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Divergent effects of quinolinic aminoxyls on mitochondrial ultrastructure and localisation in osteosarcoma 143 B cells

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In the present study we have shown that quinolinic aromatic aminoxyls are very efficient in protecting lipids of endoplasmic reticulum membranes against hydroperoxide-induced oxidation. The efficacy of these aminoxyls as protectors of lipids was much higher than the water-soluble 4-OH-TEMPO. We have also shown that QAL causes distinct changes of the morphology of mitochondria: from filamentous to granular enlarged structure via the folding of the former. QAL induces also perinuclear clustering of mitochondria. C-QAL as well as 4-OH-TEMPO treated cells revealed filamentous and scattered pattern of mitochondria. Antioxidant activity of QAL as well as morphological changes of mitochondrial raise the possibility that this drug can affect cell physiology via changes of mitochondrial function.

key words: QAL, C-QAL, 4-OH-TEMPO, oxidative stress, mitochondrial ultrastructure

INTRODUCTION

It is generally accepted that harmful reactive oxygen species (ROS) are constantly formed during cellular metabolism and the integrity of the cell system mostly depends on their removal by antioxidant defences [10]. Recently, stable aminoxyls radicals have been used successfully as general protectors of biological systems against oxidative stress. Antioxidant reactivity of aliphatic piperidine aminoxyls has been ascribed to their efficient trapping of carbon–centred radicals [6] as well as to their superoxide dismutase (SOD) mimic activity [16]. On the other hand lipid soluble quinolinic aminoxyls revealed far more extended reactivity since they react not only with C-centred radicals [5] but also with O-centred radicals, namely alkoxyl [9] and peroxyl radicals [4]. Some studies revealed the potentiality of synthetic antioxidants to change the morphology of cells exposed on them due to proapoptotic signalling [18]. Until now data concerning aromatic aminoxyl effects on cell biology and morphology are largely unknown.

MATERIAL AND METHODS

Cell culture

143 B human osteosarcoma cells (ATCCCRL8303) were grown at 37°C in a humidified atmosphere with 5% CO_2 in Dulbecco's modified Eagles medium (Nissui Co. Ltd, Tokyo, Japan) containing 1 mM pyruvate supplemented with 10% foetal bovine serum and 50 μg/ml kanamycin. The cells were kindly provided by Dr M. Tanaka, Department of Gene Therapy, Gifu

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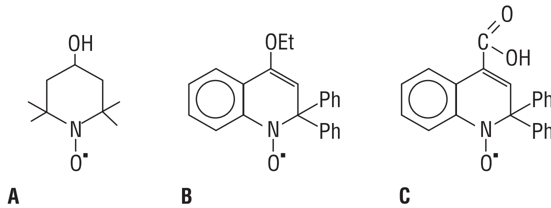


Figure 1. Structures of aminoxyls: **A.** 4-OH-TEMPO; **B.** QAL; **C.** c-QAL.

International Institute of Biotechnology, Yagi Memorial Park, Gifu, Japan.

Treatment of cells with chemicals

Cells were cultured in the presence of chemicals specified below: 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (4-OH-TEMPO) — the final concentration 100 μM; 1,2-dihydro-2,2-diphenyl-4-ethoxy-quinoline-1-oxyl (QAL) — the final concentration 100 μM, 1,2-dihydro-2,2-diphenyl-4-carboxy-quinoline-1-oxyl (c-QAL) — the final concentration 100 μM. All reagents were prepared as 1000x-concentrated stock solutions dissolved in DMSO and stored at –20°C. Aminoxyls were synthesised in our laboratories (Fig. 1).

Staining and visualisation of mitochondria by confocal microscopy

Cells growing on 10 × 10 mm-glass coverslips under various experimental conditions were incubated with 100 nM Mito Tracker Red CMXRos (CMXRos) (Molecular Probes, Inc.) for 30 minutes at 37°C in the atmosphere of 5% CO_2. Cells were then fixed with a fixative containing 2% glutaraldehyde and 2% formaldehyde in PBS for 30 minutes at room temperature, washed in PBS and permeabilized with 0.1% Triton X-100 in PBS for 10 minutes at room temperature followed by 4 washes in PBS. Coverslips with cells were placed on glass slides in a Perma Fluor mounting solution (Immunon, Pittburg, PA), and analyzed by a confocal scanning system MicroRadiance (Bio-Rad, UK), equipped with an Argon ion laser and mounted on a light microscope Eclipse 600 (Nikon, Japan), using the software LaserSharp 2000 (Bio-Rad, UK) as described before [13].

