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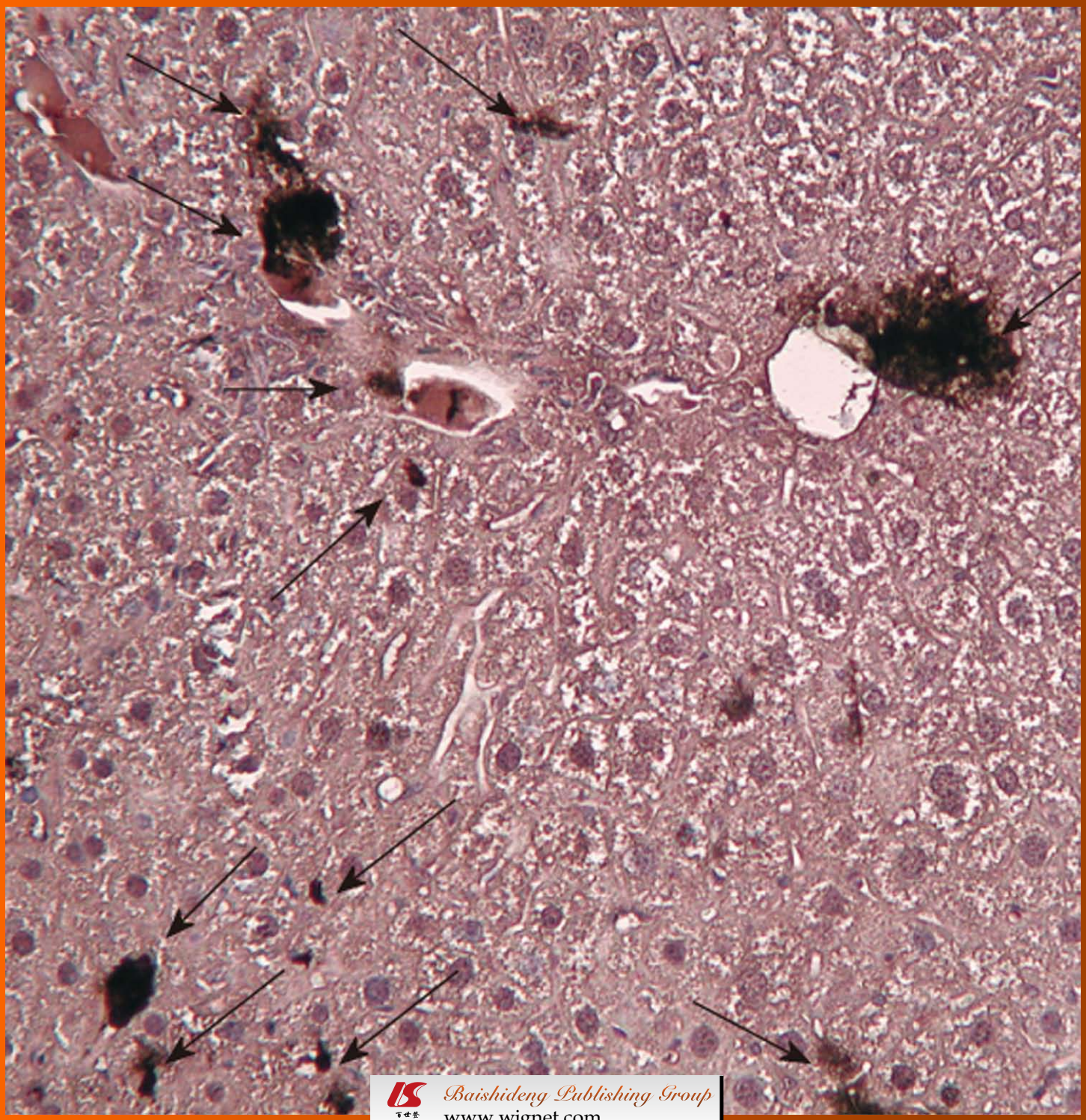

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ORIGINAL ARTICLES

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ORIGINAL ARTICLE

Regulation of heme oxygenase expression by alcohol, hypoxia and oxidative stress

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Abstract

AIM: To study the effect of both acute and chronic alcohol exposure on heme oxygenases (HOs) in the brain, liver and duodenum.

