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Intracellular glutathione plays important roles in pyrrolizidine alkaloids-induced growth inhibition on hepatocytes

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ABSTRACT

Pyrrolizidine alkaloids (PAs) are well-known natural hepatotoxins distributed widely in thousands of plants in the world. Adonifoline (Adon), senecionine (Sene) and monocrotaline (Mono) are retronecine-type PAs, and the present study is designed to observe the effects of intracellular glutathione on toxicity of these three PAs in human normal liver L-02 cells. The ratio of cellular reduced glutathione (GSH) and oxidized glutathione (GSSG) was assayed after L-02 cells were incubated with these three PAs for various times. Results showed that Adon, Sene and Mono all significantly decreased the ratio of GSH/GSSG in L-02 cells in the time- and concentration-dependent manner. The results of 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) and trypan blue staining assay showed that these three PAs all significantly decreased cell viability in L-02 cells when pretreated with 10 μM BSO (L-Buthionine-S-R-Sulfoximine) for 24 h to deplete intracellular GSH. Further results showed that anti-oxidant compounds such as NAC (N-Acetyl-Cysteine) and GSH could rescue the cytotoxicity caused by these three PAs with BSO pretreatment. Taken together, those results suggest that intracellular GSH plays important roles in regulating the cytotoxicity induced by PAs.

1. Introduction

Pyrrolizidine alkaloids (PAs) are commonly existed natural toxins, and more than half of which are hepatotoxins and carcinogens (Roeder, 2000; Stegelmeier et al., 1999). Moreover there are reports that PAs also lead to pneumotoxicity (Huxtable, 1990; Taylor et al., 1997), neurotoxicity (Huxtable et al., 1996) and embryotoxicity (Huxtable, 1989; Peterka et al., 1994). Plants containing PAs mainly exist in Compositae, Boraginaceae and Leguminosae. As PAs-containing plants so commonly affect livestock, wildlife and humans that the potential human health risk posed by exposure to PAs has been concerned intimately (Fu et al., 2004), and recently British Medicines Healthcare Products Regulatory Agency (MHRA) alerts to pay attention to the safe usage of PAs containing plants. Adon and Sene were isolated from the genus of *Senecio* (Compositae), and Mono was abundant in *Crotalaria sessiliflora* L. PAs sene, ado and mono belong to retronecine-type PAs whose structure is characteristic with 8-membered heterocyclic nicine base. Moreover, there are already reports about the toxicity of Mono and Sene after metabolic activation *in vivo* (Kasahara et al., 1997; Huan et al., 1998).

Glutathione is the most abundant low molecular-weight-thiol tripeptide in mammalian cells and it exists in either a reduced (GSH) or an oxidized (GSSG) form. Generally it is mainly in the reduced form in cells, which can be converted to the oxidized form during oxidative stress. The maintenance of cellular reducing environment is mainly dependent on the relative ratio of GSH and GSSG, which is a critical indicator for evaluating the oxidative stress in the cells (Ghosh and Sil, 2008; Kaviarasan et al., 2006). There are many reports that cellular GSH is directly involved in the detoxification of exogenous toxins such as heavy metals, solvents and pesticides (Sies, 1999). The detoxification of GSH can be due to its direct binding to the toxins or as the cofactor for anti-oxidant enzymes such as GSH-peroxidase and GSH-S transferase, and then accelerates the excretion of toxins in urine or bile (Hayes and Pulford, 1995). Furthermore, many studies have pointed out that intracellular GSH

Abbreviations: GSH, reduced glutathione; GSSG, oxidized glutathione; PAs, pyrrolizidine alkaloids; MTT, 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide; ROS, reactive oxygen species; DTNB, 5,5′-dithio-bis (2-nitrobenzoic acid); TNB, 5-thio-2-nitrobenzoic acid; Adon, adonifoline; Sene, senecionine; Mono, monocrotaline; BSO, L-buthionine-S-R-Sulfoximine; NAC, N-acetyl-L-cysteine.

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depletion was involved in many heptotoxins-induced cytotoxicity such as acetaminophen, cisplatin, etc. (Lu and Cederbaum, 2006; Schmieder et al., 2003).

The present study is designed to observe the cytotoxicity of Adon, Sene and Mono on L-02 cells and the possible involvement of intracellular GSH.

2. Materials and methods

2.1. Chemicals and reagents

The structures of the observed three PAs are shown in Fig. 1. Senecionine was isolated from *Senecio vulgaris*, Adonifoline was from *Senecio scandens* Buch.-Ham. ex D. Don, and Monocrotaline was obtained from *Orotalaria sessilufkira* L. These three PAs was structurally elucidated based on ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectral evidences. The purity of the compounds was more than 98% as determined by high-pressure liquid chromatography (HPLC) analysis. RPMI1640, new born calf serum, penicillin and streptomycin were purchased from Gibco BRL (Gaithersburg, MD, USA). GSH and NADPH were purchased from Roche (Switzerland). 5, 5′-dithio-bis (2-nitrobenzoic acid) (DTNB), Trypan Blue, Glutathione reductase and other reagents unless indicated were from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Cell culture

Human normal liver L-02 cell line was derived from adult human normal liver (Cell Bank, Type Culture Collection of Chinese Academy of Sciences, Shanghai). Cells were cultured in RPMI1640 medium supplemented with 10% [v/v] heat-inactivated new born calf serum, 2 mM glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were incubated at 37 °C in a humidified atmosphere (5% CO_2).