Isolation of microsomal membranes

Cells after washing several times in KCl were homogenised in 0.15 M KCl and 25 mM Tris-HCl pH 7.4. The homogenate was then centrifuged at 10,000 g and the obtained supernatant was centrifuged at 100,000 g for 1 h. The microsomes were resuspended in 0.15 KCl. Protein concentration was determined according to the Lowry method [15] using BSA as a standard.

Microsomal lipid oxidation

Microsomal membranes (1.5 mg of protein suspended in 2.0 ml of phosphate buffer pH 7.4) were treated with 2.5 mM Bu^tOOH for 30 min. Aminoxyls were added to the samples as ethanol solution before addition of Bu^tOOH. The control samples were treated with Bu^tOOH, and instead of aminoxyls, appropriate amounts of ethanol were added. The extent of TBAR-s production was measured by the method of Buege and Aust with 0.1 mM butylated hydroxytoluene (BHT) to prevent eventual peroxidation of microsomal lipids during assay [3].

RESULTS

Aminoxyls, when added as ethanolic solution to microsomes, inhibited lipid peroxidation as assessed by the number of TBAR-s produced (Fig. 2). At 100 μM concentration QAL and C-QAL inhibited lipid peroxidation almost completely. On the other hand efficacy of 4-OH-TEMPO was much lower in comparison with the lipid soluble aromatic aminoxyls QAL and C-QAL. Incubation of osteosarcoma cells with QAL resulted in distinct changes in the shape and intracellular redistribution of mitochondria. Mitochondria become initially rounded in shape, being not only granular but also distinctly enlarged (Fig. 3). C-QAL and 4-OH-TEMPO treated cells behaved like control ones i.e. mitochondria were filamentous and finally scattered throughout the cytosol (Fig. 3). QAL, being an inducer of morphological changes of aminoxyl-treated cells, revealed another interesting peculiarity. It can be easily noticed that mitochondria in QAL-treated cells display different intracellular localisation, being clustered perinuclearly (Fig. 3).

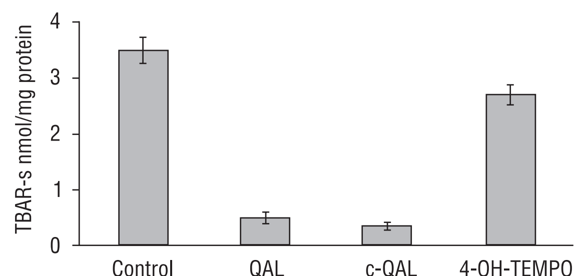


Figure 2. Effects of aminoxyls on TBAR-s formation in microsomes isolated from human osteosarcoma cells 143B. The incubation mixture contained 1.5 mg of microsomal protein 2.0 ml phosphate buffer pH 7.4 and 2.5 mM Bu^tOOH. After 30 min incubation at 25°C 0.5 ml of the mixture was assayed for TBAR-s determination. Aminoxyls were added as ethanol solution and appropriate amounts of ethanol were added to control samples (n = 5).

