox C acts as an effective free radical scavenger. Addition of TPGS was much more effective against CCl_4 induced lipid peroxidation than the addition of α-tocopherol; it was found to be more effective when the cytosolic fraction is present and with extended times of incubation (data not shown).

Table 1: Carbon tetrachloride (a) and Iron-cysteine (b) induced lipid peroxidation.

Antioxidant Conctn.	% change from control (no antioxidant) α-tocopherol*		α-tocopherol/SDS**	Trolox C	
	(a)	(b)	(b)	(a)	(b)
100 μM	-13	+7	-70	-79	-98
50 μM	-10	+3	-64	-75	-93
25 μM			-52	-65	-63
10 μM			-27	-45	-40

* added in ethanol of final concentration 5.7 mM (a), 11.4 mM (b)

** final concentration of SDS was 0.5 mM

Discussion

These observations indicate that the inefficacy of α-tocopherol in aqueous systems is not because it is a weak free radical scavenger, but because when dissolved in ethanol it does not easily integrate into the microsomal membrane. However, the addition of a detergent (SDS) disperses α-tocopherol and allows the vitamin to act more powerfully as an antioxidant. Since the detergent disrupts the membrane this procedure can not be used when the intact microsomal electron transport chain is required. Pulse radiolysis studies of Trolox C have shown that the phenoxy radical spectrum is identical to that obtained with α-tocopherol and it may act with the same mechanism. TPGS requires an esterase to release Vitamin E and thus it is more effective when the cytosolic fraction is present and with prolonged times of incubation. This gradual release of α-tocopherol during the hydrolysis of the ester may prevent its aggregation, and permit its incorporation into the membrane.

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