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Curcumin pretreatment protects against acute acrylonitrile-induced oxidative damage in rats

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ABSTRACT

Acrylonitrile (AN) is widely used in the manufacturing of fibers, plastics and pharmaceuticals. Free radical-mediated lipid peroxidation is implicated in the toxicity of AN. The present study was designed to examine the ability of curcumin, a natural polyphenolic compound, to attenuate acute AN-induced lipid peroxidation in the brain and liver of rats. Male Sprague–Dawley rats were orally administered curcumin at doses of 0 (olive oil control), 50 or 100 mg/kg bodyweight daily for 7 consecutive days. Two hours after the last dose of curcumin, rats received an intraperitoneal injection of 50 mg AN/kg bodyweight. Acute exposure to AN significantly increased the generation of lipid peroxidation products, reflected by high levels of malondialdehyde (MDA) both in the brain and liver. These increases were accompanied by a significant decrease in reduced glutathione (GSH) content and a significant reduction in catalase (CAT) activity in the same tissues. No consistent changes in superoxide dismutase (SOD) activity were observed between the control and AN-treatment groups in both tissues. Pretreatment with curcumin reversed the AN-induced effects, reducing the levels of MDA and enhancing CAT activity and increasing reduced GSH content both in the brain and liver. Furthermore, curcumin effectively prevented AN-induced decrease in cytochrome c oxidase activity in both liver and brain. These results establish that curcumin pretreatment has a beneficial role in mitigating AN-induced oxidative stress both in the brains and livers of exposed rats and these effects are mediated independently of cytochrome P450 2E1 inhibition. Accordingly, curcumin should be considered as a potential safe and effective approach in attenuating the adverse effects produced by AN-related toxicants.

1. Introduction

Acrylonitrile (AN), an organic nitrile, is used mainly as a monomer or co-monomer in the production of synthetic fibers, plastics and elastomers. Human exposure to AN predominantly occurs in occupational settings via inhalation. However, oral and dermal routes of exposure have also been recognized. AN has been found in drinking water, food, cigarette smoke and occupational environments (Rubio et al., 1990; Miller et al., 1998; IARC, 1999).

AN is metabolized in humans by two principle pathways: glutathione conjugation and oxidation by cytochrome P450. The main product of glutathione (GSH) conjugation with AN is 2-cyanoethylmercapturic acid (N-acetyl-S-(2-cyanoethyl)cysteine) (IPCS, 1983, 2002). Oxidation by cytochrome P450 2E1 (CYP2E1) forms 2-cyanoethylene oxide, which can also undergo GSH conjugation to yield a series of metabolites, which include cyanide and thiocyanate (IPCS, 1983, 2002). A study in wild-type mice showed that administration of 40 mg AN/kg led to the formation of a maximum of 110 nmol of cyanide/g liver weight, indicating a release of about 2.9 mg of cyanide per kg liver (Wang et al., 2002). Though cyanide may play a major role in acute AN toxicity of AN, it is not considered to be solely responsible for AN's toxicity (Campian and Benz, 2008).

Metabolism of AN proceeds via conjugation with glutathione or epoxidation via CYP2E1 to cyanoethyleneoxide (CEO). It was hypothesized that CEO metabolism via epoxide hydrolase is the primary pathway for cyanide formation (Ahmed et al., 1982; Wang et al., 2002). GSH conjugation has been shown to be depleted after AN treatment *in vivo* and may decrease the antioxidant capacity of the cells, resulting in an overall increase in intracellular reactive oxygen species (ROS) and oxidative damage. Cyanide has been

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shown to induce oxidative stress (lipid peroxidation) in the brain of acutely treated mice and in several cell lines (Johnson et al., 1987; Ardelt et al., 1989; Mills et al., 1996; Shou et al., 2000; Li et al., 2002; Hariharakrishnan et al., 2009). Subchronic effects of AN include oxidative damage in the brain (Jiang et al., 1998; Kamendulis et al., 1999a,b; Whysner et al., 1998; Enongene et al., 2000; Mahalakshmi et al., 2003; Esmat et al., 2007), and supplementation with antioxidants may represent an efficacious treatment modalities against AN toxicity in humans.

Curcumin, a natural polyphenolic compound abundant in the rhizome of the perennial herb turmeric, *Curcuma longa*, is known to possess comprehensive anti-inflammatory and anti-cancerous properties following topical or oral administration. It is commonly used as a dietary spice and coloring agent in cooking and anecdotally as an herb in traditional Indian and Chinese medicine (Miquel et al., 2002; Maheshwari et al., 2006).

