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The novel mitochondrial iron chelator 5-((methylamino)methyl)-8-hydroxyquinoline protects against mitochondrial-induced oxidative damage and neuronal death

Natalia P. Mena [a, *], Olimpo García-Beltrán [b], Fernanda Lourido [a], Pamela J. Urrutia [a], Raúl Mena [a], Vicente Castro-Castillo [c], Bruce K. Cassels [d], Marco T. Núñez [a]

[a] Iron and Biology of Aging Laboratory, Department of Biology, Faculty of Sciences, University of Chile, Chile
[b] Facultad de Ciencias Naturales y Matemáticas, Universidad de Ibagué, Ibagué 730001, Colombia
[c] Faculty of Basic Sciences, Universidad Metropolitana de Ciencias de la Educación, Santiago, Chile
[d] Department of Chemistry, Faculty of Sciences, University of Chile, Santiago, Chile

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ABSTRACT

Abundant evidence indicates that iron accumulation, oxidative damage and mitochondrial dysfunction are common features of Huntington's disease, Parkinson's disease, Friedreich's ataxia and a group of disorders known as Neurodegeneration with Brain Iron Accumulation. In this study, we evaluated the effectiveness of two novel 8-OH-quinoline-based iron chelators, Q1 and Q4, to decrease mitochondrial iron accumulation and oxidative damage in cellular and animal models of PD. We found that at submicromolar concentrations, Q1 selectively decreased the mitochondrial iron pool and was extremely effective in protecting against rotenone-induced oxidative damage and death. Q4, in turn, preferentially chelated the cytoplasmic iron pool and presented a decreased capacity to protect against rotenone-induced oxidative damage and death. Oral administration of Q1 to mice protected substantia nigra pars compacta neurons against oxidative damage and MPTP-induced death. Taken together, our results support the concept that oral administration of Q1 is a promising therapeutic strategy for the treatment of NBIA.

1. Introduction

Iron is highly redox-active, and its overload seems to be a critical event in the pathology of several neurodegenerative disorders that includes Huntington's disease, Parkinson's disease (PD) and a group of less characterized disorders known as Neurodegeneration with Brain Iron Accumulation (NBIA) [1–4].

Magnetic resonance imaging and ultrasound studies demonstrated iron accumulation in the substantia nigra pars compacta (SNpc) of PD patients [5–10], specifically in dying dopaminergic neurons and associated microglia [11]. Furthermore, an increase in the Fe^{3+}/Fe^{2+} ratio was observed in PD subjects [5,8]. Iron accumulation in the SNpc of PD patients has been replicated in animal models of PD, as shown in treatments with inhibitors of mitochondrial complex I 6-OHDA, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone and the proteasomal activity inhibitor lactacystin [9,10,12–15]. Accordingly, iron chelation has been proposed as a new promising alternative for PD treatment [16–20] with the caveat that iron is an essential element for normal neurological function [21,22]. Hence, iron chelation must be carefully controlled to prevent toxicity and side effects and new chelators with enhanced cell selectivity and metal affinity may have the ability to reduce secondary effects in a putative iron chelation therapy.

Recently, new iron chelators based on 8-hydroxyquinoline (8-HQ) have been developed. The ancient and antiprotozoal drug clioquinol (vioform, 5-chloro-7-iodo-8-hydroxyquinoline) was shown to prevent MPTP-induced neurotoxicity in mice and reversed the motor deficits in tau KO mice [23,24], and blood–brain barrier-permeable chelators VK28 and M30 prevented the neurotoxicity induced by 6-OHDA and MPTP [25–27]. In addition, orally

* Corresponding author. Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Santiago 780024, Chile.
E-mail address: natalia.paz.menaj@gmail.com (N.P. Mena).

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given M30 prevented the loss of tyrosine hydroxylase (TH)-positive neurons and attenuated iron accumulation and microglial activation in lactacystin-treated mice [10,28].

Using cellular and animal models of PD, in this study we characterized the antioxidant and neuroprotective capacity of two new 8-HQ-based iron chelators, 5-((methylamino)methyl)-8-hydroxy quinoline (Q1) and 5-(morpholinomethyl)-8-hydroxyquinoline (Q4), and determined their sub-cellular locus of chelation activity.

2. Materials and methods

2.1. Animals

C57Bl/6 male mice were used. All experiments were performed under a protocol approved by the Ethics Committee of the Faculty of Sciences, Universidad de Chile and the Advisory Committee on Bioethics of the Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT). Mice were housed under a 12-h light, 12-h dark cycle, and were sacrificed using CO_2 narcosis.

2.2. Cells and cell viability

Human neuroblastoma SH-SY5Y cells (CRL-2266, American Type Culture Collection, Rockville, MD) were cultured in MEM-F12 medium supplemented with 10% FBS, non-essential amino acids, antibiotic–antimycotic mixture, and 20 mM HEPES buffer, pH 7.2. The medium was replaced every two days. Cell viability was assessed in 96-well microplates using an LDH release assay [29].

