Modulation of Cellular Response to Arsenic Trioxide Toxicity by Resveratrol

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ABSTRACT: Arsenic trioxide (As$_2$O$_3$) is an environmental carcinogen and a putative endocrine disruptor. Resveratrol has been shown to reverse As$_2$O$_3$-induced oxidative damage. In immortalized but nontransformed estrogen receptor $\alpha$-negative human breast cells (MCF10A), we observed that 25 $\mu$M resveratrol ameliorated As$_2$O$_3$-induced cytotoxicity. As$_2$O$_3$, in the presence or absence of 25 $\mu$M resveratrol, induced quinone reductase (NAD(P)H quinone dehydrogenase 1, via the induction of NFE2-related factor 2. As$_2$O$_3$ caused a repression of cytochrome P450 (CYP)1B1, but the addition of 25 $\mu$M resveratrol rescued the expression of cytochrome P450 1B1 and kept it at a constant level. Therefore, 25 $\mu$M resveratrol can modulate the effects of As$_2$O$_3$ on enzymes involved in estrogen metabolism.

INTRODUCTION

Arsenic is the 20th most abundant element in the earth’s crust, and trivalent arsenic trioxide (As$_2$O$_3$) contaminates groundwater in many places, leading to arsenic poisoning or arsenicosis. Arsenic and inorganic arsenic compounds have been classified as IARC Group 1 carcinogens with lung, bladder, kidney, and liver as the predominant targets. At the same time, As$_2$O$_3$ (Trisenox or arsenic trioxide) is being used as a chemotherapy drug against acute promyelocytic leukemia (APL), where it selectively kills the leukemic cells but allows the proper development of regular blood cells. Interestingly, arsenic has long been implicated in endocrine disruption. Target genes for glucocorticoids, androgens, mineralocorticoids, and progestin receptors have also been reported to be regulated by inorganic arsenic in a biphasic dose-response fashion. To address such documented carcinogenicity and putative endocrine disruption, the U.S. Environmental Protection Agency has set the maximum contaminant level for As$_2$O$_3$ at 10 ppb.

The principal pathways implicated in As$_2$O$_3$ toxicity result from reactive oxygen species, oxidative DNA damage, and induction of apoptosis. Resveratrol, a well-known dietary stilbene, has been shown to protect normal human bronchial epithelial cells from As$_2$O$_3$ toxicity by maintaining glutathione homeostasis. Cardiotoxicity, a major side effect of using As$_2$O$_3$ for APL, could be ameliorated in Wistar rats by resveratrol, via the maintenance of a balanced expression of the NFE2-related factor 2 (Nrf2)-heme oxygenase (HO) 1 pathway, and by promoting arsenic efflux from cells. Employing similar mechanisms, resveratrol has been reported to protect from As$_2$O$_3$-induced nephrotoxicity in male Wistar rats and from hepatotoxicity in Chinese Dragon-Li cats. Finally, research by at least one group has demonstrated the ability of inorganic arsenic to promote carcinogenesis via a nonestrogen receptor (ER)-mediated pathway: chronic exposure (18 weeks) to environmentally relevant 0.5 $\mu$M arsenite promoted cancer cell phenotypes in human prostate epithelial stem/progenitor cells (WPE-stem) and in their isogenic parental RWPE-1 cells (30 weeks). Chronic exposure (24 weeks) to low-level arsenite (500 nM) has also been reported to do the same thing in human breast epithelial cells (MCF10A) via the overexpression of aromatase. To the best of our knowledge, no studies have explored any protective role of resveratrol in As$_2$O$_3$-induced carcinogenesis via non-ER-mediated pathways.

