the addition of fullerenol at a concentration of 0.1 mM. After 45 min, aliquots of cell suspensions were incubated with rhodamine 123 (1 μM) at 37 °C for 15 min. Following this incubation, hepatocytes pellets were obtained by the centrifugation at 100×*g* for 5 min and were washed with Krebs–Henseleit buffer and then centrifuged again. The pellets obtained were resuspended in the same buffer containing 0.1 % Triton X-100. After 10 min, samples were centrifuged 1,600×*g* for 5 min to remove any cellular debris. The concentration of rhodamine 123 in the supernatant medium was measured in a CytoFluor 4000 multiwell fluorescence plate reader (PerSeptive Biosystems Inc., Framingham, MA, USA): The extinction and emission filters were set at 485 nm (bandwidth ±10 nm) and 530 nm (bandwidth ±12.5 nm), respectively. The results are expressed as percentages of the fluorescence values for the untreated hepatocytes.

Detection of reactive oxygen species produced by fullerenol

A fluorescence-based microplate assay as described by Wang and Joseph (1999) was used for the evaluation of ROS in hepatocytes treated with $C_{60}(OH)_{24}$. The formation of intracellular ROS was measured by DCHF-DA. Viable cells deacetylate DCHF-DA to DCHF, which is not fluorescent, but reacts quantitatively with ROS (H_2O_2 and O_2^-) in cells to form fluorescent dye. Hepatocytes (1×10^6 cells/ml) were preincubated with NAC dissolved in Krebs–Henseleit buffer (final concentrations 1, 2.5, and 5 mM) for 20 min and with DCHF-DA dissolved in ethanol (final concentration 5 μM) for 15 min prior to the addition of $C_{60}(OH)_{24}$ at a concentration of 0.1 mM, respectively. After 1-h incubation, the hepatocyte suspensions were centrifuged at 100×*g* for 5 min, and the fluorescence intensity in supernatant fraction (100 μl) obtained was measured in a CytoFluor 4000 multiwell fluorescence plate reader (PerSeptive Biosystems Inc., Framingham, MA, USA): The extinction and emission filters were set at 485 nm (bandwidth ±10 nm) and 530 nm (bandwidth ±12.5 nm), respectively. The results are expressed as percentages of the fluorescence values for the untreated hepatocytes.

Isolation of mitochondria from isolated rat hepatocytes

After the desired time of incubation with fullerenol and/or NAC, hepatocytes were collected by centrifugation at 100×*g* for 5 min and homogenized with isolation medium (pH 7.4) containing 70 mM sucrose, 220 mM mannitol, 2.5 mM HEPES, 2 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride in a Potter–Elvehjem glass homogenizer with a loose-fitting (clearance about 0.8 mm) Teflon pestle. (Cain and Skilleter 1987). After ten up-and-down strokes, the homogenate obtained was centrifuged at 2,000×*g* for 10 min, and then, the resulting supernatant was centrifuged at 10,000×*g* for 10 min to collect the mitochondrial pellet. The pellet was resuspended in the same medium and was centrifuged to obtain a more purified mitochondrial fraction in same condition again, and the mitochondrial pellet was finally suspended in the medium before measurement of GSH.

Statistical analysis

Statistical analysis was performed with a one-way analysis of variance, followed by Dunnett's or Duncan's multiple comparison test. Statistical significance was assumed at $p < 0.05$.

Results

Effects of NAC on $C_{60}(OH)_{24}$-induced toxicity in rat hepatocytes

To investigate the effects of NAC on $C_{60}(OH)_{24}$-induced cytotoxicity, rat hepatocytes were preincubated with NAC (1, 2.5, and 5 mM) for 20 min prior to the addition of the fullerenol at a concentration of 0.1 mM, a moderately toxic concentration (Nakagawa et al. 2011). As shown in Fig. 1, the exposure of isolated rat hepatocytes to $C_{60}(OH)_{24}$ caused time (0–3 h)-dependent cell death, as assayed by trypan blue dye exclusion, this was accompanied by a rapid depletion of the intracellular ATP level and formation of cell surface blebs. The determination of cellular ATP content rather than the trypan blue dye exclusion assay is a relatively sensitive assessment of cell viability (Stone et al. 2009). Despite this, $C_{60}(OH)_{24}$-induced cell death and ATP loss were effectively prevented by treatment with NAC ranging in concentration from 1 to 5 mM during the 3-h incubation period. The onset of the formation of plasma membrane blebs caused by the fullerenol was delayed by NAC at a concentration of 1 mM, whereas NAC at concentrations of greater than 2.5 mM prevented bleb formation.

Effects of NAC on $C_{60}(OH)_{24}$-induced intracellular levels of GSH, GSSG, reduced protein thiols, and MDA in rat hepatocytes

After 1 h of incubation with the fullerenol at a concentration of 0.1 mM, the intracellular GSH level was depleted rapidly, and the depletion of GSH was subsequently followed by an increase in glutathione disulfide (GSSG, glutathione-oxidized form) and a loss of reduced protein thiols with a significant increase in the cellular level of MDA, an index of lipid peroxidation, during the incubation period (Fig. 2).