2.3. Antibodies

Antibodies and dilutions used in this study were rabbit anti-TH antibody T8700, Sigma–Aldrich (1:1,000); mouse monoclonal anti-4-HNE J-2, Abcam (1:500 for cells, 1:200 for tissue); and anti-8-hydroxy-2′-deoxiguanosine (8-OHdG) mouse monoclonal antibody 15A3 from Santa Cruz Biotechnology (1:200).

2.4. Measurement of cytosolic and mitochondrial labile iron pools

The cytoplasmic and mitochondrial labile or reactive iron pools were determined as described [30,31]. Briefly, cells were loaded for 20 min at 37 °C with 0.5 μM of the cytosolic iron chelator calcein-AM (Molecular Probes-Invitrogen, Eugene, OR) or the mitochondrial iron chelator rhodamine B-[(1,10-phenanthrolin-5-yl)aminocarbonyl]benzyl ester (RPA) [31]. After washing, the cells were placed in a fluorescence microplate reader and the basal calcein (excitation 488 nm; λ emission 517 nm) or RPA (excitation 543 nm; emission 585 nm) fluorescence was determined for 3 min. Chelators Q1or Q4 (0.25 μM) were added and the increase in fluorescence was recorded for 5 min. Both calcein and RPA decrease their fluorescence when bound to iron. The increase in fluorescence upon addition of chelators is directly proportional to the cytoplasmic (calcein) or mitochondria (RPA) labile iron pools [30–32].

2.5. Mitochondrial superoxide production

To assess mitochondrial superoxide production, cells were incubated for 10 min at 37 °C with 5 μM of the mitochondrial superoxide indicator MitoSOX red (Invitrogen, Catalogue Number M36008). Changes in MitoSOX red fluorescence (Ex. 510 nm/Em.580 nm) was evaluated in a microplate reader following manufacturer's instructions.

2.6. DNA oxidation

Oxidative damage to DNA was assessed with monoclonal antibody 15A3, which recognizes 8-hydroxy-2′-deoxyguanosine (8-OHdG), a highly specific product of DNA damage. The antibody was developed with the peroxidase-based Vectastain ABC kit (Vector Labs., Burlingame, CA). No peroxidase reaction product was observed if the primary antibodies were omitted or if it they were replaced by non-immune serum.

2.7. Immunohistochemistry

Immunohistochemistry was performed as described [33]. Briefly, mice brains were fixed by transcardiac perfusion of 4% paraformaldehyde in PBS, dissected, and post-fixed for 24 h in the same medium. Slices of 20 μm thickness were obtained in a cryostat as described [34]. Free-floating sections were permeabilized, blocked for nonspecific binding sites and incubated with a primary antibody. Immunolabeling was visualized using Alexa-488 or Alexa-532 conjugated secondary antibodies. For detection of the SNpc, the slice with the highest tyrosine hydroxylase (TH) labeling intensity was selected as representative.

2.8. Quantification of dopaminergic neurons of the SNpc

Total numbers of TH-positive cells were calculated using unbiased stereological methods with Explora Nova software.

2.9. Statistics

The data presented are representative of at least three independent experiments. A one-way ANOVA was used to test for significant differences among mean values and Turkey's post hoc test was used for comparisons (InStat, GraphPad Software, San Diego, CA). Differences were considered significant if $P < 0.05$.

3. Results

3.1. Synthesis of Q1 and Q4

The preparation of Q1 and Q4 is outlined in Supplemental Figure 1. 8-Hydroxyquinoline was converted to 5-chloromethyl-8-quinolinol hydrochloride (1) according to standard methods [35], by treating with hydrochloric acid and formaldehyde. The reaction of 5-chloromethyl-8-quinolinol either with methylamine or morpholine yielded the target compounds Q1 and Q4, respectively.

3.2. Assessment of the capacity of Q1 and Q4 to chelate cytosolic and mitochondrial iron pools

To evaluate the capacity of Q1 and Q4 to chelate iron in cytosolic and mitochondrial compartments, SH-SY5Y cells were loaded with the fluorescent iron sensors calcein or RPA. Calcein is a probe with a preferential cytoplasmic destination [30], whereas RPA is a probe with mitochondrial localization [31]. The fluorescence of both probes is quenched upon iron binding. After calcein or RPA loading, the cells were challenged with Q1 or Q4 and the changes in fluorescence were determined. The increase in fluorescence indicates the ability of the compounds to chelate either cytoplasmic iron (calcein fluorescence) or mitochondrial iron (RPA fluorescence). Fig. 1A shows that after RPA loading, the addition of 0.25 μM Q1 produced an increase in RPA fluorescence. When the cells were loaded with calcein, only a minor change in calcein fluorescence was observed (Fig. 1B). In contrast, Q4 did not change the intensity of RPA fluorescence in RPA-loaded cells (Fig. 1A). Instead, we

