Prooxidant-Induced Glutathione Antioxidant Response in Vitro and in Vivo: A Comparative Study between Schisandrin B and Curcumin

Pou Kuan Leong, Po Yee Chiu, and Kam Ming Ko*

Division of Life Science, Hong Kong University of Science & Technology; Clear Water Bay, Hong Kong SAR, China.

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We investigated whether two naturally-occurring prooxidants, namely, schisandrin B (Sch B) and curcumin, can elicit equivalent cyto/hepatoprotective responses against oxidant-induced injury. Results showed that (-)-Sch B (a potent enantiomer of Sch B, 15 μM), curcumin (7.5 μM) and menadione (2 μM) induced a similar range of protective antioxidant effects in AML12 cells. The relative potencies of cytoprotection in vitro were in a descending order of curcumin > menadione > (-)-Sch B, which were parallel to the extent of stimulation in cellular reduced glutathione level. We further examined their hepatoprotection in vivo. Pretreatment with Sch B (800 mg/kg) and curcumin (737 mg/kg), but not menadione (344 mg/kg), protected against CCl4 toxicity, with the degree of protection afforded by Sch B being much larger than that of curcumin. The attenuated hepatoprotection afforded by curcumin may be attributed to its low bioavailability in vivo. This postulation is supported by the findings that intraperitoneal injections of Sch B (400 mg/kg) and curcumin (368 mg/kg) and the long term, low dose treatment with Sch B (20 mg/kg/d×15) and curcumin (18 mg/kg/d×15) induced glutathione antioxidant response and hepatoprotection to similar extents in vivo. The inability of menadione to induce hepatoprotection may be related to its extensive intestinal metabolism and/or hepatotoxicity. Taken together, prooxidants can invariably induce the glutathione antioxidant response and confer cytoprotection in vitro. Whether or not the prooxidant can produce a similar response in vivo would depend on its bioavailability and potential toxic effect.

Key words schisandrin B; hromesis; curcumin; glutathione antioxidant response; oxidative stress; hepatoprotection

Aerobic organisms are equipped with an antioxidant network of glutathione and glutathione-related enzymes, namely glutathione antioxidant system, to defend against oxidative stress challenge.5) Our laboratory has shown that the induction of glutathione antioxidant response is essential to confer cellular/tissue protection against various oxidant-induced injuries.2–6) Daily consumption of antioxidants, such as ascorbic acid and α-tocopherol, has been recommended for enhancing cellular antioxidant defense capacity. However, results obtained from epidemiological studies as well as clinical trials have failed to demonstrate the beneficial effects of antioxidant intake.7) Strikingly, the daily consumption of antioxidants may even produce harmful effect. In this regard, the over-supplementation of α-tocopherol, when in the presence of catalytically active transition metal ions, could paradoxically induce lipid peroxidation.8) Consistent with this, an epidemiological study showed that a high dosage of α-tocopherol supplementation increased the occurrence of fatal myocardial infarction.9) The search for an alternate way to boost up the cellular antioxidant defense is therefore an area of intense research.

Reactive oxygen species (ROS) have recently been found to be important signal transduction molecules for redox signaling in cellular systems.10) The persistent consumption of antioxidants may disrupt the redox signaling, or even down-regulate the antioxidant gene expression.11) On the other hand, accumulating evidence has shown that low concentrations of prooxidant or oxidant can induce the expression of antioxidant proteins and confer a protective effect characteristic of hormetic response.12) Presumably, low concentrations of oxidant stimulated an adaptive enhancement of antioxidant system via the redox signal transduction pathway such as the nuclear factor erythroid-2-related factor-2 (Nrf2)/electrophile response element (EpRE) pathway.13) However, as to whether prooxidants can invariably induce a protective antioxidant response remains to be investigated.