The polyphenolic structure and its ability to readily cross the blood–brain barrier and bind to redox metal ions led to studies on the efficacy of curcumin as prophylactic treatment for neurodegenerative diseases (Garcia-Alloza et al., 2007). Curcumin administration was reported to exert neuroprotective effects *in vitro*, in animal models, as well in humans; it can protect against damage associated with ischemia-reperfusion, free radicals and brain injury, and epidemiologically, moderate consumption of curcumin was associated with low incidence of Alzheimer's diseases, Parkinson's disease and other neurodegenerative diseases (Cole et al., 2007). It was also shown that curcumin reverses the effects of lead (Shukla et al., 2003), aluminum (Sethi et al., 2009), cadmium (Eybl et al., 2004) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Rajeswari, 2006) toxicity. However, no studies have investigated the efficacy of curcumin in attenuating the adverse effects of AN. Accordingly, the aim of this study was to assess the ability of curcumin to maintain optimal redox status both in brain and liver of AN-treated rats. Specifically, we measured the effects of curcumin on lipid peroxidation, the activities of the enzymatic antioxidants, superoxide dismutase (SOD) and catalase (CAT), and the non-enzymatic antioxidant GSH. To understand the effect of AN on mitochondrial function and the protective effect of curcumin, if any, we further investigated the activity of cytochrome c oxidase in modulating AN-induced toxicity.

2. Materials and methods

2.1. Chemicals

Curcumin, cytochrome c and NADPH were purchased from Sigma (St. Louis, MO, USA). AN (chemical purity >99%) was provided by the Acrylonitrile Plant of Shanghai Petrochemical Company (Shanghai, China). All chemicals were of the highest purity grade available.

2.2. Animals and treatment

Adult male Sprague–Dawley rats were purchased from the Laboratory Animal Center of Jiangsu University. The rationale of selection of male rats is based on the desire to avoid confounder such as sex hormone, lactation and pregnancy, which are inherent to females. All animals were housed under standard temperature ($24 \pm 1\,^{\circ}C$) and illumination (12 h light/12 h dark cycles), and water and standard rodent chow were available *ad libitum*. Animal care and experimental protocol was consistent with the guidelines set by the Laboratory Animal Committee of Jiangsu University. Twenty-eight adult rats (180–220 g) were randomly assigned into four groups, the doses of AN and curcumin were based on our previous study as well as literature reports (Shukla et al., 2003; Eybl et al., 2004; Rajeswari, 2006; Rongzhu et al., 2009; Sethi et al., 2009). With regard to the preferred route of administration, curcumin was administrated by oral gavage, mimicking dietary intake. While inhalation exposure best mimics occupational exposures, the toxicokinetics of i.p. administered AN should be similar in both exposure routes. Furthermore, we do not have an inhalation facility and therefore opted for an i.p. injection protocol. It should also be pointed that the literature is replete with studies using AN injections, making it much easier to compare our data with previously published reports. There are only few studies on the effects of AN upon inhalation (Sapota, 1982; Nerland et al., 1989; Benz et al., 1990; Felten et al., 1998).

Group I received 1 ml of olive oil per 200 g body weight by oral gavage daily for 7 consecutive days and served as the control group.

Group II received 1 ml of olive oil per 200 g body weight by oral gavage daily for 7 days. Rats were injected intraperitoneally (i.p.) with AN at a dose of 50 mg/kg body weight 2 h after the last dose of olive oil.

Group III received curcumin (prepared in olive oil) at a dose of 50 mg/kg body weight by oral gavage daily for 7 days. Rats were injected i.p. with AN at a dose of 50 mg/kg body weight 2 h after the last dose of curcumin.

Group IV received curcumin (prepared in olive oil) at a dose of 100 mg/kg body weight by oral gavage daily for 7 days. Rats were injected i.p. with AN at a dose of 50 mg/kg body weight 2 h after the last dose of curcumin.

2.3. Blood and tissue collection

Rats were anesthetized and killed by decapatication 1 h after AN administration. Blood was collected in a heparinized syringe by cardiac puncture. One milliliter whole blood from each animal was immediately collected into Conway diffusion cell for the measurement of CN^- concentrations.

The brain represents the main target organ of acute AN toxicity and the liver serves as main organ for oxidative metabolism (Ghanayem et al., 1991; Benz et al., 1997; Benz and Nerland, 2005). After acute single exposure to AN, within an hour, we noted a transition from cholinomimetic neurotoxicity to other toxic signs such as depression, convulsion and respiratory failure (Ghanayem et al., 1991). In the disposition study, Sapota revealed that the radioactivity in the tissues of rats 1 h after single intraperitoneal injection of [^{14}C] acrylonitrile was the highest, indicating the highest internal dose of AN following acute administration (Sapota, 1982). Therefore, we opted to perform the remainder of analysis at this time point. One hour after the injection of AN the rats were decapitated and the brain and liver quickly removed, cleared of meninges and affiliated blood vessels and fat, respectively, washed with ice-cold normal saline solution and placed on ice. The samples were weighed and sonicated in chilled normal saline to produce a 10% homogenate (w/v). Next, the samples were centrifuged at $719 \times g$ (2000 rpm, Megafuge 1.0R, Heraeus, Germany) for 10 min at $4\,^{\circ}C$ and the resultant supernatants were collected and used for biochemical analyses. Assays were performed within 24 h of sample preparation.

2.4. Lipid peroxidation products

Lipid peroxidation products, namely the amount of MDA formed by the 2-thiobarbituric acid reaction as thiobarbituric acid reactive substances, were measured by the method of Utley et al. (1967), using a commercial MDA kit (Nanjing Jiancheng Bioengineering Company, Nanjing, China). The spectrophotometric absorbance was assessed at 532 nm in accordance with the manufacturer's instructions. The results were expressed as nmol MDA per mg protein.

