REVIEW

Folia Microbiol. **52** (6), 585–592 (2007) **http://www.biomed.cas.cz/mbu/folia/**

Cell-Protective and Antioxidant Activity of Two Groups of Synthetic Amphiphilic Compounds – Phenolics and Amine *N*-Oxides

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Received 14 August 2007
Revised version 29 October 2007

ABSTRACT. Two classes of newly synthesized amphiphilic compounds, phenolic antioxidants ("phenolics") and *N*-oxides exert *in vivo* antioxidant effects on live *S. cerevisiae* cells. Both groups have low toxicity, phenolics being more toxic than *N*-oxides and compounds with a longer alkyl chain having higher toxicity than those with a shorter alkyl chain. Phenolic antioxidants protect yeast cells exposed to the superoxide producer paraquat and peroxyl generator *tert*-butylhydroperoxide better than *N*-oxides at 3-fold higher concentration. Both types of antioxidants enhance the survival of pro-oxidant-exposed cells of *S. cerevisiae* mutants deficient in cytosolic and/or mitochondrial superoxide dismutase and could be good compounds which mimic the role of superoxide dismutases. The results of measurement of antioxidant activity in an *in vitro* chemiluminescence test differ from the results obtained *in vivo* with *S. cerevisiae* superoxide dismutase mutants. In contrast to their action on live cells, phenolics are less effective than *N*-oxides in preventing lipid peroxidation of an emulsion of lipids isolated from *S. cerevisiae* membranes.

Abbreviations

AAPH	1,1′-azobis(3-amidinopropane) dihydrochloride
IC_{50}	inhibitory concentration (50 % reduction of growth)
LD_{50}	lethal dose (50 % killing)
MC_{crit}	critical micelle concentration
NBD-PE	*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt ('nitrobenzoxadiazole dipalmitoylphosphatidylethanolamine')
PDR	pleiotropic drug resistance
ROS	reactive oxygen species
SOD	superoxide dismutase (EC 1.15.1.1)
TBHP	*tert*-butylhydroperoxide
TBRS	thiobarbituric acid-reactive substances
TMA-DPH	*N*,*N*,*N*-trimethyl-4-(6-phenyl-1,3,5-hexatrien-1-yl)phenylammonium 4-toluenesulfonate ('trimethylammonio-diphenylhexatriene')

CONTENTS

1 INTRODUCTION

The properties of ROS are crucial in the type, course and outcome of oxidative stress. Generally, the prime agents responsible for causing oxidative stress are primary and secondary ROS. Primary oxidants formed as products of energy metabolism include the superoxide anion, hydrogen peroxide and hydroxy radical.

Secondary oxidants are generated as products of reactions of primary ROS with major cell constituents – DNA, lipids and proteins. In living cells, production of ROS and induction of oxidative stress can be triggered by many physical and chemical factors (radiation, 'heavy metals', *etc*.) (Sigler *et al*. 1999).

To keep optimum oxidative–antioxidative balance *vis à vis* oxidative assault, cells possess a number of enzymic and non-enzymic antioxidative systems whose functions are frequently very close or overlap. Thus, although the superoxide anion is not an especially potent oxidant, it is neutralized by two or three types of superoxide dismutases; this reflects its harmful potential for the cell that is due to a number of reactions of superoxide that can yield other, highly deleterious ROS. Thus, in a reaction with hydrogen peroxide, superoxide anion produces a very active hydroxy radical that is capable of oxidizing almost all cellular constituents and can cause, *e.g*., peroxidation of lipids that is highly harmful for the cell (Sigler *et al*. 1999).

In situations involving pathological conditions or massive oxidative assaults, the activity of natural antioxidants in cells may not be sufficient to neutralize the free radical attack; synthetic antioxidants are therefore very useful in these cases. In addition to having strong antioxidative properties and being able to scavenge a wide range of reactive species, these antioxidants possess a number of other features, such as non-toxicity and activity in both lipophilic and hydrophilic compartments, and the ability to function in both intracellular and extracellular environments. Natural antioxidants, such as β-carotene, sometimes exert a pro-oxidative activity at high concentrations (Łukaszewicz *et al*. 2004). It is therefore important to have antioxidants free of pro-oxidative properties and possessing antioxidant effects at safely low concentrations. Amphiphilic properties have been recognized to be important for antioxidants that can act, *e.g*., on different regions in biological membranes (Beckman and Ames 1998).