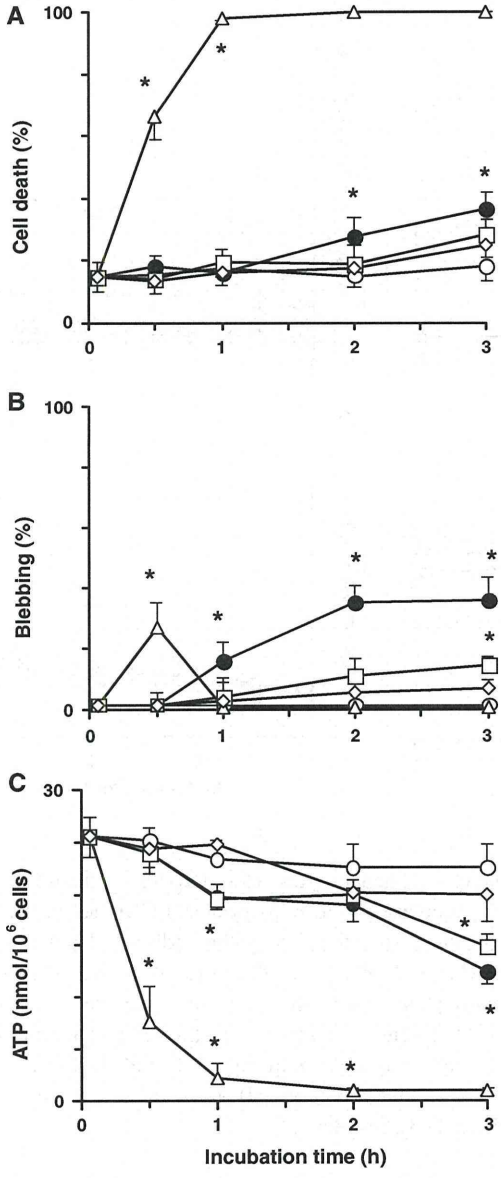

Fig. 1 Effects of NAC on cell death (**a**), blebbing (**b**), and intracellular level of ATP (**c**) in rat hepatocytes treated with $C_{60}(OH)_{24}$. Hepatocytes (10^6 cells/ml) were pretreated with different concentrations of NAC for 20 min prior to the addition of $C_{60}(OH)_{24}$ at a concentration of 0.1 mM, as described in "Materials and methods." No addition (*open circles*); $C_{60}(OH)_{24}$ alone (*open triangles*); NAC 1 mM plus $C_{60}(OH)_{24}$ 0.1 mM (*solid circles*); NAC 2.5 mM plus $C_{60}(OH)_{24}$ 0.1 mM (*open squares*); NAC 5 mM plus $C_{60}(OH)_{24}$ 0.1 mM (*open lozenges*). The results are expressed as mean ± SE of three separate experiments. *Significantly different from values for untreated (control) hepatocytes ($p < 0.05$)

On the other hand, NAC pretreatment prevented not only the decrease in the intracellular GSH and protein thiol levels, but also the increase in GSSG and MDA levels caused by $C_{60}(OH)_{24}$. NAC at concentrations of 2.5 and 5 mM in $C_{60}(OH)_{24}$-treated hepatocytes resulted in either high levels of GSH or simultaneously low levels of GSSG compared with those in untreated hepatocytes during the incubation period. After pretreatment of hepatocytes with NAC alone at concentrations of 1–5 mM, cellular GSH levels were slightly more than twofold those of untreated hepatocytes after 1-h incubation, whereas protein thiol levels were not affected by the addition of NAC (data not shown).

Changes in levels of NAC and its deacetylated intermediate, cysteine, in hepatocyte suspensions

To understand the metabolism of NAC in rat hepatocytes treated with $C_{60}(OH)_{24}$, time courses of changes in the levels of NAC and its deacetylated metabolite, free L-cysteine, were investigated (Fig. 3). The amounts of NAC and L-cysteine were quantified as described above, respectively. Although NAC itself was stable in Krebs–Henseleit buffer without hepatocytes during 3-h incubation (data not shown), initial levels (1, 2.5, and 5 mM) of NAC added to hepatocyte suspensions with $C_{60}(OH)_{24}$ decreased with time, accompanied by an increase in cellular levels of cysteine depending upon the initial concentration of NAC. Since the increase in intracellular GSH levels after the addition of NAC to hepatocyte suspensions was associated with an increase in free L-cysteine, this indicated that NAC was effectively deacetylated by isolated rat hepatocytes and is frequently employed as a source sulfhydryl group in hepatocytes as a precursor of GSH (Fig. 2). The rates of NAC loss and/or cysteine formation in hepatocytes were not affected by the presence/absence of the fullerenol during incubation (data not shown), indicating that the decrease in NAC level in hepatocyte suspensions was dependent on cellular hydrolytic (acylase) activity.

