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Toxicogenomics of resveratrol in rat liver

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Abstract

Resveratrol, a polyphenolic compound found in grape skin and peanuts has been shown to prevent many diseases including cardiovascular diseases and cancer. To better understand resveratrol's potential in vivo toxicity, we studied the dose response using cDNA stress arrays coupled with drug metabolizing enzymatic (DME) assays to investigate the expression of stress-responsive genes and Phase I and II detoxifying enzymes in rat livers. Male and female CD rats were treated with high doses of resveratrol (0.3, 1.0 and 3.0 gm/kg/day) for a period of 28 days. Total RNA from rat liver was reverse-transcribed using gene-specific primers and hybridized to stress-related cDNA arrays. Among female rats, Phase I DME genes were repressed at 0.3 and 1.0 gm/kg/day doses, while genes such as manganese superoxide dismutase, cytochrome P450 reductase, quinone oxidoreductase and thiosulfate sulfurtransferase demonstrated a dose-dependent increase in gene expression. The modulation of these liver genes may implicate the potential toxicity as observed among the rats at the highest dose level of resveratrol. Real-Time PCR was conducted on some of the Phase II DME genes and anti-oxidant genes to validate the cDNA array data. The gene expression from real-time PCR demonstrated good correlation with the cDNA array data. UGT1A genes were amongst the most robustly induced especially at the high doses of resveratrol. We next performed Phase I and Phase II enzymatic assays on cytochrome P450 2E1 (CYP2E1), cytochrome P450 1A1 (CYP1A1), NAD(P)H:quinone oxidoreductase

Abbreviations: NQO1, NAD(P)H:quinone oxidoreductase; GST, Glutathione S-transferase; UGT, UDP-glucuronosyltransferase; COX, Cyclooxygenase; SOD2, Manganese superoxide dismutase 2.

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(NQO1), glutathione S-transferase (GST) and UDP-glucuronosyl transferase (UGT). Induction of Phase II detoxifying enzymes was most pronounced at the highest dose of resveratrol. CYP1A1 activity demonstrated a decreasing trend among the 3 dose groups and CYP2E1 activity increased marginally among female rats over controls. In summary, at lower doses of resveratrol there are few significant changes in gene expression whereas the modulation of liver genes at the high dose of resveratrol may implicate the potential toxicity observed.

Keywords: Resveratrol; In vivo; Drug metabolizing enzymes; DNA arrays; Gene expression

Introduction

Resveratrol (trans-3,4′,5-trihydroxystilbene) is a polyphenolic compound found in plant sources, such as the skin of grapes (Jang et al., 1997; Soleas et al., 1997) and peanuts (Sanders et al., 2000). Resveratrol is attributed with varied biological properties including anti-oxidant and anti-inflammatory effects. These properties were observed from the beneficial effects of red wine in preventing cardiovascular disease, which contains resveratrol (1.5–3 mg/L of red wine); the phenomenon is referred to as "French Paradox" due to the consumption of cheese and other high fat foods in the daily diet and the relatively low incidence of heart disease in that geographical region (Goldberg, 1996). In addition, resveratrol was found to interfere with cellular events leading to tumor initiation, promotion and progression (Jang et al., 1997). It blocked the formation of preneoplastic lesions in mouse mammary glands (Jang et al., 1997) and has been shown to inhibit proliferation of a variety of cancer cells in culture including human colon cancer cells, breast epithelial cells and prostate cancer cells (Mgbonyebi et al., 1998; Mitchell et al., 1999; Schneider et al., 2000).

Several mechanisms have been proposed for resveratrol's anti-cancer and chemopreventive action. Resveratrol's ability to inhibit the enzyme cyclooxygenase (COX) has been proposed as one of the plausible mechanisms of action (Shin et al., 1998; Subbaramaiah et al., 1998). Resveratrol inhibits COX-1 non-competitively in a dose-dependent manner and modulates COX-2 by inhibiting the PKC signal transduction pathway. Polyphenols obtained from foods, which include resveratrol inhibit pancreatic cancer growth and cause apoptosis by the release of cytochrome c and activation of caspases (Mouria et al., 2002). Resveratrol's action on inhibiting the proliferation of cancer cells is accompanied by cell cycle arrest in a large number of cancer cell lines (Ahmad et al., 2001; Bernhard et al., 2000; Joe et al., 2002; Schneider et al., 2000).

Resveratrol is also known to have an effect on the cell's drug metabolizing enzyme systems, such as, Phase I and Phase II drug metabolizing enzymes (DME's). In particular, resveratrol was found to inhibit cytochrome P450 1A1 (CYP1A1), 1A2 (CYP1A2) and 2E1 (CYP2E1) drug metabolizing enzymes (Chang et al., 2001; Chun et al., 1999; Ciolino and Yeh, 1999; Mikstacka et al., 2002). Furthermore, another important effect of resveratrol is the induction of the Phase II enzyme NAD(P)H:quinone oxidoreductase in cultured mouse hepatoma (Hepa1c1c7) cells (Prochaska and Santamaria, 1988). The induction of Phase II enzymes plays an important role in detoxifying harmful substances. Indeed, the induction of these enzymes is an essential factor to consider when studying chemopreventive properties of a compound (Gerhauser et al., 1997; Hecht, 2000; Khan et al., 1992).