The reactions which maintain the oxidative–antioxidative balance in the cell are often interdependent. It is therefore not always simple to investigate these processes in live cells. The currently available methods for quantification of ROS production using spectroscopic and/or fluorescent probes in cellular systems often do not provide reliable pictures of what is in fact happening in the cells (Bartosz 2006). In addition, experiments with ROS generation, interconversions and effects performed in physical or chemical systems and making use of chemiluminescence, spectrophotometry, colorimetric reactions, *etc*., often provide data which do not exactly correspond with the processes and reactions in the cell. It is therefore important to compare *in vitro* and *in vivo* methods for obtaining an overview of antioxidative properties of various compounds. In agreement with this trend, we compared the *in vivo* effects of the above antioxidants on live cells of *Saccharomyces cerevisiae* and, in parallel, their *in vitro* action on isolated *S. cerevisiae* membrane lipids and their antioxidative efficiency in a luminol-based chemiluminescence test.

The yeast *S. cerevisiae* represents a eukaryotic system highly suitable for testing the activity of antioxidants. It is a standard object of genetical manipulations and expression of proteins and, unlike with animals, work with it does not involve ethical issues.

Natural phenolic antioxidants ("phenolics") have long been known to be efficient in protecting cells from oxidative stress, especially from lipid (L) peroxidation or the harmful influence of superoxide anion. Tocopherol (T; vitamin E) is one of the best known natural phenolic antioxidants that terminates the chain peroxy radical as a donor in the reaction (*1*):

$$LOO\bullet + TOH = LOOH + TO\bullet \qquad (1)$$

Tocopherol is a good chain-breaking antioxidant, because the TO• radical formed in the reaction with hydroperoxides is too weak to initiate reductive reactions (Buettner 1993). Moreover, tocopherol can undergo reactivation in a reaction with ascorbate (Packer *et al*. 1979; Hiramoto *et al*. 2002). Due to its hydrophobic properties tocopherol does not react with superoxide immediately at the site of its generation in the aqueous cytosol, but it can prevent membranes from the action of free radicals produced in reactions of superoxide with other cell molecules.

Nitric oxide •NO is a natural sacrificial antioxidant which is utilized in reaction (*2*):

$$LOO\bullet + \bullet NO = LOONO \qquad (2)$$

2 SYNTHETIC AMPHIPHILIC ANTIOXIDANTS

Synthetic nitroxides bearing an unpaired electron on the –NO group included in an aliphatic or aromatic ring system are good antioxidants that react with a wide range of free radicals (alkyl, peroxyl, alkoxyl, superoxide, *etc*.) (Stipa 1997; Damiani *et al*. 1999, 2001). The best known described nitroxides are the cyclic synthetic compounds Tempo (2,2,6,6-tetramethylpiperidine-1-oxyl) and Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) (Samuni and Barenholtz 2003).

Two groups of amphiphilic antioxidants – phenolic compounds and *N*-oxides with controlled length of alkyl chain – were recently synthesized and tested (Krasowska *et al*. 2001, 2006). They were found to be efficient antioxidants (Krasowska *et al.* 2006, 2007).

2.1 Phenolics

Quaternary ammonium salts with a phenol substituent functioning as an antioxidant synthesized in our laboratory belong to three groups (PYA, PYE and PPA) (Fig. 1). Their synthesis has been described by Krasowska *et al*. (1999, 2001).

