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HUMAN ALDO-KETO REDUCTASE-7A3 PROTECTS LO2 CELLS AGAINST APAP-INDUCED OXIDATIVE STRESS AND CELL DEATH

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ABSTRACT

Human aldo-keto reductase-7A (AKR7A3) also referred to as aflatoxin aldehyde reductases (AFAR) is NADPH dependent oxidoreductases. Previous studies have shown that AKR7A3 is capable of detoxifying liver toxin aflatoxin B1 to less toxic alcohol. This study was carried out to investigate whether AKR7A3 is involved in the detoxification of acetaminophen (APAP), a commonly used drug for the fever and pain but is capable of inducing hepatic toxicity.

Liver LO2 cells were transiently transfected with AKR7A3 over-expressing plasmid pFLAG-AKR7A3 or the control vector pFLAG-CMV. 24 hours after the transfection, cells were treated with APAP at 4 mM for 24 or 48 hours. Following the APAP treatment, cell viability (MTT assay), intracellular ROS, GSH, and mRNA of antioxidant and dehydrogenase enzymes were determined.

Results following APAP treatment, LO2 cells showed significant dose- and time-dependent increased in ROS production, decreased in GSH level and cell viability. Interestingly, APAP treatment significantly induced AKR7A3 mRNA and protein expression, suggesting AKR7A3 might be involved in the protection against APAP insults. Indeed, LO2 cells overexpressing AKR7A3 showed significantly attenuated ROS production and partially recovered GSH level, and mRNA induction of NADPH producing enzymes as well as antioxidant defense.

Conclusions this results demonstrate that AKR7A3 contributes to protection against APAP-induced hepatocellular toxicity that probably is achieved in part through enhancing the anti-oxidative defensive mechanisms and induction of NADPH producing enzymes therefore, maintained the GSH levels.

INTRODUCTION

Aldo-Keto reductases (AKRs) are a family of NAD (P) H oxidoreductases enzymes that can be capable to reduce aldehydes and ketones to their corresponding primary and secondary alcohols (Jin, Y. and Penning, 2007). They play a central role in the metabolism of sugar aldehydes, drugs, carcinogens, reactive aldehyde, ketoprostaglandins, and ketosteroids (Penning, and Drury, 2007), and this result in a harmful or beneficial effect depends on the products (1).

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Also it appears that AKR family are involved in transformations of carbonyls introduced by metabolic transformations by cytochrome P450, and detoxification of aldehydes and ketones generated endogenously during metabolism of drug because it leads to the formation of chemically less reactive products (Barski *et al*., 2008). Human AKR7A3 (aflatoxin aldehyde reductase) is a member of AKR7 family that is include, human (AKR7A3 and AKR7A2), rats (AKR7A1 and AKR7A4), and mouse AKR7A5 (Barski *et al*., 2008). Human AKR7A3 and rat AKR7A1 they play important role in the detoxification of aflatoxin B1 by reduction to its mono and dialcohols, so they can capable to protect the cells against aflatoxin induced cytotoxicity and prevent the formation of protein adduct (Bodreddigari *et al.,* 2008).

Acetaminophen (APAP; paracetamol; N-acetyl-p-aminophenol) is a widely used drug with analgesic and antipyretic activity. When consumed in large doses, it is known to cause severe centrilobular hepatic toxicity (Bessems and Vermeulen., 2001; Prescott., 1983; Black., 1984). But at therapeutic doses is rapidly metabolized in the liver principally through glucuronidation and sulfation, and only a small portion is oxidized by cytochrome P-450 2E1 to generate a highly reactive and cytotoxic intermediate, N-acetyl-p-benzoquinoneimine (NAPQI) (Lee *et al*., 1996; Vermeulen *et al.,* 1992). This metabolite is efficiently detoxified by being reduced back to APAP or covalently linked to GSH to form a 3-glutathione-S-yl-APAP conjugate (Potter and Hinson., 1986). After an overdose of APAP, the glucuronidation and sulfation routes become saturated and more extensive bioactivation of APAP occurs within 1 to 2 hour, leading to rapid depletion of hepatic GSH levels. Subsequently, covalent binding of NAPQI to cellular macromolecules, membrane lipid peroxidation, and disturbance of intracellular calcium balance (Wang *et al*., 1996), oxidative stress and reactive oxygen formation (Bajt *et al.,* 2004). Oxidative stress and reactive oxygen species (ROS) it is become known is important factors in the development of hepatotoxicity caused by APAP (Bajt *et al.,* 2004). Although production of ROS is essential for regulation of various important cellular events, including transcription factor activation, differentiation and cell proliferation (Gonzalez *et al*., 2002; Baran *et al*., 2004; Shen, and Liu., 2006), but excessive production of ROS lead to apoptosis and necrosis (Cai *et al*., 2008). Because a high level of ROS is the dangers and harmful to cell, the organisms have developed several cellular defenses to minimize the toxicity of ROS.