Schisandrin B (Sch B) is the most abundant and active dibenzocyclooctadiene lignan isolated from the fruit of Schisandra chinensis (Fructus Schisandrae), a commonly used Chinese herb for promoting health and which is also clinically prescribed for the treatment of viral and chemical hepatitis.14) The cyto/hepatoprotection afforded by Sch B pretreatment was found to be associated with the enhancement in cellular/mitochondrial glutathione antioxidant status, heat shock protein expression as well as the increased resistance of mitochondria to Ca2+-induced permeability transition.15,16) The Sch B preparation used in earlier investigations was found to be a mixture of γ-schisandrin and (-)-Sch B (or gomisin N). (-)-Sch B has been found to be more potent than γ-schisandrin in protecting against oxidative injury in culture AML12 hepatocytes.17) Recent studies have shown that ROS generated from cytochrome P-450 (CYP)-, CYP2E1 in particular, catalyzed metabolism of Sch B may induce a glutathione antioxidant response in cultured AML12 hepatocytes and mouse livers,8,18 implicating the involvement of prooxidant action. It has been further demonstrated that the Sch B-induced glutathione antioxidant response is mediated via a ROS-dependent, redox-sensitive extracellular signal-regulated kinase (ERK)/Nrf2/EpRE signal transduction pathway.19) Curcumin is one of the principal curcuminoid obtained from the root of Curcuma longa, which was widely used in ancient Indian medicine for the treatment of various diseases, including liver disorders. Consistent with the traditional belief, curcumin has been shown to protect against the iron/CCl4-induced hepatotoxicity,20,21) attenuate thioacetamide-induced hepatitis/cirrhosis22,23) as well as to relieve the symptoms of cholestasis.24) A mechanistic study of curcumin-induced pro-
tection showed that the induction of hemeoxygenase 1 expression was ROS-dependent, implying that the cytoprotection action may be attributed to its prooxidant action.

To investigate whether the prooxidant action of Sch B and curcumin can invariably contribute to the cyto/tissue protective effect, we compared between Sch B and curcumin on their drug-induced ROS production in vitro, glutathione antioxidant response induction in vitro and in vivo, as well as cyto- and hepatoprotective effects. Menadione, which can generate ROS by redox cycling during the metabolism, was also studied for comparison.

MATERIALS AND METHODS

Chemicals AML12 cell line was purchased from ATCC (Rockville, MD, U.S.A.). Cell culture medium and fetal bovine serum (FBS) were obtained from Gibco (BRL Life Technologies, Grand Island, NY, U.S.A.). Reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase (GR), 1-aminobenzotriazole (ABT), dimethylthiouuracil (DMTU), curcumin and menadione were purchased from Sigma (St. Louis, MO, U.S.A.). 2',7'-Dichlorofluorescein diacetate (DCFDA) was purchased from Fluka (Buchs, Switzerland). Menadione, which can generate ROS by redox cycling during the metabolism, was prepared by differential centrifugation, as described previously. All other chemicals were of analytical grade.

Cell Culture AML12 cells, a well differentiated, non-transformed murine hepatocyte cell line derived from transforming growth factor-α transgenic mice, were cultured as monolayer using Dulbecco’s modified Eagle’s medium/F12 (GIBCO BRL) supplemented with 10% FBS, 100 units/ mL penicillin, 0.1 mg/mL streptomycin, 40 mg/mL dexamethasone and insulin-transferrin-sodium selenite media supplement (ITS, containing 5 mg/L insulin, 5 mg/L transferrin and 5 μg/L selenium). Cells were grown in 75 cm² flasks and kept at 37°C in a humidified atmosphere of air and 5% CO₂. Cells used for experiments were seeded at a density of 1.0×10⁴ on a 12-well culture plate, and cells in each well were allowed to grow to achieve 60–80% confluence within 24 h prior to drug treatment.

Animal Care Adult female Balb/c mice and Imprinting Control Region (ICR) mice (8–10 weeks old, 20–25 g) were maintained under a 12-h dark/light cycle at about 22°C, and allowed food and water ad libitum in the Animal and Plant Care Facility at the Hong Kong University of Science and Technology (HKUST). All experimental protocols were approved by the University Committee on Research Practice at HKUST.

Measurement of Prooxidant-Induced ROS Generation in Mouse Liver Microsomes Microsomal fractions were prepared by differential centrifugation, as previously described as Chiu et al. An aliquot (40 μL) of microsomal fraction (ca. 5 μg protein) was pre-incubated with 20 μL drug solution [or vehicle, dimethyl sulfoxide (DMSO) at 0.2% (v/v) final concentration] and 60 μL DCFDA (dissolved in 0.2% DMSO at 5 μM final concentration) at 37°C for 15 min. Then, an aliquot (50 μL) of reduced nicotinamide adenine dinucleotide phosphate (NADPH) (500 μM final concentration) was added in the reaction mixture. The fluorescence intensity of each well was measured at an excitation wavelength of 485 nm and an emission wavelength of 530 nm by Victor V² Multi-label Counter (Perkin Elmer, Turku, Finland) every minute for 1 h at 37°C. The fluorescence intensity of drug-treated cells was normalized with reference to the respective time-matched vehicle (i.e., DMSO) control. The extent of drug-induced ROS production was estimated by computing the area under the curve (AUC) plotting normalized fluorescence intensity against time (min), and expressed in arbitrary unit.

