Chemical Research in Toxicology

Rapid Report

pubs.acs.org/crt

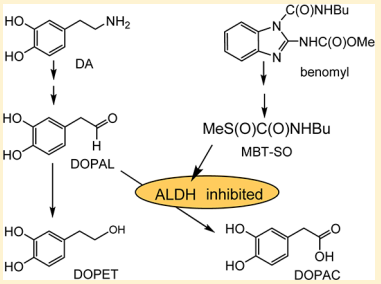
Benomyl, Aldehyde Dehydrogenase, DOPAL, and the Catecholaldehyde Hypothesis for the Pathogenesis of Parkinson's Disease

John E. Casida,*[,†] Breanna Ford,[†] Yunden Jinsmaa,[‡] Patti Sullivan,[‡] Adele Cooney,[‡] and David S. Goldstein*[,‡]

[†]Environmental Chemistry and Toxicology Laboratory, University of California, Berkeley, California 94720-3112, United States

[‡]Clinical Neurocardiology Section, Clinical Neurosciences Program, Division of Intramural Research, National Institute of Neurological Disorders and Stroke, Bethesda, Maryland 20892-1620, United States

ABSTRACT: The dopamine metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL) is detoxified mainly by aldehyde dehydrogenase (ALDH). We find that the fungicide benomyl potently and rapidly inhibits ALDH and builds up DOPAL *in vivo* in mouse striatum and *in vitro* in PC12 cells and human cultured fibroblasts and glial cells. The *in vivo* results resemble those noted previously with knockouts of the genes encoding ALDH1A1 and 2, a mouse model of aging-related Parkinson's disease (PD). Exposure to pesticides that inhibit ALDH may therefore increase PD risk via DOPAL buildup. This study lends support to the "catecholaldehyde hypothesis" that the autotoxic dopamine metabolite DOPAL plays a pathogenic role in PD.



Pesticides have long been implicated in the pathogenesis of Parkinson's disease (PD).[1−3] A recent epidemiologic study noted increased PD risk from occupational or residential exposure to the fungicide benomyl.[4,5] Benomyl undergoes bioactivation to *S*-methyl *N*-butylthiocarbamate sulfoxide (MBT-SO), a potent aldehyde dehydrogenase (ALDH) inhibitor,[6,7] and ALDH is the main enzyme metabolizing the toxic dopamine metabolite, 3,4-dihydroxyphenylacetaldehyde (DOPAL). Thus, a potential mechanism for increased PD risk from benomyl exposure is ALDH inhibition and consequent DOPAL build-up,[4] in line with the "catecholaldehyde hypothesis" (Figure 1).[8−13]

In this study, we tested whether benomyl produces a neurochemical pattern indicating ALDH inhibition, *in vivo* in mouse striatum and *in vitro* in rat pheochromocytoma PC12 cells and in human cultured fibroblasts and glial cells.

Mice received intraperitoneally (ip) administered benomyl at 40 mg/kg and were sacrificed 2 h later and the brains recovered. Frozen striatum samples were homogenized in 20:80 0.2 M phosphoric acid/0.2 M acetic acid and the supernate transferred to plastic cryotubes and stored at −80 °C until assayed by batch alumina extraction followed by liquid chromatography with serial electrochemical detection[14,15] (Figure 2A). Three and only three of the seven catechols analyzed showed significant changes relative to controls without benomyl (Figure 2B). DOPAL increased by 3.1-fold and DOPET by 2.5-fold, while DOPAC decreased. ALDH inhibition was evident by the decrease in DOPAC with respect to elevations of both DOPAL and DOPET. The tissue concentration ratio of DOPAC/(DOPAL + DOPET) was therefore used as a neurochemical index of ALDH activity. The magnitude of the benomyl-induced decrease in the striatal DOPAC/(DOPAL + DOPET) ratio (Figure 2C1 and D1) was not greatly changed when benomyl was coadministered with reserpine (40 mg/kg) and L-DOPA (20 mg/kg) (Figure 2C2 and D2) and closely resembled that reported previously in *Aldh1a1*−/− × *Aldh2*−/− knockout mice (Figure 2C3 and D3), an animal model of aging-related PD.[16]