The tripeptide reduced glutathione is a key substance in the network of antioxidants enzymes induction through activation the Nrf2 pathway, which is most important detoxifying against oxidative stress (Copple *et al*., 2008). Thus, the regulatory ability to maintain the cellular GSH balance is crucial to confer the resistance to oxidative stress. These systems depend on NADPH productions, which is an important cofactor for regeneration of reduced glutathione from oxidized form (Circu and Aw., 2010). So the NADPH producing enzymes such as glucose-6-phosphate dehydrogenase (G6PD), malic enzyme (ME), and NADP dependent Isocitrate dehydrogenase are also key regulatory to the survival of organisms exposed to oxidative stress (Lee *et al*., 2002). The present study was, therefore, undertaken to determine whether APAP induced AKR7A3 expression have protective role during the apoptosis and oxidative injury induced by APAP, and whether overexpression of AKR7A3 in LO2 can capable to protect this cells against APAP induced oxidative stress, considering that AKR7A3 is NADPH dependent enzyme. The results obtained show that overexpression of AKR7A3 protected LO2 liver normal cell line from APAP induced oxidative stress and cell death probably by induction of NADPH producing enzymes as well as antioxidative defense.

MATERIALS AND METHODS

Chemicals

RPMI-1640, fetal bovine serum (FBS), dimethylsulfoxide (DMSO), APAP, DCFH-DA, 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Aldrich. AKR7A3 Rabbit polyclonal antibodies, beta-Actin from Santa Cruz. GSH kit from Jianchen, Nanjing, P.R. China.

Cell culture

Human normal liver cell line LO2 cells were obtained from ATCC. Cells were maintained in RPMI-1640 medium (sigma) supplemented with 10% fetal bovine serum and incubated in a humidified incubator at 37ºC in 5% CO2 until semi-confluent. Different concentrations of APAP were dissolved in serum free culture medium.

Plasmid construction

The human AKR7A3 cDNA was amplified from HepG2 (human liver cancer cell line). The AKR7A3 gene coding region of about 999 bp was prepared by PCR using a forward primer that contains a *HindIII* and *claI* site. The PCR product was gel-purified and digested with *HindIII* and *claI* restriction enzymes, and then ligated into the pFLAG-CMV2 plasmid at the *HindIII* and *claI* sites. The plasmid was transformed into *E. coli* DH5-α cell, creating pFLAG- AKR7A3.

Sense Primer: CACAAGCTTGTCATGTCCCGGCAGCTGT.
Antisense Primer: CACATCGAT CGA TG GG CC TA GC G G AAGT

Cell transfection and experimental design

Twenty-four hours after cell plating, the plasmid pFLAG-AKR7A3 or the empty vector pFLAG-CMV2 were transfected into LO2 cells using the lipofectamine 2000 transfection reagent (invitrogen) according to the instructions provided by the manufacturer. Then after 24 hour the culture media were replaced with a serum-free medium containing or not 4 mM APAP. After incubation for 24 hour with APAP or not, the cells were washed twice with phosphate-buffered saline (PBS), and whole-cell lysates were prepared for western blot, real time PCR, GSH, ROS, and MTT analysis. For time course study cells were treated with APAP 4 mM for different time points.

Assessment of cytotoxicity

Cell viability was determined by measuring the ability of cells to transform MTT to a purple formazan dye. Cells were seeded in 96-well tissue culture plates at 1×10^4 cells/well for 24 hour. The cells were then incubated with APAP at different concentrations for different periods of time. For transfection experiment, the cells transfected with pFLAG-AKR7A3 or control vector pFLAG-CMV for 24 hour followed by incubation with APAP 4 mM for 24 hour. After incubation, 20 µl/well of MTT solution (5 mg/ml phosphate buffered saline) was added and incubated for 4 hour in incubator. The medium was aspirated and replaced with 200 µl/well of DMSO to dissolve the formazan salt formed. The plates were then shaken for 1 h to extract the blue products. And the color intensity of the formazan solution, which reflects the cell growth condition, was measured at 490 nm using a microplate spectrophotometer

Western blotting

Cell lysates were prepared by scraping the cells by PBS followed by lysis the cell into lysis buffer, followed by centrifugation. The protein concentration of the supernatant was determined by the BCA protein assay kit and then 30μg of denatured protein was resolved on 12% SDS-PAGE and electroblotted onto nitrocellulose membranes (Amersham). After blocking, membranes were incubated with following antibodies: AKR7A3 at a dilution of (1:1000 or 1:2000) at 4 C overnight followed by further incubation with secondary antibody (1:2000). After washing with TBST, the blots were detected by the chemiluminescence. Followed by exposure to Kodak-X-Omat x-ray film.