METHODS: Wild-type C57BL/6 mice, heterozygous Sod2 knockout mice, which exhibit attenuated manganese superoxide dismutase activity, and liver-specific ARNT knockout mice were used to investigate the role of alcohol-induced oxidative stress and hypoxia. For acute alcohol exposure, ethanol was administered in the drinking water for 1 wk. Mice were pair-fed with regular or ethanol-containing Lieber De Carli liquid diets for 4 wk for chronic alcohol studies. HO expression was analyzed by real-time quantitative polymerase chain reaction and Western blotting.

RESULTS: Chronic alcohol exposure downregulated HO-1 expression in the brain but upregulated it in the duodenum of wild-type mice. It did not alter liver HO-1 expression, nor HO-2 expression in the brain, liver or duodenum. In contrast, acute alcohol exposure decreased both liver HO-1 and HO-2 expression, and HO-2 expression in the duodenum of wild-type mice. The decrease in liver HO-1 expression was abolished in ARNT$^{+/-}$ mice. Sod2$^{+/-}$ mice with acute alcohol exposure did not exhibit any changes in liver HO-1 and HO-2 expression or in brain HO-2 expression. However, alcohol inhibited brain HO-1 and duodenal HO-2 but increased duodenal HO-1 expression in Sod2$^{+/-}$ mice. Collectively, these findings indicate that acute and chronic alcohol exposure regulates HO expression in a tissue-specific manner. Chronic alcohol exposure alters brain and duodenal, but not liver HO expression. However, acute alcohol exposure inhibits liver HO-1 and HO-2, and also duodenal HO-2 expression.

CONCLUSION: The inhibition of liver HO expression by acute alcohol-induced hypoxia may play a role in the early phases of alcoholic liver disease progression.

Key words: Alcohol; Brain; Duodenum; Heme oxygenase; Hypoxia; Iron; Liver; Mitochondria; Oxidative stress; Reactive oxygen species

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INTRODUCTION

Heme (iron protoporphyrin IX) is a tetrapyrole contain-

ing a central iron. Heme biosynthesis takes place in both mitochondria and the cytosol. The synthesis of 5-aminolevulinic acid (ALA) catalyzed by the first enzyme of heme synthesis, ALA synthase-1, is the rate-limiting step in heme synthesis[1]. On the other hand, heme oxygenases (HOs) catalyze the first and rate-limiting step in the oxidative degradation of heme, which produces ferrous iron, CO and biliverdin[2,3]. Biliverdin is subsequently converted to bilirubin by an NAD(P)H-dependent biliverdin reductase[2]. Both bilirubin and CO exert cytoprotective and anti-inflammatory effects[2]. Furthermore, HO-derived CO has been shown to act as a second messenger by increasing cGMP levels in the intestine and brain[4,5].

In mammals, there are two HOs, HO-1 and HO-2, which are products of distinct genes but catalyze the same reaction[2,6,7]. HO-1 is the inducible form and is expressed in several tissues[2,8]. HO-1 expression is regulated by inflammation, heme and hypoxia[2]. The enhancer regions in HO-1 promoter mediate its inducer-dependent transcriptional regulation *via* the binding of NF-E2-related factor 2 (Nrf2), activator protein 1 (AP-1), nuclear factor κ-light-chain-enhancer of activated B cells (NFκB) and E26 transformation-specific (Ets) family of transcription factors, and the repressor Bach-1[2,9-12]. HO-2 expression, which is constitutive, occurs at high levels in the brain and testes, and at lower levels in the liver and myenteric plexus of the gut[2,7,13]. HO-2 is also expressed in the interstitial cells of Cajal in the intestine and takes part in neurotransmission and intestinal peristalsis[14]. Glucocorticoids have been shown to modulate HO-2 expression in the brain[4,15].

Both HO-1 and HO-2 exert antioxidant properties, and are also regulated by oxidative stress[16-18]. Humans and transgenic mice deficient in HO-1 are vulnerable to oxidative-stress-mediated injury, and develop chronic inflammation and iron accumulation[19-21]. Knockout mouse studies have also shown a role for HO-2 in oxidative-stress-induced tissue injury[22-24]. Alcohol consumption is well known to induce inflammation and oxidative stress in the brain, liver and duodenum[25-28]. Induction of HO-1 has been reported to prevent alcohol-induced inflammation[29-32]. HO-1 has also been suggested to play a protective role in fatty liver, which is also observed in patients with alcoholic liver disease[33].