2.3. MTT assay

Cells were seeded in 96-well microplates (10^4 cells/well), while for the long time incubation of PAs, the cell density was adjusted to 3×10^3/well. Cells were incubated with 10, 25, 50 and 100 μM three PAs for 24, 48, 72 or 144 h, respectively. After treatments, cells were incubated with 500 μg/ml MTT for 4 h in the CO_2 incubator. The functional mitochondrial succinate dehydrogenases in survival cells can convert MTT to formazan that generates a blue color (Hansen et al., 1989). At last the formazan was dissolved in 10% SDS–5% iso-butanol–0.01 M HCl. The optical density was measured at 570 nm with 630 nm as a reference and cell viability was normalized as a percentage of control.

2.4. Trypan blue staining assay

Cells were seeded in 6-well microplates and incubated with Adon, Sene or Mono for various times. After treatment, cells were mixed with 0.4% trypan blue-PBS for 10 min, and the dead cells were stained blue by trypan blue. The number of stained and unstained cells was counted using a hemocytometer and the values were expressed as the percentage of total cells including survival and dead cells.

2.5. Measurement of cellular GSH

Cellular GSH and GSSG were determined by DTNB assay according to reported method (Sies and Akerboom, 1984) with a minor modification. Briefly, after treatment cells were harvested in metaphosphoric acid (5%) buffer. The reaction mixture contained 1 mM EDTA, NADPH (0.24 mM), glutathione reductase (0.06 U), DTNB (86 μM) and samples. Yellow 5-thio-2-nitrobenzoic acid (TNB) formation is monitored at 412 nm. GSSG was determined after elimination of GSH with 2-vinylpyridine. The levels of GSH were calculated from the difference between concentrations of total glutathione (GSH + GSSG) and GSSG. The intracellular levels of GSH and GSSG were calculated based on cellular protein concentration.

2.6. Statistical analyses

All values were expressed as means ± standard deviation (SD). Differences between groups were assessed by one-way analysis of variance (ANOVA) using the SPSS software package for windows. Post hoc multiple comparison tests were performed for inter-group comparisons using the least-significant difference (LSD) test. $P \leq 0.05$ was considered as statistically significant difference.

3. Results

3.1. Effects of PAs on GSH/GSSG in L-02 cells

Intracellular GSH functions as the major anti-oxidant and provides a first line of defense against oxidative and chemical injury (Wu et al., 2004). As shown in Fig. 2A, after L-02 cells were treated with Adon, Sene and Mono (100 μM) for 24, 48 and 72 h, the ratio of intracellular GSH/GSSG decreased unambiguously in a time-dependent manner. The reason leading to the decrease in GSH/GSSG in PAs-treated cells is due to the significant decrease in cellular GSH (data not shown). To further confirm the effects of PAs on cellular GSH/GSSG, we treated L-02 cells with these three PAs of various concentrations (10, 25, 50 and 100 μM). As shown in Fig. 2B, these three PAs decreased GSH/GSSG in the concentration-dependent manner. All these data suggest that these three PAs changed intracellular GSH and GSSG redox balance, which may play some roles in regulating their cytotoxicity on hepatocytes.

3.2. BSO augmented the cytotoxicity of the three PAs on L-02 cells

BSO is an irreversible inhibitor of intracellular GSH synthesis (Griffith, 1999). In our study, we found that cellular GSH was depleted after cells were treated with 10 μM BSO for 24 h, while BSO itself had no significant toxicity on L-02 cells (data not shown). Further we pre-incubated L-02 cells with 10 μM BSO for 24 h to deplete cellular GSH, and then added various concentrations of these three PAs for another 72 h to observe PAs-induced inhibition of cell growth. The results of MTT and trypan blue staining assay (Figs. 3 and 4) showed that the inhibition of cell growth in PAs-treated cells was very weak, while with BSO pretreatment significantly augmented the inhibition of cell growth induced by these three PAs. These results further confirmed the important roles of GSH in regulating the cytotoxicity of these three PAs.

3.3. PAs induced-inhibition of cell growth after long time incubation

As these three PAs decreased cellular GSH after 72 h incubation and with BSO depleted cellular GSH obviously augmented the PAs-induced cell growth inhibition, so we observed whether these three PAs inhibit the cell growth with long time incubation. The MTT and trypan blue staining assay (Fig. 5A and B) showed that these three PAs induced significant inhibition of cell growth in the concentration-dependent manner after 144 h incubation, which may be due to the depletion of cellular GSH after long time incubation.