Measurement of Prooxidant-Induced ROS Generation in AML12 Cells AML12 cells (1×10⁴) were seeded on a 96-well black multi-titer plate with clear bottom, and they were allowed to grow overnight. The cells were washed twice with phosphate-buffered saline (PBS) and then incubated with 0.1% bovine serum albumin (BSA) in PBS containing 5 μM (final concentration) DCFDA (dissolved in 0.2% (v/v) ethanol) for 30 min at 37°C. The cells were washed twice with PBS again and then incubated with fresh medium containing 500 μM NADPH (final concentration) and drug (i.e. 15 μM Sch B, 7.5 μM or 15 μM curcumin and 2 μM or 15 μM menadione, final concentration). The fluorescence intensity of each well was measured for 6 h as described above.

Time Course Study of Prooxidant-Induced Changes in Cellular GSH Level in Vitro AML12 cells were incubated with 15 μM (−)Sch B, 7.5 μM curcumin or 2 μM menadione for 6 h, followed by the incubation with drug-free medium for increasing periods of time (i.e. 0, 2, 4, 8, 12, 16, 20 h). Cell lysates were prepared at the indicated period of time. The GSH level in cell lysates was measured using an enzymatic method of Griffith.

Effect of ABT or DMTU on Prooxidant-Induced Glutathione Antioxidant Response in Vitro To investigate the involvement of CYP and ROS in the cellular GSH stimulation, AML12 cells were pre-incubated with ABT (a broad range CYP inhibitor, 10 μM final concentration, for 2 h), or DMTU (a free radical scavenger, 20 μM, 1 h) prior to the addition of 15 μM (−)Sch B, 7.5 μM curcumin or 2 μM menadione. The vehicle (PBS for ABT and DMTU) was added for the respective control. Then cells were co-incubated with ABT/DMTU and (−)Sch B (15 μM) or curcumin (7.5 μM)/menadione (2 μM) for 6 h. The drug-containing medium was removed, and the cellular GSH level was measured at 16 h post-drug exposure for Sch B or 12 h post-drug exposure for curcumin and menadione.

Effect of Prooxidant Pretreatment on Menadione Toxicity in Vitro AML12 cells were incubated with (−)Sch B (15 μM), curcumin (7.5 μM) or menadione (2 μM) for 6 h. Then the drug-containing medium was replaced with drug-free medium for further incubation. The drug-pretreated cells were challenged with menadione (50 μM) at 16 h post-drug exposure for Sch B or 12 h post-drug exposure for curcumin and menadione. Menadione-induced cytotoxicity was assessed by the measurement of lactate dehydrogenase (LDH) leakage at 4 h post-challenge. Cellular GSH was also measured using the enzymatic method.

Effect of Single Bolus Dose of Prooxidant on CCl₄ Hepatotoxicity in Vivo Female Balb/c mice were randomly divided into groups of five. For the sake of comparison among tested prooxidants, it was logical to treat the mice at a same molar dose. Mice were orally administered with 800 mg/kg Sch B, 737 mg/kg curcumin or 344 mg/kg menadione (all at an equivalent molar dose of 2 mmol/kg). Control mice received olive oil (vehicle) only. Twenty-four hours post-dosing, mice were orally administered with 158 mg/kg CCl₄ [1% (v/v) in
olive oil]. Twenty-four hours after the CCl₄ intoxication, heparinized blood samples were drawn from anesthetized mice by cardiac puncture, and the mice were then killed by cardiac excision. Hepatic mitochondrial fractions were prepared as described. Plasma samples as well as hepatic mitochondrial fractions were obtained for biochemical analysis.

Effect of Antioxidant Pretreatment on Prooxidant-Induced Glutathione Antioxidant Response in Vivo To investigate the effect of antioxidant on the Sch B/curcumin-induced glutathione antioxidant response, female Balb/c mice were pre-treated orally with α-tocopherol at 1.3 g/kg (dissolved in olive oil) 24 h before Sch B/curcumin treatment. Control group received olive oil only. Then, mice were subjected to Sch B/curcumin treatment followed by CCl₄ intoxication as mentioned earlier. Plasma samples and hepatic mitochondrial samples obtained from mice were subjected to biochemical analysis.