However, the effect of alcohol on the expression of HOs is not well understood. The studies in the literature are inconclusive and have been mainly performed in the liver[29,34,35]. The aim of this study therefore was to understand how acute and chronic alcohol exposure, hypoxia, and mitochondrial reactive oxygen species (ROS) accumulation regulate the expression of HO-1 and HO-2 in the brain, liver and duodenum *in vivo*.

MATERIALS AND METHODS

Animal experiments

Animal experiments were approved by the Animal Ethics Committee at the University of Nebraska Medical Center. C57BL/6 wild-type and transgenic mice were used for these studies. $Sod2^{+/-}$ mice, on a C57BL/6 genetic background, lack the expression of mitochondrial manganese superoxide dismutase (Sod2) enzyme on one allele and were generated, as described previously[36]. Aryl hydrocarbon receptor nuclear translocator (ARNT) floxed transgenic mice, on a C57BL/6 genetic background, expressing the floxed allele of ARNT gene were generated, as described previously[37]. ARNT floxed mice were crossed with Albumin-Cre transgenic mice (Jackson Laboratories, Bar Harbor, ME, United States), on a C57BL/6 genetic background and expressing Cre recombinase under the control of liver-specific albumin promoter, to create liver-specific ARNT heterozygous knockout mice ($ARNT^{+/-}$/$Alb\text{-}Cre^{+/-}$).

Alcohol treatment: For acute alcohol studies, wild-type and transgenic mice, maintained on a regular chow diet (Harland Teklad 7012), were housed in individual cages. Mice were then exposed to either 10% (w/v) ethanol in the drinking water or plain water (control) for 7 d, as described previously[38,39]. For chronic alcohol studies, wild-type mice, housed individually, were pair-fed with either regular or ethanol-containing Lieber De Carli liquids diets (Dyets, Bethlehem, PA, United States). The ethanol content of the diet was gradually increased over a 9 d period to 5% (no ethanol for 3 d, 1% for 2 d, 2% for 2 d and 3% for 2 d). Mice were exposed to 5% ethanol for 4 wk.

Mice were sacrificed at the end of experiments to harvest the organs. Before harvesting, the livers were perfused *via* the portal vein with warm (37 ℃) PBS buffer (pH 7.4) to eliminate blood. Duodenums, which were rinsed clean with RNAase-free ice-cold PBS, were placed in RNAlater (Ambion, Austin, TX, United States) solution. All the organs isolated from mice were snap frozen in liquid nitrogen and stored at -80 ℃ until further use.

RNA isolation and cDNA synthesis

For RNA isolation, frozen organs were lysed in TRIzol reagent (Invitrogen, Carlsbad, CA, United States) and total RNA was isolated according to the manufacturer's specifications. cDNA was synthesized using 2-4 μg isolated RNA, 2.5 μmol/L random primers (Applied Biosystems, Carlsbad, CA, United States) and 200 U Superscript II RNAase H reverse transcriptase enzyme (Invitrogen). To exclude possible genomic DNA contamination, control samples were used, in which the reverse transcriptase enzyme was omitted from the cDNA synthesis reaction.

Real-time quantitative polymerase chain reaction

Gene expression was analyzed by real-time quantitative polymerase chain reaction (PCR), using an ABI Prism 7700 Sequence Detection System (Applied Biosystems). Specific primers and Taqman fluorescent probes [5' 6-(FAM); 3' (TAMRA-Q)] flanking about 70 base pairs of the open reading frame sequences were designed by the Primer Express 1.5 program (Applied Biosystems). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

Table 1 Mouse-specific sequences of real-time quantitative polymerase chain reaction probe and primers

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Taqman probe (5'-3')
HO-1	CCTGGAGCAGGACATGGC	AATCATCCCTTGCACGCC	TTCTGGTATGGGCCTCACTGGCAGG
HO-2	GAGGCAGCAACTGCCCC	TGAGCTGCAGGCTAGGCTTC	TCCAGACAACCGTGGCTGTGCTGA
GAPDH	TTGACCTCAACTACATGG	TCATACCAGGAAATGAGC	TTTGGTCGTATTGGGCGCCTGG