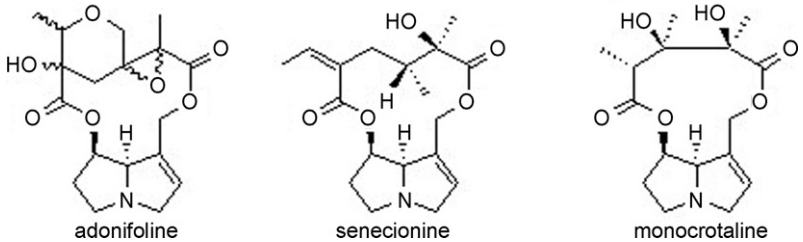

Fig. 1. The chemical structure of PAs. (A) Adonifoline (Adon), (B) Senecionine (Sene), and (C) Monocrotaline (Mono).

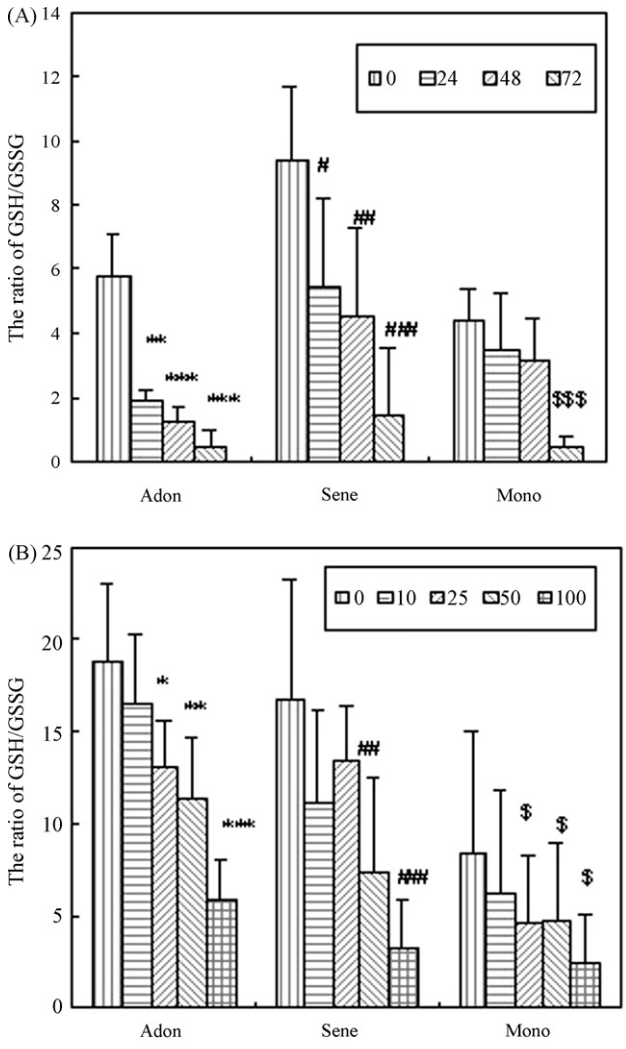

Fig. 2. Effects of Sene, Adon and Mono on the ratio of GSH/GSSG in L-02 cells. (A) Cells were treated with 100 μM Adon, Sene and Mono for various times, and then cellular GSH and GSSG were determined according to the materials and methods. (B) Cells were treated with various concentrations of Adon, Sene and Mono for 72 h, and then cellular GSH and GSSG were determined according to the materials and methods. All values are Mean ± SD ($n = 5$). $^{*}P$, $^{\#}P$, $^{\$}P \leq 0.05$; $^{\#\#}P$, $^{**}P \leq 0.01$; $^{***}P$, $^{\#\#\#}P$, $^{\$\$\$}P \leq 0.001$ compared with absence of PAs.