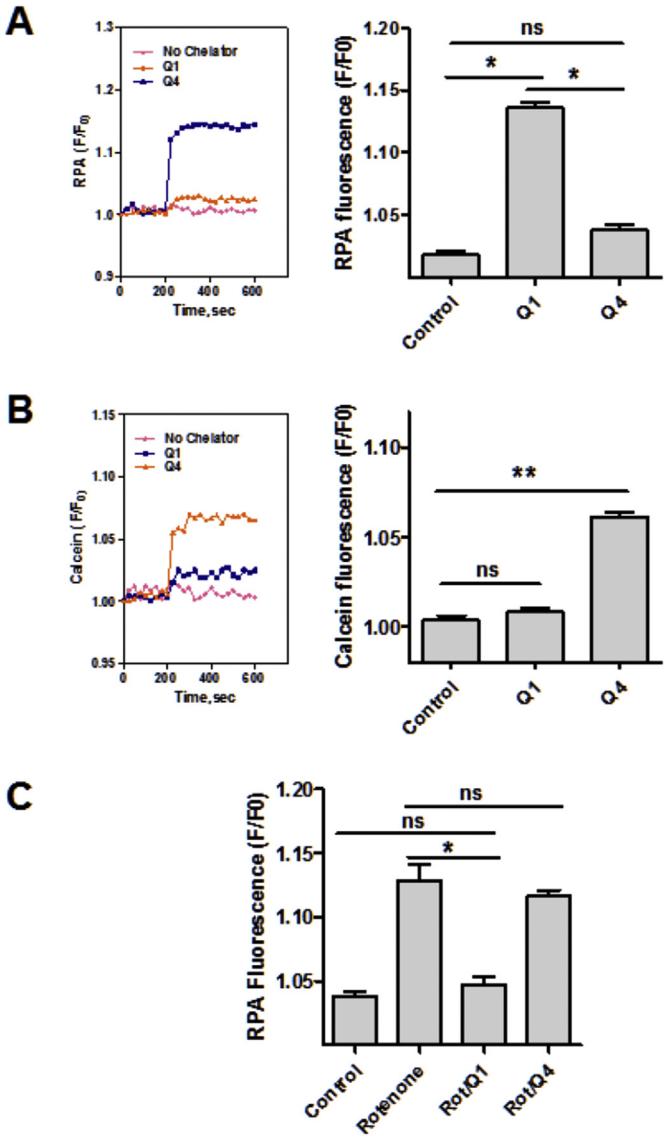

Fig. 1. Capacity of Q1 and Q4 to chelate mitochondrial and cytoplasmic iron. SH-SY5Y cells were loaded with the mitochondrial iron sensor RPA (A) or the cytoplasmic iron sensor calcein (B), after which the cells were challenged with 0.25 μM Q1 or Q4. Chelation activity in mitochondria is evidenced by an increase in RPA fluorescence, while chelation activity in the cytoplasm is evidenced by an increase in calcein fluorescence. The control condition corresponds to cells loaded with RPA or calcein but without chelator post-treatment. Left panels in A and B show representative tracings of RPA (A) and calcein (B) fluorescence upon the addition of Q1 or Q4. Right panels show the quantification of the changes in fluorescence (mean ± SEM) after addition of chelators. C) SH-SY5Y cells were incubated for 24 h in medium containing 5 μM rotenone with or without 0.25 μM Q1 or Q4. The cells were then loaded with RPA and the basal fluorescence (F0) was measured, after which, 5 mM deferiprone was added and the change in fluorescence (F) was determined. Shown is the change in fluorescence (mean ± SEM), expressed as F/F0, as a function of treatment. *P < 0.05, **P < 0.01.

observed that Q4 addition resulted in a significant increase in the calcein fluorescence of calcein-loaded cells (Fig. 1B).

To further evaluate the capacity of Q1 and Q4 to chelate iron from mitochondria, RPA-loaded cells were treated with either rotenone or with rotenone plus Q1 or Q4. Previous work from our laboratory showed that 5 μM rotenone produces an increase in mitochondrial labile iron pool levels (Mena et al., unpublished; Fig. 1C). The permeant iron chelator deferiprone was used to de-quench RPA. We observed that rotenone treatment resulted in an increase in RPA fluorescence upon de-quenching, while the co-incubation of rotenone with Q1 resulted in no change in de-quenched RPA, as compared to the control (Fig. 1C). Q4 was unable to reverse the rotenone effect. These results strongly suggest that Q1 is an effective mitochondrial iron chelator and that it suppresses the increase in the mitochondrial labile iron pool induced by rotenone.

3.3. Q1 protects SH-SY5Y cells from death induced by rotenone

We then evaluated the capacity of Q1 and Q4 to protect SH-SY5Y cells from rotenone-induced cell death as determined by LDH released to the culture medium (Fig. 2). Co-incubation of rotenone with Q1 largely blocked the deleterious effect of rotenone on cell viability. Q4 also reduced rotenone toxicity but this reduction did not reach a statistically significant level.

3.4. Q1 and Q4 protect SH-SY5Y cells from oxidative damage

Inhibition of complex I results in increased superoxide radical production by mitochondria [36] with the consequent oxidative damage to lipids, proteins and nucleic acid. We evaluated the putative effects of Q1 and Q4 on mitochondrial superoxide production induced by rotenone (Fig. 3A). The increased mitochondrial superoxide production resulting from rotenone treatment was blocked when cells were co-incubated with either Q1 or Q4. Oxidative damage was also evaluated determining the effect of Q1 and Q4 on lipid peroxidation and protein modification, determined by the formation of 4-HNE adducts (Fig. 3B). Q1 and Q4 were highly effective in preventing the formation of 4-HNE adducts induced by rotenone. Therefore, sub-micromolar concentrations of Q1 or Q4 are extremely effective in protecting cells against oxidative damage derived from complex I inhibition.