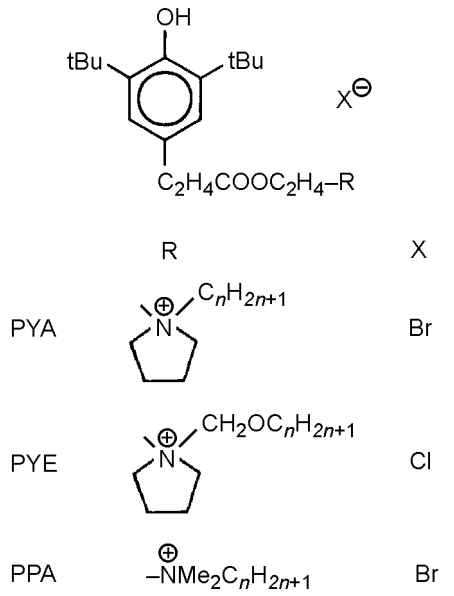

Fig. 1. Structures of phenolic antioxidants: quaternary ammonium salt derivatives of 3,5-di-*t*-butyl-4-hydroxydihydrocinnamic acid 2-aminoethyl esters: *N*-(*n*-alkyl) pyrrolidinium bromides (PYA), *N*-(*n*-alkoxymethyl)-pyrrolidinium chlorides (PYE), and *N*-(*n*-alkyl)-*N*-dimethylammonium bromides (PPA); n = 12 or 16.

Table I. Effect of 10 μmol/L phenolic and 30 μmol/L *N*-oxide antioxidants on survival (%) of *S. cerevisiae* parent strains SP4 and US50-18C

Antioxidant	SP4	US50-18C
Phenolics		
12-PYA	79 ± 2.1	76 ± 1.5
16-PYA	55 ± 1.6	51 ± 2.4
12-PYE	76 ± 1.8	79 ± 3.3
16-PYE	71 ± 2.7	69 ± 2.0
12-PPA	73 ± 2.3	70 ± 1.3
16-PPA	83 ± 2.1	75 ± 5.2
***N*-Oxides**		
11-EDA	94 ± 2.7	99 ± 2.4
13-EDA	83 ± 3.1	85 ± 8.4
11-PDA	71 ± 2.4	75 ± 1.5
13-PDA	60 ± 1.8	53 ± 3.2
12-MPDA	105 ± 2.1	108 ± 2.8
14-MPDA	106 ± 1.6	102 ± 1.6
12-MEDA	101 ± 2.4	99 ± 10.5
14-MEDA	99 ± 2.6	95 ± 5.5

These compounds have a low toxicity not only in *S. cerevisiae* (Table I) but also in higher organisms; the LD_{50} for the representative compound 16-PPA administered orally to rats was 1722 mg/kg body mass; when injected hypodermally, it was >2000 mg/kg. 16-PPA was found to moderately irritate the rabbit skin (*Toxicological Report* 2000).

2.2 N-Oxides

The synthesis of amine-*N*-oxides of the EDA, PDA, MEDA and MPDA series (Fig. 2) was described by Krasowska *et al*. (2006, 2007).

These compounds have a medium or low toxicity for both *S. cerevisiae* (Table I) and for higher organisms; LD_{50} for the representative compound 14-MEDA administered orally to rats was 1000 mg/kg body mass; when injected hypodermally, the LD_{50} was 2000 mg/kg. 14-MEDA was found to moderately irritate the rat skin but it did not irritate rabbit skin (*Toxicological Report* 2006).

3 EFFECT OF ANTIOXIDANTS ON *S. cerevisiae* PARENT STRAINS

Because of their minor toxicity for humans and considerable antibacterial properties, *N*-oxides have long been used as detergents and components of cosmetics. They have recently been proposed to be active as bioreductive drugs (McKeown *et al*. 2007). On the other hand, phenolic antioxidants (natural and synthetic) could be potentially toxic for humans; for instance, some flavonoids interact with drug-metabolizing enzymes or have apoptosis-inducing properties (Galati and O'Brien 2004).

We examined the effect of both groups of antioxidants on the survival of SOD-competent *S. cerevisiae* strains SP4 (MATα, leu, arg) (Biliński 1979) and US50-18C (MATα, PDR1-3, ura3, his1) (Balzi 1987). Our aim was to establish if a mutation in the gene encoding the transcriptional factor PDR1 that affects among other things the performance of the detoxication PDR efflux pumps in *S. cerevisiae* could also influence the sensitivity of *S. cerevisiae* strains to our antioxidants. The mutation was found to have no effect.