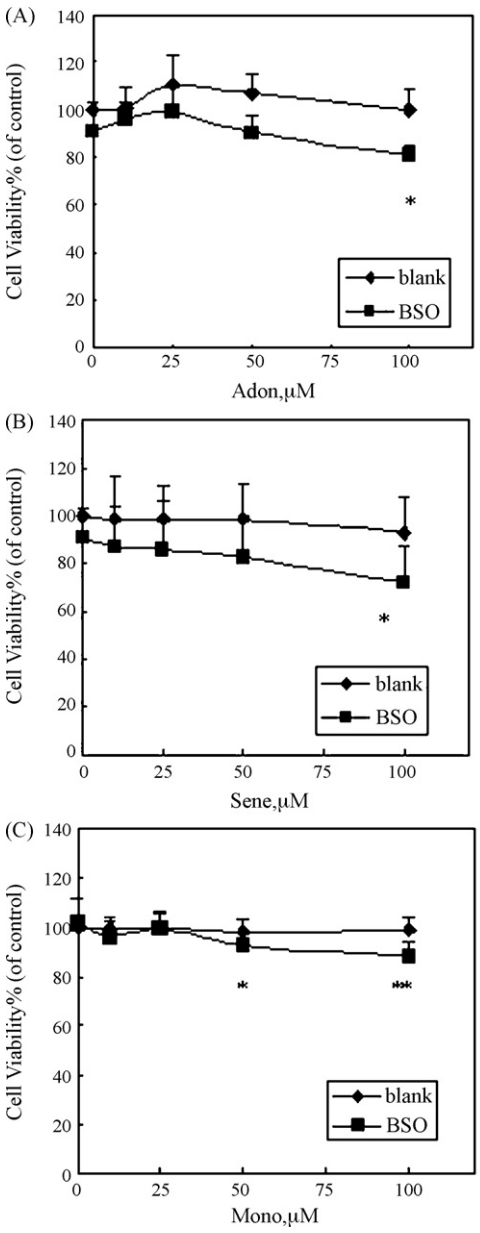

Fig. 3. MTT assay of Adon (A), Sene (B), Mono (C) induced inhibition of cell growth in BSO pretreated L-02 cells. Cells were pretreated with BSO 10 μM for 24 h, and then incubated with different concentrations of Adon, Sene and Mono for 72 h. The survival cells were determined by MTT assay and the results are expressed in percentage of control and presented as the Means ± SD ($n = 6$). $^{*}P \leq 0.05$; $^{**}P \leq 0.01$ compared with absence of PAs.

3.4. Effect of NAC and GSH on BSO-augmented cytotoxicity induced by PAs

NAC is a well-known precursor of cellular GSH synthesis. Further we observed the effects of NAC and exogenous GSH on BSO-augmented cell growth inhibition induced by PA Sene. L-02 cells were pre-incubated with 10 μM BSO for 24 h followed by incubation with 100 μM Sene for 72 h in the present of 5 mM NAC and GSH, respectively. As shown in Fig. 6, the cell viability of MTT and trypan blue assay significantly decreased in 100 μM Sene-treated cells with 10 μM BSO pretreatment, while 5 mM NAC and GSH both reversed this cell growth inhibition induced by PA Sene plus BSO pretreatment.

4. Discussion

GSH is an important intracellular anti-oxidant, which conjugates toxic substances and participates in the detoxification of carcinogens, free radicals and peroxides, ultimately protecting cells and organs against oxidative stress-induced toxicity (Park and Park, 2007; Sakurai et al., 2005; Yadav et al., 2008; Dukhande et al., 2006). The ratio of GSH/GSSG is an important indicator for evaluating cellular oxidative stress, and the decrease in GSH/GSSG predicts the onset of oxidative damage (Macho et al., 1997; Beaver and Waring, 1995). In the present study, 100 μM Adon, Sene and Mono all significantly decreased the ratio of GSH/GSSG in L-02 cells after 72 h incubation (Fig. 2). These results suggest the potential roles of GSH in regulating the toxicity of these three PAs.

BSO is reported to inhibit γ-glutamylcysteine synthetase (γ-GCS), which is the rate-limiting enzyme of cellular GSH synthesis, specifically and irreversible (Griffith, 1982). There are many reports that depletion of cellular GSH with BSO makes cells more sensitive to many toxic chemicals (Hristova et al., 2007; Friesen et al., 2004; Gómez-Quiroz et al., 2008). Our results showed that with 10 μM BSO pre-incubation for 24 h obviously augmented PA Adon,