Effects of NAC on $C_{60}(OH)_{24}$-induced loss of mitochondrial membrane potential and generation of ROS in rat hepatocytes

The reduction in the cellular ATP levels caused by $C_{60}(OH)_{24}$ at a concentration of 0.1 mM was related to a decrease in the mitochondrial membrane potential (MMP; $\Delta\Psi$) to approximately 20 % of the control value after 45-min incubation (Fig. 4). On the other hand, MMP of hepatocytes preincubated with NAC at concentrations of 1 and 2.5 mM showed apparent protective effects against the loss of membrane potential caused by the fullerenol under the same conditions.

2′,7′-Dichlorodihydrofluorescein diacetate is widely used to measure ROS generation in various cells (Shen et al. 1996). Viable cells deacetylate DCHF-DA to 2′,7′-Dichlorodihydrofluorescein, which is not fluorescent,

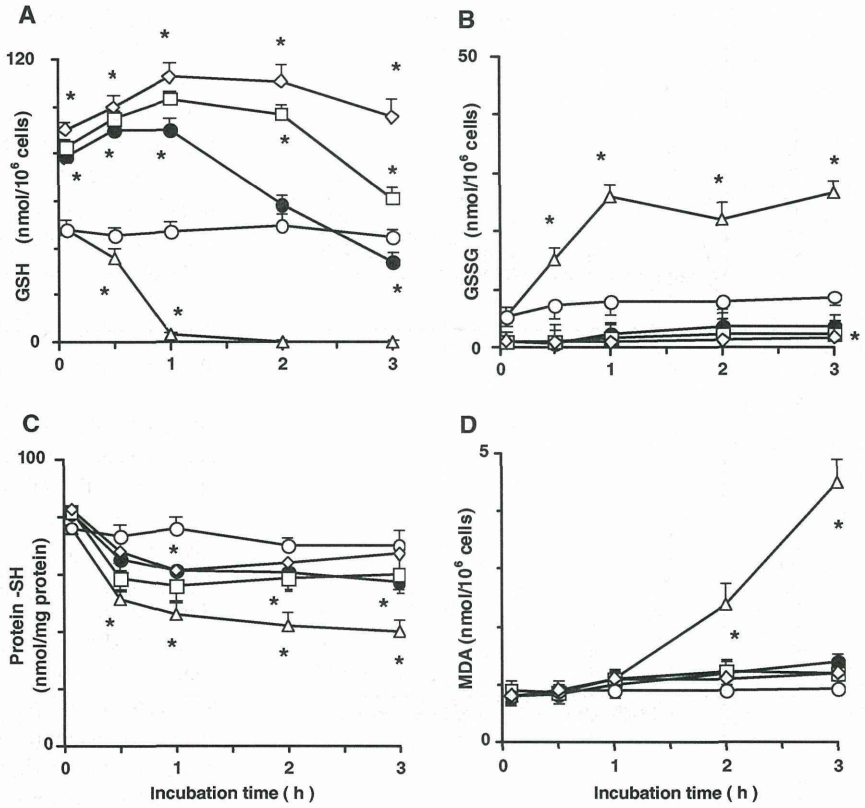

Fig. 2 Effects of NAC on intracellular levels of GSH (**a**), GSSG (**b**), protein thiols (**c**), and MDA (**d**) in isolated rat hepatocytes treated with $C_{60}(OH)_{24}$. Hepatocytes (10^6 cells/ml) were pretreated with different concentrations of NAC for 20 min prior to the addition of $C_{60}(OH)_{24}$ at a concentration of 0.1 mM, as described in "Materials and methods." No addition (*open circles*); $C_{60}(OH)_{24}$ alone (*open triangles*); NAC 1 mM plus $C_{60}(OH)_{24}$ 0.1 mM (*solid circles*); NAC 2.5 mM plus $C_{60}(OH)_{24}$ (*open squares*); NAC 5 mM plus $C_{60}(OH)_{24}$ (*open lozenges*). The results are expressed as mean ± SE of three separate experiments. *Significantly different from values for untreated (control) hepatocytes ($p < 0.05$)

but reacts quantitatively with ROS (H_2O_2 and O_2^-) within cells to form fluorescent dye, which remains trapped within the cells and can be measured to provide an index of intracellular oxidation. The levels of ROS following 1-h incubation with $C_{60}(OH)_{24}$ are shown in Fig. 5. The fluorescence intensity increased in hepatocytes incubated with $C_{60}(OH)_{24}$ at a concentration of 0.1 mM, but preincubation with NAC significantly prevented the increase in ROS levels.