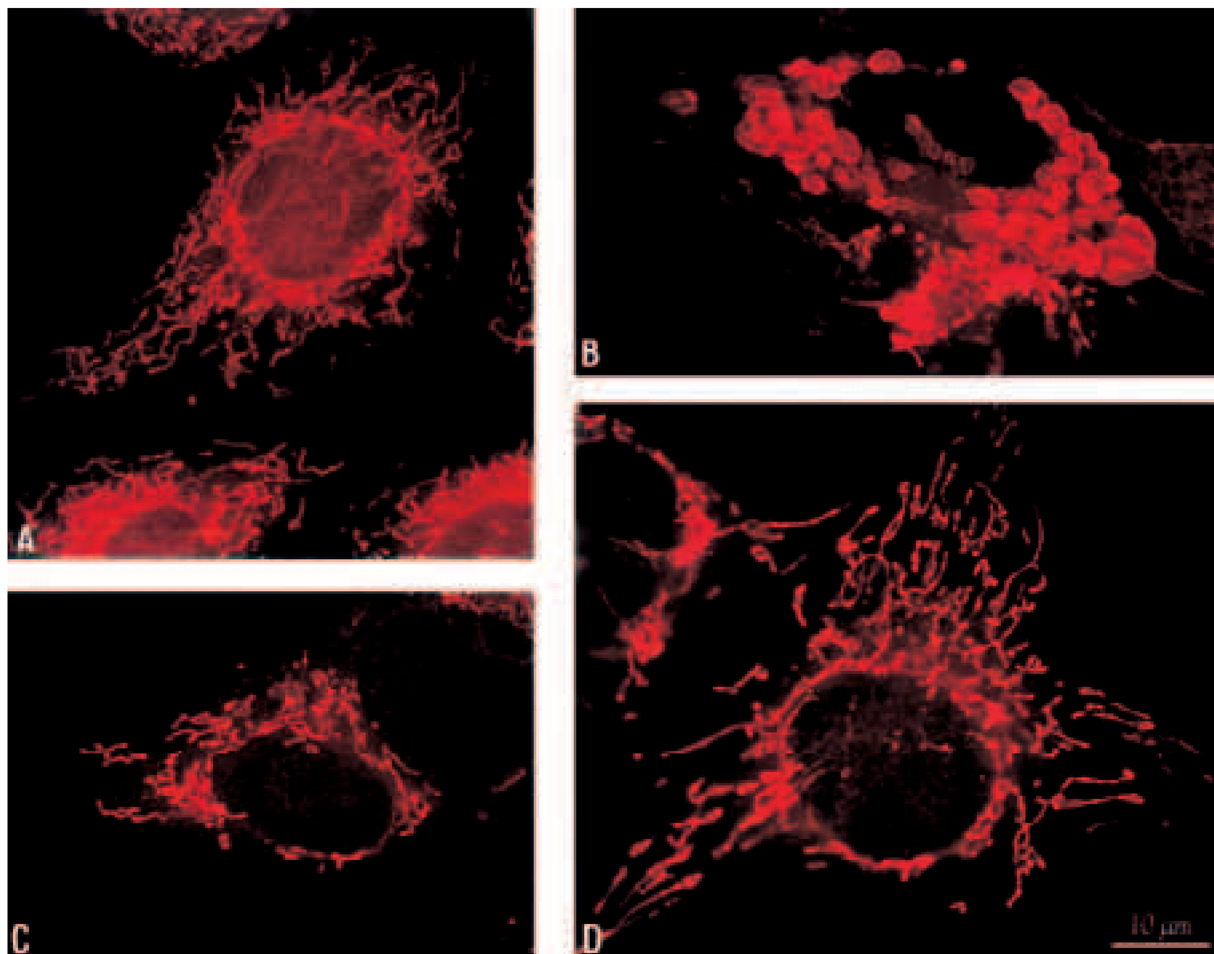
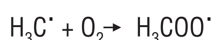


Figure 3. Morphological changes of mitochondria in 143B cells. Cells were treated with aminoxyls dissolved in DMSO during 24 h. Appropriate amounts of DMSO were added to control samples. **A.** Control cells; **B.** 100 μM QAL; **C.** 100 μM c-QAL; **D.** 100 μM 4-OH-TEMPO.

DISCUSSION

Our results prove that quinolinic aromatic aminoxyls are extremely efficient antioxidants protecting endoplasmic reticulum phospholipids from radicals arising from Bu^tOOH decomposition which provide mainly methyl carbon centred radicals $^{\bullet}CH_3$ [1, 11] from which peroxyl species initiating lipid peroxydation can be formed according to the equation:



$$H_3C^{\bullet} + O_2 \rightarrow H_3COO^{\bullet}$$

Methyl peroxyl radicals can be regarded as the main destructive species in Bu^tOOH dependent cytotoxicity [17]. Aromatic aminoxyls possess the ability to react with peroxyl radicals [4] while piperidine ring dependent 4-OH-TEMPO can intercept only carbon centred radicals [2].

Mitochondria are both the major ATP producer and also the main intracellular source of ROS of the mammalian cells [14]. Several structural characteristics of mitochondria can be changed during apoptosis [12] and antioxidants can now be regarded as one of the most efficient apoptosis inducers [18]. Taking into account this assumption we inspected morphology of mitochondria and their localisation inside the cell after exposition on aminoxyl antioxidants. After 24 h of treatment of osteosarcoma 143B cells with 100 μM QAL the spatial redistribution of mitochondria evolved in the majority of the treated cells from scattered in non-exposed control cells on highly asymmetric perinuclear clustered distribution can be seen (Fig. 3). Mitochondria in the control cells, as well as exposed on C-QAL while stained with fluorescent dye CMX Ros revealed thin, filamentous structures under the confocal laser microscope. Exposition of cells on QAL results in distinct changes in shape of mitochondria into the population of enlarged granular structures. QAL induced modifications of the spatial redistribution of mitochondria as well as their morphology from

control filamentous to enlarged granular ones seem to be topics of special interest. Our results provide the first evidence that some antioxidants can act as tumour necrosis factor (TNF) mimic in spite of mitochondrial ultrastructure and redistribution. It has already been shown that TNF initiates hyperphosphorylation of kinesin light chain [8] and induces perinuclear clustering of granular mitochondria via 55 kDa receptor [7].