3.5. Q1 protects dopaminergic neurons from cell death and oxidative stress in MPTP intoxicated mice

We tested whether oral dosages of Q1 protect SNpc neurons from MPTP-induced death (Fig. 4A). Three-month-old mice were given one oral dose of Q1 (2.5 mg/kg) per day for two days prior to MPTP intoxication. Subsequently, on day three, the mice were subjected to acute intoxication with MPTP followed by oral doses of Q1 from days four to seven. At day eight, the animals were

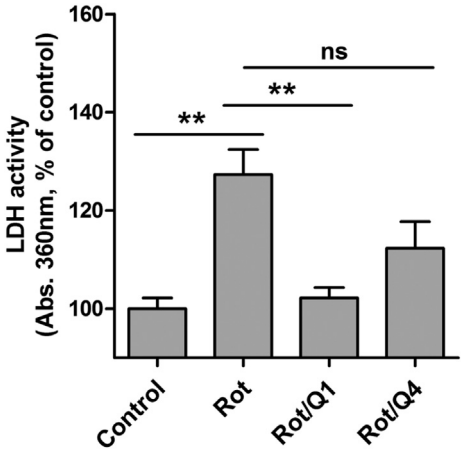

Fig. 2. Q1 protects SH-SY5Y cells from rotenone-induced cell death. SH-SY5Y cells were incubated for 24 h with 5 μM rotenone with or without 0.25 μM of Q1 or Q4. Cell viability was measured by LDH activity in the culture medium. The control of 100% viability was LDH activity in the culture medium of cells without rotenone treatment. Shown are means ± SEM. N = 3 independent determinations, (*P < 0.05; **P < 0.01).

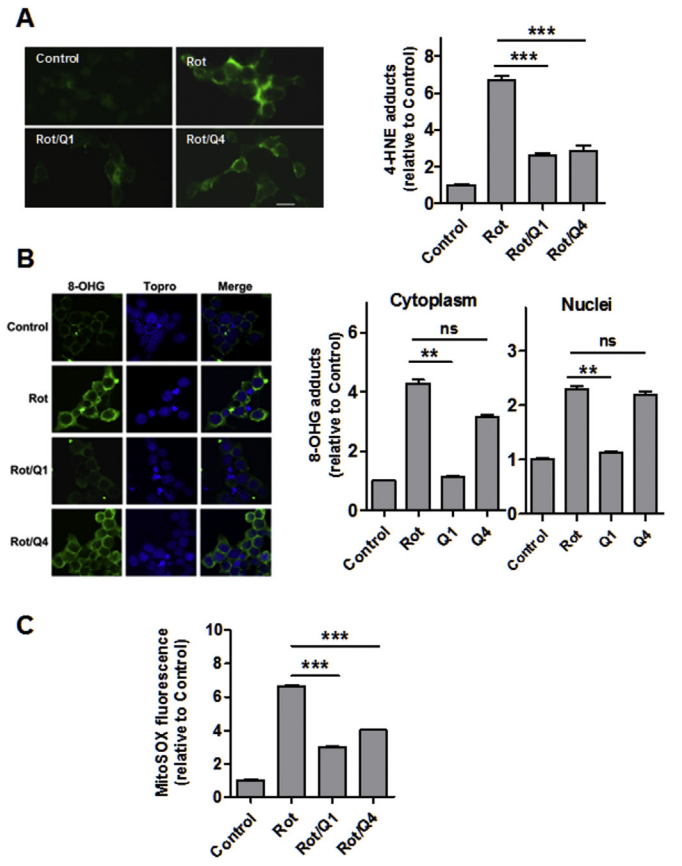

Fig. 3. Q1 protects SH-SY5Y cells from rotenone-induced oxidative damage. SH-SY5Y cells grown in 24-well plates were incubated for 24 h with 5 μM rotenone with or without 0.25 μM Q1 or Q4. (A) Protein oxidation determined by the formation of 4-HNE adducts. Left panel shows representative images upon treatment with rotenone and rotenone plus Q1 or Q4. Right panel show quantification of 4-HNE immunodetection in 40–55 individual cells per experimental condition. Values are mean ± SEM. Scale bar = 25 μm (B) Nucleic acid modification detected by the formation of 8-OHdG. Left panel shows representative images upon treatment with rotenone a rotenone plus Q1 or Q4. Right panel show quantification of 4-HNE immunodetection in 40–55 individual cells per experimental condition. Values are mean ± SEM of a typical experiment. **P < 0.01. Scale bar = 25 μm. (C) Changes in MitoSOX Red fluorescence evaluated in a microplate reader. Shown are mean ± SEM. N = 3 independent determinations. *P < 0.05 compared to rotenone.