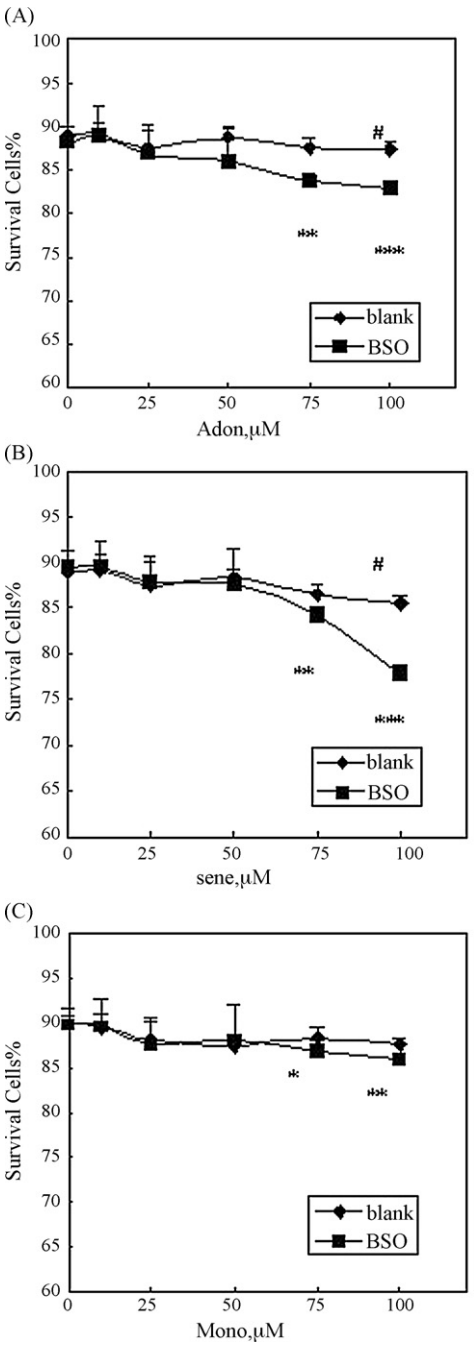

Fig. 4. Trypan blue staining assay of Adon (A), Sene (B), Mono (C) induced inhibition of cell growth in BSO pretreated L-02 cells. Cells were pretreated with BSO 10 μM for 24 h, and then incubated with different concentrations of Adon, Sene and Mono for 72 h. The number of viable cells was determined by trypan blue staining assay, and the results are expressed in percentage of total cells and presented as the Means ± SD ($n = 6$). $^{*}P$, $^{\#}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$ compared with absence of PAs.

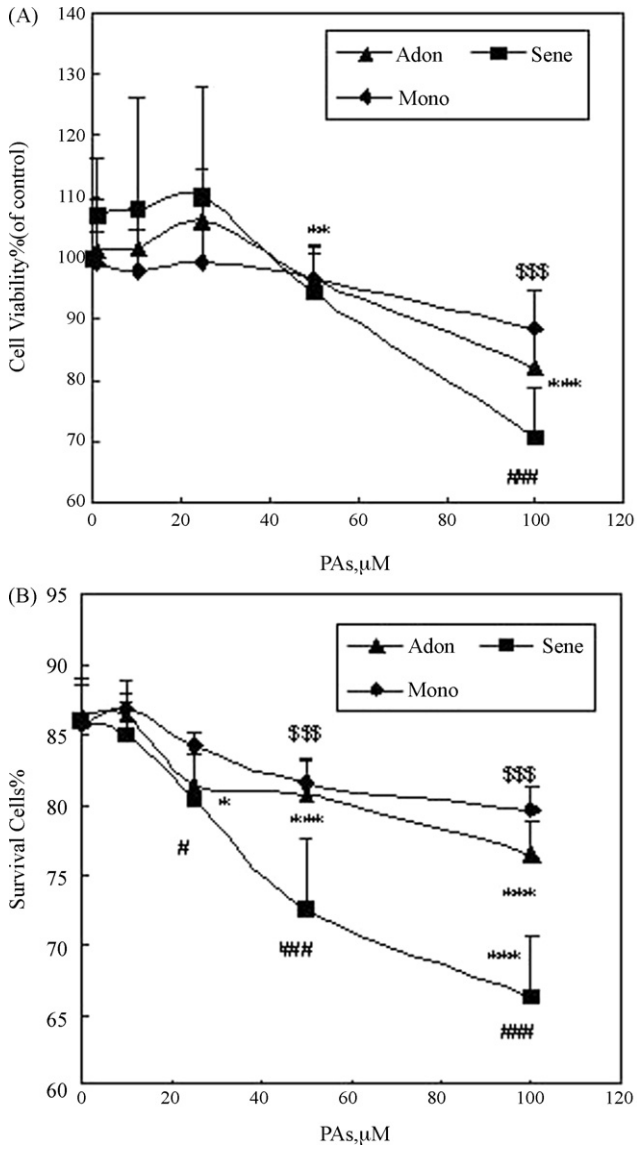

Fig. 5. Adon, Sene and Mono induced inhibition of cell growth in L-02 cells. (A) Cells were incubated with various concentrations of Adon, Sene and Mono for 144 h, and then the survival cells were determined by MTT assay. The results are expressed in percentage of control and presented as the Means ± SD ($n = 6$). (B) Cells were incubated with various concentrations of Adon, Sene and Mono for 144 h, and then the survival cells were determined by trypan blue staining assay. The results are expressed in percentage of total cells and presented as the Means ± SD ($n = 6$). $^{*}P$, $^{\#}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P$, $^{\#\#\#}P$, $^{\$\$\$}P \leq 0.001$ compared with absence of PAs.

Sene and Mono-induced decrease in cell viability in L-02 cells, while these three PAs had no significant toxic effects on L-02 cells without BSO pretreatment (Figs. 3 and 4). All these results further confirmed the important roles of GSH in regulating the toxicity of these three PAs.

As Adon, Sene and Mono all had obvious effects on cellular GSH amounts; in the further study we observed the protection of NAC and exogenous GSH on the toxicity induced by PA Sene. NAC, N-acetyl derivative of the amino acid L-cysteine, is a precursor of cellular GSH synthesis. There are reports about the protection of NAC against hepatotoxicity induced by some exogenous toxins such as acetaminophen, alcohol, etc. (Wu et al., 2008; Terneus et al., 2008; Wang et al., 2006; Liao et al., 2008). Exogenous GSH was also reported to prevent the hepatotoxicity induced by some toxins (Kim et al., 2004; Milchak and Douglas, 2002). Our results showed that 5 mM NAC and 5 mM GSH both significantly reversed PA Sene plus BSO decreased cell viability (Fig. 6), which further demonstrated that the toxicity of these three PAs could be due to the depletion of cellular GSH, and when enhancing cellular GSH amounts via adding NAC or GSH can alleviate the cytotoxicity.