Estrogens have been implicated in the development of a variety of cancers. Several types of evidence suggest the role of estrogen in tumor development in an ER knock-out transgenic mouse model of breast cancer, as well as in the transformation of ER$\alpha$-negative breast epithelial cells (MCF10F). To explain such receptor-independent pathways for cancer initiation, it has been hypothesized that the metabolism of estrogens/androgens generates catechol quinones that can react with DNA, leading to the formation of apurinic sites and mutations, to initiate oncogenic transformation. In high-risk conditions.
groups and cancer patients (women: breast, ovarian, and thyroid; men: prostate and non-Hodgkin lymphoma), an imbalance in estrogen metabolism stems from irregular expression patterns of one or more of the four key estrogen-metabolizing enzymes: cytochrome P450 19 or aromatase, cytochrome P450 1B1 (CYP1B1), catechol-O-methyltransferase, and quinone reductase (NAD(P)H quinone dehydrogenase 1 (NQO1)). Certain naturally occurring or synthetic compounds have the ability to alter the expression of some of these enzymes; for example, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) can induce CYP1B1, whereas resveratrol antagonizes this effect. At the same time, resveratrol can induce NQO1.

Our laboratory has shown that preincubation with 25 μM resveratrol for 48 h significantly lowered the production of estrogen-DNA adduct formation in ERα-negative human breast epithelial MCF10F cells. Therefore, in this study, the effects of As₂O₃ on the expression levels of key estrogen-metabolizing enzymes (NQO1 and CYP1B1), and on the signaling molecule Nrf2, were studied in the presence or absence of 25 μM resveratrol in immortalized but nontransformed ERα-negative human breast epithelial cells (MCF10A).

**RESULTS AND DISCUSSION**

As₂O₃ exhibited cytotoxicity at both 48 and 72 h, with an IC₅₀ at 1.45 μM for 48 h treatment (Figure 1A), which is an environmentally significant exposure. However, the presence of resveratrol, which also has some cytotoxicity (Figure 1B) at 25 μM, reduced this cytotoxicity by shifting the survival curves to the right. The IC₅₀ for the combined 48 h As₂O₃ and resveratrol treatment was 28.69 μM. The rightward shift in the survival curve was lesser for 72 h treatment compared to that for 48 h treatment (Figure 1C). This indicates that the rescue effect from resveratrol is more pronounced at 48 h.

As₂O₃, with or without 25 μM resveratrol, induced the enzyme NQO1 in a dose-dependent manner, but no additive or synergistic effects were observed (Figure 2A,B). Resveratrol has...
been shown to act via the signaling molecule Nrf2, and NQO1 has been implicated as a downstream gene of Nrf2. This prompted us to study the effect of As$_2$O$_3$ on the expression level of Nrf2. As$_2$O$_3$, in both the presence and absence of 25 μM resveratrol, induced Nrf2 in a dose-dependent fashion, but again no additive or synergistic effects were observed (Figure 2C,D). Our finding is in accordance with a study in mouse hepatoma cell line hepa1c1c7, in which arsenic was shown to induce Nrf2 protein in a dose-dependent fashion, leading to a robust induction of NQO1. Furthermore, in MCF10A cells the extent of induction was much greater for Nrf2 compared to that for NQO1, but this may stem from the fact that resting cells have a very low-level basal expression of Nrf2 ($t_{1/2} \approx 10-20$ min). Similarly, disproportionate induction of NQO1 and Nrf2 by As$_2$O$_3$ has been reported in male Kunming mouse testis in both the presence and absence of antioxidants such as lutein.

CYP1B1 is another key estrogen-metabolizing enzyme that is overexpressed in a variety of cancers, including breast cancer, and is subject to regulation through both hormonal and a putative aryl hydrocarbon receptor (AhR) pathways; the latter can be modulated by resveratrol. Previous studies on the male C57Bl/6 mouse heart have reported that 12.5 mg/kg As(III) induces CYP1B1 mRNA levels by 150%. We observed, however, that As$_2$O$_3$ treatment (0.5−5 μM) of human breast epithelial MCF10A cells caused a repression of CYP1B1 protein in a dose-dependent manner. Resveratrol was earlier shown to downregulate TCDD-induced CYP1B1 expression in MCF10F cells. Interestingly, the addition of 25 μM resveratrol rescued As$_2$O$_3$-suppressed CYP1B1 expression and