Effects of NAC on $C_{60}(OH)_{24}$-induced mitochondrial GSH loss in rat hepatocytes

Mitochondrial GSH in hepatocytes is found mainly in the reduced form and represents a minor fraction of the total intracellular GSH pool (10–15 %) (Marí et al. 2009). Because previous studies indicated that mitochondria are target organelles for fullerenols (Nakagawa et al. 2011; Ueng et al. 1997), the effects of NAC on mitochondrial GSH levels in rat hepatocytes treated with $C_{60}(OH)_{24}$ at a concentration of 0.1 mM for 1 h were investigated (Fig. 6). In this study, mitochondrial pellets obtained by the first centrifugation from rat hepatocyte suspensions were centrifuged under the same conditions to obtain more purified mitochondria prior to the measurement of GSH. Although incubation of hepatocytes with $C_{60}(OH)_{24}$ resulted in a marked decrease in the mitochondrial GSH level, that loss was significantly prevented by the addition of NAC at concentrations of 1 and 2.5 mM. On the other hand, exposure of hepatocytes to NAC alone caused a concentration (1 and 2.5 mM)-dependent increase in the mitochondrial GSH level, which was approximately 1.8- and 2.5-fold greater than in the untreated (control) group (8.8 ± 1.1 nmol/mg protein; $n = 3$), respectively.

Effects of diethyl maleate (DEM) on rat hepatocytes treated with $C_{60}(OH)_{24}$

To investigate the effects of GSH depletion on $C_{60}(OH)_{24}$-induced cytotoxicity, rat hepatocytes were preincubated with DEM (1.25 mM), a GSH-depleting agent, for 15 min prior to the addition of the fullerenol at a concentration of 50 μM, a mildly toxic concentration. As shown in Fig. 7, severe depletion of cellular GSH levels occurred after preincubation with DEM led to the enhancement of cytotoxicity caused by the fullerenol, accompanied by a rapid loss of ATP levels and cell death. In addition, DEM treatment stimulated the formation of cell surface blebs caused by the fullerenol (data not shown). Although DEM at 1.25 mM elicited an abrupt depletion of the cellular GSH level, DEM

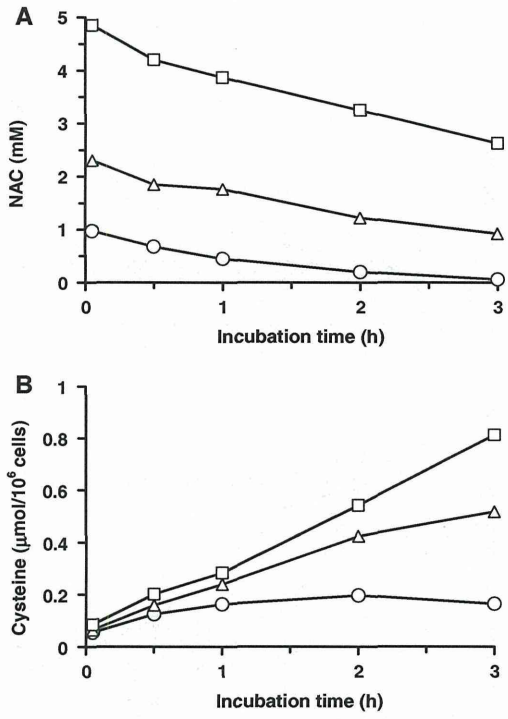

Fig. 3 Changes in the levels of NAC (**a**) and its deacetylated intermediate, L-cysteine (**b**), in rat hepatocyte suspensions. Hepatocytes (10^6 cells/ml) were preincubated with different concentrations of NAC for 20 min prior to the addition of $C_{60}(OH)_{24}$ at a concentration of 0.1 mM. The amounts of NAC and L-cysteine were determined as described in "Materials and methods." Initial concentrations of NAC added to hepatocyte suspensions were 1 mM (*open circles*), 2.5 mM (*open triangles*), and 5 mM (*open squares*). The increase in cysteine levels (**b**) depended upon the initial concentrations of NAC. The results are averages of two experiments

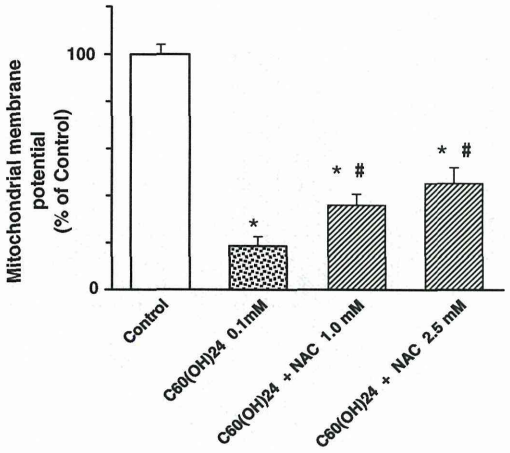

Fig. 4 Effects of NAC on the MMP in rat hepatocytes treated with $C_{60}(OH)_{24}$. Hepatocytes (10^6 cells/ml) were preincubated with different concentrations of NAC for 20 min prior to the incubation of $C_{60}(OH)_{24}$ 0.1 mM for 45 min and subsequently incubated with rhodamine 123 (1 μM) for 15 min as described in "Materials and methods." The results are expressed as mean ± SE of three determinations. *Significant difference from values for untreated (control) hepatocytes ($p < 0.05$). #Significant difference between $C_{60}(OH)_{24}$- and NAC plus $C_{60}(OH)_{24}$-treated hepatocytes ($p < 0.05$)