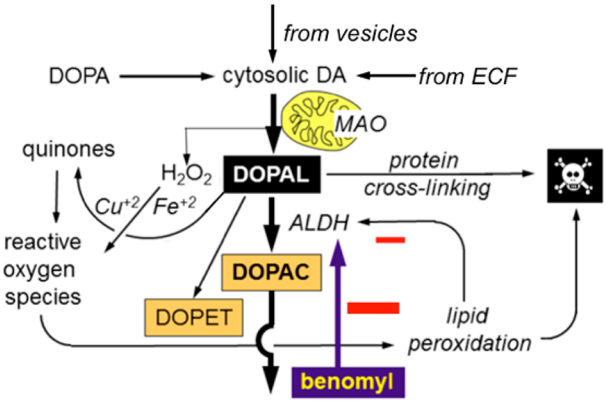


Figure 1. Catecholaldehyde hypothesis for PD. The toxic dopamine (DA) metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL) is formed from mitochondrial monoamine oxidase (MAO) acting on cytoplasmic DA. DOPAL cytotoxicity occurs via oxidative injury and protein cross-linking. DOPAL is detoxified by aldehyde dehydrogenase (ALDH) to form 3,4-dihydroxyphenylacetic acid (DOPAC). An alternative metabolite is 3,4-dihydroxyphenylethanol (DOPET).

Received: June 5, 2014
Published: July 21, 2014

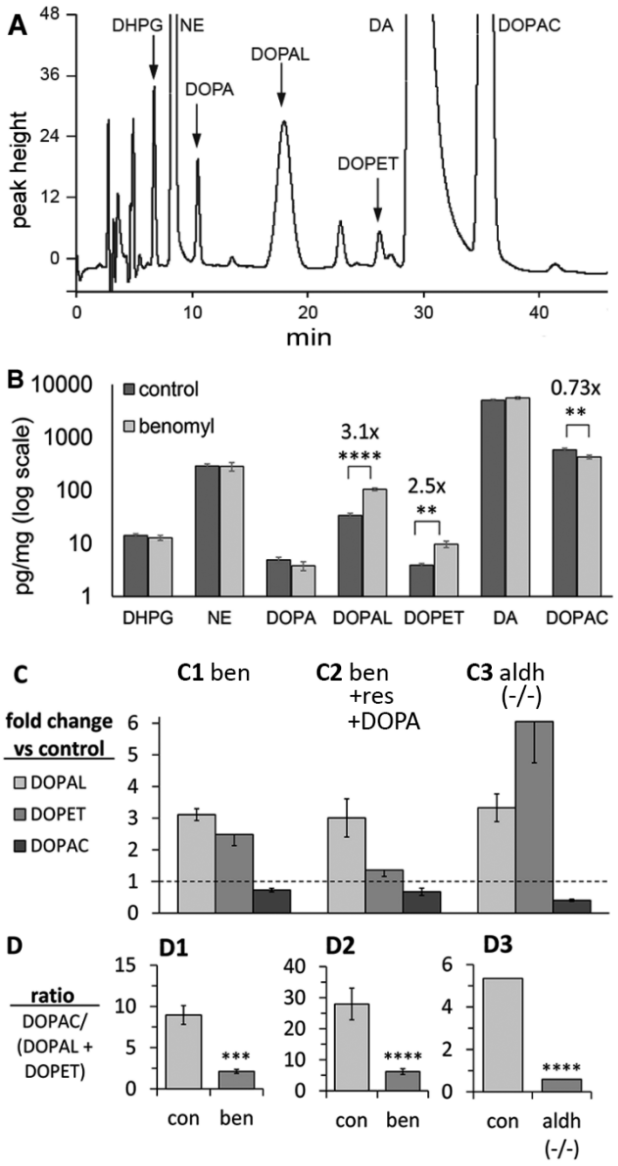


Figure 2. Effects of benomyl (40 mg/kg, ip, 2 h after treatment) and ALDH −/− gene knockout on DA-derived catechols in mouse striatum. (A) Chromatogram of extracted catechols from a control mouse. (B) Catechol levels on a log scale showing benomyl-induced increase in DOPAL and DOPET and decrease in DOPAC (mean ± SEM). (C1−C3) DOPAL, DOPET, and DOPAC levels on a linear scale and D1−D3 the same data expressed as DOPAC/(DOPAL + DOPET) ratios. C1 and D1: 2 h after benomyl. C2 and D2: 2 h after benomyl with reserpine and L-DOPA. C3 and D3: ALDH −/− mice data from Wey et al.[16] Levels are relative to no benomyl (C1 and C2) or ALDH −/− (C3). Different from control (mean ± SEM, $n = 5–6$), $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

To supplement the *in vivo* data, three types of cells were used to determine effects of benomyl *in vitro* on contents of catechols. Nonadherent rat PC12 cells[17,18] were obtained from ATCC (Manassas, VA). The PC12 cells were cultured in F12 media containing 15% human serum (HS) plus 2.5% fetal calf serum (FCS) and pretreated for 24 h with 10 μM tolcapone to block catechol-*O*-methyltransferase.[13] Human fibroblasts were from a skin biopsy of a PD patient. The fibroblasts were cultured in minimal essential medium (MEM)-alpha containing 10% FCS and penicillin/streptomycin. Human glioblastoma cells were from ATCC. The glial cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% FCS.