Quantitative Real time PCR analysis

Total RNA was isolated from LO2 using TRIzol reagent according to manufacturer's instructions. Then the total RNA was reverse transcribed to cDNA using ReverTra Ace (TOYOBO, Tokyo, Japan) according to the manufacturer's instruction. Real-time PCR was performed with the SYBR Green assay. The condition of cycles consisted of a denaturation step at 95Cº for 5 min, 40 cycles at 95 Cº for 15s, annealing step between 58Cº to 60 Cº for 30s, and finally a holding temperature at 72 Cº for 30s. Quantitative results of real-time fluorescence PCR were assessed by a cycle threshold (Ct) value. The relative gene expression was determined by calculated ΔCt values by substraction of the Ct value from control primer. The primers and genes name listed in Table 1.

Flow cytometry measurement of intracellular ROS

DCFH-DA is a cell-permeable compound. When it enters the cell its acetate group is cleaved by cellular esterases and nonfluoresent DCFH is trapped inside. Subsequent oxidation by ROS DCFH yields the fluorescent product DCF and upon excitation at 488 nm emits green fluorescence, proportional to the intracellular level of ROS. Therefore DCFH-DA is an ROS-sensitive probe that can be used to detect oxidative activity in living cells. In the present experimental, LO2 cells were grown in 6-well plates for 24 hours to reach 90% confluence. Then transfected with pFLAG-AKR7A3 or empty vector pFLAG-CMV for additional 24 hours. They were then exposed to APAP for 24 hours in culture medium. Cells were then washed, resuspended in PBS before introducing DCFDA for 45 minutes in the dark in an incubator at a final concentration of 10 μmol/L and immediate detection by flow cytometry.

Quantification of intracellular GSH levels

The GSH in cells was measured using a GSH quantification kit (Jianchen, Nanjing, P.R. China). Briefly, after treatment, cells were scraped an centrifuged at 2500 rpm for 5 minutes at 4C, and the cell pellets were resuspended, sonicated in 1ml PBS containing 1mM EDTA and centrifuged at 14000 rpm for 15 minute at 4C. Then removed the supernatant and stored on ice, and measured the GSH concentration according to the manufacturer's instructions. Then the concentrations of GSH were calculated. Cellular protein was determined by BCA assay kit.

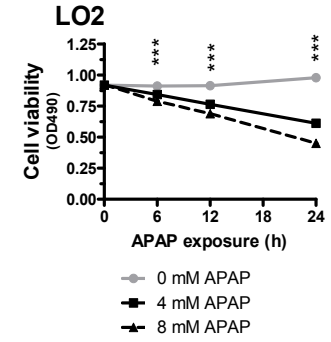
Statistical Analysis

All values reported in the text are mean ± standard error mean. Comparisons between multiple groups or, results form two different treatments were performed with one-way ANOVA followed by t-test for multiple statistical comparisons. A p-value <0.05 was determined to be significant. All statistical analyses were performed with statistical GraphPad Prism software.

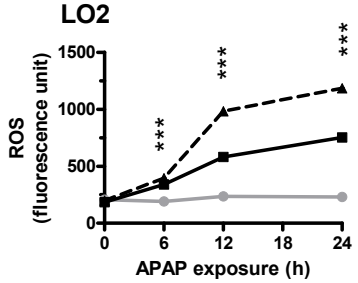
RESULTS

Acetaminophen-induced hepatotoxicity

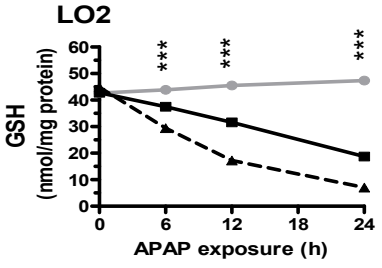
We investigated the time/concentration point for APAP-induced toxicity in LO2 liver cell line using the MTT assay. The viability of LO2 was inhibited after treatment with APAP in dose and time dependent manner (Figure 1A).



A



B



C

Fig.1 LO2 cells are sensitive to APAP-induced hepatotoxicity

Cells were seeded and allowed to grow for 24 hour to about 80% confluency. Subsequently, cells were exposed to 4 mM or 8 mM APAP and collected at different time points for MTT assays, ROS and GSH determinations. Values were expressed as the means ± SEM, n = 3-4. The indicated results of statistical analyses at 6-, 12-, and 24-h represented the comparisons between 4 mM APAP-treated cells and the untreated cells. ***, $P < 0.001$. The comparisons between 8 mM APAP-treated cells and the untreated cells at corresponding time points were all significant but not indicated. (A) Exposure to APAP reduced LO2 cell viability in a time- and dose-dependent manner as determined by the MTT assays. (B) APAP-induced ROS production in LO2 cells. (C) APAP-induced GSH depletion.