Figure 2. Induction of NQO1 (A, B) and Nrf2 (C, D) by As$_2$O$_3$ with and without resveratrol. (A, C) Representative images. (B, D) Quantitative data. Data are presented as mean ± standard error of the mean protein expression (%) from three independent experiments (n = 3), X = mean of two experiments. A † indicates significant difference (p < 0.05) between As$_2$O$_3$ treatment groups and control, whereas an * indicates significant difference (p < 0.05) between As$_2$O$_3$ treatment groups and control in the resveratrol group.
kept it at a nearly constant level (Figure 3A,B). To confirm this interesting observation, the expression of CYP1B1 enzyme was further studied simultaneously in three different nontransformed cell lines derived from human breast epithelia, namely MCF10A, MCF10F, and MCF12F cells. In this study, the cells were also treated with a higher dose of 7.5 μM As₂O₃. The repressive effect of As₂O₃ on CYP1B1 expression was less pronounced in MCF10F cells, leaving little room for resveratrol to exert any rescue effect (Supporting Information, Figure S1C). Collectively, these results suggest the induction of the signaling protein Nrf2. To the best of our knowledge, however, this is the first time resveratrol has been shown to rescue As₂O₃-suppressed CYP1B1 expression back to the baseline levels. Collectively, these results suggest that 25 μM resveratrol has the ability to modulate the effects of As₂O₃ on the expression of estrogen-metabolizing enzymes in MCF10A cells. Whether such modulation leads to any potential benefit in estrogen metabolism and carcinogenesis needs to be addressed in future studies of the effects of As₂O₃ and resveratrol on estrogen metabolism.

**Conclusions.** In the present study involving nontransformed breast epithelial MCF10A cells, 25 μM resveratrol was shown to ameliorate As₂O₃-induced cytotoxicity, with the beneficial effect being more prominent after 48 h treatment. As expected, both As₂O₃ and resveratrol induced the expression of the catechol quinone-quenching enzyme NQO1, possibly via the induction of the signaling protein Nrf2. To the best of our knowledge, however, this is the first time resveratrol has been shown to rescue As₂O₃-suppressed CYP1B1 expression back to the baseline levels. Collectively, these results suggest that 25 μM resveratrol has the ability to modulate the effects of As₂O₃ on the expression of estrogen-metabolizing enzymes in MCF10A cells. Whether such modulation leads to any potential benefit in estrogen metabolism and carcinogenesis needs to be addressed in future studies of the effects of As₂O₃ and resveratrol on estrogen metabolism.

**EXPERIMENTAL PROCEDURES**

**Cytotoxicity.** Cells were seeded in a 96-well plate (seeding density: MCF10A 3000 cells per well, MCF12F 5000 cells per well) in estrogen- and phenol-red-indicator-free media and treated with 0.5−50 μM total concentration of As₂O₃ (purity ≥ 99.5%; Sigma-Aldrich, St. Louis, MO), 12.5−200 μM total concentration of resveratrol (purity ≥ 98%; Cayman Chemical, Ann Arbor, MI), or 0.5−50 μM total concentration of As₂O₃ + 25 μM resveratrol for two time points (48 and 72 h). Finally, MTT (Calbiochem, San Diego, CA) was used to assay the cell viability.

**Protein Expression Studies.** Expression levels of the key estrogen-metabolizing enzymes NQO1 and CYP1B1 and of cell signaling protein Nrf2 were studied under different treatment regimens. Cells were seeded in a 6-well plate (seeding density: MCF10A and MCF10F 0.8 × 10⁶ cells per well, MCF12F 1 × 10⁶ cells per well) in estrogen- and phenol-red-indicator-free media and treated with 0.5−5 μM total concentration of As₂O₃ ± 25 μM resveratrol for 48 h. Following cell lysis with radioimmunoprecipitation assay buffer containing protease inhibitor cocktail (Roche Diagnostics, Basel, Switzerland), total protein was estimated by the Bradford assay, and 10−30 μg of total protein was resolved on a 15% polyacrylamide gel and transferred to poly(vinylidene difluoride) membranes. The membranes were probed with anti-NQO1, anti-Nrf2 (Abcam, Cambridge, MA), anti-CYP1B1 (LSBio, Seattle, WA), or anti-β-actin (Santa Cruz, Dallas, TX) primary antibodies overnight at 4 °C and secondary antibodies for 1 h at room temperature. Detection was performed with ECL Western Blotting Detection Reagents (GE Healthcare/Amersham, Little Chalfont, U.K.). Both the treatment and western blotting were performed three times to achieve statistical significance.

