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Mechanism of Phase 2 Detoxifying Enzyme Induction by Isoalantolactone Isolated from *Inula helenium*

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Objectives

This study has been performed to confirm the potential of isoalantolactone to induce phase 2 detoxifying (antioxidant) enzymes and elucidate the mechanism how the compound caused the enzyme induction.

Materials and Methods

Materials

Isoalantolactone was purified from *Inula helenium* (root) by bioassay-guided fractionation and its structure was identified by NMR (Lim, Soon Sung from Hallym University). Mouse hepatoma cells hepa1c1c7 and its mutant cell line BPRc1, and HepG2-C8, which is a human hepatoma transfectant carrying vector constructed with antioxidant response element (ARE) linked to reporter gene (luciferase), were used.

Methods

Quinone reductase (QR) was assyed according to Benson's method using 2,6-dichlorophenolindophenol as a substrate (Benson et al., 1980). Intracellular levels of antioxidant enzymes including QR, GST-phi, gamma-cysteine ligase (GCL), HO-1 were measured by Western blot. Involvement of ARE in promoter regions of relevant genes in the induction of phase 2 detoxifying (antioxidant) enzymes was tested by assaying leciferase activity of cells before and after treatment of cells with isoalantolactone at various levels and exposure periods. Nuclear translocation of Nrf2 by isoalantolactone was also measured by western blot for nuclear fraction. Involvement of PI3K, Akt, mTOR in nuclear translocation of Nrf2 was assessed by examining nuclear levels of Nrf2 before and after treatment the respective inhibitor.

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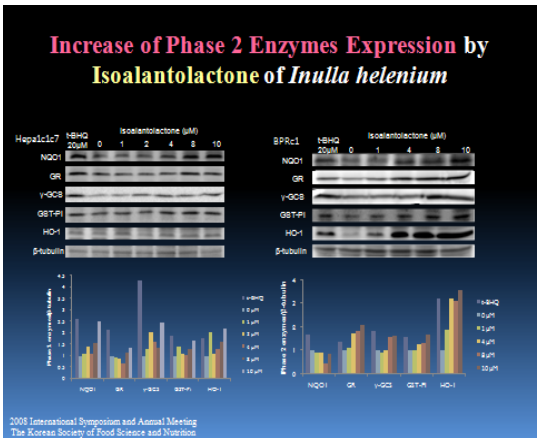
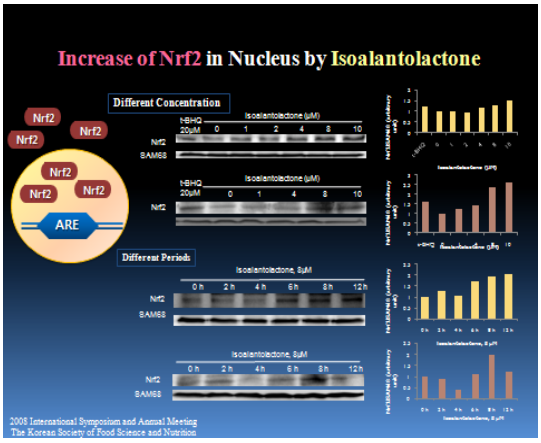
Results

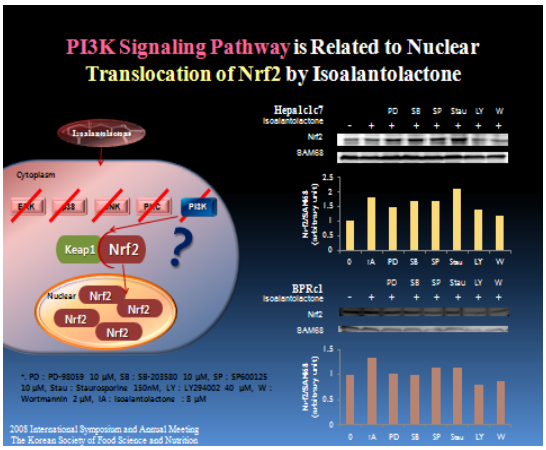
Isoalantolactone caused a dose-dependent induction of antioxidant enzymes including QR, GST, GCL, HO-1 in hepa1c1c7 mouse hepatoma cells and its mutant BPRc1 cells. The compound increased the luciferase activity of HepG2-C8 cells, transfectants carrying antioxidant response element (ARE)-luciferase gene, in a dose-dependent manner, suggesting ARE-mediated transcriptional activation of antioxidant enzymes. Isoalantolactone also stimulated nuclear accumulation of Nrf2 that was inhibited by phosphatidylinositol 3-kinase (PI3K) inhibitors. In conclusion, isoalantolactone appears to activate PI3K signaling pathway, promote nuclear translocation of Nrf2 and interaction between Nrf2 and ARE in the relevant genes, eventually leading to induction of detoxifying enzymes.

(A) (B)





(C) (D)



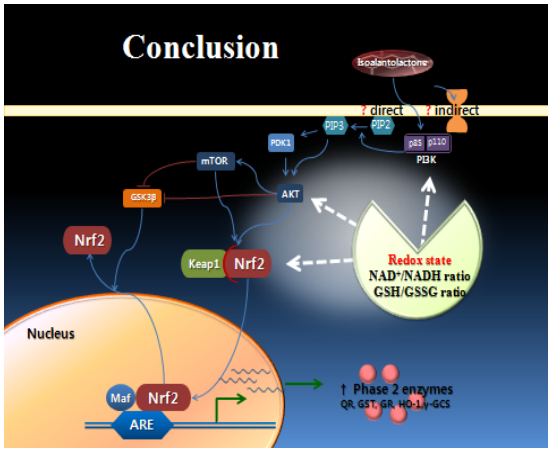


Figure 1. Induction of phase 2 antioxidant enzymes (A) and nuclear translocation of Nrf2 by isoalantolactone (B), and involvement of PI3K signalig pathway in nuclear Nrf2 accumulation by the compound (C), and a proposed mechanism for phase 2 antioxidant enzyme induction (D).