Previous researches have reported that PA Mono caused hepatic venoocclusive disease by causing a significant GSH depletion in sinusoidal endothelial cells (DeLeve et al., 1996). Additionally, rats injected with Mono had a lower hepatic GSH, which indicated that GSH might protect against hepatotoxicity induced by Mono (Yan and Huxtable, 1995, 1996). Furthermore, *in vitro* metabolism study showed GSH could interact with a carcinogenic metabolite of

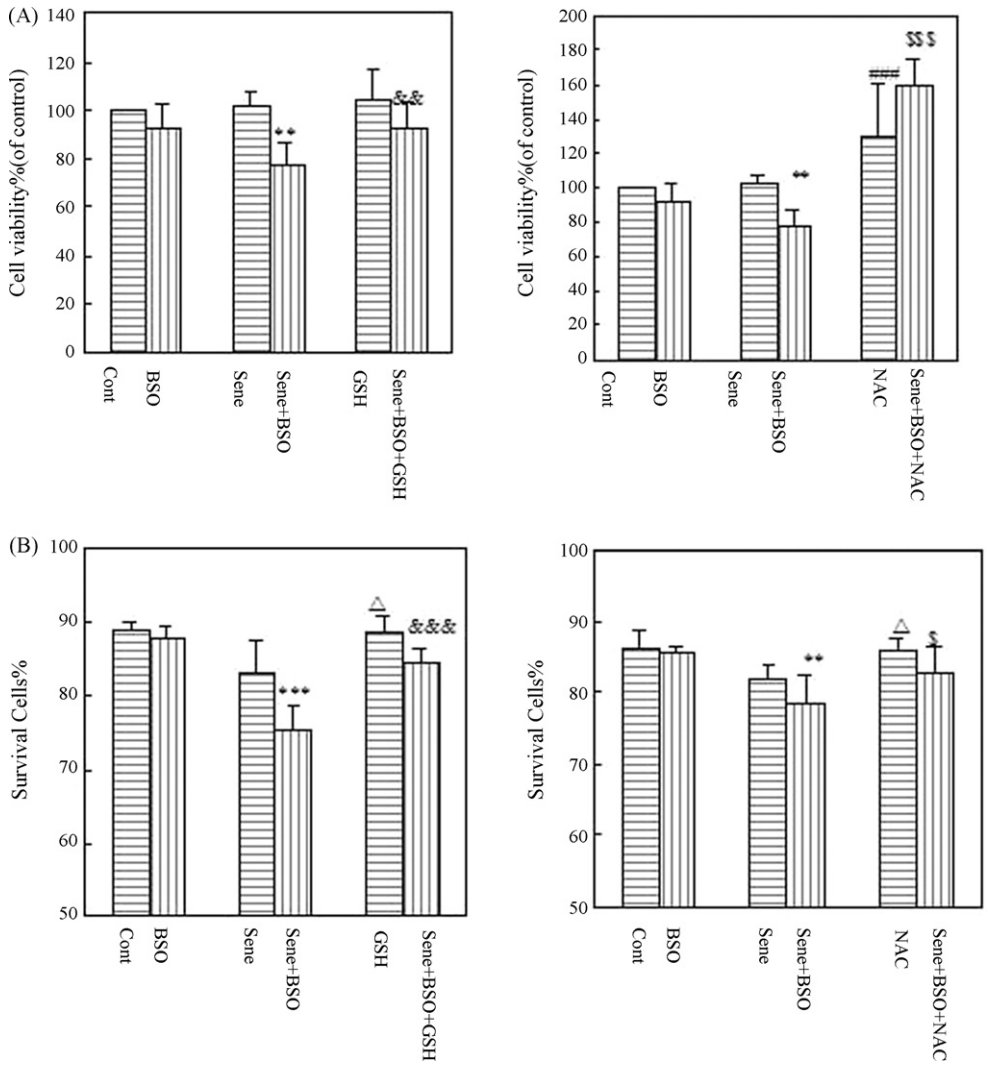

Fig. 6. Effects of GSH and NAC on PA Sene-induced cytotoxicity on L-02 cells pretreated with BSO. After cells were pre-incubated with 10 μM BSO for 24 h, 100 μM Sene and 5 mM GSH were added and incubated with cells for another 48 h, and then 5 mM NAC was added and further incubated cells for another 24 h. After treatment, the survival cells were determined by MTT (A) or trypan blue staining assay (B). The results are expressed in percentage of control (A) or total cells (B), respectively, and presented as the Means ± SD ($n = 6$). $^{\triangle}P$, $^{\$}P \leq 0.05$; $^{\&\&}P$, $^{**}P \leq 0.01$; $^{***}P$, $^{\#\#\#}P$, $^{\$\$\$}P$, $^{\&\&\&}P \leq 0.001$.

monocrotaline (Robertson et al., 1977). The present study further demonstrated that intracellular GSH imposed important regulating effects on not only Mono but also Adon and Sene induced cytotoxicity on human normal liver L-02 cells.

