292. INDUCTION OF MURINE NAD(P)H:QUINONE OXIDOREDUCTASE BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN REQUIRES THE CNC BASIC LEUCINE ZIPPER TRANSCRIPTION FACTOR NRF2. CROSS-INTERACTION BETWEEN AHR AND NRF2 SIGNAL TRANSDUCTION

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2,3,7,8-tetrachlorodibenzo-*p*-dixoin induces phase II drug-metabolizing enzyme NAD(P)H:quinone oxidoreductase (EC 1.6.99.2, NQO1, DT-diaphorase) in a wide range of mammalian tissues and cells. We analyzed the molecular pathway mediating NQO1 induction by TCDD in mouse hepatoma cells. Inhibition of protein synthesis with cycloheximide (CHX) completely blocks induction of NQO1 by TCDD as well as the basal expression and induction by phenolic antioxidant 2-t-butylbenzene-1,4-diol (tBHQ), implicating a labile factor in NQO1 mRNA expression. The inhibition is both time and concentration-dependent, requires inhibition of protein synthesis, and occurs at a transcriptional level. Inhibition of NQO1 transcription by CHX correlates with a rapid reduction of CNC bZip transcription factor Nrf2 through the 26S proteasome pathway. Moreover, blocking Nrf2 degradation with proteasome inhibitor MG132 increases the amount of Nrf2 and superinduces NQO1 in the presence of TCDD or tBHQ. Finally, genetic experiments using AhR, Arnt, or Nrf2-deficient cells reveal that, while induction of NQO1 by TCDD depends on the presence of AhR and Arnt, the basal and inducible expression of NQO1 by either TCDD or tBHQ requires functional Nrf2. The findings demonstrate a novel role of Nrf2 in the induction of NQO1 by TCDD and provide new insights into the mechanism by which Nrf2 regulates the induction of phase II enzymes by both phenolic antioxidants and AhR ligands.

293. DOWN-REGULATION OF CYP1A1 EXPRESSION BY EUGENOL

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Eugenol (4-ally-2-methoxy phenol) is naturally occurring compound in plant that has been extensively used as a flavoring agent and fragrance. Human exposure to eugenol also occurs though its use as an analgesic and from clove cigarettes. Compared with related allylbenzenes derivatives such as safrole and estragole, which are hepatocarcinogenic in mouse models, eugenol has not shown similar evidence for carcinogenecity. In the present study, we investigated the effect of eugenol on 2,3,7,8-Tetrachlorodibenzo-p-dioxine (TCDD)-inducible CYP1A1 gene expression in mouse hepatoma Hepa-1c1c7 cells. TCDD-induced cytochrome CYP1A1-specific 7-ethoxyresorufin *O*-deethylase (EROD) activity was markedly reduced in the concomitant treatment of TCDD and eugenol in a dose dependent manner. TCDD-induced CYP1A1 mRNA level was also markedly suppressed in the concomitant treatment of TCDD and eugenol. A transient transfection assay using dioxin-response element (DRE)-linked luciferase and electrophoretic mobility shift assay revealed that eugenol reduced transformation of the aryl hydrocarbons (Ah) receptor to a form capable of specifically binding to the DRE sequence in the promoter of the CYP1A1 gene. These results suggest the down regulation of the CYP1A1 gene expression by eugenol in Hepa-1c1c7 cells might be antagonism of the DRE binding potential of nuclear Ah receptor.