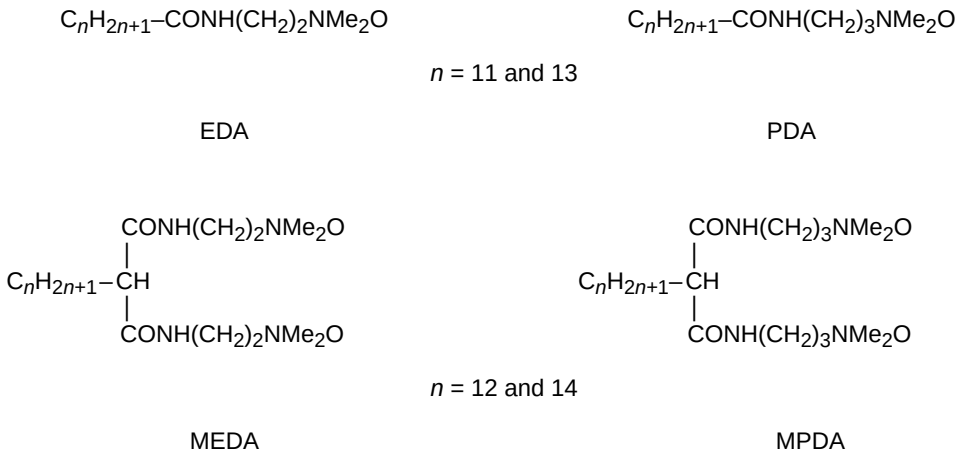

Fig. 2. Structures of tested alkane mono- and di-*N*-oxides; 2-(alkanoylamino)ethyldimethylamine-*N*-oxides (EDA), 3-(alkanoylamino)propyl-dimethylamine-*N*-oxides (PDA), 1,1-bis{[2-(*N*,*N*-dimethylamino)ethyl]amido}alkane-di-*N*-oxides (MEDA), and 1,1,-bis{[3-(*N*,*N*-dimethylamino)propyl]amido}alkane-di-*N*-oxides (MPDA).

N-Oxides were found to be mostly less toxic than phenolics even at a 3-fold higher concentration. The toxic effects of both groups of antioxidants varied with the length of the alkyl chain; in most cases compounds with 11 or 12 carbon atoms in the alkyl chain tended to have lower toxicity than compounds with 14- or 16-membered alkyl chain, with the exception of MPDA class compounds, which were nontoxic and even slightly stimulated cell growth (Krasowska *et al.* 2007). The most toxic seemed to be two groups of antioxidants, PYA (phenolics) and PDA (*N*-oxides) (Krasowska *et al.* 1999, 2006, 2007) (Table I).

3.1 Protection of yeast cells against hydrophilic and amphiphilic oxidants

The amphiphilic oxidant TBHP strongly decreases the colony-forming ability of *S. cerevisiae* strain already after two generations (Krasowska *et al.* 2002) and causes strong TBRS production in intact cells (Krasowska *et al.* 2000).

Paraquat, a hydrophilic oxidant, has no toxic effect on parental strain US 50-18c and its Δ*sod1* and Δ*sod1* Δ*sod2* mutants with deletions in superoxide dismutases under aerobic conditions except at high concentration (100 μmol/L). Under anaerobic conditions it was toxic for Δ*sod1* and Δ*sod1* Δ*sod2* mutants already at 50 μmol/L (Krasowska *et al*. 2003). Under microaerophilic conditions 100 μmol/L of both paraquat and TBHP were almost nontoxic for the parental strain, but highly toxic for Δ*sod1* and Δ*sod1* Δ*sod2* mutants (Table II). The Δ*sod2* mutant was relatively resistant to paraquat but not to TBHP (Krasowska *et al*. 2006).

Table II. Survival (%) of *S. cerevisiae* US50-18c[a] parent strain and its Δ*sod* mutants in the presence of 100 μmol/L pro-oxidants paraquat and TBHP

Strain	Control	Paraquat	TBHP
US50-18c (JS-C66)	100 ± 3.5	80 ± 6.5	26 ± 3.1
Δ*sod1* (JS-C50)	100 ± 4.1	10 ± 2.5	6 ± 1.2
Δ*sod2* (JS-C55)	100 ± 3.9	73 ± 5.4	15 ± 2.0
Δ*sod1* Δ*sod2* (JS-C128)	100 ± 2.5	3 ± 0.4	2 ± 0.2

[a]Strain designations used in some of the previous publications are given in brackets.