**Statistical Analysis.** To determine whether the differences observed were statistically significant, a 2-sample t-test was performed. α was set at 0.05 for all statistical tests, and data with p < 0.05 were considered to be significantly different.
The expression of CYP1B1 by As$_2$O$_3$ with and without resveratrol in MCF10A, MCF10F, and MCF12F cells; the viability of MCF12F cells treated with As$_2$O$_3$ (PDF)

**REFERENCES**

(1) Saha, J. C.; Dikshit, A. K.; Bandyopadhyay, M.; Saha, K. C. A review of arsenic poisoning and its effects on human health. Crit. Rev. Environ. Sci. Technol. 1999, 29, 281–313.

(2) Straif, K.; Benbrahim-Tallaa, L.; Baan, R.; Grosse, Y.; Secretan, B.; El Ghissassi, F.; Bouvard, V.; Guha, N.; Freeman, C.; Galichet, L.; Cogliano, V. A review of human carcinogens—Part C: metals, arsenic, dusts, and fibres. Lancet Oncol. 2009, 10, 453–454.

(3) Soignet, S. L.; Maslak, P.; Wang, Z. G.; Jhanwar, S.; Calleja, E.; Dardashti, L. J.; Corso, D.; DeBlasio, A.; Gabrilove, J.; Scheinberg, D. A.; Pandolfi, P. P. Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. N. Engl. J. Med. 1998, 339, 1341–1348.

(4) Bodwell, J. E.; Gosse, J. A.; Nomikos, A. P.; Hamilton, J. W. Arsenic disruption of steroid receptor gene activation: complex dose–response effects are shared by several steroid receptors. Chem. Res. Toxicol. 2006, 19, 1619–1629.

(5) United States Environmental Protection Agency Office of Water (4607) The Technical Fact Sheet: Final Rule for Arsenic in Drinking Water, 815-F-00-016; EPA, 2001.

(6) Chen, C.; Jiang, X.; Lai, Y.; Liu, Y.; Zhang, Z. Resveratrol protects against arsenic trioxide-induced oxidative damage through maintenance of glutathione homeostasis and inhibition of apoptotic progression. Environ. Mol. Mutagen. 2015, 56, 333–346.

(7) Zhang, W.; Guo, C.; Gao, R.; Ge, M.; Zhu, Y.; Zhang, Z. The protective role of resveratrol against arsenic trioxide-induced cardiotoxicity. J. Evidence-Based Complementary Altern. Med. 2013, 3, 1–8.

(8) Zhang, W.; Liu, Y.; Ge, M.; Jing, J.; Chen, Y.; Jiang, H.; Yu, H.; Li, N.; Zhang, Z. Protective effect of resveratol on arsenic trioxide-induced nephrotoxicity in rats. Nutr. Res. Pract. 2014, 8, 220–226.

(9) Zhang, Z.; Gao, L.; Cheng, Y.; Jiang, J.; Chen, Y.; Jiang, H.; Yu, H.; Shan, A.; Cheng, B. Resveratrol, a natural antioxidant, has a protective effect on liver injury induced by inorganic arsenic exposure. BioMed Res. Int. 2014, 1, 1–7.

(10) Tokar, E. J.; Diwan, B. A.; Waalkes, M. P. Arsenic exposure transforms human epithelial stem/progenitor cells into a cancer stem-like phenotype. Environ. Health Perspect. 2010, 118, 108.

(11) Achnazar, W. E.; Brambila, E. M.; Diwan, B. A.; Webber, M. M.; Waalkes, M. P. Inorganic arsenite-induced malignant transformation of human prostate epithelial cells. J. Natl. Cancer Inst. 2002, 94, 1888–1891.