2.5. Reduced glutathione

The total GSH content in the brain and liver samples was determined with Ellman's reagent, 5,5′-dithiobis-(2-nitrobenzoic acid) (Sedlak and Lindsay, 1968). Briefly, the 10% (w/v) tissue homogenate was immersed in 5% (w/v) trichloroacetic acid and centrifuged at $719 \times g$ (2000 rpm, Megafuge 1.0R) for 20 min. The GSH content in the deproteinized supernatant was then estimated using Ellman's reagents and the spectrophotometric absorbance was determined at 420 nm using a GSH kit (Nanjing Jiancheng Bioengineering Company). The results were expressed as nmol GSH per mg protein.

2.6. Enzymatic antioxidants

Analysis of SOD activity was based on SOD-mediated inhibition of nitrite formation from hydroxylammonium in the presence of O_2^- generators (xanthine and xanthine oxidase) (Elstner and Heupel, 1976). One unit of SOD is defined to as reducing nitrite formation in 40 min at $37\,^{\circ}C$ by 50%. A commercial SOD kit (Nanjing Jiancheng Bioengineering Company) was used for the analyses and spectrophotometric absorbance was assessed at 500 nm. Results were expressed as units of SOD activity per mg protein.

CAT activity was measured by the ammonium molybdate spectrophotometric method (Góth, 1991). Ammonium molybdate rapidly terminates the activity of CAT to decompose H_2O_2 and reacts with the remaining H_2O_2 to form a yellow-colored adduct. The compound is monitored by measuring absorbance at 405 nm. CAT activity is calculated using a formula and the control is distilled water. The results are expressed as units of CAT activity per mg protein (U/mg protein), where 1 U of CAT is defined as the amount of enzyme that decomposes 1 μmol of H_2O_2 per second. A commercial CAT kit (Nanjing Jiancheng Bioengineering Company) was used.

2.7. Cytochrome c oxidase activity

Cytochrome c oxidase activity was determined by mixing 0.5 ml of cytochrome c (0.09 mmol/l), 2.4 ml phosphate buffer (0.03 mol/l, pH 7.4) and appropriate amounts of sodium hydrosulfite ($Na_2S_2O_4$) to convert oxidized cytochrome c to the reduced

form. Samples of the tissue homogenates (1% liver, 2% brain, w/v) and 30 μl of n-dodecyl-β-D-maltoside (2 M) were added to the reaction mixture to initiate the reaction. The change in spectrophotometric absorbance at 550 nm was recorded between 0 and 3 min at interval of 30 s. The results were expressed as the rate of oxidation of reduced cytochrome c per mg wet tissue (Ikegaya et al., 2000, 2001).

2.8. Cyanide (CN^-) content assay

CN^- concentrations in whole blood, brain and liver homogenates were determined by Conway microdiffusion (Ballantyne et al., 1974). Specimens (200–300 mg) of brain and liver were homogenized in ice-cold normal saline (0.9%, w/v, NaCl). 1.0 ml of 0.1 M NaOH was added to the central well of a Conway diffusion cell (Bel-Art Products, USA). Three milliliters of 15% sulfuric acid were added to one side of the middle well, and 1.0 ml tissue homogenate (20% w/v) was added to the opposite side of the middle well. The Conway dish was then sealed with silicone grease and kept on a horizontal shaker at 70 rpm for 3 h at room temperature. At the end of incubation, 0.5 ml of NaOH from the central cell of the dish was added to a cuvette containing 2 ml of 0.1 M potassium phosphate buffer (pH 7.4). 0.2 ml of 10 g/L chloramine-T were added to the cuvette and allowed to react at 37 °C for 5 min. 2.0 ml of pyridine–barbituric acid reagent were added to the cuvette, and, after 20 min at 25 °C, the maximum absorbance at 580 nm was determined by monitoring over 5 min. Serially diluted standards of potassium CN^- in 0.1 M NaOH were used to construct a standard curve. The limit of the CN^- detection of this method was found to be less than 1.0 nmol. Results were expressed as μg CN^-/g tissue or blood.

2.9. Cytochrome P450 2E1 (CYP2E1) activity

CYP2E1 activity was measured based on the rate of oxidation of p-nitrophenol (PNP) to p-nitrocatechol in the presence of NADPH by modification of the methods of Reinke and Moyer (1985) and Koop (1986). The reaction was performed with 100 μg of liver microsomal protein for 30 min at 37 °C. The results were expressed as nmol per minute per mg of microsomal protein. Briefly, liver homogenate (10%) was prepared in chilled potassium phosphate (5 mM; pH 7.4) then centrifuged at 11,800 × *g* for 20 min at 4 °C. The supernatant (1.5 ml) was added to ice-cold $CaCl_2$ (final concentration: 8 mmol/l) and, 30 min later, was centrifuged at 11,800 × *g* for 20 min. The resulting pellets (microsomes) were resuspended in 50 mM sodium phosphate buffer (pH 7.4) and kept at −80 °C for further study. The activity was determined in a final volume of 500 μl containing 100 μg liver microsomes, 100 mM potassium phosphate, pH 6.8, 0.1 mM PNP p-nitrophenol, 1 mM NADPH and 5 mM $MgCl_2$ followed by incubation at 37 °C for 30 min. The reaction was terminated by the addition of 100 μl of chilled trichloroacetic acid (20%, w/v) and centrifuged at 10,000 × *g* for 5 min. After vortexing and centrifugation, 100 μl of the supernatant was added to a 96-well plate with 50 μl of 10 M sodium hydroxide. The plate was read at 550 nm and quantified by reference to p-nitrocatechol standards.