Table. 1 A list of real-time RT-PCR primers

Accession No	Gene symbol	Forward	Reverse
NM_009804	CAT	AGCGACCAGATGAAGCAGTG	TCCGCTCTCTGTCAAAGTGTG
NM_008160	GPx-1	ATGTCGCGTCTCTCTGAGG	CCGAACTGATTGCACGGGAA
NM_000402	G6pdh	CAAACAGAGTGAGCCCTTCTTC	CTCATGCAGGACTCGTGAATG
NM_005896	Idh1	TGTGGTAGAGATGCAAGGAGA	TGACGCCAACATTATGCTTCT
NM_001145412	Nrf2	AGTGGATCTGCCAACTACTC	CATCTACAAACGGGAATGTCTG
NR-003286	18S-rRNA	CGACGACCCATTCGAACGTCT	CTCTCCGGAATCGAACCCTGA

At treatment of APAP for different time points, there is significant cell damage was evidenced in LO2 when compared with the control as indicated by cell viability assay. To establish whether APAP induced reduction in cell viability and toxicity is associated with ROS production and hepatic GSH in the LO2, the effect of APAP on ROS production and GSH depletion was determined. We found that APAP treatment showed times and concentrations a significant decreases in GSH level and increases in ROS production compared to control (Figure 1B, C).

APAP treatment significantly induced the expression of AKR7A3

In order to evaluate the effect of APAP on hepatic expression of xenobiotic detoxification and oxidative stress related genes, western blotting and qPCR analysis performed in the LO2 liver normal cell line for AKR7A3. After 4 hour treatment with APAP 4 mM Western blot and qPCR showed significant increases in the AKR7A3 protein and mRNA expression levels, Akr7a3 mRNA expression appeared at 4 hours and peaked at 24 hours (with a maximum induction of ~ 6-fold) and fell slightly subsequently (Figure 2A). In line with the induction of Akr7a3 mRNA, apparent induction of AKR7A3 protein in LO2 was observed at 4 hours, was sustained at least up to 36 hours, then decreased (Figure 2B). Together, these results indicated that hepatic AKR7A3 expression is responsive to APAP exposure.

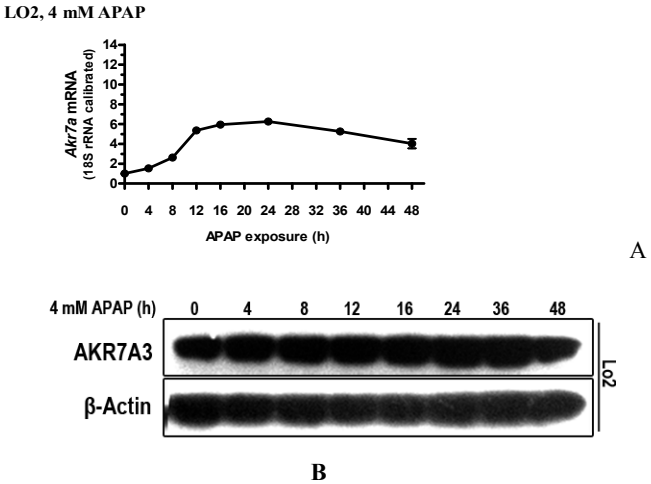


Fig.2 APAP-induced up-regulation of Akr7a3 mRNA and AKR7A3 protein

Cells were seeded and allowed to grow for 24 hours to approximately 80% confluency. Subsequently, LO2 cells were exposed to 4 mM of APAP. Cells collected at 0, 4, 8, 12, 16, 24, 36, and 48 hours were used for mRNA and protein analyses. Values represented the means ± standard error of the mean (SEM) for three to four independent experiments. 18S ribosomal RNA was used for calibration in real-time RT-PCR analyses of mRNA, and β-actin served as a loading control for western blotting. (A) APAP-induced time-dependent up-regulation of Akr7a3 mRNA in LO2 cells. (B) APAP-induced time-dependent up-regulation of AKR7A3 protein in LO2 cells.

AKR7A3 is capable of ameliorating APAP-induced oxidative stress and cell death

Because oxidative stress is central to APAP-induced hepatotoxicity and its protection, we generated a human Akr7a3-overexpressing plasmid (pFlag-AKR7A3) to overexpress Akr7a3 in LO2 cells to explore the contribution of AKR7A3 to oxidative stress of APAP-treated LO2 cells marked by ROS level, and GSH. Cells were transfected with pFlag- AKR7A3 or control vector pFlag-CMV for 24 h, then exposed to 4 mM APAP for additional 24 h. The time-dependent APAP-induced increase in ROS and the APAP-induced depletion of GSH in AKR7A3-overexpressing cells were significantly diminished, but not completely inhibited (Figure 3A, B).

