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Note

Suppression of the Menadione-Induced Cytotoxicity toward Hepa1c1c7 Murine Hepatoma by Quinone Reductase Inducers

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The obligatory two-electron reduction of quinones by quinone reductase (NAD(P)H:quinone oxidoreductase) competes with the one-electron reduction of quinones and protects cells against the cytotoxicity of quinones. We assessed the inhibitory effects of quercetin and curcumin against the menadione-induced cytotoxicity toward murine Hepa1c1c7 cells. tert-Butylhydroquinone, a positive control, induced the quinone reductase activity and suppressed the menadione-induced cytotoxicity. Both quercetin and curcumin induced the quinone reductase activity. While quercetin suppressed the menadione-induced cytotoxicity, curcumin showed no such suppressive effect.

Keywords: quinone reductase, quercetin, curcumin, enzyme induction, detoxification, cell culture

A quinone like menadione can be reduced by accepting one electron from a donor with an enzyme such as NADPH-cytochrome P450 oxidoreductase to give the semiquinone radical. This radical can be recycled back to menadione through a rapid reaction with molecular oxygen to yield the superoxide radical. The superoxide radical is changed to a hydrogen peroxide by the enzyme, superoxide dismutase. These reactive oxygen species cause oxidative injury. The obligatory two-electron reduction of quinones catalyzed by NAD(P)H: quinone oxidoreductase [quinone reductase (QR) which is also known as DT-diaphorase] competes with the conversion of quinones to semiquinone radicals and with the generation of reactive oxygen species. It has been proposed that the induction of QR may have a protective effect against carcinogenicity, mutagenicity and other types of toxicity caused by quinones and their metabolic precursors (Lind *et al*., 1982; Thor *et al*., 1982; Morrison *et al*., 1984; Di Monte *et al*., 1984a, b). Hepa1c1c7 murine hepatoma cells provide a rapid, reliable, and convenient system for assaying the inducer potency for QR. Using this system, inducer activities of many polyphenols including quercetin (Q) and curcumin (C) have been reported (Uda *et al*., 1997; Dinkova-Kostova & Talalay, 1999). Quercetin derivatives are present in a high concentration in onions, apples, broccoli, wine and tea (Hertog *et al*., 1993), and an increased consumption of onions has resulted in a reduced risk of stomach carcinoma (Dorant *et al*., 1996). The biological effects of Q have been reviewed (Formica & Regelson, 1995; Cook & Samman, 1996). C is the major component of turmeric, and its anticarcinogenic effect *in vivo* and antioxidative effect *in vitro* have been reported (Lu *et al*., 1994; Tanaka *et al*., 1994; Osawa *et al*., 1995; Sugiyama *et al*., 1996). In this present study, the effects of these QR inducers on the menadione (MEN) cytotoxicity to Hepa1c1c7 cells were examined.

MEN and C were obtained from Wako Chemicals (Osaka) and Q was from Kanto Chemical (Tokyo). All other chemicals were of reagent grade. Hepa1c1c7 cells were seeded in 96-well plates (4.0×10^4 cells/well) and incubated in 200 μl of a minimum essential medium (MEM) supplemented with 10% Serum Plus (JRH Biosciences, KS), a serum substitute, in a humidified atmosphere of 5% CO_2 in air at 37°C for 24 h. Each assay sample was dissolved in DMSO and added to the medium, the final concentration of DMSO in the medium being less than 0.1%. After a 24-h period of incubation, the QR activity and cell viability were measured. The QR activity was measured as the reduction of menadione to menadiol, coupled to the non-enzymic reduction of MTT to formazan dye (Uda *et al*, 1997). The cytotoxic effect of menadione on Hepa1c1c7 cells and the protective effect of each assay sample against menadione-induced cytotoxicity were determined with the Alamar Blue assay reagent (Trek Diagnostic Systems, OH). Each result is expressed as the mean and standard deviation of four separately treated cultures.

We first determined the dose effect of MEN on the survival of the cells. The survival of the cells decreased with increasing concentration of MEN and reached almost zero at 50 μM (Fig. 1), the LD_{50} value being about 9 μM. We next investigated the protective effects of Q and C. The protective effect of tert-butylhydroquinone (TBHQ) was also measured as a positive control. At the concentration used in this experiment, TBHQ, Q and C, gave 2.76±0.11, 1.61±0.10 and 2.10±0.16 -fold increase in QR activity, respectively. As shown in Fig. 2, TBHQ and Q each exerted a protective effect against the cytotoxicity of MEN. On the other hand, C had no effect on the survival of Hepa1c1c7 cells. To investigate the possible role of QR in the protective effects of TBHQ and quercetin, we assayed their effect on the cytotoxicity of MEN in the presence of 20 μM of dicumarol, a specific inhibitor of QR activity. Adding dicumarol to the medium resulted in the protective effects of TBHQ and Q against the cytotoxicity of MEN being almost completely suppressed (Fig. 2). Dicumarol itself was not toxic at the concentration used in these experiments (survival of the dicumarol-treated cells was 104±8%), so these results indicate that Q reduced the quinone-mediated cytotoxicity