PC12 cells (500,000/well) were placed in 12 well plates and incubated for 24 h at 37 °C. The medium was then changed to F12 containing benomyl (0, 5, 10, 50, 100, 500, and 1000 nM). The medium was removed and the cells collected at 180 min, transferred into Eppendorf sample tubes, centrifuged, and resuspended in 400 μL of 20:80 0.2 M phosphoric acid/0.2 M acetic acid, frozen in dry ice, and stored at −80 °C before thawing for catechol assays. Human fibroblasts (50,000/well) were cultured for 3 days and glioblastoma cells (100,000/well) for 2 days in 12 well plates at 37 °C in a 5% CO_2 incubator. Then, the medium was replaced with FCS-free MEM-alpha for fibroblasts and DMEM for glia containing no benomyl (control) or 1000 nM benomyl and preincubated for 10 min before adding 3 μM DOPAL to determine its metabolism as the substrate. The samples were collected at 10, 20, 30, 60, 120, and 180 min. The medium was removed, and cells were washed with 1× PBS and then scraped into 400 μL of 20:80 phosphoric acid/acetic acid as described above, frozen in dry ice, and kept at −80 °C before thawing for catechol assays.

In all three cell types, benomyl greatly decreased the DOPAC/(DOPAL + DOPET) ratios (Figure 3). The

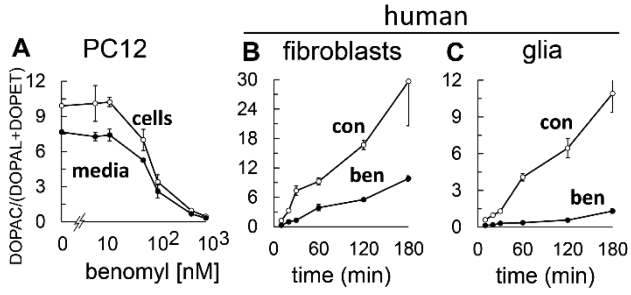


Figure 3. Effects of benomyl on DOPAC/(DOPAL + DOPET) ratios in three cell types. (A) PC12 cells and media at 180 min as a function of benomyl concentration (0−1000 nM). (B and C) Human cultured fibroblasts and glial cells at 0 nM (control) or 1000 nM benomyl as a function of time after adding DOPAL as the substrate (mean ± SEM, $n = 3–5$).

concentration for half-maximal effect with PC12 cells was about 50 nM, based on an analysis of the cells or the media at 180 min (Figure 3A). Time-series studies established the rapid action of 1000 nM benomyl in both human fibroblasts and glia (Figure 3B and C). Thus, in the cells as in the mouse striatum, the principal catechols affected were DOPAL and DOPET increasing and DOPAC decreasing, as expected for ALDH inhibition.

ALDH inhibitors are of both pharmacological and toxicological interest and include several pesticides.[19,20] The mouse and cell systems described here are potential *in vivo* and *in vitro* models to assay pesticides, environmental chemicals, and pharmaceuticals as candidate contributors to PD by disrupting DOPAL detoxification (Figure 1).

An alternative to the catecholaldehyde hypothesis of PD that still involves ALDH inhibition is *trans*-4-hydroxy-2-nonenal as the neurotoxicant formed on membrane lipid peroxidation and serving as an ALDH inhibitor for both its own detoxification and that of DOPAL.[21−23] The catecholaldehyde hypothesis predicts straightforwardly that inhibition of MAO should attenuate pesticide-evoked cytotoxicity. This has been demonstrated for the insecticide rotenone with pathophysiological

dx.doi.org/10.1021/tx5002223 | *Chem. Res. Toxicol.* 2014, 27, 1359−1361

mechanisms involving mitochondrial dysfunction and oxidative stress[1−3] and for benomyl as an ALDH inhibitor.[4]

AUTHOR INFORMATION

Corresponding Authors

*(J.E.C.) Phone: 510-642-5424. Fax: 510-642-6497. E-mail: ectl@berkeley.edu.