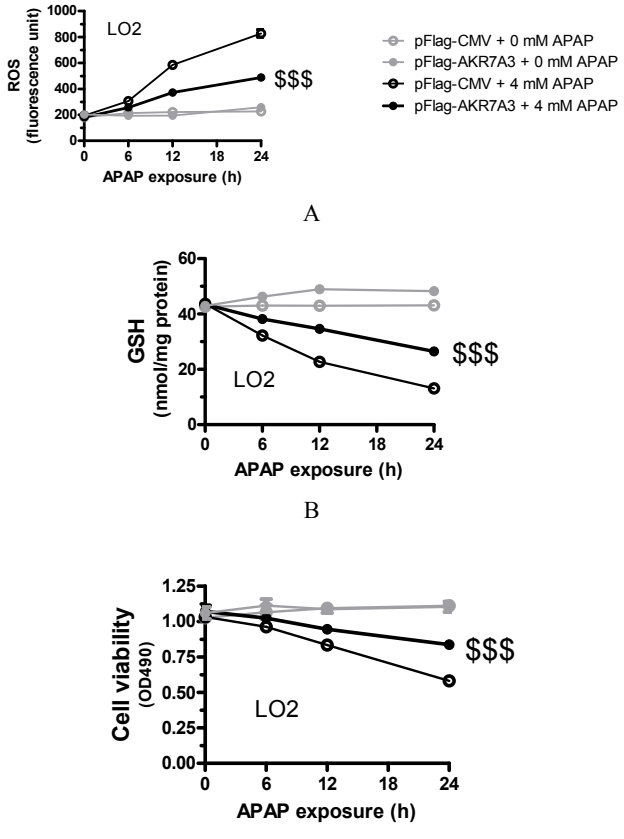


Fig. 3. overexpression of Akr7a3 protects LO2 cells against APAP induced oxidative stress and cell death

Cells were transfected with pFlag-AKR7A3 or pFlag-CMV for 24 hours, then treated with 4 mM of APAP or vehicle. Cells were then collected at designated time points for analyses. Values represented the means ± SEM for three to four independent experiments. Statistical comparisons were made between the APAP- treated groups ($). NS, not significant; $$$ $P < 0.001$. (A) Overexpression of Akr7a3 greatly attenuated APAP-induced ROS production. (C) Overexpression of Akr7a3 significantly attenuated APAP-induced GSH depletion. (D) Overexpression of Akr7a3 significantly increased cell viability after APAP exposure, as determined by MTT assay

Furthermore, cell viability was greatly increased in AKR7A3-overexpressing cells after APAP exposure (Figure 3C). Increased AKR7A3, therefore, might protect liver cells from APAP-induced oxidative stress and cell death.

Overexpression of AKR7A3 enhanced antioxidant and NADPH producing enzymes

To evaluate whether the overexpression of AKR7A3 in LO2 would affect antioxidant or dehydrogenase defense, total RNA extracted form LO2 and examined the expression of *Nrf2* transcriptional factor, antioxidant enzymes, Catalase (*Cat*), glutathione peroxidase (*GPx*-1), and dehydrogenases enzymes, glucose-6-phosphate dehydrogenase (*G6pdh*), cytosolic NADP dependent isocitrate dehydrogenase (*Idh*1), and normalized to 18s rRNA levels. In the AKR7A3-overexpressing LO2 infected with pFLAG-AKR7A3 and LO2 infected with pFLAG-AKR7A3 treated with 4 mM APAP, the mRNA expression levels of dehydrogenases enzymes, including G6PD, IDH1, and Nrf2 transcriptional factor and its downstream antioxidative enzymes, including catalase, and glutathione peroxidase (GPx-1), were significantly elevated (Figure 4). This result indicate that overexpression of AKR7A3 enhanced antioxidative defense and reproduction of NADPH by induction of dehydrogenase enzymes.

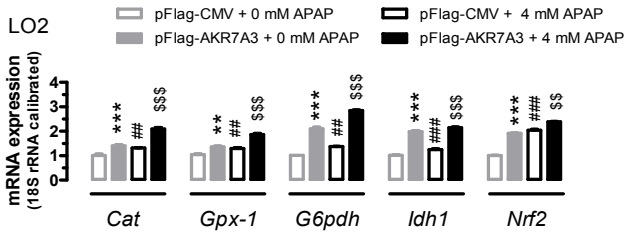


Fig.4 Effects of overexpression of Akr7a3 on antioxidant and NADPH-producing enzymes in LO2 cells

Cells were transfected with pFlag-AKR7A3 or pFlag-CMV for 24 hours, then treated with 4 mM of APAP or vehicle. Cells were then collected at designated time points for analyses. Values represented the means ± SEM for three to four independent experiments. Statistical comparisons were made between the APAP-untreated groups (*) or between the APAP-treated groups ($) or between (pFlag-CMV þ 0 mM APAP) versus (pFlag-AKR7A3 þ 4 mM APAP) (#). NS, not significant; * or #P < 0.05; ** or ## or $$P < 0.01; *** or $$$ or ###P < 0.001. Effects of overexpression of Akr7a3 on mRNA expression of Nrf2, antioxidant enzymes, and NADPH-producing enzymes in LO2 cells, as determined by quantitative real-time RTPCR