Effect of Intraperitoneal Injection with Sch B or Curcumin on Hepatic Mitochondrial GSH Level in Vivo Sch B and curcumin were prepared freshly by dissolving in DMSO (100%). Female Balb/c mice were intraperitoneally injected with 400 mg/kg Sch B or 368 mg/kg curcumin (both at an equivalent molar dose of 1 mmol/kg, 0.1 mL/mice). Vehicle control mice received 0.1 mL DMSO. Twenty-four hours post-injection, mice were killed and hepatic mitochondrial GSH levels of mice were measured.

Effect of Long Term and Low Dose Treatment with Sch B or Curcumin on CCl₄ Hepatotoxicity in Vivo Balb/c mice are relatively small in size and therefore too delicate to receive multiple intragastric administration of drug by intubation. To avoid a high mortality during the experimental period, female ICR mice, which are bigger in size, were used in the study. Female ICR mice were orally administered with Sch B (20 mg/kg/d) or curcumin (18 mg/kg/d) for 15 d (i.e. 50 nmol/kg/d×15). The hepatotoxicity was induced by the oral administration of CCl₄ at 158 mg/kg 24 h after the last dosing with the tested drug. Twenty-four hours post-challenge, plasma samples and hepatic mitochondrial samples obtained from mice were subjected to biochemical analysis.

Biochemical Analysis The extent of hepatic oxidative damage was assessed by measurements of plasma sorbitol dehydrogenase (SDH) activity and hepatic mitochondrial malondialdehyde (MDA) level, as described previously. LDH activity was measured in the culture media and cell lysates prepared from unchallenged/challenged cells, with or without drug pretreatment, as described by Chui et al. Cellular or hepatic mitochondrial GSH level was measured using the enzymatic method of Griffith. Protein concentrations of samples were determined using a Bio-Rad protein assay kit.

Statistical Analysis Data were analyzed by one-way analysis of variance (ANOVA). Post-hoc multiple comparisons were performed using Least Significant Difference (LSD). p values <0.05 were regarded as statistically significant.

RESULTS

Prooxidant-Induced ROS Generation in Mouse Liver Microsomes and AML12 Cells Menadione, a well-known ROS-generating quinone, was used as a positive control in the study. As shown in Fig. 1A, (−)Sch B, curcumin and menadione caused a concentration-dependent increase in ROS production in mouse liver microsomes. At the concentration of 20 μM, the relative potencies in ROS production were in the descending order of curcumin>menadione>(−)Sch B, with the extent of stimulation being 11 fold, 270 and 214%, respectively, when compared with the vehicle control. The incubation with (−)Sch B, curcumin and menadione (all at 15 μM) significantly increased the extent of ROS production in AML12 cells, with the relative potencies being in the descending order of menadione (106%)>curcumin (91%)>(−)Sch B (30%), when compared with the vehicle control.

Time Course of Prooxidant-Induced Changes in Cellular GSH Level in Vitro Cells were exposed to 15 μM (−)Sch B, 7.5 μM curcumin or 2 μM menadione for 6 h, during which a similar extent of ROS was produced (Fig. 1B). As shown in Fig. 2, all tested compounds caused a time-dependent increase in cellular GSH level in AML12 cells. The (−)Sch B incubation increased the GSH level maximally (64%) at 16 h post-drug exposure, while curcumin and menadione stimulated the GSH level maximally (278, 148%, respectively) at 12 h post-drug exposure.
Effects of ABT and DMTU Pretreatment on Prooxidant-Induced Glutathione Antioxidant Response in Vitro

To investigate the role of CYP and ROS in the prooxidant-induced glutathione antioxidant response, specific inhibitors were used. As shown in Fig. 3, pre/co-incubation with ABT suppressed the Sch B/curcumin-induced GSH stimulation. Menadione, which is not mainly metabolized by CYP, increased the GSH level in the presence ABT pre/co-incubation. Pre/co-incubation with DMTU, a ROS scavenger, attenuated the prooxidant-induced GSH stimulation for all three tested compounds (Fig. 3).

Effect of Prooxidant Pretreatment on Menadione Toxicity in AML12 Cells

As indicated in Fig. 4A, a high concentration of menadione (50 μM) caused cell injury, as indicated by an increase (ca. 3.2 fold) in LDH release. Pretreatment with (−)Sch B (15 μM), curcumin (7.5 μM) and menadione (2 μM) protected against menadione toxicity, as evidenced by a reduced extent of LDH release. The relative potencies of cytoprotection were in the descending order of curcumin>(−)Sch B, menadione, with the degree of protection being 74, 24 and 29%, respectively. The cytoprotection was found to be associated with the increase in cellular GSH level (14 fold, 174, 150%, respectively), when compared with menadione-challenged cells (Fig. 4B).