In summary, cellular GSH plays important roles in regulating the cytotoxicity induced by PAs Adon, Sene and Mono on hepatocytes. GSH synthesis inhibitor BSO can make hepatocytes more sensitive to the cytotoxicity induced by these three PAs, while anti-oxidants GSH and NAC reversed PA Sene-induced cytotoxicity. Furthermore, our investigation indicated that GSH might play an important role to protect against cytotoxicity developed by PAs, which got worldwide attraction because of extensive distribution PA-containing plant and their potential hepatotoxicity.

Conflict of interest

Nothing declared.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.etap.2009.06.002.

References

Beaver, J.P., Waring, P., 1995. A decrease in intracellular glutathione concentration precedes the onset of apoptosis in murine thymocytes. Eur. J. Cell Biol. 68, 47–54.
DeLeve, L.D., Wang, X., Kuhlenkamp, J.F., Kaplowitz, N., 1996. Toxicity of azathioprine and monocrotaline in murine sinusoidal endothelial cells and hepatocytes: the role of glutathione and relevance to hepatic venoocclusive disease. Hepatology 23, 589–599.
Dukhande, V.V., Malthankar-Phatak, G.H., Hugus, J.J., Daniels, C.K., Lai, J.C., 2006. Manganese-induced neurotoxicity is differentially enhanced by glutathione depletion in astrocytoma and neuroblastoma cells. Neurochem. Res. 31, 1349–1357.