2.10. Protein content assays

The protein concentration of the samples was estimated with the BCA kit from Bio-Rad Laboratories Inc. (Hercules, CA, USA). Standards and blanks were assessed simultaneously.

2.11. Statistical analysis

All values were expressed as mean ± standard deviation. Differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by the Student Newman–Keuls test for multiple post hoc comparison tests. The alpha level for the analyses was set at $p < 0.05$. All analyses were performed using SPSS 10.0 software (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Curcumin prevented increases in MDA levels in AN-treated rats

The lipid peroxidation status in the brain and liver are shown in Fig. 1. A significant increase in levels of MDA was observed in the AN-treated group both in the brain ($F = 11.37$, $p < 0.05$) and liver ($F = 3.37$, $p < 0.05$) compared with the control group. However, the magnitude of the brain increment in MDA levels was different from that in the liver, corresponding to 38.6% and 10.8%, respectively. Curcumin pretreatment at 100 mg/kg significantly decreased ($p < 0.05$) the level of lipid peroxidation products in both the brain and the liver compared with the AN-treated group. However, the protective effect in the brain was not as pronounced as in the liver. Pretreatment with 50 mg/kg curcumin prevented the increase in hepatic MDA and it remained indistinguishable compared with the control group ($p > 0.05$). However, the same caroming pretreatment did not inhibit the AN-induced increase in brain MDA levels, which remained significantly higher compared with the control group ($p < 0.05$).

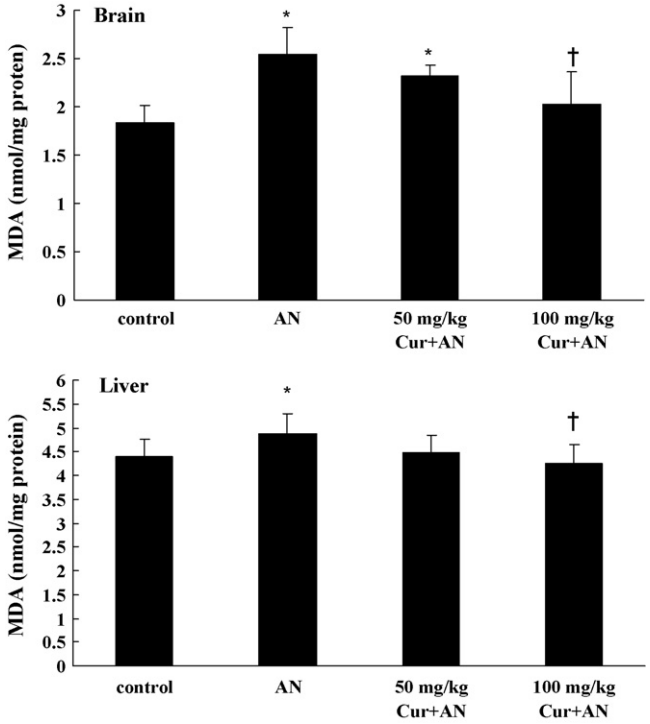

Fig. 1. Effects of AN and curcumin on lipid peroxide (MDA) levels in rat brain and liver. Results were the mean ± standard deviation from seven rats per group and are expressed as nmol MDA per mg protein. *$p < 0.05$ versus control; †$p < 0.05$ versus AN treatment. AN: acrylonitrile and CUR: curcumin.

3.2. Curcumin prevented the decrease in GSH contents in the brain and liver of AN-treated rats

AN treatment led to a significant decrease in GSH levels in the brain ($F = 18.15$, $p < 0.05$) and liver ($F = 48.89$, $p < 0.05$) (Fig. 2) compared with controls (38.6% and 70.5%, respectively). Pretreatment with curcumin increased the GSH content in both brain and liver compared with the AN-treated group. These increases were significant ($p < 0.05$) at both doses of curcumin in the brain, while in the liver only the 100 mg/kg curcumin pretreatment attained statistical significance ($p < 0.05$). At 100 mg/kg curcumin, the brain level of GSH returned to 83.2% of the control level, whereas hepatic GSH recovery was only 46.6%.

3.3. Curcumin prevented the decrease in CAT, but not SOD activity in AN-treated rats

SOD activity in the brain and liver was reduced upon AN treatment; however, these effects did not reach statistical significance ($F = 0.337$, $p > 0.05$ and $F = 1.89$, $p > 0.05$, respectively), Although curcumin pretreatment moderately increased the SOD activity in both the brain and liver compared with the AN-treated group, the changes were not statistically significant (Fig. 3).