*(D.S.G.) Phone: 301-496-2103. Fax: 301-402-0180. E-mail: goldstein@ninds.nih.gov.

Funding

B.F. was supported by Sponsored Projects for Undergraduates Program at the University of California, Berkeley. Research at Bethesda was supported by Intramural Research Program of the National Institute of Neurological Disorders and Stroke.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Madhur Garg, Liane Kuo, and Amanda Ly for assistance.

ABBREVIATIONS

ALDH, aldehyde dehydrogenase; DA, dopamine; DHPG, 3,4-dihydroxyphenylglycol; DMEM, Dulbecco's modified Eagle's medium; DOPA, 3,4-dihydroxyphenylalanine; DOPAC, 3,4-dihydroxyphenylacetic acid; DOPAL, 3,4-dihydroxyphenylacetaldehyde; DOPET, 3,4-dihydroxyphenylethanol; ECF, extracellular fluid; FCS, fetal calf serum; HS, human serum; ip, intraperitoneal; MAO, monoamine oxidase; MBT-SO, *S*-methyl *N*-butylthiocarbamate sulfoxide; MEM, minimal essential medium; NE, norepinephrine; PD, Parkinson's disease

REFERENCES

(1) Di Monte, D. A. (2003) The environment and Parkinson's disease: is the nigrostriatal system preferentially targeted by neurotoxins? *Lancet Neurol. 2*, 531−538.

(2) Betarbet, R., Sherer, T. B., MacKenzie, G., Garcia-Osuna, M., Panov, A. V., and Greenamyre, J. T. (2000) Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat. Neurosci. 3*, 1301−1306.

(3) Tanner, C. M., Kamel, F., Ross, G. W., Hoppin, J. A., Goldman, S. M., Korell, M., Marras, C., Bhudhikanok, G. S., Kasten, M., Chade, A. R., Comyns, K., Richards, M. B., Meng, C., Priestly, B., Fernandez, H. H., Cambi, F., Umbach, D. M., Blair, A., Sandler, D. P., and Langston, J. W. (2011) Rotenone, paraquat, and Parkinson's disease. *Environ. Health Perspect. 119*, 866−872.

(4) Fitzmaurice, A. G., Rhodes, S. L., Lulla, A., Murphy, N. P., Lam, H. A., O'Donnell, K. C., Barnhill, L., Casida, J. E., Cockburn, M., Sagasti, A., Stahl, M. C., Maidment, N. T., Ritz, B., and Bronstein, J. M. (2013) Aldehyde dehydrogenase inhibition as a pathogenic mechanism in Parkinson disease. *Proc. Natl. Acad. Sci. U.S.A. 110*, 636−641.

(5) Fitzmaurice, A. G., Rhodes, S. L., Cockburn, M., Ritz, B., and Bronstein, J. M. (2014) Aldehyde dehydrogenase variation enhances effect of pesticides associated with Parkinson disease. *Neurology 82*, 419−426.

(6) Staub, R. E., Quistad, G. B., and Casida, J. E. (1998) Mechanism for benomyl action as a mitochondrial aldehyde dehydrogenase inhibitor in mice. *Chem. Res. Toxicol. 11*, 535−543.

(7) Staub, R. E., Quistad, G. B., and Casida, J. E. (1999) *S*-Methyl *N*-butylthiocarbamate sulfoxide. Selective carbamoylating agent for mouse mitochondrial aldehyde dehydrogenase. *Biochem. Pharmacol. 58*, 1467−1473.

(8) Burke, W. J., Li, S. W., Williams, E. A., Nonneman, R., and Zahm, D. S. (2003) 3,4-Dihydroxyphenylacetaldehyde is the toxic dopamine metabolite in vivo: implications for Parkinson's disease pathogenesis. *Brain Res. 989*, 205−213.

(9) Rees, J. N., Florang, V. R., Eckert, L. L., and Doorn, J. A. (2009) Protein reactivity of 3,4-dihydroxyphenylacetaldehyde, a toxic dopamine metabolite, is dependent on both the aldehyde and the catechol. *Chem. Res. Toxicol. 22*, 1256−1263.

(10) Panneton, W. M., Kumar, V. B., Gan, Q., Burke, W. J., and Galvin, J. E. (2010) The neurotoxicity of DOPAL: behavioral and stereological evidence for its role in Parkinson disease pathogenesis. *PLoS One 5*, e15251.