Effect of a Single Bolus Dose of Prooxidant on CCl4 Hepatotoxicity in Mice

To confirm the experimental observation in AML12 cells, the effects of prooxidant pretreatment on CCl4 hepatotoxicity were examined in mice. SDH is a house keeping enzyme in the liver, and the plasma SDH activity can serve as a biochemical index of liver damage. MDA is a product of lipid peroxidation, therefore an increased MDA level is indicative of oxidative stress. As shown in Fig. 5, CCl4 caused hepatic oxidative injury, as indicated by significant increases in plasma SDH activity and mitochondrial MDA level. Pretreatments with Sch B (400 mg/kg) and curcumin (737 mg/kg) protected against CCl4 toxicity, as indicated by decreases in plasma SDH activity (93, 20%, respectively) and mitochondrial MDA level (67, 27%, respectively), when compared with the untreated CCl4-challenged control. The hepatoprotection afforded by Sch B and curcumin was associated with the increase in mitochondrial GSH level (102, 63%, respectively), when compared with those of CCl4-intoxicated mice.

Menadione pretreatment did not protect against CCl4 toxicity or increase mitochondrial GSH level in mice.

Effect of Antioxidant Pretreatment on the Sch B/ Curcumin-Induced Hepatoprotection against CCl4 Toxicity in Mice

To investigate the role of ROS in Sch B/curcumin-
induced hepatoprotection in vivo, the effect of α-tocopherol pretreatment was examined. Pretreatment with α-tocopherol conferred a hepatoprotection, as indicated by a slight but significant suppression on CCl₄-induced hepatic SDH leakage (17%) (Fig. 6). α-Tocopherol pretreatment attenuated the Sch B-induced hepatoprotection, as indicated by a partial reversal of Sch B-induced decrease in plasma SDH activity, with the extent of protection being 49% in α-tocopherol and Sch B co-treated mice (vs. 94% in Sch B-treated mice). α-Tocopherol pretreatment completely abrogated the curcumin-induced hepatoprotection. The α-tocopherol-induced attenuation on Sch B/curcumin-induced hepatoprotection was found to be associated with a reversal of Sch B/curcumin-induced increase in mitochondrial GSH level in CCl₄-intoxicated mice.

**DISCUSSION**

The enzyme-catalyzed detoxification of xenobiotics by liver cells is often associated with a concomitant production of oxidant species like ROS. In the present study, the three compounds of interest were found to induce the production of ROS in both mouse liver microsomes and AML12 cells. While (−)Sch B is the least potent compound in stimulating ROS production, the relative potencies of curcumin and menadione varied in the two assay systems. This observation may be related to the differential expression of detoxifying enzymes such as CYP and NAD(P)H-ubiquinone oxidoreductase in mouse liver and cultured hepatocytes and/or the differential membrane permeability of these two compounds in hepatocytes. Previous studies in our laboratory have shown that the cumulative amount of ROS generated from a 6-h exposure to 15 μM (−)Sch B was found to maximally stimulate

Fig. 5. Effects of Prooxidant Treatment on CCl₄-Induced Hepatotoxicity in Balb/c Mice

Mice were orally administered with Sch B, curcumin or menadione (2 mmol/kg). Twenty-four hours after the drug treatment, drug-pretreated mice were orally administered with CCl₄ (158 mg/kg). Twenty-four hours post-challenge, the extent of CCl₄-induced injury was assessed by plasma sorbitol dehydrogenase (SDH) activity (A). Hepatic mitochondrial malondialdehyde (MDA) level (B) and GSH level (C) were also measured. Values given are means±S.E.M. * Significantly different from the non-CCl₄ control; # Significantly different from the CCl₄ control.
cellular GSH at 16 h post-drug exposure in AML12 cells. Consistent with this, a 6-h exposure of the tested compounds [namely, (−)Sch B, curcumin and menadione] at appropriate concentrations that produced a similar amount of ROS caused an increase in GSH level post-drug exposure in AML12 cells. Despite the similar extent of ROS production, curcumin was found to cause a larger degree of GSH stimulation than those of Sch B and menadione. The greater effect of curcumin on GSH stimulation may be related to its ability to directly induce a dissociation of kelch-like ECH-associated protein 1 (Keap 1, a repressor of Nrf2) with a resultant synergistic induction of glutathione antioxidant response with ROS. The finding that both CYP inhibitor and antioxidant pretreatments were able to suppress the GSH stimulation induced by the tested compounds suggest the involvement of CYP-catalyzed reaction in the prooxidant-induced glutathione antioxidant response.