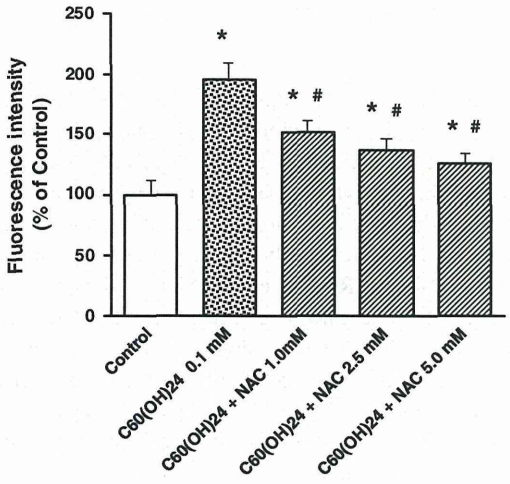

Fig. 5 Effects of NAC on ROS generation by $C_{60}(OH)_{24}$ in isolated rat hepatocytes. Hepatocytes (10^6 cells/ml) were preincubated with NAC (final concentrations 1, 2.5 and 5 mM) for 20 min and with DCHF-DA (5 μM) for 15 min and subsequently incubated with $C_{60}(OH)_{24}$ at a concentration of 0.1 mM for 1 h as described in "Materials and methods." The results are expressed as mean ± SE of three determinations. *Significant difference from values for untreated (control) hepatocytes ($p < 0.05$). #Significant difference between $C_{60}(OH)_{24}$- and NAC plus $C_{60}(OH)_{24}$-treated hepatocytes ($p < 0.05$)

itself caused neither cell death nor a decrease in cellular ATP level during the incubation period. Further, treatment with DEM did not induce lipid peroxidation (data not shown). This result indicates that the initial GSH level is an essential factor in the modulation of cytotoxicity observed in hepatocytes exposed to $C_{60}(OH)_{24}$.

Discussion

Effects of NAC on target sites of $C_{60}(OH)_{24}$-induced cytotoxicity

The results of the present study showed that in isolated rat hepatocytes, $C_{60}(OH)_{24}$ at a concentration of 0.1 mM elicited time-dependent acute cell death, which was accompanied by the formation of cell blebs and decreased in intracellular ATP levels and MMP (Figs. 1, 4). In our previous study, fullerenols such as $C_{60}(OH)_{12}$ and $C_{60}(OH)_{24}$ also elicited mitochondrial dysfunction related to oxidative phosphorylation and the MPT (Nakagawa et al. 2011). These results support the concept that mitochondria as an

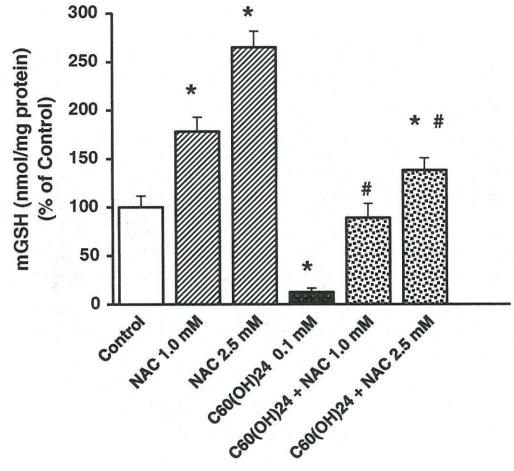

Fig. 6 Effects of NAC on mitochondrial GSH (mGSH) levels in rat hepatocytes treated with $C_{60}(OH)_{24}$. Hepatocytes (10^6 cells/ml) were preincubated with different concentrations of NAC for 20 min prior to incubation with $C_{60}(OH)_{24}$ 0.1 mM for 1 h, and subsequently mitochondria were collected by centrifugation followed by homogenization of hepatocytes. The amounts of mitochondrial GSH were determined as described in "Materials and methods." Values are expressed as mean ± SE of three determinations ($p < 0.05$). *Significant difference from values for untreated (control) hepatocytes ($p < 0.05$). #Significant difference between $C_{60}(OH)_{24}$- and NAC plus $C_{60}(OH)_{24}$-treated hepatocytes ($p < 0.05$)

ATP-generating site are important targets organelles for the fullerenol, resulting in energy stress caused by the rapid depletion of the cellular ATP pool. $C_{60}(OH)_{24}$-induced cell death was also initiated, at least in part, by oxidative stress, indicating that the depletion of cellular GSH caused by $C_{60}(OH)_{24}$ was concurrently associated with an increase in GSSG, MDA, and ROS levels, and a decrease in the levels of reduced protein thiol and mitochondrial GSH (Figs. 2, 5, 6). Despite these, the addition of NAC to rat hepatocyte suspensions effectively inhibited the cytotoxic and/or mitochondrial damage caused by $C_{60}(OH)_{24}$, suggesting the possibility that fullerenol-induced hepatotoxicity is ameliorated by the addition of NAC.