(11) Anderson, D. G., Santhana Mariappan, S. V., Buettner, G. R., and Doorn, J. A. (2011) Oxidation of 3,4-dihydroxyphenylacetaldehyde, a toxic dopaminergic metabolite, to a semiquinone radical and an *ortho*-quinone. *J. Biol. Chem. 286*, 26978−26986.

(12) Goldstein, D. S., Sullivan, P., Holmes, C., Kopin, I. J., Basile, M. J., and Mash, D. C. (2011) Catechols in post-mortem brain of patients with Parkinson disease. *Eur. J. Neurol. 18*, 703−710.

(13) Goldstein, D. S., Sullivan, P., Cooney, A., Jinsmaa, Y., Sullivan, R., Gross, D. J., Holmes, C., Kopin, I. J., and Sharabi, Y. (2012) Vesicular uptake blockade generates the toxic dopamine metabolite 3,4-dihydroxyphenylacetaldehyde in PC12 cells: relevance to the pathogenesis of Parkinson's disease. *J. Neurochem. 123*, 932−943.

(14) Holmes, C., Eisenhofer, G., and Goldstein, D. S. (1994) Improved assay for plasma dihydroxyphenylacetic acid and other catechols using high-performance liquid chromatography with electrochemical detection. *J. Chromatogr. B: Biomed. Sci. Appl. 653*, 131−138.

(15) Goldstein, D. S., Sullivan, P., Holmes, C., Miller, G. W., Alter, S., Strong, R., Mash, D. C., Kopin, I. J., and Sharabi, Y. (2013) Determinants of buildup of the toxic dopamine metabolite DOPAL in Parkinson's disease. *J. Neurochem. 126*, 591−603.

(16) Wey, M.C.-Y., Fernandez, E., Martinez, P. A., Sullivan, P., Goldstein, D. S., and Strong, R. (2012) Neurodegeneration and motor dysfunction in mice lacking cytosolic and mitochondrial aldehyde dehydrogenases: implications for Parkinson's disease. *PLoS One 7*, e31522.

(17) Lamensdorf, I., Eisenhofer, G., Harvey-White, J., Hayakawa, Y., Kirk, K., and Kopin, I. J. (2000) Metabolic stress in PC12 cells induces the formation of the endogenous dopaminergic neurotoxin, 3,4-dihydroxyphenylacetaldehyde. *J. Neurosci. Res. 60*, 552−558.

(18) Ohsawa, I., Nishimaki, K., Yasuda, C., Kamino, K., and Ohta, S. (2003) Deficiency in a mitochondrial aldehyde dehydrogenase increases vulnerability to oxidative stress in PC12 cells. *J. Neurochem. 84*, 1110−1117.

(19) Marchitti, S. A., Deitrich, R. A., and Vasiliou, V. (2007) Neurotoxicity and metabolism of the catecholamine-derived 3,4-dihydroxyphenylacetaldehyde and 3,4-dihydroxyphenylglycolaldehyde: the role of aldehyde dehydrogenase. *Pharmacol. Rev. 59*, 125−150.

(20) Koppaka, V., Thompson, D. C., Chen, Y., Ellermann, M., Nicolaou, K. C., Juvonen, R. O., Petersen, D., Deitrich, R. A., Hurley, T. D., and Vasiliou, V. (2012) Aldehyde dehydrogenase inhibitors: a comprehensive review of the pharmacology, mechanism of action, substrate specificity, and clinical application. *Pharmacol. Rev. 64*, 520−539.

(21) Leiphon, L. T., and Picklo, M. J., Sr. (2007) Inhibition of aldehyde detoxification in CNS mitochondria by fungicides. *Neurotoxicology 28*, 143−149.

(22) Doorn, J. A., Hurley, T. D., and Peterson, D. R. (2006) Inhibition of human mitochondrial aldehyde dehydrogenase by 4-hydroxynon-2-enal and 4-oxonon-2-enal. *Chem. Res. Toxicol. 19*, 102−110.

(23) Jinsmaa, Y., Florang, V. R., Rees, J. N., Anderson, D. G., Strack, S., and Doorn, J. A. (2009) Products of oxidative stress inhibit aldehyde oxidation and reduction pathways in dopamine catabolism yielding elevated levels of a reactive intermediate. *Chem. Res. Toxicol. 22*, 835−841.