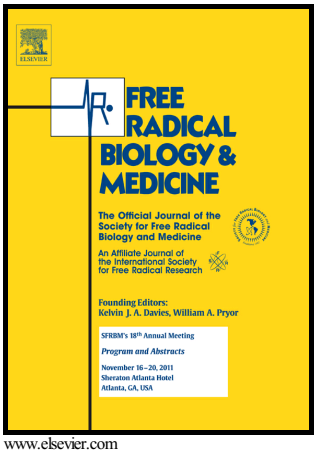


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# Dr. Jekyll and Mr. Hyde: Oxidizable phenol-generated reactive oxygen species enhance sulforaphane's antioxidant response element activation, even as they suppress Nrf2 protein accumulation

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**Abstract**

The transcription factor Nrf2 is a master regulator of antioxidant and cytoprotective genes, binding to antioxidant response elements (AREs) in their promoter regions. Due to the therapeutic role of the Nrf2/ARE system in oxidative homeostasis, its activation has been investigated in many pre-clinical and clinical trials for common chronic diseases. One of the most promising Nrf2 activators is sulforaphane, the subject of over 50 clinical trials. In this work, we examine the effect of reactive oxygen species (ROS) on sulforaphane's Nrf2/ARE activation in the non-tumorigenic keratinocyte cell line HaCaT, with the non-arylating oxidizable phenol, 2,5-di-*tert*-butylhydroquinone (dtBHQ), as the source of ROS. We find that, in combination with 2.5 μM sulforaphane, dtBHQ markedly enhances ARE-regulated gene expression, including expression of the cytoprotective proteins aldo-keto reductase family 1 member C1 (AKR1C1) and heme oxygenase-1 (HO-1). Additionally, sulforaphane's therapeutic window is widened by 12.5 μM dtBHQ. Our data suggest that $H_2O_2$ generated by dtBHQ oxidation is responsible for these effects, as shown by inclusion of catalase and by co-treatment with sulforaphane and $H_2O_2$. While sulforaphane treatment causes Nrf2 protein to accumulate as expected, interestingly, dtBHQ and $H_2O_2$ appear to act on targets downstream of Nrf2 protein accumulation to enhance sulforaphane's ARE-regulated gene expression. Inclusion of dtBHQ or $H_2O_2$ with sulforaphane does not increase Nrf2 protein levels, and catalase has little effect on Nrf2 protein levels in the presence of sulforaphane and dtBHQ. Surprisingly, dtBHQ suppresses Nrf2 protein synthesis. Inclusion of a superoxide dismutase mimetic with sulforaphane and dtBHQ partly rescues Nrf2 suppression and significantly further increases sulforaphane's efficacy for ARE-reporter expression. Thus, there is a "Dr. Jekyll and Mr. Hyde" effect of ROS: ROS enhance sulforaphane's ARE-regulated gene expression even as they also inhibit Nrf2 protein synthesis. This unexpected finding reveals the degree to which targets in the ARE pathway downstream of Nrf2 protein accumulation contribute to gene expression. The results presented here provide a model system for significant enhancement of sulforaphane's potency with small molecule co-treatment.

Abbreviations: AKR1C1, aldo-keto reductase family 1 member C1; DMEM, sodium pyruvate free Dulbecco's modified Eagle's medium; DMNQ, 2,3-dimethoxy-1,4-naphthoquinone; dtBHQ, 2,5-di-tert-butylhydroquinone; EGCG, epigallocatechin-3-gallate; FBS, fetal bovine serum; HEPES, 4-(2-hydroxyethyl)-1-

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