Previous in vitro studies about the subcellular distribution of nanomaterials showed that: (1) the water-soluble fullerene derivative $C_{61}(COOH)_2$ added to monkey kidney COS-7 cells was able to accumulate within the mitochondria through the cell membrane and preferentially affected mitochondrial function such as its electron transport chain (Foley et al. 2002); and (2) when nanoparticles consisting of organic carbon and polycyclic aromatic hydrocarbons were incorporated by macrophages and/or epithelial cells, they localized in the mitochondria and induced structural damage through oxidative stress (Li et al. 2003). Mitochondrial structural damage caused by fullerenols is supported by our previous results showing that fullerenols, especially

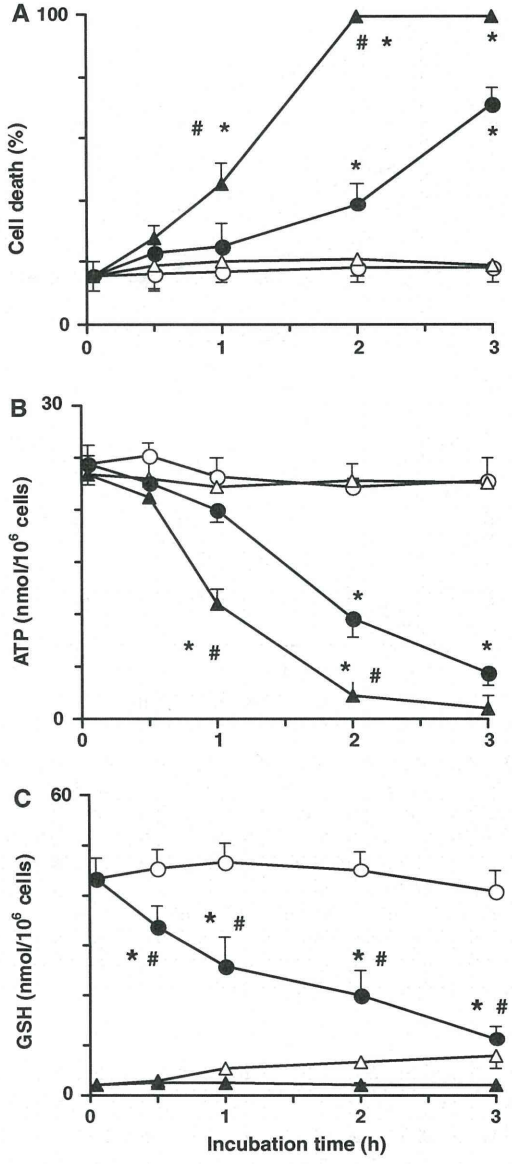

Fig. 7 Effects of DEM on cell death (**a**) and intracellular levels of ATP (**b**) and GSH (**c**) in isolated rat hepatocytes treated with $C_{60}(OH)_{24}$. Hepatocytes (10^6 cells/ml) were preincubated with DEM 1.25 mM for 15 min prior to the addition of $C_{60}(OH)_{24}$ at a concentration of 50 μM, as described in "Materials and methods." No addition (*open circles*); DEM alone (*open triangles*); $C_{60}(OH)_{24}$ alone (*solid circles*); DEM plus $C_{60}(OH)_{24}$ (*solid triangles*). The results are expressed as mean ± SE of three separate experiments. *Significantly different from values for untreated (control) hepatocytes ($p < 0.05$). #Significant difference between $C_{60}(OH)_{24}$- and DEM plus $C_{60}(OH)_{24}$-treated hepatocytes ($p < 0.05$)

$C_{60}(OH)_{24}$, resulted in the induction of organelle swelling, as assayed by the mitochondrial MPT which was monitored by the absorbance changes at 540 nm (Nakagawa et al. 2011). It is well known that the homeostasis of MMP

is disturbed by chemicals and/or xenobiotics (Lemasters et al. 1993). In addition, the depletion of intracellular ATP caused by xenobiotics is known to be a critical factor in the development of plasma membrane blebbing prior to cell death (Nicotera et al. 1992), and the maintenance of cellular ATP levels is necessary for the polymerization of microfilaments and microtubules, which is related to the interaction between the cytoskeletal organization and cellular morphology (Bellomo et al. 1990). In this regard, Johnson-Lyles et al. (2010) observed that in LLC-PK1 cells, the fullerenol $C_{60}(OH)_n$ caused cytotoxicity through depletion of the intracellular ATP levels accompanied by cytoskeletal disruption. After the exposure of rat hepatocytes to $C_{60}(OH)_{24}$, the onset of bleb formation associated with the depletion of cellular ATP levels was effectively prevented by pretreatment with NAC (Fig. 1). In this connection, Zaragoza et al. (2001) observed that cocaine-induced cytotoxicity via mitochondrial damage was prevented by NAC pretreatment in rat hepatocytes. Based on the results of previous and the present studies, it seems that $C_{60}(OH)_{24}$ penetrates the hepatocyte membrane and accumulates in mitochondria to elicit organelle dysfunction, which could be effectively attenuated by pretreatment with NAC.