DISCUSSION

In the present study, we have shown that APAP induced AKR7A3 protein expression and overexpression of AKR7A3 protein capable to protect from APAP induced oxidative stress and cell death in LO2 normal human liver cell line. The major finding in this study that is APAP induced AKR7A3 protein expression. AKR7A3 is a member of the aldo-keto-reductase family which exhibits broad substrate specificity for a variety of reactive aldehydes such as aflatoxin, acrolein, and methylglyoxal (Barski *et al*., 2008; Bodreddigari *et al* 2008). AKR7A3 recognized as detoxification enzyme, Previous studies demonstrated that human AKR7A3 and rat AKR7A1 can protect against aflatoxin (AFB1) induced toxicity by converting aflatoxin to less toxic substance and prevent formation of protein adduct (Bodreddigari *et al* 2008). Moreover AKR7A3 are involved in detoxification of acrolein and methylglyoxal (Barski *et al*., 2008). Thus the up-regulation of KAR7A3 in LO2 following treated with 4 mM APAP it has suggested that AKR7A3 could play a protective role by providing a mechanism for the cellular detoxification of APAP. APAP a commonly used drug for decrease the fever and pain but is capable to induced hepatotoxicity at over dose. Oxidative stress, production of reactive oxygen species, GSH depletion, and apoptosis is the major mechanism of APAP induced hepatotoxicity (Reid *et al*., 2005). The results obtained from the present study agreed with those of the previous studies. Moreover, LO2 cells overexpressing AKR7A3 showed significantly attenuated ROS production and partially recovered GSH level, and increased cell viability. GSH and reactive oxygen species (ROS) it is become known is important factors in the development of hepatotoxicity caused by APAP. Therefore, the regulatory ability to maintain the cellular GSH balance and ROS is crucial to confer the resistance to oxidative stress. Intracellular ROS levels are regulated by a series of antioxidative enzymes such as catalase, SOD, GPx-1, glutathione S-transferase and the small-molecule antioxidant GSH. And all theses antioxidant and xenobiotic detoxification molecules under the control of Nrf2 transcription factor (Ferret *et al*., 2001; Kensler *et al*., 2007). Therefore, we investigated the mRNA expression levels for Nrf2, antioxidative defense, and NADPH producing enzymes. Interestingly, the results obtained from our study showed that, overexpression of AKR7A3 induced significantly mRNA expression levels of Nrf2, catalase, Glutathione peroxidase, glucose-6-phosphate dehydrogenase, and cytosolic NADP dependent Isocitrate dehydrogenase. Nrf2 is an important component of the cellular detoxification against oxidative stress, it's well known that Nrf2 regulates antioxidant defenses, and a variety of genes involved in detoxification and drug metabolism (Aleksunes and Manautou., 2007).

Previous study demonstrated the importance of Nrf2 in the detoxification of APAP, they showed that the toxicity of APAP is markedly augmented in Nrf2 knock out mice. Moreover, increased expression level of Nrf2 mRNA in the liver regulated the antioxidant response element (ARE) in response to APAP (Chan *et al*., 2001). Furthermore, Nrf2 knock down by siRNA in HepG2 overexpressing CYP2E1 were more sensitive to Arachidonic acid induced toxicity, and overexpression of Nrf2 by transient transfection more resistance to toxicity, indeed, this resistance related to induction of glutamate cysteine ligase the rate-limiting enzyme for GSH synthesis, thus increase of GSH levels (Gong and Cederbaum., 2006). Moreover, antioxidant enzymes its known can protect against ROS, previous studies demonstrated that catalase and intracellular glutathione capable to protect against hydrogen peroxide induced apoptosis (Simon *et al*., 2000). It's more interesting to note that, in this study we demonstrated the induction of NADPH producing enzymes, G6PD, IDH1, which were essential for regenerating NADPH a coenzyme involved in the reduction of GSSG to GSH which is catalyzed by glutathione reductase. Moreover, NADPH is not only important for regeneration of GSH but also used as cofactor for catalase (Fico *et al*., 2004). Therefore, maintenance of the cytosolic pool of NADPH is crucial for the cellular redox balance, which is important mechanism against electrophiles and oxidative damage.

Previous study in HepG2 showed that, knock down of G6PD attenuated GSH regeneration and augmented the cytotoxicity induced by oxidant (Gao *et al*., 2009). Furthermore, oxidant stress induced ROS production attenuated by overexpression of G6PD in vascular endothelial cells (Leopold *et al*., 2003). Recent study showed that G6PD-defeicient mice more acceptable to oxidative stress (Xu *et al*., 2010). Concerning these results, G6PDH plays a critical role in the cell death.