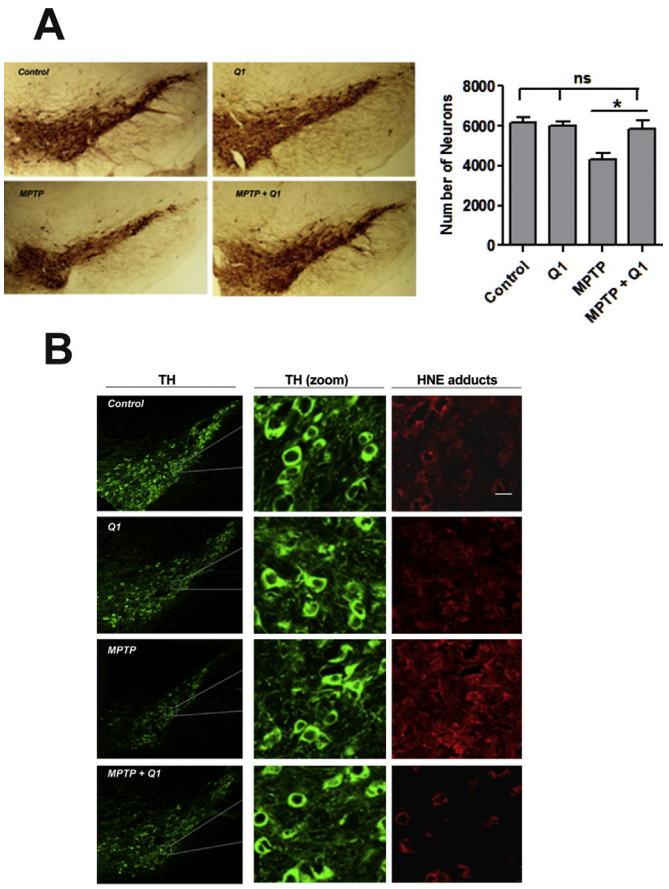

Fig. 4. Q1 protects dopaminergic neurons in MPTP intoxicated mice. (A) Mice (five per group) were treated on days one and two with oral doses (2.5 mg/kg) of Q1. On day three, the mice were intoxicated with MPTP (four doses separated by two hours, acute protocol). Subsequently, on days four, five, six and seven, the mice were again treated with oral doses (2.5 mg/kg) of Q1. The animals were sacrificed on day eight and the substantia nigra was analyzed using immunohistochemistry for TH. Scale bar: 100 μm. Right panel shows quantification of the dopaminergic neurons of the SNpc. *P < 0.05 compared to the MPTP condition. Values represent mean ± SEM of one of two similar experiments. *P < 0.05 compared to the MPTP condition. (B) Mice were treated with MPTP and Q1, as described above. The animals were sacrificed on day eight and the sustantia nigra was analyzed using immunohistochemistry for TH (green) and the HNE adducts (red), Scale bar: 20 μm.

sacrificed and the number of TH^+ neurons was analyzed using immunohistochemistry. After MPTP intoxication, TH^+ neurons of SNpc were significantly decreased compared to the control ($P < 0.05$; Fig. 4A). Conversely, when mice were treated with MPTP plus Q1, no TH + neuronal loss was apparent. Additionally, to determine possible protection by Q1 against oxidative damage, the formation of HNE adducts was evaluated in control, as well as in Q1-treated, MPTP-treated and Q1+MPTP-treated mice (Fig. 4B). MPTP treatment resulted in a marked increase in the HNE adducts in SNpc TH + neurons, whereas Q1+MPTP-treated mice showed a markedly decreased HNE immunostaining compared with MPTP-treated mice ($P < 0.05$).

4. Discussion

Several chronic neurodegenerative disorders, such as PD, Alzheimer's disease, multiple sclerosis, Amyotrophic lateral sclerosis, and Friedreich's ataxia, display increased brain iron levels [37–41]. Iron is defined as a "double-edged sword" since it is an essential nutrient while also being a highly reactive and potentially toxic element. Redox-active iron is a key player in the pathogenesis of PD, and therefore, iron chelators are potential therapeutic agents for the treatment of the disease [42–44]. In this study, we evaluated the capacity of two new 8-HQ-derivated iron chelators, Q1 and Q4, to selectively chelate mitochondrial and cytoplasmic iron pools, and to protect against oxidative damage and cell death induced by complex I inhibition. We also tested the effectiveness of oral doses of Q1 to protect SNpc neurons in MPTP-intoxicated mice.

The synthesis of the most abundant iron-containing prosthetic groups, heme and iron–sulfur clusters, takes place in the mitochondrion, and hence, significant amounts of iron enter this organelle [45,46]. Additionally, mitochondria are the principal source of reactive oxygen species generation, derived from electron leak originating in the electron transport chain [47]. The co-existence of both ROS and iron in a confined space makes this organelle particularly prone to oxidative damage and therefore susceptible to dysfunction. Accordingly, chelators that preferably chelate mitochondrial iron should be more effective in reducing iron-induced oxidative damage to mitochondria. We found that sub-micromolar concentrations of Q1 selectively chelated the mitochondrial labile iron pool of SHSY-5Y cells, while Q4 did not.