Metabolism of NAC and its effects on $C_{60}(OH)_{24}$-induced cytotoxicity

Freshly isolated rat hepatocytes retain intact membranes and have high levels of various drug-metabolizing enzymes and their cofactors associated with phase I and phase II reactions, and multiple defense systems against intracellular oxidative stress produced by various chemicals (Moldéus et al. 1978). As shown in Fig. 3, the amounts of NAC added to hepatocyte suspensions decreased rapidly with time, accompanied by an increase in cysteine levels, indicating that NAC incorporated into hepatocytes was enzymatically *N*-deacetylated by cytosolic aminoacylase and was frequently employed as a source sulfhydryl group in hepatocytes as a precursor of GSH (Fig. 2), which is well known as a major antioxidant and free radical scavenger that protects cells from ROS in isolated rat hepatocytes and other cell lines treated with drugs and xenobiotics (Reed 1990; Nakagawa et al. 2005; Marí et al. 2009). When NAC led to the enhancement of cellular and mitochondrial levels of GSH (Figs. 2, 6), $C_{60}(OH)_{24}$-induced ROS generation and MMP loss were apparently prevented (Figs. 4, 5). Despite this, a certain amount of NAC incorporated into hepatocytes may also reduce free radical species, because Zhang et al. (2011) reported that in renal proximal tubule epithelial cells exposed to a hydroquinone derivative, NAC may exert cytoprotective effects in part by directly scavenging ROS. It was observed that the ability of cysteine to react with ROS was less than that of NAC in a phosphate buffer without cells (Aruoma et al. 1989), while free cysteine produced in rat hepatocytes as shown in Fig. 3 cannot be disregarded as an effective antioxidant and/or a protector of thiols (Takashima et al. 2012). Pretreatment of rat hepatocyte suspensions with L-cysteine is known to prevent the decrease in cellular levels of GSH and protein thiols caused by hydroxylated biphenyls (Nakagawa et al. 1992). The accumulation of proteins with irreversible thiol oxidation, which is a hallmark of oxidative stress-induced cellular damage (Kettenhofen and Wood 2010), caused by $C_{60}(OH)_{24}$ was also prevented by the addition of NAC (Fig. 2). Since the mechanism of fullerenol-induced oxidative stress within hepatocytes may be attributed to a sulfhydryl redox-state imbalance, further studies are necessary to determine the multiple defense systems of NAC.

Effects of NAC on mitochondrial toxicity induced by $C_{60}(OH)_{24}$

Because mitochondria in hepatocytes, as in other organ cells, are the main site of energy production, their dysfunction directly/indirectly affects cellular ATP synthesis and cell viability, and thus, the mechanisms of cytotoxicity caused by a wide range of chemicals and a number of pathologic conditions (Kehrer et al. 1990; Wallace et al. 1997). The synthesis of GSH from its constituent amino acids, cysteine, glutamate, and glycine involves two ATP-requiring enzymatic systems and thus depends on the continuous supplementation of ATP. The distribution of reduced GSH in the cytosolic and mitochondrial fractions is approximately 85–90 and 10–15 %, respectively (Marí et al. 2009). It is well known that GSH can easily cross the mitochondrial outer membrane via porin channels and the mitochondrial inner membrane (Smith et al. 1996). Thus, the mitochondrial GSH level depends on (1) cytosol mitochondrial level and (2) specific transporters on the mitochondrial membrane. In this study, the loss of the MMP caused by $C_{60}(OH)_{24}$ was partially recovered after NAC addition in a concentration-dependent manner, accompanied by the restoration of depleted mitochondrial GSH levels (Figs. 4, 6). Because NAC may exert cytoprotective effects by increasing the mitochondrial GSH level, alterations in the GSH pool may also participate in the mitochondrial bioenergetics of fullerenol-treated hepatocytes. On the other hand, pretreatment with DEM (1.25 mM), which caused an abrupt depletion of cytosolic and mitochondrial GSH levels and did not elicit cytotoxicity (Ku and Billings 1986), enhanced $C_{60}(OH)_{24}$-induced cytotoxicity, suggesting that intracellular factors, especially the mitochondrial reduced GSH pool, play an important role in the onset of cytotoxicity (Fig. 7). Several studies concluded that exposure to most chemicals exposures is associated with the depletion of mitochondrial GSH rather than cytosolic GSH

to regulate the apoptotic pathway (Mithöfer et al. 1992; Lluis et al. 2005; Fernandez-Checa and Kaplowitz 2005). In addition, mitochondrial GSH is also a critical factor regulating the redox status of protein thiols that regulate the mitochondrial MPT (Chernyak and Bernardi 1996; Armstrong and Jones 2002), the disturbance of which is induced by hydroxylated fullerenes. Therefore, the finding that NAC elicits increases in the levels of mitochondrial GSH as well as cytosolic GSH in rat hepatocytes suggests that maintenance of the mitochondrial GSH level could prevent, at least partially, mitochondrial dysfunction such as ATP generation caused by the fullerenol.