Though more toxic (Table I), phenolic antioxidants protect yeast cells against reactive oxygen species better than *N*-oxides at 3-fold higher concentration (Table III). An exception is the paraquat-exposed cells

of the Δ*sod2* mutant deficient in mitochondrial Mn-SOD, in which phenolics have a weaker growth-enhancing effect than *N*-oxides. In terms of individual ROS, both phenolics and *N*-oxides were more efficient against peroxy radicals generated by TBHP than against superoxide anion produced by paraquat (Table III).

Table III. Efficiency of antioxidants (10 μmol/L phenolics and 30 μmol/L *N*-oxides) against paraquat and TBHP (100 μmol/L) in SOD-deleted *S. cerevisiae* strains under microaerophilic conditions measured by survival enhancement (%)

Antioxidant	Δ*sod1*		Δ*sod2*		Δ*sod1* Δ*sod2*	
	paraquat	TBHP	paraquat	TBHP	paraquat	TBHP
Phenolics						
12-PYA	+25 ± 6.1	+92 ± 2.1	+25 ± 3.5	+76 ± 6.5	+9 ± 1.3	+40 ± 2.1
16-PYA	+15 ± 4.3	+69 ± 3.1	+14 ± 4.5	+67 ± 3.4	+6 ± 1.0	+34 ± 3.5
12-PYE	+16 ± 4.0	+91 ± 1.5	+12 ± 2.7	+70 ± 4.5	+7 ± 0.9	+30 ± 3.0
16-PYE	+9 ± 2.7	+30 ± 2.4	+9 ± 3.7	+39 ± 5 4	+5 ± 0.8	+22 ± 2.9
12-PPA	+5 ± 2.4	+93 ± 2.8	+7 ± 4.2	+82 ± 5.0	+4.5 ± 1.7	+36 ± 2.0
16-PPA	+2 ± 1.5	+80 ± 3.5	+15 ± 4.0	+80 ± 3.2	+3 ± 0.3	+28 ± 1.8
***N*-Oxides**						
11-EDA	+3 ± 0.4	+10 ± 0.3	+27 ± 2.0	+6 ± 0.7	+2 ± 0.5	+11 ± 1.9
13-EDA	+1 ± 0.9	+9 ± 0 4	+27 ± 1.3	+4.5 ± 1.3	+3 ± 0.5	+10 ± 0.5
11-PDA	+10 ± 0.6	+2 ± 0.1	+22 ± 3.1	+1 ± 1.0	+1.5 ± 0.2	+21.5 ± 2.1
13-PDA	+7 ± 0.4	+5 ± 0.03	+21 ± 2.5	+3 ± 0.8	+1 ± 0.3	+8 ± 2.0
12-MPDA	–2 ± 0.6	+10 ± 1.5	+27 ± 0.6	0	+5 ± 0 2	+6.5 ± 0.7
14-MPDA	–4 ± 1.0	+3.5 ± 1.4	+20 ± 1.7	–2 ± 2.0	0	+7 ± 1.3
12-MEDA	–3 ± 0.9	+11 ± 0.9	+27 ± 2.5	+4.5 ± 0.6	+5 ± 0.9	+10.5 ± 1.5
14-MEDA	–1 ± 1.1	+11 ± 1.0	+22 ± 4.0	–1 ± 0.4	–1 ± 0.1	+8 ± 1.4

4 INCORPORATION OF SYNTHETIC ANTIOXIDANTS INTO MEMBRANES

Both synthetic phenolics and *N*-oxides incorporate into biological membranes, as was documented for most of them *in vitro* on liposomes (Krasowska *et al*. 2001, 2006). Almost all compounds with a longer alkyl chain were found to incorporate into the membrane lipid matrix deeper than those with shorter chains. Differences in the incorporation of various compounds into the membrane lipids of the parent strain US 50-18c and its different Δ*sod* deletants reflect, in addition, the fatty acid composition of the membrane (Krasowska *et al*. 2006). The most efficient incorporation of the compounds into membrane lipids was observed in the case of the double Δ*sod1* Δ*sod2* mutant.

Kleszczynska *et al*. (2005, 2006) also found various incorporation of some synthetic *N*-oxides into model membranes (DPPC liposomes). The compounds were found to cause hemolysis of erythrocyte membranes and this hemolysing action increased with elongation of the alkyl chain. A similar increase in hemolysis with increasing length of the alkyl chain was found in phenolic amphiphilic antioxidants (Kleszczynska *et al*. 2001).

