



Aged Garlic Extract Reduces ROS Production and Cell Death Induced by 6-Hydroxydopamine through Activation of the Nrf2-ARE Pathway in SH-SY5Y Cells

PDF (Size: 530KB) PP. 31-40 DOI: 10.4236/pp.2013.41004

Author(s)

Kohfuku Kohda, Hitomi Goda, Kei Itoh, Keiji Samejima, Tomoko Fukuuchi

ABSTRACT

Many degenerative or pathological processes, such as aging, cancer and coronary heart disease, are related to reactive oxygen species (ROS) and radical-mediated reactions. We examined the effectiveness of aged garlic extract (AGE), a garlic preparation rich in water-soluble cysteinyl moieties, for protection of cells from ROS produced by 6-hydroxy-dopamine (6-OHDA) using human neuroblastoma SH-SY5Y cells. Concomitant treatment of cells with AGE (2 and 4 mg/ml) showed the dose-dependent protective effect on the cell death induced by 6-OHDA. In addition, the AGE treatment significantly suppressed the increase of ROS generation by 6-OHDA. Furthermore, the protective effect of AGE was accompanied by activation of the nuclear factor erythroid 2-related factor 2 (Nrf2)-antioxidant response element (ARE) pathway and the increase of mRNAs of heme oxygenase-1 and NAD(P)H quinone oxidoreductase 1. These two enzymes are important in the cellular antioxidant system. These results indicated that AGE protected cells from ROS damage by not only capturing ROS directly but also activating the cellular antioxidant system by stimulating antioxidant gene expression via the Nrf2-ARE pathway. The present study suggested that AGE may be useful for prevention and treatment of cell damage caused by ROS.

KEYWORDS

Aged Garlic Extract (AGE); 6-OHDA; ROS; Nrf2-ARE Pathway; SH-SY5Y Cells

Cite this paper

K. Kohda, H. Goda, K. Itoh, K. Samejima and T. Fukuuchi, "Aged Garlic Extract Reduces ROS Production and Cell Death Induced by 6-Hydroxydopamine through Activation of the Nrf2-ARE Pathway in SH-SY5Y Cells," *Pharmacology & Pharmacy*, Vol. 4 No. 1, 2013, pp. 31-40. doi: 10.4236/pp.2013.41004.

References

- [1] J. G. Dausch and D. W. Nixon, "Garlic: A Review of Its Relationship to Malignant Disease," *Preventive Medicine*, Vol. 19, No. 3, 1990, pp. 346-361. doi:10.1016/0091-7435(90)90034-H
- [2] H. Amagase, B. L. Petesch, H. Matsuura, S. Kasuga and Y. Itakura, "Intake of Garlic and Its Bioactive Components," *The Journal of Nutrition*, Vol. 131, No. 3S, 2001, pp. 955S-962S.
- [3] S. Nakagawa, K. Masamoto, H. Sumiyoshi, K. Kunihiro and T. Fuwa, "Effect of Raw and Extracted-Aged Garlic Juice on Growth of Young Rats and Their Organs after Peroral Administration," *The Journal of Toxicological Sciences*, Vol. 5, No. 1, 1980, pp. 91-112. doi:10.2131/jts.5.91
- [4] M. Ushijima, I. Sumioka, N. Kashimoto, K. Yokoyama, N. Uda, H. Matsuura, E. Kyo, A. Suzuki, S. Kasuga, Y. Itakura, B. L. Petesch and H. Amagase, "Effect of Garlic and Garlic Preparations on Physiological and Psychological Stress in Mice," *Phytotherapy Research*, Vol. 11, No. 3, 1997, pp. 226-230. doi:10.1002/(SICI)1099-1573(199705)11:3<226::AID-PTR85>3.0.CO;2-E
- [5] N. Morihara, M. Ushijima, N. Kashimoto, I. Sumioka, T. Nishihama, M. Hayama and H. Takeda, "Aged Garlic Extract Ameliorates Physical Fatigue," *Biological & Pharmaceutical Bulletin*, Vol. 29, No. 5, 2006, pp. 962-966. doi:10.1248/bpb.29.962
- [6] E. Kyo, N. Uda, S. Kasuga and Y. Itakura, "Immunomodulatory Effects of Aged Garlic Extract," *The*

- [Open Special Issues](#)
- [Published Special Issues](#)
- [Special Issues Guideline](#)

[PP Subscription](#)
[Most popular papers in PP](#)
[About PP News](#)
[Frequently Asked Questions](#)
[Recommend to Peers](#)
[Recommend to Library](#)
[Contact Us](#)