Mitochondria are also the major source of the generation of toxic ROS, which is enhanced by the actions of various mitochondrial inhibitors from complexes I to IV (Mehta et al. 2008; Zhang et al. 2001). Although it is uncertain whether the generation of ROS in hepatocytes incubated with $C_{60}(OH)_{24}$ depends on disturbance of the mitochondrial respiration chain, fullerenols are known to be free radical scavengers and/or antioxidants in biological systems (Dugan et al. 1996; Kamat et al. 2000; Saitoh et al. 2010). Further investigations are necessary to clarify the mechanisms of their harmful and/or protective effects and to study nanomaterial-biological interactions.

In conclusion, the results of the current study show that the exposure of hepatocytes to $C_{60}(OH)_{24}$ caused cytotoxicity, accompanied by losses of intracellular ATP, GSH, and protein thiols, and an increase in GSSG and ROS levels. On the other hand, NAC ameliorated (a) mitochondrial dysfunction linked to the depletion of ATP and the MMP and (b) induction of oxidative stress as assessed by ROS generation, losses of cellular and/or mitochondrial GSH and protein thiol levels, and MDA formation. NAC taken up hepatocytes was effectively converted to GSH through deacetylated cysteine. The cytotoxicity caused by the fullerenol was enhanced by the addition of DEM, which continuously depleted cellular GSH. Taken collectively, these results indicate that the onset of the toxic effects of $C_{60}(OH)_{24}$ is, at least in part, affected by intracellular and/or mitochondrial GSH levels as well as mitochondrial dysfunction.

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International Journal of Nanomedicine

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ORIGINAL RESEARCH

Magnetic nanoparticles of Fe_3O_4 enhance docetaxel-induced prostate cancer cell death

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Abstract: Docetaxel (DTX) is one of the most important anticancer drugs; however, the severity of its adverse effects detracts from its practical use in the clinic. Magnetic nanoparticles of Fe_3O_4 (MgNPs-Fe_3O_4) can enhance the delivery and efficacy of anticancer drugs. We investigated the effects of MgNPs-Fe_3O_4 or DTX alone, and in combination with prostate cancer cell growth in vitro, as well as with the mechanism underlying the cytotoxic effects. MgNPs-Fe_3O_4 caused dose-dependent increases in reactive oxygen species levels in DU145, PC-3, and LNCaP cells; 8-hydroxydeoxyguanosine levels were also elevated. MgNPs-Fe_3O_4 alone reduced the viability of LNCaP and PC-3 cells; however, MgNPs-Fe_3O_4 enhanced the cytotoxic effect of a low dose of DTX in all three cell lines. MgNPs-Fe_3O_4 also augmented the percentage of DU145 cells undergoing apoptosis following treatment with low dose DTX. Expression of nuclear transcription factor κB in DU145 was not affected by MgNPs-Fe_3O_4 or DTX alone; however, combined treatment suppressed nuclear transcription factor κB expression. These findings offer the possibility that MgNPs-Fe_3O_4–low dose DTX combination therapy may be effective in treating prostate cancer with limited adverse effects.

Keywords: prostate cancer, magnetic nanoparticles, docetaxel, reactive oxidative species

Introduction

Prostate cancer is the most common cancer affecting men, and the second leading cause of cancer death in the United States.[1] The incidence and mortality rates of prostate cancer vary greatly among different geographic areas and ethnic groups. Although the incidence of prostate cancer in Japan remains low compared with that in the United States, it has been increasing in recent years. However, by 2020, prostate cancer is projected to surpass stomach cancer as the most frequently diagnosed cancer in Japanese men.[2]

Several management options are available when prostate cancer is diagnosed at an early stage, including watchful waiting, surgery, cryosurgery, radiation therapy, and hormonal therapy. For advanced prostate cancers, surgical or medical ablation of androgens is regarded the optimal first-line treatment. In most patients treated by androgen deprivation, disease progression will continue until reaching a stage referred to as castration-resistant prostate cancer (CRPC). Progression to a hormonal refractory state is a complex process, involving both selection and outgrowth of preexisting clones of androgen-independent cells as well as adaptive upregulation of genes that help cancer cells survive and grow after androgen ablation.[3]

Although the effects of several anticancer drugs for prostate cancer have been evaluated in vitro and in animal experiments in vivo, most have little or no impact

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http://dx.doi.org/10.2147/IJN.S40766