5 ANTIOXIDANT ACTIVITY *in vitro* DETERMINED BY CHEMICAL METHODS

The *in vitro* antioxidant activity of various compounds against different ROS can be tested by a variety of methods (Bartosz 2006). We used the chemiluminescence test with luminol as the luminescent compound and the hydrophilic oxidant AAPH as the pro-oxidant. AAPH was previously found to have a weak or no effect on *S. cerevisiae*, both parental and Δ*sod* mutants, causing only a temporal inhibition of colony-forming ability (Krasowska *et al.* 1999, 2002). Production of TBRS in intact *S. cerevisiae* cells during a 15-min incubation with AAPH was low in comparison to other yeast species, such as *Rhodotorula glutinis* or *Candida albicans.*

The test revealed differences between the antioxidant activities of the two groups of compounds against peroxy radicals generated from AAPH. With a few exceptions (*viz*. the highly active 12-MEDA and 14-MEDA), *N*-oxides were less efficient than phenolic antioxidants (Table IV). The high antioxidant activity of MEDA against peroxy radicals in the chemiluminescence test, *i.e*. its low IC_{50} value, was at variance with

the results obtained with *S. cerevisiae* Δ*sod* mutants exposed to TBHP-induced peroxy radicals. In contrast, these antioxidants had a very low effect on paraquat (*i.e*. superoxide)-exposed mutant cells (Table III). *N*-Oxides, which had high IC_{50} in the chemiluminescence test, *e.g*., MPDA or PDA, were less active in protecting *S. cerevisiae* cells than phenolic antioxidants which exhibited low IC_{50} in the chemiluminescence test (Tables III and IV).

Table IV. IC_{50} values for phenolic antioxidants and *N*-oxides obtained in chemiluminescence test against AAPH-generated peroxyl radicals

Compound	IC_{50} (μmol/L)
12-PYA	1.92 ± 0.03
16-PYA	1.68 ± 0.20
12-PYE	2.1 ± 0.26
16-PYE	1.90 ± 0.15
12-PPA	5.3 ± 0.95
16-PPA	4.3 ± 1.63
11-EDA	–[a]
13-EDA	–[a]
11-PDA	6040 ± 0.95
13-PDA	43 ± 0.35
12-MPDA	1100 ± 0.05
14-MPDA	600 ± 0.90
12-MEDA	6.0 ± 0.43
14-MEDA	0.12 ± 0.60

[a](–) – not detectable.

The *in vitro* antioxidant activity of the compounds was also checked by determining the TBRS level in lipids extracted from *S. cerevisiae* plasma membranes in their presence. Surprisingly, *N*-oxides were more active in this respect than phenolic antioxidants; di-*N*-oxides had an especially high activity against TBHP-generated peroxy radicals (Table V).

Although the antioxidant activity within the lipid emulsion is strongly dependent on the amphiphilic character of individual compounds, experiments with small unilamellar (SUV) liposomes and fluorescent probes TMA-DPH or NBD-PE reporting on membrane fluidity (Lakowicz 1983; Cambpell and Dwek 1984; Lentz 1988) indicated an effect of the hydrophilic moiety on the ability of the compounds to incorporate into lipids (Krasowska *et al*. 2001, 2006). With phenolics, compounds with 16 carbon atoms in the alkyl chain incorporated less readily than those with 12-membered chains. PYA and PYE compounds incorporated better than PPA ones, which did not influence the fluorescence of any of the two fluorescent probes in liposomes (Krasowska *et al*. 2001). Di-*N*-oxides incorporated less easily than mono-*N*-oxides (Krasowska *et al*. 2006, 2007), apparently due to their bulkier structure. The effect of incorporation into liposomes or lipid emulsion can depend on MC_{crit}. In general, compounds with longer alkyl chains have a lower MC_{crit} than compounds with shorter chains. Except for PPA, which did not affect the cells negatively and

Table V. Percent inhibition of TBHP (100 μmol/L) induced peroxidation (TBRS level) of plasma membrane lipids isolated from individual strains by 30 μmol/L antioxidants