AN treatment led to a significant decrease in CAT activity both in the brain ($F = 19.01$, $p < 0.05$) and in the liver ($F = 11.86$, $p < 0.05$) versus controls (Fig. 4). CAT activity in the 100 mg/kg curcumin pretreatment group was significantly greater in both the brain and the liver compared with the AN-treated group, approaching con-

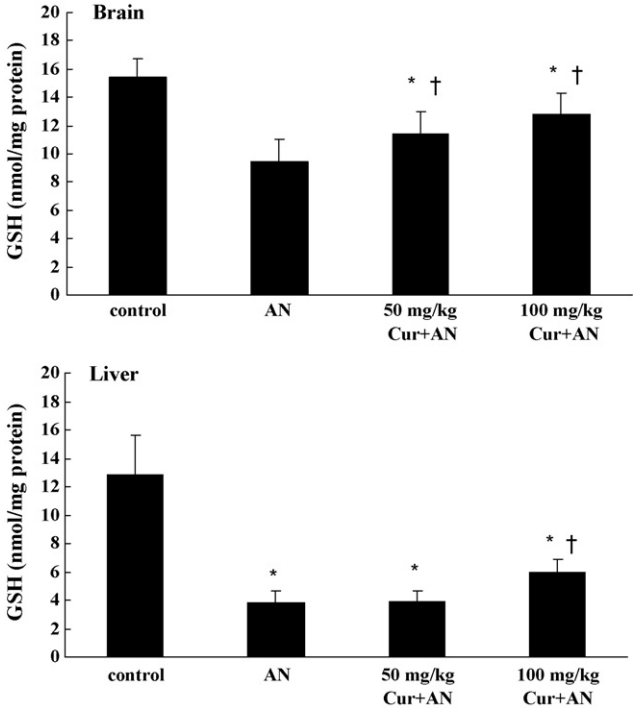

Fig. 2. Effects of AN and curcumin on GSH content in rat brain and liver. Results were the mean ± standard deviation from seven rats per group and are expressed as nmol of GSH per mg protein: $^{*}p < 0.05$ versus control; $^{\dagger}p < 0.05$ versus AN treatment. AN: acrylonitrile and CUR: curcumin.

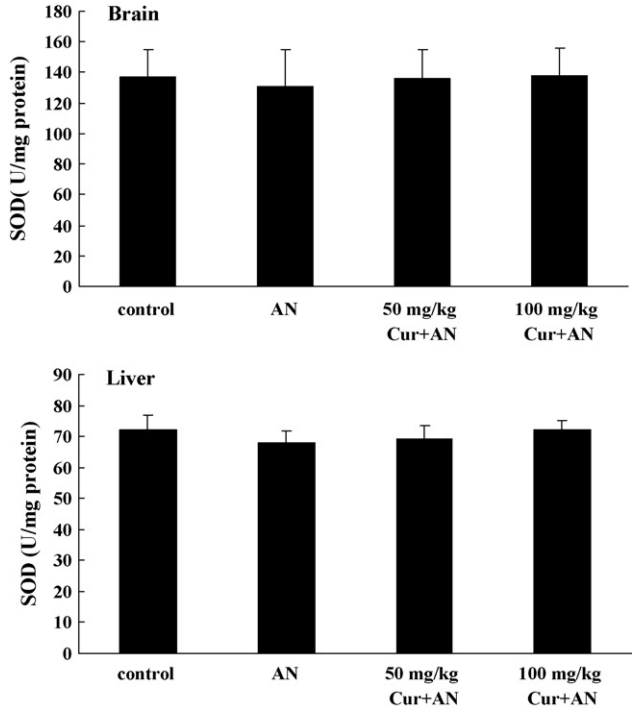

Fig. 3. Effects of AN and curcumin on SOD activity in the rat brain and liver. Results were the mean ± standard deviation from seven rats per group and are expressed as units of SOD per mg protein. AN: acrylonitrile and CUR: curcumin.

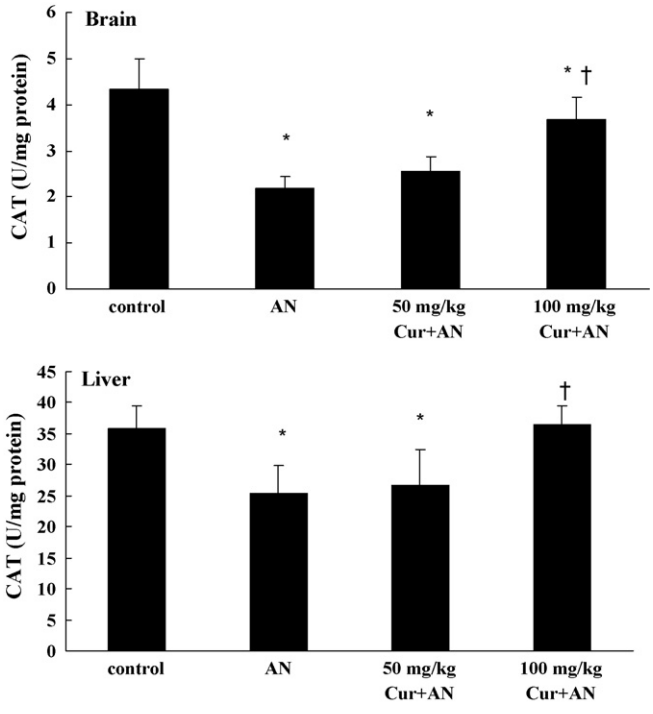

Fig. 4. Effects of AN and curcumin on CAT activity in rat brain and liver. Results were the mean ± standard deviation from seven rats per group and are expressed as units of CAT per mg protein: $^{*}p < 0.05$ versus control; $^{\dagger}p < 0.05$ versus AN treatment. AN: acrylonitrile and CUR: curcumin.