REFERENCES

1. Barr DP, Mason RP (1995) Mechanism of radical production from the reaction of cytochrome c with organic hydroperoxides. An ESR spin trapping investigation. J Biol Chem, 270: 12709–12716.
2. Beckwith ALJ, Bowry VW, Ingold KU (1988) Kinetics of nitroxide coupling reactions of the nitroxyl radical 1,1,3,3-tetra-methylisonidoline-2-oxyl with carbon-centered radicals. J Org Chem, 53: 1632–1641.
3. Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol, 52: 302–310.
4. Cardellini L, Carloni P, Greci L, Stipa P, Faucitano A (1989) Homolytic substitution in indolinone nitroxide radicals. Part 5: Reactions with tert-butylperoxyl radicals. Gazz Chim Ital, 119: 621–625.
5. Carloni P, Greci L, Stipa P, Eberson L (1991) Electron-transfer reactions. Oxidation of Gringard reagents in the presence of an aminoxyl as radical-trapping agent. J Org Chem, 56: 4733–4737.
6. Chateauneuf J, Lusztyk J, Ingold K (1988) Absolute rate constants for the reactions of some carbon-centered radicals with 2,2,6,6-tetramethylpiperidine-N-oxyl. J Org Chem, 53: 1629–1632.
7. De Vos K, Goossens V, Boone E, Vercammen D, Vancompernolle K, Vandenabeele P, Haegeman G, Fiers W, Grooten J (1998) The 55-kDa tumor necrosis factor receptor induces clustering of mitochondria through its membrane-proximal region. J Biol Chem, 273: 9673–9680.
8. De Vos K, Severin F, Van Herreweghe F, Vancompernolle K, Goossens V, Hyman A, Grooten J (2000) Tumor necrosis factor induces hyperphosphorylation of kinesin light chain and inhibits kinesin-mediated transport of mitochondria. J Cell Biol, 149: 1207–1214.
9. Greci L (1982) Homolytic substitution in indolinone nitroxide radicals: Part III. Reactions with terbutoxy and methyl radicals. Tetrahedron 38: 2435–2437.
10. Halliwell B and Gutteridge JMC eds (2000) Free radicals in biology and medicine. Clarendon Press. Oxford
11. Hix S, Kadiiska MB, Mason RP, Augusto O (2000) *In vivo* metabolism of tert-butyl hydroperoxide to methyl radicals. EPR spin-trapping and DNA methylation studies. Chem Res Toxicol, 13: 1056–1064.
12. Karbowski M, Kurono C, Wozniak M, Ostrowski M, Teranishi M, Nishizawa Y, Usukura J, Soji T, Wakabayashi T (1999) Free radical-induced megamitochondria formation and apoptosis. Free Radic Biol Med, 26: 396–409.
13. Karbowski M, Spodnik JH, Teranishi M, Wozniak M, Nishizawa Y, Usukura J, Wakabayashi T (2001) Opposite effects of microtubule-stabilizing and microtubule-destabilizing drugs on biogenesis of mitochondria in mammalian cells. J Cell Sci, 114: 281–291.
14. Lee HC, Wei YH (2000) Mitochondrial role in life and death of the cell. J Biomed Sci, 7: 2–15.
15. Lowry O, Rosebrough N, Farr A, Randall R (1951) Protein measurement with the folin phenol reagent. J Biol Chem, 193: 265–275.
16. Mitchell JB, Samuni A, Krishna MC, DeGraff WG, Ahn MS, Samuni U, Russo A (1990) Biologically active metal-independent superoxide dismutase mimics. Biochemistry, 29: 2802–2807.
17. Nieminen AL, Saylor AK, Tesfai SA, Herman B, Lemasters JJ (1995) Contribution of the mitochondrial permeability transition to lethal injury after exposure of hepatocytes to t-butylhydroperoxide. Biochem J, 307: 99–106.
18. Serrano A, Palacios C, Roy G, Cespon C, Villar ML, Nocito M, Gonzalez-Porque P (1998) Derivatives of gallic acid induce apoptosis in tumoral cell lines and inhibit lymphocyte proliferation. Arch Biochem Biophys, 350: 49–54.