Cytosolic NADP dependent Isocitrate dehydrogenase (IDH1 or IDPc) plays a protective role during oxidative damage. Overexpression of cytosolic ICDH provided a protective mechanism against oxidant induced oxidative stress and apoptosis by regenerating GSH (Lee *et al*., 2002; Kim *et al*., 2007). Moreover, previous study demonstrated that IDPc attenuated oxidative damage induced by ultraviolet radiation (Jo *et al*., 2002). Furthermore, recently study showed that, knock down of IDPc by siRNA augmented staurosporine induced apoptosis in HeLa (Lee *et al*., 2009).

Conclusion

The APAP induced transcription and protein expression of AKR7A3 in LO2, and the overexpression of AKR7A3 protect against APAP induced cell death, this is protection almost attributed to augmentation of Nrf2 which probably induced the activity and mRNA expression of antioxidative defense and NADPH regenerating enzymes, and maintained the GSH levels which can regulate the apoptosis, oxidative stress and reactive oxygen production producing during APAP induced toxicity, and also critical for prevention against electrophiles in the present of GST. But also may the protection by overexpression of AKR7A3 could be due to its broad substrate specificity for reactive aldehydes generated in the cell during APAP mediated cellular damage.

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REFERENCES

Aleksunes, L. M. and Manautou, J. E. 2007. Emerging role of Nrf2 in protecting against hepatic and gastrointestinal disease. *Toxicologic pathology,* 35:459-473.

Bajt, M. L., Knight, T. R., Lemasters, J. J. and Jaeschke, H. 2004. Acetaminophen-induced oxidant stress and cell injury in cultured mouse hepatocytes: protection by N-acetyl cysteine. *Toxicol Sci.,* 80**:**343-349.

Baran, C. P., Zeigler, M. M., Tridandapani, S. and Marsh, C. B. 2004. The role of ROS and RNS in regulating life and death of blood monocytes. *Current pharmaceutical design* 10**:**855-866.

Barski, O. A., Tipparaju, S. M. and Bhatnagar, A. 2008. The aldo-keto reductase superfamily and its role in drug metabolism and detoxification. Drug metabolism reviews 40:553-624.

Black, M. 1984. Acetaminophen hepatotoxicity. Annual review of medicine 35:577-593. Nitrogen species. Free radical biology and medicine 40:928-939.

Bodreddigari, S., Jones, L. K., Egner, P. A., Groopman, J.D., Sutter, C. H., Roebuck, B. D., Guengerich, F. P., Kensler, W., Sutter, T. R. 2008. Protection against aflatoxin B1-induced cytotoxicity by expression of the cloned aflatoxin B1-aldehyde reductases rat AKR7A1 and human AKR7A3. Chemical research in toxicology 21:1134-1142.

Cai, Y. J., Lu, J. J., Zhu, H., Xie, H., Huang, M., Lin, L. P., Zhang, X. W. and Ding, J. 2008. Salvicine triggers DNA double-strand breaks and apoptosis by GSH-depletion-driven H2O2 generation and topoisomerase II inhibition. Free radical biology and medicine 45:627-635.

Chan, K., Han, X. D. and Kan, Y. W. 2001. An important function of Nrf2 in combating oxidative stress: detoxification of acetaminophen. Proceedings of the National Academy of Sciences of the United States of America 98:4611-4616.

Circu, M. L. and Aw, T. Y. 2010. Reactive oxygen species, cellular redox systems and apoptosis. Free radical biology and medicine 48:749-762.

Copple, I. M., Goldring, C. E., Jenkins, R. E., Chia, A. J., Randle, L. E., Hayes, J. D., Kitteringham, N. R. and Park, B. K. 2008. The hepatotoxic metabolite of acetaminophen directly activates the Keap1-Nrf2 cell defense system. Hepatology (Baltimore, Md 48:1292-1301.

Ferret, P. J., Hammoud, R., Tulliez, M., Tran, A., Trebeden, H., Jaffray, P., Malassagne, B., Calmus, Y., Weill, B. and Batteux, F. 2001. Detoxification of reactive oxygen species by a nonpeptidyl mimic of superoxide dismutase cures acetaminophen-induced acute liver failure in the mouse. Hepatology (Baltimore, Md 33:1173-1180.

Fico, A., Paglialunga, F., Cigliano, L., Abrescia, P., Verde, P., Martini, G., Iaccarino, I. and Filosa, S. 2004. Glucose-6-phosphate dehydrogenase plays a crucial role in protection from redox-stress-induced apoptosis. Cell death and differentiation 11:823-831.

Gao, L. P., Cheng, M. L., Chou, H. J., Yang, Y. H., Ho, H. Y. and Chiu, D. T. 2009. Ineffective GSH regeneration enhances G6PD-knockdown Hep G2 cell sensitivity to diamide-induced oxidative damage. Free radical biology and medicine 47:529-535.