trol levels. The hepatic CAT activity in the 100 mg/kg curcumin pretreatment group was indistinguishable from controls (101.8% of control), while in the brain CAT activity (84.7% of the control) in the same group was greatly increased; however, it remained significantly lower versus the control group ($p < 0.05$).

3.4. Curcumin partially prevented the AN-induced inhibition of cytochrome c oxidase in livers and brains of rats

As shown in Fig. 5, AN treatment significantly reduced the cytochrome c oxidase activity in the brain ($F = 22.21$, $p < 0.05$) and liver ($F = 50.96$, $p < 0.05$) compared with the control group, and these effects were significantly mitigated by 100 mg/kg curcumin pretreatment both in the brain and liver. Though the pattern of decreased of cytochrome c oxidase activity in brain and liver by AN were similar, the magnitude of the brain decrease (46.3% of control) was lower versus the liver (28.9% of control). The extents of recovery of activity of cytochrome c oxidase upon curcumin pretreatment in the brain was relatively higher compared with the liver, reaching approximately 75% and 58% of the control values, for brain and liver, respectively.

3.5. Curcumin did not inhibit CN^- formation by AN in rat brain and whole blood

Hepatic CN^- formation was not detectable. Fig. 6 shows CN^- formation in brain and whole blood 1 h after injection of AN in rats with or without curcumin pretreatment. There were no significant changes between the AN alone and the AN plus curcumin pretreatment groups ($p > 0.05$).

3.6. Hepatic CYP2E1 activity was not affected by AN and curcumin in rat liver

Fig. 7 demonstrates the hepatic CYP2E1 activity in the control and the various treatment groups. Neither the change between the

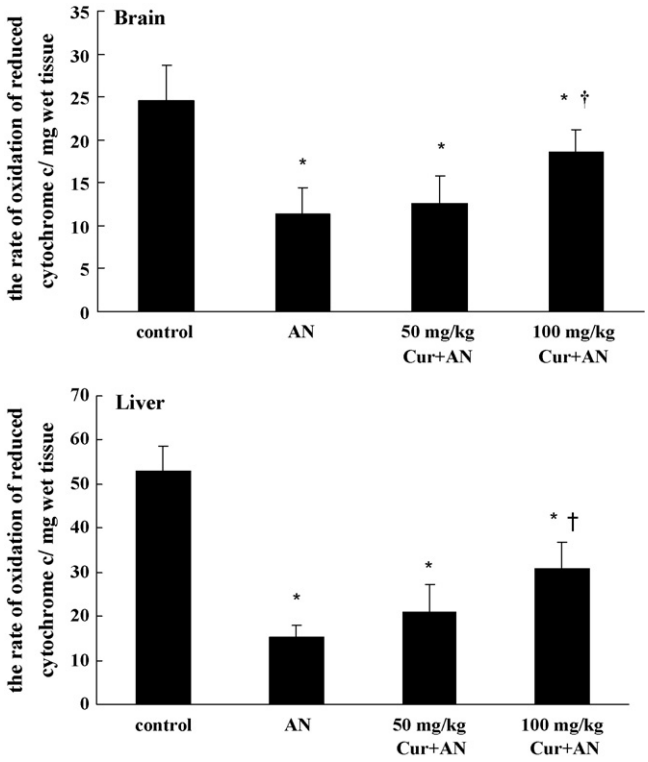

Fig. 5. Effects of AN and curcumin on cytochrome c oxidase activities in rat brain and liver. Results were the mean ± standard deviation from seven rats per group and are expressed as the rate of oxidation of reduced cytochrome c/mg wet tissue. $^{*}p < 0.05$ versus control; $^{\dagger}p < 0.05$ versus AN treatment. AN: acrylonitrile and CUR: curcumin.