Downloads:	83,612
------------	--------

Visits:	195,220
---------	---------

[Sponsors >>](#)

- [7] M. J. Budoff, N. Ahmadi, K. M. Gul, S. T. Liu, F. R. Flores, J. Tiano, J. Takasu, E. Miller and S. Tsimikas, "Aged Garlic Extract Supplemented with B Vitamins, Folic Acid and L-Arginine Retards the Progression of Subclinical Atherosclerosis: A Randomized Clinical Trial," *Preventive Medicine*, Vol. 49, No. 2-3, 2009, pp. 101-107. doi:10.1016/j.ypmed.2009.06.018
- [8] N. Morihara, N. Ide and N. Weiss, "Aged Garlic Extract Inhibits CD36 Expression in Human Macrophages via Modulation of the PPAR γ Pathway," *Phytotherapy Research*, Vol. 24, No. 4, 2010, pp. 602-608. doi:10.1002/ptr.3008
- [9] N. Morihara, I. Sumioka, T. Mori-guchi, N. Uda and E. Kyo, "Aged Garlic Extract Enhances Production of Nitric Oxide," *Life Sciences*, Vol. 71, No. 5, 2002, pp. 509-517. doi:10.1016/S0024-3205(02)01706-X
- [10] I. Sumioka, T. Matura and K. Yamada, "Therapeutic Effect of S-Allylmercaptocysteine on Acetaminophen-Induced Liver Injury in Mice," *European Journal of Pharmacology*, Vol. 433, No. 2-3, 2001, pp. 177-185. doi:10.1016/S0014-2999(01)01503-5
- [11] J. Imai, N. Ide, S. Nagae, T. Moriguchi, H. Matsuura and Y. Itakura, "Antioxidant and Radical Scavenging Effects of Aged Garlic Extract and Its Constituents," *Planta Medica*, Vol. 60, No. 5, 1994, pp. 417-420. doi:10.1055/s-2006-959522
- [12] K. Kohda, K. Itoh, H. Goda, K. Samejima, T. Fukuuchi, N. Morihara, K. Imamura, Y. Kodera and T. Oka, "Enhancing Effect of Aged Garlic Extract on Induction of Morphological Differentiation with Neurite Out-growth in NGF-Treated PC12 Cells," *Pharmacology & Pharmacy*, Vol. 3, No. 1, 2012, pp. 37-43. doi:10.4236/pp.2012.31006
- [13] D. Anderson, "Antioxidant Defences against Reactive Oxygen Species Causing Genetic and Other Damage," *Mutation Research*, Vol. 350, No. 1, 1996, pp. 103-108. doi:10.1016/0027-5107(95)00096-8
- [14] K. F. Gey, "The Antioxidant Hypothesis of Cardiovascular Disease: Epidemiology and Mechanisms," *Biochemical Society Transactions*, Vol. 18, No. 6, 1990, pp. 1041-1045. doi:10.1042/bst0181041
- [15] D. Harman, "Role of Antioxidant Nutrients in Aging: Overview," *AGE*, Vol. 18, 1995, pp. 51-62. doi:10.1007/BF02432519