(12) Xu, Y.; Tokar, E. J.; Waalkes, M. P. Arsenic-induced cancer cell phenotype in human breast epithelia is estrogen receptor-independent but involves aromatase activation. Arch. Toxicol. 2014, 88, 263–274.

(13) Yue, W.; Wang, J. P.; Li, Y.; Bocchinfuso, W. P.; Korach, K. S.; Devanesan, P. D.; Rogan, E.; Cavaleri, E.; Santen, R. J. Tamoxifen versus aromatase inhibitors for breast cancer prevention. Clin. Cancer Res. 2005, 11, 925s–930s.

(14) Russo, J.; Lareef, M. H.; Balogh, G.; Guo, S.; Russo, I. H. Estrogen and its metabolites are carcinogenic agents in human breast epithelial cells. J. Steroid Biochem. Mol. Biol. 2003, 87, 1–25.

(15) Cavaleri, E.; Rogan, E. The molecular etiology and prevention of estrogen-initiated cancers: Ockham’s Razor: Pluralitas non est ponenda sine necessitate. Plurality should not be posited without necessity. Mol. Aspects Med. 2014, 36, 1–55.

(16) Zahid, M.; Gaiwad, N. W.; Ali, M. F.; Lu, F.; Saeed, M.; Yang, L.; Rogan, E. G.; Cavaleri, E. L. Prevention of estrogen–DNA adduction formation in MCF-10F cells by resveratrol. Free Radical Biol. Med. 2008, 45, 136–145.

(17) Ungvari, Z.; Bagi, Z.; Feher, A.; Recchia, F. A.; Sonntag, W. E.; Pearson, K.; De Cabo, R.; Csiszar, A. Resveratrol confers endothelial protection via activation of the antioxidant transcription factor Nrf2. Am. J. Physiol. 2010, 299, H18–H24.

(18) He, X.; Chen, M. G.; Lin, G. X.; Ma, Q. Arsenic induces NAD(P)H-quinone oxidoreductase 1 by disrupting the Nrf2: Keap1-Cul3 complex and recruiting Nrf2 to the antioxidant response element enhancer. J. Biol. Chem. 2006, 281, 23620–23631.

(19) Itoh, K.; Wakabayashi, N.; Katoh, Y.; Ishii, T.; O’connor, T.; Yamamoto, M. Keap1 regulates both cytoplasmic-nuclear shuttling and degradation of Nrf2 in response to electrophiles. Genes Cells 2003, 8, 379–391.

(20) Li, S. G.; Xu, S. Z.; Niu, Q.; Ding, Y. S.; Pang, L. J.; Ma, R. L.; Jing, M. X.; Wang, K.; Ma, X. M.; Feng, G. L.; Liu, J. M.; et al. Lutein alleviates arsenic-induced reproductive toxicity in male mice via Nrf2 signaling. Hum. Exp. Toxicol. 2016, 35, 491–500.

(21) Spink, D. C.; Spink, B. C.; Cao, J. Q.; DePasquale, J. A.; Pentecost, B. T.; Fasco, M. J.; Li, Y.; Sutter, T. R. Differential expression of CYP1A1 and CYP1B1 in human breast epithelial cells and breast tumor cells. Carcinogenesis 1998, 19, 291–298.

(22) Anwar-Mohamed, A.; El-Sherbeni, A. A.; Kim, S. H.; Althuwri, H. N.; Zordoky, B. N.; El-Kadi, A. O. Acute arsenic toxicity alters cytochrome P450 and soluble epoxyeicosatrienoic acid and their associated arachidonic acid metabolism in C57Bl/6 mouse liver. Xenobiotica 2012, 42, 1235–1247.

(23) Lu, F.; Zahid, M.; Wang, C.; Saeed, M.; Cavaleri, E. L.; Rogan, E. G. Resveratrol prevents estrogen-DNA adduct formation and neoplastic transformation in MCF-10F cells. Cancer Prev. Res. 2008, 1, 135–145.