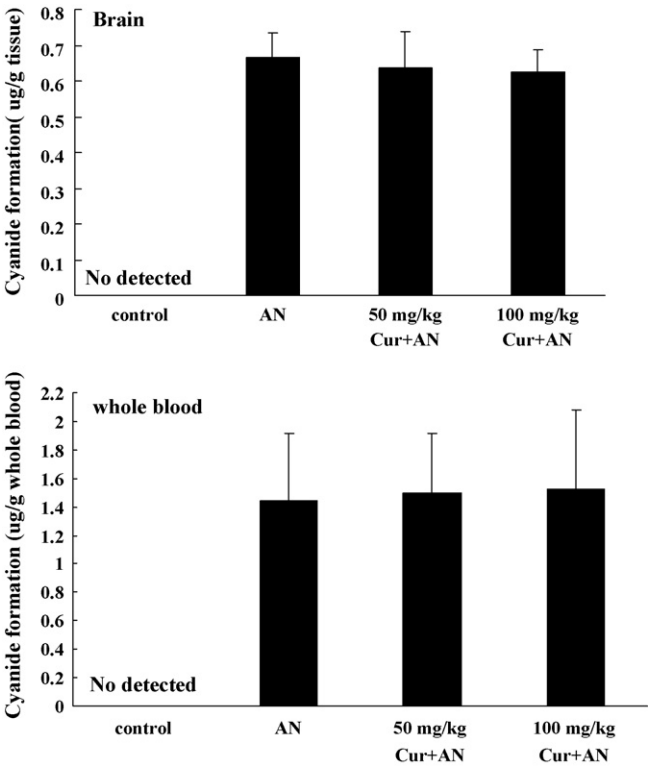

Fig. 6. Curcumin did not prevent CN^- formation by AN in rat brain and whole blood. Results were the mean ± standard deviation from seven rats per group. CN^- concentrations were expressed microgram CN^-/g tissue. AN: acrylonitrile and CUR: curcumin.

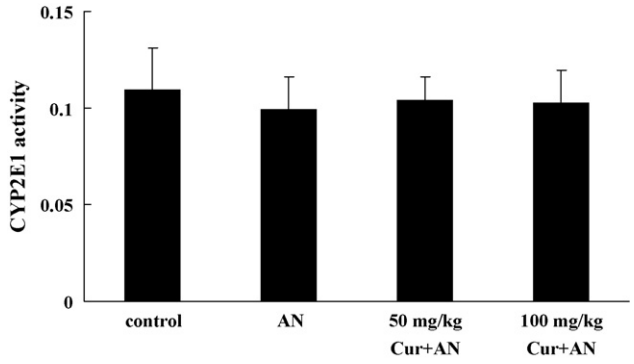

Fig. 7. Effects of AN and curcumin on CYP2E1 activity in rat liver. Results are mean ± standard deviation from seven rats per group and are expressed as nmol p-nitrocatechol/min/mg microsomal protein. AN: acrylonitrile and CUR: curcumin.

AN alone and the control group, nor changes between the AN alone and the AN with curcumin pretreatment groups at doses of 50 and 100 mg/kg were statistically significant ($p > 0.05$).

4. Discussion

The present study establishes increased levels of lipid peroxidation end-products (MDA) and decreased antioxidative enzyme activities, non-enzymatic antioxidants content and cytochrome c oxidase levels both in the liver and brain of AN-treated rats. Additional data also demonstrate AN-induced tissue-specific alterations in lipid peroxidation and antioxidant profiles in rat liver and brain, providing novel evidence on the selective effects associated with acute exposure to AN (Silver and Szabo, 1982). This selective effect was confirmed by observations on the differential alterations in cytochrome c oxidase activity in brain and liver. Given the key role of cytochrome c oxidase in mitochondrial ATP synthesis, and its inhibition by AN within an hour after acute AN exposure, the results establish the importance of immediate mitochondrial protection from accidental exposure to high levels of AN.

Several studies have indicated the role of oxidative stress in the toxicity of AN (Jiang et al., 1998; Esmat et al., 2007; Kamendulis et al., 1999a,b; Mahalakshmi et al., 2003). The major pathway of AN elimination is via conjugation with GSH. Accordingly GSH depletion may ensue, decreasing antioxidant capacity and the redox status, resulting in increased intercellular ROS production and oxidative damage (Ivanov et al., 1989). Metabolism of AN also results in the production of cyanide (Wang et al., 2002). Cyanide has been shown to induce oxidative stress (lipid peroxidation) in the brain of acutely treated mice and in cell lines by inhibiting the mitochondrial respiratory chain, CAT and glutathione peroxidase activities (Johnson et al., 1987; Ardelt et al., 1989; Mills et al., 1996; Kanthasamy et al., 1997; Shou et al., 2000; Li et al., 2002; Hariharakrishnan et al., 2009). Therefore, oxidative stress likely plays a causal role in the toxicity of AN. However, in general, data obtained to date was derived from subchronic exposure studies in animals or *in vitro* cell culture models, whereas results presented herein demonstrate, for the first time, that even a single exposure to AN can produce acute oxidative damage and disturb mitochondria energy metabolism.

GSH, a non-enzymatic free radical scavenger, participates in the detoxification of ROS (Reed, 1990; Franco et al., 2007). GSH participates in the enzymatic reduction of membrane hydroperoxy-phospholipids and prevents the formation of secondary alkoxyl radicals when organic peroxides are homolyzed (Franco et al., 2007). Binding of AN to GSH results in the induction of oxidative stress and impaired regeneration of other antioxidants (Pilon et al., 1988; Benz et al., 1997). Studies with 1^{-14}C-AN have shown that AN covalently binds to the sulfhydryl groups of pro-

tein and to tissue macromolecules and nucleic acids (Ahmed et al., 1982). This explains the reduction in GSH content of tissues. However, in this acute study, the reduction of GSH content was tissue-specific; namely, AN treatment reduced brain GSH to a lower extent versus liver, corroborating several earlier studies (Szabo et al., 1977; Cote et al., 1984). Benz et al. (1997) showed that AN can differentially bind to proteins from different tissues. Accordingly, the tissue specificity of AN toxicity may reflect variations in AN's binding to proteins in liver and brain. Alternatively, the lessened liver sensitivity upon AN exposure may reflect the higher binding to proteins rich in –SH group in this tissue versus the brain or differential distribution of AN in these tissues (Benz et al., 1997; Nerland et al., 2001, 2003).