Consistent with this finding, upon rotenone treatment, Q1 exhibited higher efficiency than did Q4 in suppressing the increase of mitochondrial superoxide production. Both Q1 and Q4 were effective in suppressing the formation of 4-HNE-protein and of 8-OHdG adducts. Taken together, the data strongly suggest that chelation of mitochondrial or cytoplasmic iron by sub-micromolar concentrations of Q1 or Q4 reduce rotenone-induced oxidative stress and oxidative damage to lipids, proteins and DNA.

Additionally, we found that low oral doses of Q1 (2.5 mg/kg body weight) prevented SNpc neuronal death in MPTP-intoxicated mice. This suggests that Q1 is a resilient molecule that crosses both the intestinal epithelium and blood–brain barriers, preserving its chelating activity.

The high mitochondrial iron chelating capacity of Q1 compared with Q4 can be tentatively ascribed to the different substitutions at C5 of the quinoline ring: Q1 bears a methylaminomethyl group while Q4 has a morpholinomethyl moiety. Arguably, the methylaminomethyl group confers on Q1 its mitochondrial targeting capacity, while the morpholinomethyl group targets Q4 to the cytoplasm. Further studies are needed to unequivocally demonstrate the mitochondrial destination of Q1 and the role of the methylaminomethyl group in this process.

In summary, this study describes the properties of Q1 and Q4, two new 8-hydroxyquinoline derivatives with iron chelating capacity. Q1, with a methylaminomethyl substitution at C5, chelates mitochondrial iron, protecting dopaminergic cells from rotenone-induced mitochondrial oxidative stress and oxidative damage. Q4, a cytoplasmic targeted chelator was also effective in protecting cells against rotenone-induced oxidative damage. Administered to mice, Q1 probably crosses the intestinal epithelium and blood–brain barriers, protecting dopaminergic neurons against MPTP-induced death.

Acknowledgments

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Transparency document

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.06.014.