Antioxidant	Δ*sod1*	Δ*sod2*	Δ*sod1* Δ*sod2*
12-PYA	21.4 ± 2.5	19.3 ± 1.7	16.7 ± 2.1
16-PYA	30.8 ± 2.8	16.6 ± 1.0	13.4 ± 1.3
12-PYE	17.7 ± 3.6	15.4 ± 2.1	14.7 ± 0.6
16-PYE	14.2 ± 0.9	12.3 ± 1.2	11.4 ± 1.2
12-PPA	10.8 ± 1.1	7.8 ± 0.8	6.2 ± 0.4
16-PPA	5.5 ± 0.3	4.1 ± 0.5	3.5 ± 0.2
11-EDA	20.0 ± 2.4	14.0 ± 1.2	13.2 ± 4.6
13-EDA	37.3 ± 3.7	22.1 ± 5.2	24.2 ± 7.5
11-PDA	25.4 ± 1.6	20.4 ± 3.4	44.0 ± 2.1
13-PDA	28.1 ± 3.2	31.3 ± 2.5	51.9 ± 3.1
12-MPDA	79.1 ± 1.3	56.4 ± 5.7	86.8 ± 3.6
14-MPDA	45.0 ± 6.1	28.0 ± 8.5	68.0 ± 6.6
12-MEDA	78.0 ± 3.9	67.0 ± 5.8	39.0 ± 6.5
14-MEDA	46.0 ± 6.3	40.0 ± 7.8	19.0 ± 4.7

its incorporation into the membranes could not be reliably determined (Krasowska *et al*. 2001), in experiments with live cells, all antioxidants had a protective effect at concentrations below MC_{crit}, at which the compounds are in a monomeric form (*see*, *e.g.*, Warisnoicharoen *et al.* 2003), and a similar dependence was observed in the chemiluminescence test (Table IV) and with pure lipids (Table V). Antioxidants of the PPA group, which have the lowest MC_{crit} values of all tested compounds (Krasowska *et al*. 2001), and hence a low proportion of the monomeric form, were less active in lipid emulsion (Table V). On the other hand, we did not observe a lower activity of PPA compounds in live cells (Table III) or in the chemiluminescence test when compared to *N*-oxides (Table IV).

6 CONCLUSIONS

Comparison of the two groups of amphiphilic antioxidants mentioned above, *i.e.* phenolics and *N*-oxides recently synthesized in our group, and their antioxidant properties *in vivo* and *in vitro* had the following results: *N*-oxides can act as preventive antioxidants for a wide range of free radicals. The presence of alkyl chain in their molecule lends them amphiphilic properties that enable them to incorporate into the plasma membrane of live cells at different level depending on the length of the alkyl chain. Compounds with more than 12 carbon atoms incorporate deeply into the membrane matrix, presumably tending to disturb the membrane structure. They are therefore more toxic and have lower protective efficiency for live cells. In chemical systems such as the chemiluminescence test, where the membrane-associated effects play no role, longer-chain compounds with lower IC_{50} were more active.

Although phenolic antioxidants do not react immediately with the superoxide anion, all groups of these compounds provided strong protection when the superoxide inducer paraquat was used for producing free radicals in live cells (Table II), and a still higher protection against TBHP. Their protective action on live cells, which is higher than that of *N*-oxides (Table II), can be augmented by their potential for reactivation. In contrast, when oxidant-induced peroxidation of pure lipids is blocked in an experimental system, phenolic antioxidants cannot undergo reactivation and then *N*-oxides are more efficient, especially those with two antioxidant active –NO groups (Table IV). Hence it seems that both groups of antioxidants could act as acceptable compounds that mimic the role of superoxide dismutases. Further investigation of their properties can be important for shedding light on the various mechanisms of action of individual groups of antioxidants, and can lead to their practical use in cosmetics, health care and other fields.

This work was supported by *Polish Committee for Scientific Research* (grant 3 T09B 075 27), by *Wroclaw University* (grant 2483/W/IGIM), Research Center 1M0570 (*Ministry of Education, Youth and Sports of the Czech Republic*), and by the *Institutional Research Concept* of the *Institute of Microbiology* (AV 0Z 5020 0510).

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