- [16] V. M. Adhami, A. Malik, N. Zaman, S. Sarfaraz, I. A. Siddiqui, D. N. Syed, F. Afaq, F. S. Pasha, M. Saleem and H. Mukhtar, "Combined Inhibitory Effects of Green Tea Polyphenols and Selective Cyclooxygenase-2 Inhibitors on the Growth of Human Prostate Cancer Cells both in Vitro and in Vivo," *Clinical Cancer Research*, Vol. 13, No. 5, 2007, pp. 1611-1619. doi:10.1158/1078-0432.CCR-06-2269
- [17] I. Tahirovic, E. Sofic, A. Sapcanin, I. Gavrankapetanovic, L. Bach-Rojecky, M. Salkovic-Petrisic, Z. Lackovic, S. Hoyer and P. Riederer, "Brain Antioxidant Capacity in Rat Models of Betacytotoxic-Induced Experimental Sporadic Alzheimer's Disease and Diabetes Mellitus," *Journal of Neural Transmission. Supplement*, No. 72, 2007, pp. 235-240.
- [18] H. Tsumoto, S. Kawahara, Y. Fujisawa, T. Suzuki, H. Nakagawa, K. Kohda and N. Miyata, "Syntheses of Water-Soluble [60]Fullerene Derivatives and Their Enhancing Effect on Neurite Outgrowth in NGF-Treated PC12 Cells," *Bioorganic & Medicinal Chemistry Letters*, Vol. 20, No. 6, 2010, pp. 1948-1952. doi:10.1016/j.bmcl.2010.01.142
- [19] T. Baati, F. Bourasset, N. Gharbi, L. Njim, M. Abderrabba, A. Kerkeni, H. Szwarc and F. Moussa, "The Prolongation of the Lifespan of Rats by Repeated Oral Administration of [60]Fullerene," *Biomaterials*, Vol. 33, No. 19, 2012, pp. 4936-4946. doi:10.1016/j.biomaterials.2012.03.036
- [20] H. Hara, M. Ohta, K. Ohta, S. Kuno and T. Adachi, "Apomorphine Attenuates 6-Hydroxydopamine-Induced Apoptotic Cell Death in SH-SY5Y Cells," *Redox Report*, Vol. 8, No. 4, 2003, pp. 193-197. doi:10.1179/135100003225002682
- [21] W. S. Choi, S. Y. Yoon, T. H. Oh, E. J. Choi, K. L. O' Malley and Y. J. Oh, "Two Distinct Mechanisms Are Involved in 6-Hydroxydopamine-and MPP+-Induced Dopaminergic Neuronal Cell Death: Role of Caspases, ROS, and JNK," *Journal of Neuroscience Research*, Vol. 57, No. 1, 1999, pp. 86-94. doi:10.1002/(SICI)1097-4547(19990701)57:1<86::AID-JNR9>3.0.CO;2-E
- [22] R. von Coelln, S. Kugler, M. Bahr, M. Weller, J. Dichgans and J. B. Schulz, "Rescue from Death but Not from Functional Impairment: Caspase Inhibition Protects Dopaminergic Cells against 6-