The present findings show that curcumin pretreatment attenuated AN-induced lipid peroxidation in the rat brain and liver. Specifically, curcumin prevented AN-induced increases in MDA levels, and concomitantly restored GSH content and CAT activity in both tissues, albeit to a different degrees. These effects may reflect the ability of curcumin to enhance the scavenging and inactivation of H_2O_2 and hydroxyl radicals. In addition, curcumin may serve as a chelator and directly bind to Fe^{2+}, which catalyzes formation of free radicals via the Fenton reactions (Jiao et al., 2006, 2009). Curcumin may also terminate lipid peroxidation by induction of enzymatic and non-enzymatic antioxidants, such as GSH, SOD and CAT (Miquel et al., 2002). Accordingly, the protection afforded by curcumin against AN-induced ROS generation is likely attributable to its antioxidant effects. Our findings are in agreement with those by El-Demerdash et al. (2009), demonstrating curcumin's efficacy in restoring GSH content in the liver and kidney of arsenite treated rats. Moreover, our results are consistent with those of Kaur et al. (2006), who found that curcumin attenuated lipopolysaccharide-induced hepatotoxicity in rats by reducing NO and oxygen free radical levels.

The protective effects of curcumin may also involve the promotion of mitochondrial respiratory function (Raza et al., 2008). Our data demonstrate that curcumin to a certain degree was effective in reversing the decline in cytochrome c oxidase activity both in rat brain and liver produced by single acute AN exposure. Cytochrome c oxidase is sensitive to cyanide ions (Ostermeier et al., 1996; Brzezinski and Gennis, 2008), thus it was reasonable to accept that changes in cytochrome c oxidase activity would be reflected by increased cyanide levels. However, levels of cyanide were indistinguishable between AN-treated rats in the presence or absence of curcumin pretreatment. Since cyanide ions are released when oxidative metabolism is mediated by CYP2E1, we also determined the activity of CYP2E1 in rat liver. Consistent with the lack of changes in cyanide ions in the treatment groups, we also failed to show significant changes in CYP2E1 in rats treated with AN in the presence or absence of curcumin pretreatment. Notably, Cyp2E1 can be regulated both by translational and post-translational means, the former via enhancement of *cyp2e1* mRNA transcription and the latter via stabilization of CYP2E1 protein and reduced mRNA and/or protein degradation (Lieber, 1997). However, the most important mechanism is its catalyzing capacity, which directly determines oxidative metabolisms of AN. Thus, it can be concluded that CYP2E1 does not mediate the protective effect of curcumin against AN-induced toxicity, despite earlier reports on the modifying effects of curcumin on CYP2E1 activity (Oetari et al., 1996; Masubuchi et al., 2008; Valentine et al., 2006; Sugiyama et al., 2006). Our findings corroborate the limited role of cyanide in acute AN toxicity (Benz and Nerland, 2005; Campian and Benz, 2008), reflecting that the ability of cyanide antidotes to only minimally shift the LD50 of AN (~1.3-fold in rats) (Gut et al., 1981) as well as the fact that even when blood cyanide levels declined to near zero, AN-induced death cannot be prevented (Hashimoto and Kanai, 1965).

In summary, the present biochemical data confirm that curcumin may protect against acute AN-induced oxidative stress via its antioxidant and free radical-scavenging properties. Oxidative stress is a key mechanism in the toxicity of AN. A number of compounds, such as taurine, vitamin E and N-acetyl-cysteine have been used to prevent AN-induced injury *in vitro* and *in vivo* (Kamendulis et al., 1999a,b; Mahalakshmi et al., 2003; El-Sayed et al., 2008). In the present study, we assessed whether the oxidative effects caused by acute administration to AN could be attenuated by pretreatment with curcumin. Our findings provide biochemical evidence for the efficacy of curcumin in attenuating the adverse effects of AN, both in the brain and liver of rats. The amount of curcumin that would be required for physiologic effects is well below the maximum tolerable pharmacological level (equivalent to 12 g/day for humans) (Shoba et al., 1998; Cheng et al., 2001; Lao et al., 2006). Moreover, curcumin is a natural polyphenolic compound that is already used clinically and is approved by the Food and Drug Administration as a safe food additive. Future studies should examine the hypothesis that curcumin administration is a safe and effective approach to preventing broad chemical toxicity which is mediated via oxidative stress. As a first step, it would seem profitable to conduct additional studies to address whether curcumin supplementation in smokers and individuals prone to occupational exposure to AN can effectively block oxidative stress and adverse health effects.

Conflict of interest

None declared.

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