GSH is critically involved in protecting the cells against oxidative injury, directly by reacting with ROS and/or indirectly through the intermediacy of enzyme-catalyzed reactions. Menadione-induced oxidative injury, which is a well-established model of oxidant injury in our laboratory, can reproducibly induce a similar extent of injury in AML12 cells. While an exposure to a high concentration of menadione caused the depletion of cellular GSH and thus cell injury in AML12 cells, the glutathione antioxidant response induced by the tested compounds was accompanied by the cytoprotection against menadione toxicity, with the extent of cytoprotection afforded by curcumin being most potent. However, pretreatment with Sch B (400 mg/kg) or curcumin (737 mg/kg), but not menadione...
one (344 mg/kg), protected against CCl4 hepatotoxicity, with the degree of protection afforded by Sch B being much larger than that of curcumin. We found that α-tocopherol treatment conferred a hepatoprotection against CCl4 toxicity at 24 h but not 48 h post-dosing with α-tocopherol (data not shown), indicating that α-tocopherol treatment enhanced the antioxidant capacity of liver tissue at 24 h post-dosing when Sch B or curcumin treatment was commenced. The ability to α-tocopherol pretreatment to partly suppress the hepatoprotection afforded by Sch B and curcumin also implicates the involvement of ROS-mediated process in the induction of hepatoprotective response in vivo. The inability of menadione to induce the hepatoprotection, as compared with that in AML12 cells, may be attributed to its low bioavailability due to intestinal absorption, extensive intestinal metabolism and rapid elimination from the liver. In this regard, a recent study has shown that dietary quinones are subjected to intestinal NAD(P)H quinone oxidoreductase 1-catalyzed reduction and a subsequent glucuronidation. Alternatively, menadione may be toxic to the liver at the administered dose. It has been reported that menadione overdose caused anemia, polycythemia, splenomegaly, renal and hepatic damages.

A recent study in another laboratory showed that Sch B was absorbed in all segments of intestine, particularly in duodenum, using an in situ single pass intestinal perfusion model. This observation is consistent with our finding that Sch B could be detected in plasma 30 min after the oral administration in rats (unpublished data). In addition, treatment with Sch B at multiple doses (200 mg/kg twice daily, 30 d) or dietary supplementation (0.012% w/w, starting from 9 months of age until death) in mice were found to be effective in inducing protective effects, which indirectly support a good oral bioavailability of Sch B. The reduced potency of curcumin in hepatoprotection, as compared with that in AML12 cells, may be attributed to its low bioavailability due to low intestinal absorption, extensive intestinal metabolism and rapid elimination from the body. In this connection, intraperitoneal injections of curcumin (368 mg/kg) and Sch B (400 mg/kg), which did not require intestinal absorption of the compound, were found to increase hepatic mitochondrial GSH level (referred to as glutathione antioxidant response) to a similar extent in mice. However, an intraperitoneal injection of menadione produced a hepatotoxic effect. In addition, the long-term, low dose pretreatments with Sch B and curcumin was found to induce a glutathione antioxidant response and conferred a hepatoprotection against CCl4 toxicity to a similar degree, suggesting that a sustained low blood concentration of curcumin by repetitive oral administration may culminate in the induction of a glutathione antioxidant response in the liver, presumably
via the ROS-mediated processes. The underlying mechanism is yet to be investigated.

The prooxidant-induced glutathione antioxidant response, as observed in the present study, is consistent with the recent findings in our laboratory that a low concentration of ROS arising from CYP-catalyzed metabolism of schisandrin B can trigger the redox-sensitive signaling pathway, with resultant induction of Nrf2-activated expression of antioxidant proteins.\textsuperscript{20} Taken together, results obtained from the present study support the postulation that prooxidants can invariably induce the glutathione antioxidant response and conferred cytoprotection against oxidant injury \textit{in vitro}. Whether or not the prooxidant of interest can produce a similar response \textit{in vivo} would depend on the bioavailability of the compound \textit{via} oral route and the potential toxic effect of its prooxidant action.

REFERENCES