- [23] G. Walkinshaw and C. M. Waters, "Neurotoxin-Induced Cell Death in Neuronal PC12 Cells Is Mediated by Induction of Apoptosis," *Neuroscience*, Vol. 63, No. 4, 1994, pp. 975-987. doi:10.1016/0306-4522(94)90566-5
- [24] R. Venugopal and A. K. Jaiswal, "Nrf1 and Nrf2 Positively and c-Fos and Fra1 Negatively Regulate the Human Antioxidant Response Element-Mediated Expression of NAD(P)H:Quinone Oxidoreductase 1 Gene," *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 93, No. 25, 1996, pp. 14960-14965. doi:10.1073/pnas.93.25.14960
- [25] J. Alam, D. Stewart, C. Touchard, S. Boinapally, A. M. K. Choi and J. L. Cook, "Nrf2, a Cap'n' Collar Transcription Factor, Regulates Induction of the Heme Oxygenase-1 Gene," *The Journal of Biological Chemistry*, Vol. 274, No. 37, 1999, pp. 26071-26078. doi:10.1074/jbc.274.37.26071
- [26] H. Motohashi and M. Yamamoto, "Nrf2-Keap1 Defines a Physiologically Important Stress Response Mechanism," *Trends in Molecular Medicine*, Vol. 36, No. 11, 2004, pp. 549-557. doi:10.1016/j.molmed.2004.09.003
- [27] A. K. Jaiswal, "Nrf2 Signaling in Coordinated Activation of Antioxidant Gene Expression," *Free Radical Biology and Medicine*, Vol. 36, No. 10, 2004, pp. 1199-1207. doi:10.1016/j.freeradbiomed.2004.02.074
- [28] K. Itoh, N. Wakabayashi, Y. Katoh, T. Ishii, K. Igarashi, J. D. Engel and M. Yamamoto, "Keap1 Represses Nuclear Activation of Anti-oxidant Responsive Elements by Nrf2 through Binding to the Amino-Terminal Neh2 Domain," *Genes & Development*, Vol. 13, No. 1, 1999, pp. 76-86. doi:10.1101/gad.13.1.76
- [29] J. M. Lee, J. D. Moehlenkamp, J. M. Hanson and J. A. Johnson, "Nrf2-Dependent Activation of the Antioxidant Responsive Element by Tert-Butylhydroquinone Is Independent of Oxidative Stress in IMR-32 Human Neuroblastoma Cells," *Biochemical Biophysical Research Communications*, Vol. 280, No. 1, 2001, pp. 286-292. doi:10.1006/bbrc.2000.4106
- [30] T. Mosmann, "Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays," *Journal of Immunological Methods*, Vol. 65, No. 1-2, 1983, pp. 55-63. doi:10.1016/0022-1759(83)90303-4
- [31] A. S. Keston and R. Brandt, "The Fluorometric Analysis of Ultramicro Quantities of Hydrogen Peroxide," *Analytical Biochemistry*, Vol. 11, No. 1, 1965, pp. 1-5. doi:10.1016/0003-2697(65)90034-5
- [32] H. Hara and T. Adachi, "Contribution of Hepatocyte Nuclear Factor-4 to Down-Regulation of CYP2D6 Gene Expression by Nitric Oxide," *Molecular Pharmacology*, Vol. 61, No. 1, 2002, pp. 194-200. doi:10.1124/mol.61.1.194
- [33] M. M. Bradford, "A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding," *Analytical Biochemistry*, Vol. 72, No. 1-2, 1976, pp. 248-254. doi:10.1016/0003-2697(76)90527-3
- [34] K. Kohda, Y. Noda, S. Aoyama, M. Umeda, T. Sumino, T. Kaiya, W. Maruyama and M. Naoi, "Cytotoxicity of 1-Amino-4-phenyl-1,2,3,6-tetrahydropyridine and 1-Amino-4-phenylpyridinium Ion, 1-Amino Analogues of MPTP and MPP+, to Clonal Pheochromocytoma PC12 Cells," *Chemical Research in Toxicology*, Vol. 11, No. 11, 1998, pp. 1249-1253. doi:10.1021/tx980032o
- [35] K. Mineura, I. Izumi, K. Watanabe, M. Kowada, K. Kohda, K. Koyama, I. Terashima and M. Ikenaga, "Enhancing Effect of O6-Alkylguanine Derivatives on Chloroethylnitrosourea Cytotoxicity toward Tumor Cells," *International Journal of Cancer*, Vol. 58, No. 5, 1994, pp. 706-712. doi:10.1002/ijc.2910580515
- [36] N. Morihara, N. Ide, I. Sumioka and E. Kyo, "Aged Garlic Extract Inhibits Peroxynitrite-Induced Hemolysis," *Redox Report*, Vol. 10, No. 3, 2005, pp. 159-165. doi:10.1179/135100005X57364
- [37] N. Morihara, M. Hayama and H. Fujii, "Aged Garlic Extract Scavenges Superoxide Radicals," *Plant Foods for Human Nutrition*, Vol. 66, No. 1, 2011, pp. 17-21. doi:10.1007/s11130-011-0216-6
- [38] R. Venugopal and A. K. Jaiswal, "Nrf2 and Nrf1 in Association with Jun Proteins Regulate Antioxidant Response Element-Mediated Expression and Coordinated Induction of Genes Encoding Detoxifying Enzyme," *Oncogene*, Vol. 17, No. 24, 1998, pp. 3145-3156.