References

[1] H.H. Andersen, K.B. Johnsen, T. Moos, Iron deposits in the chronically inflamed central nervous system and contributes to neurodegeneration, Cell. Mol. Life Sci. 71 (2014) 1607–1622.
[2] R.J. Ward, F.A. Zucca, J.H. Duyn, R.R. Crichton, L. Zecca, The role of iron in brain ageing and neurodegenerative disorders, Lancet Neurol. 13 (2014) 1045–1060.
[3] S. Levi, D. Finazzi, Neurodegeneration with brain iron accumulation: update on pathogenic mechanisms, Front. Pharmacol. 5 (2014) 99.
[4] M.A. Kurian, S.J. Hayflick, Pantothenate kinase-associated neurodegeneration (PKAN) and PLA2G6-associated neurodegeneration (PLAN): review of two major neurodegeneration with brain iron accumulation (NBIA) phenotypes, Int. Rev. Neurobiol. 110 (2013) 49–71.
[5] E. Sofic, P. Riederer, H. Heinsen, H. Beckmann, G.P. Reynolds, G. Hebenstreit, M.B. Youdim, Increased iron (III) and total iron content in post mortem substantia nigra of parkinsonian brain, J. Neural Transm. 74 (1988) 199–205.
[6] D.T. Dexter, F.R. Wells, F. Agid, Y. Agid, A.J. Lees, P. Jenner, C.D. Marsden, Increased nigral iron content in postmortem parkinsonian brain, Lancet 2 (1987) 1219–1220.
[7] E.C. Hirsch, J.P. Brandel, P. Galle, F. Javoy-Agid, Y. Agid, Iron and aluminum increase in the substantia nigra of patients with Parkinson's disease: an X-ray microanalysis, J. Neurochem. 56 (1991) 446–451.
[8] J. Sian-Hulsmann, S. Mandel, M.B. Youdim, P. Riederer, The relevance of iron in the pathogenesis of Parkinson's disease, J. Neurochem. 118 (2011) 939–957.
[9] J.M. Gorell, R.J. Ordidge, G.G. Brown, J.C. Deniau, N.M. Buderer, J.A. Helpern, Increased iron-related MRI contrast in the substantia nigra in Parkinson's disease, Neurology 45 (1995) 1138–1143.
[10] D. Berg, C. Grote, W.D. Rausch, M. Maurer, W. Wesemann, P. Riederer, G. Becker, Iron accumulation in the substantia nigra in rats visualized by ultrasound, Ultrasound Med. Biol. 25 (1999) 901–904.
[11] E.C. Hirsch, Altered regulation of iron transport and storage in Parkinson's disease, J. Neural Transm. Suppl. (2006) 201–204.
[12] M. Gerlach, K.L. Double, M.B. Youdim, P. Riederer, Strategies for the protection of dopaminergic neurons against neurotoxicity, Neurotox. Res. 2 (2000) 99–114.
[13] M.B. Youdim, G. Stephenson, D. Ben Shachar, Ironing iron out in Parkinson's disease and other neurodegenerative diseases with iron chelators: a lesson from 6-hydroxydopamine and iron chelators, desferal and VK-28, Ann. N. Y. Acad. Sci. 1012 (2004) 306–325.
[14] W. Zhu, X. Li, W. Xie, F. Luo, D. Kaur, J.K. Andersen, J. Jankovic, W. Le, Genetic iron chelation protects against proteasome inhibition-induced dopamine neuron degeneration, Neurobiol. Dis. 37 (2010) 307–313.
[15] P.G. Mastroberardino, E.K. Hoffman, M.P. Horowitz, R. Betarbet, G. Taylor, D. Cheng, H.M. Na, C.A. Gutekunst, M. Gearing, J.Q. Trojanowski, M. Anderson, C.T. Chu, J. Peng, J.T. Greenamyre, A novel transferrin/TfR2-mediated mitochondrial iron transport system is disrupted in Parkinson's disease, Neurobiol. Dis. 34 (2009) 417–431.
[16] B. Ghosh, T. Antonio, M.E. Reith, A.K. Dutta, Discovery of 4-(4-(2-((5-Hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino)ethyl)piperaz in-1-yl)quinolin-8-ol and its analogues as highly potent dopamine D2/D3 agonists and as iron chelator: in vivo activity indicates potential application in symptomatic and neuroprotective therapy for Parkinson's disease, J. Med. Chem. 53 (2010) 2114–2125.
[17] S. Gogoi, T. Antonio, S. Rajagopalan, M. Reith, J. Andersen, A.K. Dutta, Dopamine D(2)/D(3) agonists with potent iron chelation, antioxidant and neuroprotective properties: potential implication in symptomatic and neuroprotective treatment of Parkinson's disease, ChemMedChem 6 (2011) 991–995.
[18] D. Devos, C. Moreau, J.C. Devedjian, J. Kluza, M. Petrault, C. Laloux, A. Jonneaux, G. Ryckewaert, G. Garcon, N. Rouaix, A. Duhamel, P. Jissendi, K. Dujardin, F. Auger, L. Ravasi, L. Hopes, G. Grolez, W. Firdaus, B. Sablonniere, I. Strubi-Vuillaume, N. Zahr, A. Destee, J.C. Corvol, D. Poltl, M. Leist, C. Rose, L. Defebvre, P. Marchetti, Z.I. Cabantchik, R. Bordet, Targeting chelatable iron as a therapeutic modality in Parkinson's disease, Antioxid. Redox Signal. 21 (2014) 195–210.
[19] R.J. Ward, D.T. Dexter, R.R. Crichton, Neurodegenerative diseases and therapeutic strategies using iron chelators, J. Trace Elem. Med. Biol. 31 (2015 Jul) 267–273, http://dx.doi.org/10.1016/j.jtemb.2014.12.012. Epub 2015 Jan.
[20] G. Grolez, C. Moreau, B. Sablonniere, G. Garcon, J.C. Devedjian, S. Meguig, P. Gele, C. Delmaire, R. Bordet, L. Defebvre, I.Z. Cabantchik, D. Devos, Ceruloplasmin activity and iron chelation treatment of patients with Parkinson's disease, BMC Neurol. 15 (2015) 74.
[21] J.M. Gutteridge, B. Halliwell, Free radicals and antioxidants in the year 2000. A historical look to the future, Ann. N. Y. Acad. Sci. 899 (2000) 136–147.
[22] C. Hidalgo, M.T. Nunez, Calcium, iron and neuronal function, IUBMB Life 59 (2007) 280–285.
[23] D. Kaur, F. Yantiri, S. Rajagopalan, J. Kumar, J.Q. Mo, R. Boonplueang, V. Viswanath, R. Jacobs, L. Yang, M.F. Beal, D. DiMonte, I. Volitaskis, L. Ellerby, R.A. Cherny, A.I. Bush, J.K. Andersen, Genetic or pharmacological iron chelation prevents MPTP-induced neurotoxicity in vivo: a novel therapy for Parkinson's disease, Neuron 37 (2003) 899–909.
[24] P. Lei, S. Ayton, A.T. Appukuttan, I. Volitakis, P.A. Adlard, D.I. Finkelstein, A.I. Bush, Clioquinol rescues Parkinsonism and dementia phenotypes of the tau knockout mouse, Neurobiol. Dis. (2015 Mar 18), http://dx.doi.org/10.1016/j.nbd.2015.03.015 pii:S0969-9961(15)00087-X.
[25] D.B. Shachar, N. Kahana, V. Kampel, A. Warshawsky, M.B. Youdim, Neuroprotection by a novel brain permeable iron chelator, VK-28, against 6-hydroxydopamine lession in rats, Neuropharmacology 46 (2004) 254–263.
[26] S. Gal, H. Zheng, M. Fridkin, M.B. Youdim, Novel multifunctional neuroprotective iron chelator-monoamine oxidase inhibitor drugs for neurodegenerative diseases. In vivo selective brain monoamine oxidase inhibition and prevention of MPTP-induced striatal dopamine depletion, J. Neurochem. 95 (2005) 79–88.
[27] S. Gal, Z.A. Abassi, M.B. Youdim, limited potentiation of blood pressure in response to oral tyramine by the anti-Parkinson brain selective multifunctional monoamine oxidase-AB inhibitor, M30, Neurotox. Res. 18 (2010) 143–150.
[28] W. Zhu, W. Xie, T. Pan, P. Xu, M. Fridkin, H. Zheng, J. Jankovic, M.B. Youdim, W. Le, Prevention and restoration of lactacystin-induced nigrostriatal dopamine neuron degeneration by novel brain-permeable iron chelators, FASEB J. 21 (2007) 3835–3844.
[29] M.A. Aras, K.A. Hartnett, E. Aizenman, Assessment of cell viability in primary neuronal cultures, Curr. Protoc. Neurosci. (2008 Jul), http://dx.doi.org/10.1002/0471142301.ns0718s44. Chapter 7:Unit 7.18.

