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TRANSCRIPTIONAL REGULATION OF HUMAN CARBOXYLESTERASE 1A BY NUCLEAR FACTOR-ERYTHROID 2 RELATED FACTOR 2 (NRF2)
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[Purpose] Human carboxylesterase (CES) 1A, which is predominantly expressed in liver and lung, plays an important role in the hydrolysis of endogenous compounds and xenobiotics. CES1A has been reported to be induced by oxidative stress in human hepatocytes. However, the molecular mechanism remains to be determined. In this study, we investigated whether CES1A is regulated by Nrf2.

[Methods] HepG2, Caco-2 and HeLa cells were treated with 80 µM tert-butylhydroquinone (tBHQ) and 10 µM sulforaphane (SFN), which are representative activators of Nrf2, for 24 hrs. The CES1A1 mRNA level, protein level, and imidapril hydrolyase activity were determined by real-time RT-PCR, Western blotting, and LC-MS/MS analysis, respectively. Luciferase assays were performed to identify the functional antioxidant response element (ARE). Electrophoretic mobility shift assays (EMSA) and chromatin immunoprecipitation (ChIP) assays were performed to examine the binding of Nrf2 to the ARE in the CES1A1 gene.

[Results and Discussion] CES1A1 mRNA was significantly induced by tBHQ and SFN in HepG2, Caco-2 and HeLa cells. The induction was abolished with small interfering RNA for Nrf2. In HepG2 cells, the CES1A protein level and imidapril hydrolyase activity were also significantly induced by tBHQ and SFN. Luciferase assays revealed that the ARE at -2,025 in the CES1A1 gene was responsible for the transactivation by Nrf2. The binding of Nrf2 to the ARE in the CES1A1 gene was clearly demonstrated by EMSA and ChIP assays.

[Conclusions] We found that CES1A1 is transcriptionally regulated by Nrf2. This is the first study to demonstrate the molecular mechanism of the induction of CES1A1.

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HUMAN ARYLACETAMIDE DEACETYLASE IS A PRINCIPAL ENZYME IN FLUTAMIDE HYDROLYSIS
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[Purpose] Flutamide, a widely used drug for the treatment of prostate cancer, occasionally causes severe hepatotoxicity. Although flutamide hydrolysis has been supposed to be a key pathway for hepatotoxicity, the enzyme responsible for the hydrolysis remains to be clarified. It has been reported that purified CES1A and CES2A could not hydrolyze flutamide. In this study, we investigated whether human arylacetamide deacetylase (AADAC) is involved in flutamide hydrolysis.

[Methods] COS7 cells transiently expressing human AADAC, CES1A1, CES1A2, and CES2A1 were established. Flutamide hydrolyase activities by the recombinant AADAC and CES, or human liver, jejunum, pulmonary, and renal microsomes were measured by HPLC. Kinetic analyses and inhibition studies by human liver microsomes (HLM) and recombinant AADAC for flutamide hydrolyase activities were performed. The expression levels of AADAC protein in individual HLM (n = 50) were determined by immunoblot analysis.

[Results and Discussion] Recombinant AADAC efficiently hydrolyzed flutamide, whereas CES1A1/2 and CES2A1 did not. Microsomes from human liver and jejunum expressing AADAC showed flutamide hydrolyase activities, but microsomes from lung and kidney in which AADAC is not expressed showed scarce activities. The Km value of flutamide hydrolyase activity by AADAC was close to that of HLM. The effects of the inhibitors such as DFP, BNPP, and eserine were similar between HLM and recombinant AADAC. In individual HLM, the flutamide hydrolyase activities were significantly (p < 0.001) correlated with the expression levels of AADAC protein.

[Conclusions] AADAC is a principal enzyme in flutamide hydrolysis.