- [39] A. L. Egger, K. A. Gay and A. D. Mesecar, "Molecular Mechanisms of Natural Products in Chemoprevention: Induction of Cytoprotective Enzymes by Nrf2," *Molecular Nutrition & Food Research*, Vol. 52, Suppl. 1, 2008, pp. S84-S94.
- [40] K. W. Kang, S. J. Lee, J. W. Park and S. J. Kim, "Phosphatidylinositol 3-Kinase Regulates Nuclear Translocation of NF-E2-Related Factor 2 through Actin Rearrangement in Response to Oxidative Stress," *Molecular Pharmacology*, Vol. 62, No. 5, 2002, pp. 1001-1010. doi:10.1124/mol.62.5.1001
- [41] A. T. Dinkova-Kostova, W. D. Holtzclaw, R. N. Cole, K. Itoh, N. Wakabayashi, Y. Katoh, M. Yamamoto and P. Talalay, "Direct Evidence That Sulfhydryl Groups of Keap1 Are the Sensors Regulating Induction of Phase 2 Enzymes That Protect against Carcinogens and Oxidants," *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 99, No. 18, 2002, pp. 11908-11913. doi:10.1073/pnas.172398899
- [42] X. Gao, A. T. Dinkova-Kostova and P. Talalay, "Powerful and Prolonged Protection of Human Retinal Pigment Epithelial Cells, Keratinocytes, and Mouse Leukemia Cells against Oxidative Damage: The Indirect Antioxidant Effects of Sulforaphane," *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 98, No. 26, 2001, pp. 15221-15226. doi:10.1073/pnas.261572998
- [43] Y. Kodera, H. Matsuura, H. Sumiyoshi and S. Sumi, "Garlic Chemistry: Chemical and Biological Properties of Sulfur-Containing Compounds Derived from Garlic," In: F. Shahadi, C. T. Ho, S. Watanabe and T. Osawa, Eds., *ACS Symposium Series 851. Food Factors in Health Promotion and Disease Prevention*, Oxford University Press, Oxford, 2003, pp. 346-357.
- [44] C. Y. Ho, Y. T. Cheng, C. F. Chau and G. C. Yen, "Effect of Diallyl Sulfide on in Vitro and in Vivo Nrf2-Mediated Pulmonic Antioxidant Enzyme Expression via Activation ERK/p38 Signaling Pathway," *Journal of Agricultural and Food Chemistry*, Vol. 60, No. 1, 2012, pp. 100-107. doi:10.1021/jf203800d
- [45] H. Y. Kay, J. Won Yang, T. H. Kim, D. Y. Lee, B. Kang, J. H. Ryu, R. Jeon and S. J. Kim, "Ajoene, a Stable Garlic By-Product, Has an Antioxidant Effect through Nrf2-Mediated Glutamate-Cysteine Ligase Induction in HepG2 Cells and Primary Hepatocytes," *The Journal of Nutrition*, Vol. 140, No. 7, 2010, pp. 1211-1219. doi:10.3945/jn.110.121277
- [46] S. Kalayarsan, N. Sriram, A. Sureshkumar and G. Sudhandiran, "Chromium (VI)-Induced Oxidative Stress and Apoptosis Is Reduced by Garlic and Its Derivative S-Allylcysteine through the Activation of Nrf2 in the Hepatocytes of Wistar Rats," *Journal of Applied Toxicology*, Vol. 28, No. 7, 2008, pp. 908-919. doi:10.1002/jat.1355
- [47] C. D. Fisher, L. M. Augustine, J. M. Maher, D. M. Nelson, A. L. Slitt, C. D. Klaassen, L. D. Lehman-McKeeman and N. J. Cherrington, "Induction of Drug-Metabolizing Enzymes by Garlic and Allyl Sulfide Compounds via Activation of Constitutive Androstane Receptor and Nuclear Factor E2-Related Factor 2," *Drug Metabolism & Disposition*, Vol. 35, No. 6, 2007, pp. 995-1000. doi:10.1124/dmd.106.014340
- [48] C. Chen, D. Pung, V. Leong, V. Hebbar, G. Shen, S. Nair, W. Li and A. N. Kong, "Induction of Detoxifying Enzymes by Garlic Organosulfur Compounds through Transcription Factor Nrf2: Effect of Chemical Structure and Stress Signals," *Free Radical Biology & Medicine*, Vol. 37, No. 10, 2004, pp. 1578-1590. doi:10.1016/j.freeradbiomed.2004.07.021
- [49] A. O. Lawal and E. M. Ellis, "The Chemopreventive Effects of Aged Garlic Extract against Cadmium-Induced Toxicity," *Environmental Toxicology and Pharmacology*, Vol. 32, No. 2, 2011, pp. 266-274. doi:10.1016/j.etap.2011.05.012
- [50] D. Martin, A. I. Rojo, M. Salinas, R. Diaz, G. Gallardo, J. Alam, C. Ruiz de Galarreta and A. Cuadrado, "Regulation of Heme Oxygenase-1 Expression through the Phosphatidylinositol 3-Kinase/Akt Pathway and the Nrf2 Transcription Factor in Response to the Antioxidant Phytochemical Carnosol," *The Journal of Biological Chemistry*, Vol. 279, No. 10, 2004, pp. 8919-8929. doi:10.1074/jbc.M309660200
- [51] M. Salazar, A. I. Rojo, D. Velasco, R. Maria de Sagarra and A. Cuadrado, "Glycogen Synthase Kinase-3b Inhibits the Xenobiotic and Antioxidant Cell Response by Direct Phosphorylation and Nuclear Exclusion of the Transcription Factor Nrf2," *The Journal of Biological Chemistry*, Vol. 281, No. 21, 2006, pp. 14841-14851. doi:10.1074/jbc.M513737200
- [52] Y. P. Hwang and H. G. Jeong, "Ginsenoside Rb1 Protects against 6-Hydroxydopamine-Induced Oxidative Stress by Increasing Heme Oxygenase-1 Expression through an Estrogen Receptor-Related PI3K/Akt/Nrf2-Dependent Pathway in Human Dopaminergic Cells," *Toxicology and Applied*