[30] S. Epsztejn, O. Kakhlon, H. Glickstein, W. Breuer, I. Cabantchik, Fluorescence analysis of the labile iron pool of mammalian cells, Anal. Biochem. 248 (1997) 31–40.
[31] F. Petrat, D. Weisheit, M. Lensen, H. de Groot, R. Sustmann, U. Rauen, Selective determination of mitochondrial chelatable iron in viable cells with a new fluorescent sensor, Biochem. J. 362 (2002) 137–147.
[32] M. Kruszewski, Labile iron pool: the main determinant of cellular response to oxidative stress, Mutat. Res. 531 (2003) 81–92.
[33] J. Salazar, N. Mena, S. Hunot, A. Prigent, D. Alvarez-Fischer, M. Arredondo, C. Duyckaerts, V. Sazdovitch, L. Zhao, L.M. Garrick, M.T. Nunez, M.D. Garrick, R. Raisman-Vozari, E.C. Hirsch, Divalent metal transporter 1 (DMT1) contributes to neurodegeneration in animal models of Parkinson's disease, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 18578–18583.
[34] C. Beurrier, Y. Ben-Ari, C. Hammond, Preservation of the direct and indirect pathways in an in vitro preparation of the mouse basal ganglia, Neuroscience 140 (2006) 77–86.
[35] H. Zheng, L.M. Weiner, O. Bar-Am, S. Epsztejn, Z.I. Cabantchik, A. Warshawsky, M.B. Youdim, M. Fridkin, Design, synthesis, and evaluation of novel bifunctional iron-chelators as potential agents for neuroprotection in Alzheimer's, Parkinson's, and other neurodegenerative diseases, Bioorg. Med. Chem. 13 (2005) 773–783.
[36] T.S. Smith, J.P. Bennett Jr., Mitochondrial toxins in models of neurodegenerative diseases. I: In vivo brain hydroxyl radical production during systemic MPTP treatment or following microdialysis infusion of methylpyridinium or azide ions, Brain Res. 765 (1997) 183–188.
[37] G. Bartzokis, D. Sultzer, J. Cummings, L.E. Holt, D.B. Hance, V.W. Henderson, J. Mintz, In vivo evaluation of brain iron in Alzheimer disease using magnetic resonance imaging, Arch. Gen. Psychiatry 57 (2000) 47–53.
[38] K.A. Jellinger, The role of iron in neurodegeneration: prospects for pharmacotherapy of Parkinson's disease, Drugs Aging 14 (1999) 115–140.
[39] L. Zecca, M.B. Youdim, P. Riederer, J.R. Connor, R.R. Crichton, Iron, brain ageing and neurodegenerative disorders, Nat. Rev. Neurosci. 5 (2004) 863–873.
[40] M.T. Carri, A. Ferri, M. Cozzolino, L. Calabrese, G. Rotilio, Neurodegeneration in amyotrophic lateral sclerosis: the role of oxidative stress and altered homeostasis of metals, Brain Res. Bull. 61 (2003) 365–374.
[41] J.M. Stankiewicz, M. Neema, A. Ceccarelli, Iron and multiple sclerosis, Neurobiol. Aging 35 (Suppl 2) (2014) S51–S58.
[42] O. Weinreb, S. Mandel, M.B. Youdim, T. Amit, Targeting dysregulation of brain iron homeostasis in Parkinson's disease by iron chelators, Free Radic. Biol. Med. 62 (2013) 52–64.
[43] R.J. Ward, D.T. Dexter, R.R. Crichton, Chelating agents for neurodegenerative diseases, Curr. Med. Chem. 19 (2012) 2760–2772.
[44] Z.I. Cabantchik, A. Munnich, M.B. Youdim, D. Devos, Regional siderosis: a new challenge for iron chelation therapy, Front. Pharmacol. 4 (2013) 167.
[45] O. Stehling, J. Mascarenhas, A.A. Vashisht, A.D. Sheftel, B. Niggemeyer, R. Rosser, A.J. Pierik, J.A. Wohlschlegel, R. Lill, Human CIA2A-FAM96A and CIA2B-FAM96B integrate iron homeostasis and maturation of different subsets of cytosolic-nuclear iron-sulfur proteins, Cell Metab. 18 (2013) 187–198.
[46] H.A. Dailey, P.N. Meissner, Erythroid heme biosynthesis and its disorders, Cold Spring Harb. Perspect. Med. 3 (2013) a011676.
[47] A.D. Romano, E. Greco, G. Vendemiale, G. Serviddio, Bioenergetics and mitochondrial dysfunction in aging: recent insights for a therapeutical approach, Curr. Pharm. Des. 20 (2014) 2978–2992.