Gong, P. and Cederbaum, A. I. 2006. Transcription factor Nrf2 protects HepG2 cells against CYP2E1 plus arachidonic acid-dependent toxicity. The Journal of biological chemistry 281:14573-14579.

Gonzalez, C., Sanz-Alfayate, G., Agapito, M. T., Gomez-Nino, A., Rocher, A. and Obeso, A. 2002. Significance of ROS in oxygen sensing in cell systems with sensitivity to physiological hypoxia. Respiratory physiology and neurobiology 132:17-41.

Jin, Y. and Penning, T. M. 2007. Aldo-keto reductases and bioactivation/detoxication. Annual review of pharmacology and toxicology 47:263-292.

Jo, S. H., Lee, S. H., Chun, H. S., Lee, S. M., Koh, H. J., Lee, S. E., Chun, J. S., Park, J. W. and Huh, T. L. 2002. Cellular defense against UVB-induced phototoxicity by cytosolic NADP (+)-dependent isocitrate dehydrogenase. Biochemical and biophysical research communications 292:542-549.

Kensler, T. W., Wakabayashi, N. and Biswal, S. 2007. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. Annual review of pharmacology and toxicology 47:89-116.

Kim, S. Y., Lee, S. M., Tak, J. K., Choi, K. S., Kwon, T. K. and Park, J. W. 2007. Regulation of singlet oxygen-induced apoptosis by cytosolic NADP+-dependent isocitrate dehydrogenase. Molecular and cellular biochemistry 302:27-34.

Lee, S. M., Koh, H. J., Park, D. C., Song, B. J., Huh, T. L. and Park, J. W. 2002. Cytosolic NADP (+)-dependent isocitrate dehydrogenase status modulates oxidative damage to cells. Free radical biology and medicine 32:1185-1196.

Lee, S. M., Park, S. Y., Shin, S. W., Kil, I. S., Yang, E. S. and Park, J. W. 2009. Silencing of cytosolic NADP (+)-dependent isocitrate dehydrogenase by small interfering RNA enhances the sensitivity of HeLa cells toward staurosporine. Free radical research 43:165-173.

Lee, S. S., Buters, J. T., Pineau, T., Fernandez-Salguero, P. and Gonzalez, F. J. 1996. Role of CYP2E1 in the hepatotoxicity of acetaminophen. The Journal of biological chemistry 271:12063-12067.

Leopold, J. A., Zhang, Y. Y., Scribner, A. W., Stanton, R. C. and Loscalzo, J. 2003. Glucose-6-phosphate dehydrogenase overexpression decreases endothelial cell oxidant stress and increases bioavailable nitric oxide. Arteriosclerosis, thrombosis, and vascular biology 23:411-417.

Penning, T. M. and Drury, J. E. 2007. Human aldo-keto reductases: Function, gene regulation, and single nucleotide polymorphisms. Archives of biochemistry and biophysics 464:241-250.

Potter, D. W. and Hinson, J. A. 1986. Reactions of N-acetyl-p-benzoquinone imine with reduced glutathione, acetaminophen, and NADPH. Molecular pharmacology 30:33-41.

Prescott, L. F. 1983. Paracetamol overdosage. Pharmacological considerations and clinical management. Drugs 25:290-314.

Reid, A. B., Kurten, R. C., McCullough, S. S., Brock, R. W. and Hinson, J. A. 2005. Mechanisms of acetaminophen-induced hepatotoxicity: role of oxidative stress and mitochondrial permeability transition in freshly isolated mouse hepatocytes. The Journal of pharmacology and experimental therapeutics 312:509-516.

Shen, H. M. and Liu, Z. G. 2006. JNK signaling pathway is a key modulator in cell death mediated by reactive oxygen and Simon, H. U., Haj-Yehia, A., Levi-Schaffer, F. 2000. Role of reactive oxygen species (ROS) in apoptosis induction. Apoptosis 5:415-418.

Vermeulen, N. P., Bessems, J. G. and Van de Straat, R. 1992. Molecular aspects of paracetamol-induced hepatotoxicity and its mechanism-based prevention. Drug metabolism reviews 24:367-407.

J. G. and Vermeulen, N. P. 2001. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. Critical reviews in toxicology 31:55-138.

Wang, E. J., Li, Y., Lin, M., Chen, L., Stein, A. P., Reuhl, K. R. and Yang, C. S. 1996. Protective effects of garlic and related organosulfur compounds on acetaminophen-induced hepatotoxicity in mice. Toxicology and applied pharmacology 136:146-154.

Xu, Y., Zhang, Z., Hu, J., Stillman, I. E., Leopold, J. A., Handy, D. E., Loscalzo, J. and Stanton, R. C. 2010. Glucose-6-phosphate dehydrogenase-deficient mice have increased renal oxidative stress and increased albuminuria. *Faseb J.*, 24:609-616.

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