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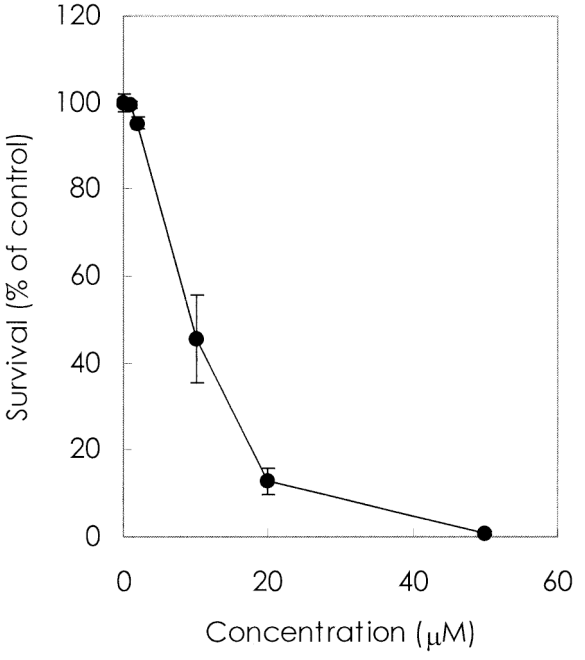

Fig. 1. Dose-effect relationship for the survival of Hepa1c1c7 cells treated with MEN.
Hepa1c1c7 cells were treated with MEN at 37°C for 24 h, before the cell viability was measured. The values shown are the means (n=4)±SD bars.

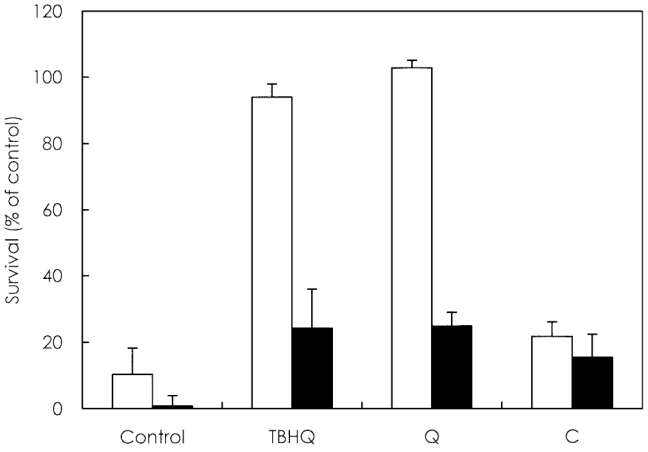

Fig. 2. Effect of QR inducers on the survival of Hepa1c1c7 cells treated with MEN.
Hepa1c1c7 cells were treated with TBHQ (50 μM), Q (20 μM) or C (20 μM) at 37°C for 24 h, and subsequently with MEN for 24 h, the cell viability then being measured. Each data bar represents the mean±SD for four samples. Unshaded bars: treated with MEN (50 μM); shaded bars, treated with MEN (50 μM) and dicumarol (20 μM).

by its QR-inducing activity. MEN may also have caused toxicity due to the depletion of GSH by conjugate formation. The effect of Q on the intracellular level of GSH needs to be examined.

C induced QR activity as well as Q, but failed to protect the cells against the MEN-mediated cytotoxicity. It is known that QR competes with cytochrome p-450 reductase, during which superoxide radical is partly formed and changed to a hydrogen peroxide by the enzyme, superoxide dismutase. On the other hand, Nakayama *et al*. (1997) reported that, despite C having an antioxidative effect *in vitro*, it showed no inhibitory effect on hydrogen peroxide-induced cytotoxicity. They suggested this may be attributable to C having no *o*-dihydroxy moiety in its molecule, because *o*-dihydroxy compounds, including quercetin, protected against the cytotoxicity of hydrogen peroxide in mammalian cells (Nakayama *et al*., 1997). The reason C could not reduce the MEN-mediated cytotoxicity in Hepa1c1c7 cells may be partly explained by these facts. Further investigation is needed to elucidate the protective role of the *o*-dihydroxy structure in mammalian cells against quinone-mediated cytotoxicity.

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