Protection by Antioxidants Against Ozone Toxicity in Mice

Ozone is a major atmospheric pollutant whose primary target is the respiratory tract. With high concentrations of ozone and prolonged exposures, pulmonary edema, hemorrhage and death ensue. The mechanisms of ozone toxicity have been extensively studied and free radical formation and injury, resulting from the interactions of ozone with water and organic substances[1], has been incriminated[2–4]. Chromosome aberrations have been induced in *Vicia faba* cells by both ozone and X-rays; these effects were additive[3]. A variety of thiol radioprotective agents also protected mice against ozone injury[4].

We report here on protection by antioxidants against ozone toxicity in mice. These experiments were designed to test further the role of free radical injury in ozone toxicity and also to explore the practicality of chemical protection against the toxic effects of ozone and oxidant atmospheric pollutants.

A total of 16 antioxidants (Table) were administered to Swiss mice (ICR/Ha), 6–8 weeks old, and of 20–22 g weight by 4 daily i.p. injections of 0.1 ml solutions or suspensions in saline or tricaprylin. Dosage of antioxidants, as determined by preliminary toxicity tests, was based on maximum sub-lethal levels. 1 h following the last injection of antioxidant, equal numbers of test and control mice were exposed to ozone for 4 h in 2 stainless steel chambers, of 112 liters capacity, supplied with ozonized air at flow rates of 10 liters/min. Ozone was produced by passing filtered compressed air through a neon tube generator to each chamber. Ozone concentrations, controlled by varying the voltage supply of the generator, were measured at 15 min intervals by the neutral potassium iodide method[5]. Ozone concentrations were maintained at 9–11 ppm, producing mortalities of 80–90% in untreated controls. Experiments were replicated on a minimum of 2 occasions, using groups of 10–20 mice in each experiment.

Protection by antioxidants against ozone toxicity was measured by the reduction in mortality of test in relation to control mice. As can be seen, of the 16 antioxidants tested, significant protection was produced by 6, particularly AHQ, primaquin and EMQ. The practical implications of such protection should be further explored.

Antioxidants have been shown to protect against photodynamic toxicity[6–8] and CCl_4 hepatotoxicity[9], in both of which the role of free radicals has been incriminated, dietary liver necrosis[10], and high pressure oxygen injury[11]. There appears to be no correlation between these effects and protection by antioxidants against ozone toxicity.

Effects of antioxidants on the survival of mice exposed to ozone

Antioxidant Chemical name	Trade name	Dose mg	Ozone ppm	No. of control and test mice	% Mortality Controls	% Mortality Test	Protection
2,5-*bis*(1,1-dimethyl propyl)hydroquinone(*tert.* amyl hydroquinone)	AHQ	15	10.6–11.1	40	78	23	55 (p 0.01)
8-(4-Amino-1-methylbutylamino)-6-methoxyquinoline	Primaquin	4	10.0–12.6	80	74	20	54 (p 0.005)
6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline	EMQ	20	10.2–10.4	40	80	33	47 (p 0.01)
Mixture of 2 and 3 *tert.* butyl-4-methoxyphenol	BHA	30	9.3–10.3	50	58	23	35 (p 0.05)
4,4′-(2,3-Dimethyltetramethylene)-dipyrocatechol	NDGA	10	10.5–10.8	40	76	43	35 (p 0.05)
Ascorbic acid	–	50	9.6–10.4	70	66	40	26 (p 0.10)
tert.-Butylhydroquinone	TBHQ	10	8.8–10.4	60	55	34	21 (n.s.)
n-Propylgallate	–	6	9.7–10.1	60	83	68	15 (n.s.)
4,4′-Diphenyl-*p*-phenylenediamine	DPPD	40	9.0–11.8	60	84	69	15 (n.s.)
3,3′,4′,5,7-Pentahydroxyflavone	Quercetin	5	9.9–12.0	60	89	77	12 (n.s.)
Sodium selenite	–	0.01	8.8–10.3	50	66	58	8 (n.s.)
α-Tocopherol	–	20	8.1–10.4	40	54	50	4 (n.s.)
Tetraethylthiuramdisulfide	Antabuse	3	8.9–11.5	80	51	49	2 (n.s.)
D-α-tocopheryl acetate	–	40	8.9– 9.2	40	25	25	0 (n.s.)
4-Dimethylaminoazobenzene	Butter yellow	4	9.1–11.7	70	67	70	−3 (n.s.)
3,7-*bis*(dimethylamino)phenazathionium chloride	Methylene blue	2	9.2–11.5	40	60	65	−5 (n.s.)

Zusammenfassung. 16 Antioxidantien wurden auf ihre Schutzwirkung gegen Ozontoxizität in Mäusen geprüft. AHQ, Primaquin und EMQ waren hochwirksam, BHA, NDGA und Ascarbinsäure mässig wirksam, und die übrigen zeigten keinen nennenswerten Effekt.

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