Friesen, C., Kiess, Y., Debatin, K.M., 2004. A critical role of glutathione in determining apoptosis sensitivity and resistance in leukemia cells. Cell Death Differ. 11, 73–85.
Fu, P.P., Xia, Q., Lin, G., Chou, M.W., 2004. Pyrrolizidine alkaloids-genotoxicity, metabolism enzymes, metabolic activation, and mechanisms. Drug Metab. Rev. 36, 1–55.
Ghosh, A., Sil, P.C., 2008. A protein from Cajanus indicus Spreng protects liver and kidney against mercuric chloride-induced oxidative stress. Biol. Pharm. Bull. 31, 1651–1658.
Gómez-Quiroz, L.E., Factor, V.M., Kaposi-Novak, P., Coulouarn, C., Conner, E.A., Thorgeirsson, S.S., 2008. Hepatocyte-specific c-Met deletion disrupts redox homeostasis and sensitizes to Fas-mediated apoptosis. J. Biol. Chem. 283, 14581–14589.
Griffith, O.W., 1982. Mechanism of action, metabolism, and toxicity of buthionine sulphoximine and its higher homologues, potent inhibitors of glutathione synthesis. J. Biol. Chem. 257, 13704–13712.
Griffith, O.W., 1999. Biologic and pharmacologic regulation of mammalian lutathione synthesis. Free Radic. Biol. Med. 27, 922–935.
Hansen, M.B., Nielsen, S.E., Berg, K., 1989. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J. Immunol. Methods 119, 203–210.
Hayes, J.D., Pulford, D.J., 1995. The glutathione Stransferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit. Rev. Biochem. Mol. Biol. 30, 445–600.
Hristova, M., Heuvelmans, S., vander, V.A., 2007. GSH-dependent regulation of Fas-mediated caspase-8 activation by acrolein. FEBS Lett. 581, 361–367.
Huan, J.Y., Miranda, C.L., Buhler, D.R., Cheeke, P.R., 1998. Species differences in the hepatic microsomal enzyme metabolism of the pyrrolizidine alkaloids. Toxicol. Lett. 99, 127–137.
Huxtable, R.J., 1989. Human embryotoxicity of pyrrolizidine-containing drugs. Hepatology 9, 510–511.
Huxtable, R.J., 1990. Activation and pulmonary toxicity of pyrrolizidine alkaloids. Pharmacol. Ther. 47, 371–389.
Huxtable, R.J., Yan, C.C., Wild, S., Maxwell, S., Cooper, R., 1996. Physicochemical and metabolic basis for the differing neurotoxicity of the pyrrolizidine alkaloids, trichodesmine and monocrotaline. Neurochem. Res. 21, 141–146.
Kaviarasan, S., Ramamurty, N., Gunasekaran, P., Varalakshmi, E., Anuradha, C.V., 2006. Fenugreek (trigonella foenum graecum) seed extract prevents ethanol-induced toxicity and apoptosis in Chang liver cells. Alcohol Alcohol. 41, 267–273.
Kim, Y.S., Jhon, D.Y., Lee, K.Y., 2004. Involvement of ROS and JNK1 in selenite-induced apoptosis in Chang liver cells. Exp. Mol. Med. 36, 157–164.
Kasahara, Y., Kiyatake, K., Tatsumi, K., Sugito, K., Kakusaka, I., Yamagata, S., Ohmori, S., Kitada, M., Kuriyama, T., 1997. Bioactivation of monocrotaline by P-450 3A in rat liver. J. Cardiovasc. Pharmacol. 30, 124–129.
Liao, Y., Lu, X., Lu, C., Li, G., Jin, Y., Tang, H., 2008. Selection of agents for prevention of cisplatin-induced hepatotoxicity. Pharmacol. Res. 57, 125–131.
Lu, Y., Cederbaum, A.I., 2006. Cisplatin-induced hepatotoxicity is enhanced by elevated expression of cytochrome P450 2E1. Toxicol. Sci. 89, 515–523.
Macho, A., Hirsch, T., Marzo, I., Marchetti, P., Dallaporta, B., Susin, S.A., Zamami, N., Kroemer, G., 1997. Glutathione depletion is an early and calcium elevation is a late event in thymocyte apoptosis. J. Immunol. 158, 4612–4619.
Milchak, L.M., Douglas, B.J., 2002. The effects of glutathione and vitamin E on iron toxicity in isolated rat hepatocytes. Toxicol. Lett. 126, 169–177.
Park, E.J., Park, K., 2007. Induction of reactive oxygen species and apoptosis in BEAS-2B cells by mercuric chloride. Toxicol. *In Vitro* 21, 789–794.
Peterka, M., Sarin, S., Roeder, E., Wiedenfeld, H., Halasková, M., 1994. Differing embryotoxic effects of senecionine and senecionine-N-oxide on the chick embryo. Funct. Dev. Morphol. 4, 89–92.
Roeder, E., 2000. Medicinal plants in China containing pyrrolizidine alkaloids. Pharmazie 55, 711–726.
Robertson, K.A., Seymour, J.L., Hsia, M.T., Allen, J.R., 1977. Covalent interaction of dehydroretronecine, a carcinogenic metabolite of the pyrrolizidine alkaloid monocrotaline, with cysteine and glutathione. Cancer Res. 37, 3141–3144.
Sakurai, T., Ochiai, M., Kojima, C., Ohta, T., Sakurai, M.H., Takada, N.O., Qu, W., Waalkes, M.P., Himeno, S., Fujiwara, K., 2005. Preventive mechanism of cellular glutathione in monomethylarsonic acid-induced cytolethality. Toxicol. Appl. Pharmacol. 206, 54–65.
Schmieder, P.K., Tapper, M.A., Kolanczyk, R.C., Hammermeister, D.E., Sheedy, B.R., Denny, J.S., 2003. Discriminating redox cycling and arylation pathways of reactive chemical toxicity in trout hepatocytes. Toxicol. Sci. 72, 66–76.
Sies, H., 1999. Glutathione and its role in cellular functions. Free Radic. Biol. Med. 27, 916–921.
Sies, H., Akerboom, T.P., 1984. Glutathione disulfide (GSSG) efflux from cells and tissues. Meth. Enzymol. 105, 445–451.
Stegelmeier, B.L., Edgar, J.A., Colegate, S.M., 1999. Pyrrolizidine alkaloid plants, metabolism and toxicity. J. Nat. Toxins 8, 95–116.
Taylor, D.W., Wilson, D.W., Lame, M.W., Dunston, S.D., Jones, A.D., Segall, H.J., 1997. Comparative cytotoxicity of monocrotaline and its metabolites in cultured pulmonary artery endothelial cells. Toxicol. Appl. Pharmacol. 143, 196–204.
Terneus, M.V., Brown, J.M., Carpenter, A.B., Valentovic, M.A., 2008. Comparison of S-adenosyl-L-methionine (SAMe) and N-acetylcysteine (NAC) protective effects on hepatic damage when administered after acetaminophen overdose. Toxicology 244, 25–34.
Wang, A.L., Wang, J.P., Wang, H., Chen, Y.H., Zhao, L., Wang, L.S., Wei, W., Xu, D.X., 2006. A dual effect of N-acetylcysteine on acute ethanol-induced liver damage in mice. Hepatol. Res. 34, 199–206.
Wu, G., Fang, Y.Z., Yang, S., Lupton, J.R., Turner, N.D., 2004. Glutathione metabolism and its implications for health. J. Nutr. 134, 489–492.
Wu, Y.L., Piao, D.M., Han, X.H., Nan, J.X., 2008. Protective effects of salidroside against acetaminophen-induced toxicity in mice. Biol. Pharm. Bull. 31, 1523–1529.
Yadav, U.C., Ramana, K.V., Awasthi, Y.C., Srivastava, S.K., 2008. Glutathione level regulates HNE-induced genotoxicity in human erythroleukemia cells. Toxicol. Appl. Pharmacol. 227, 257–624.
Yan, C.C., Huxtable, R.J., 1995. The effect of the pyrrolizidine alkaloids, monocrotaline and trichodesmine, on tissue pyrrole binding and glutathione metabolism in the rat. Toxicon 33, 627–634.
Yan, C.C., Huxtable, R.J., 1996. Effects of taurine and guanidinoethane sulfonate on toxicity of the pyrrolizidine alkaloid monocrotaline. Biochem. Pharmacol. 51, 321–329.