HO: Heme oxygenase.

gene probe was used as the endogenous control. Species-specific sequences of the Taqman fluorescent probes, sense and antisense primers are listed in Table 1. cDNA was used as a template in PCRs. Following PCR [50ºC for 2 min, 95ºC for 10 min (one cycle), 95ºC for 15 s, 60ºC for 1 min (40 cycles)], the data were analyzed using Sequence Detection Systems software (Applied Biosystems), and the cycle number at the linear amplification threshold (Ct) of the endogenous control (GAPDH) gene and the target gene was recorded. Relative gene expression (the amount of target, normalized to the endogenous control gene) was calculated using the comparative Ct method formula $2^{-\Delta\Delta Ct}$.

Western blotting and immunohistochemistry

Whole cell or nuclear lysates were prepared, as described previously[40]. Proteins resolved by SDS-PAGE were transferred onto PVDF membranes (Bio-Rad Laboratories, Hercules, CA, United States). The membranes were incubated with anti-hypoxia inducible factor (HIF)-1α polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, United States), anti-heme oxygenase-1 monoclonal antibody (Santa Cruz Biotechnology), anti-TATA-binding protein monoclonal antibody (Abcam, Cambridge, MA, United States) or GAPDH monoclonal antibody (Chemicon International, Temecula, CA, United States), followed by alkaline phosphatase (AP)-conjugated anti-rabbit or anti-mouse secondary antibodies (Bio-Rad Laboratories). Immunoreactive bands were detected by the ImmunStar anti-rabbit or anti-mouse-AP kits (Bio-Rad Laboratories).

Immunostaining of paraffin-embedded liver sections was performed by using the Vectastain ABC kit (Vector Labs, Burlingame, CA, United States), as described previously[41]. The sections were incubated with an anti-8-OHdG antibody (Abcam) overnight at 4 ℃ in an enclosed humidified chamber. Peroxidase activity was detected by nickel-enhanced diaminobenzidine (DAB) reaction, as described previously[42]. The slides were subsequently counterstained with hematoxylin.

Statistical analysis

Statistical analysis of differences in treatment groups was performed by using the Student's *t* test. $P < 0.01$ was considered statistically significant. Data are presented as mean ± SD.

RESULTS

We investigated the effect of both chronic and acute alcohol exposure, hypoxia, and mitochondrial superoxide anion ($O_2^{\cdot-}$) accumulation on the expression of HO-1 and HO-2 in the liver, brain and intestine.

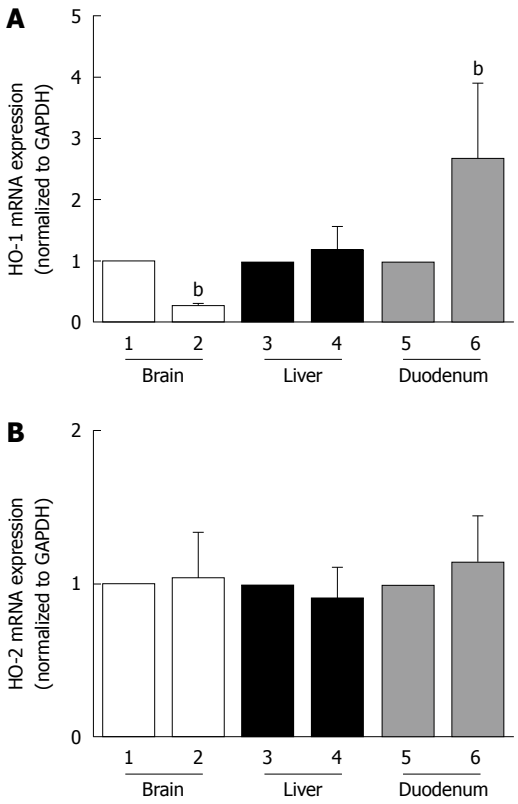

Figure 1 Effect of chronic alcohol exposure on heme oxygenase expression. Heme oxygenase (HO)-1 (A) and HO-2 (B) mRNA expression in the brain (lanes 1 and 2), liver (lanes 3 and 4) and duodenum (lanes 5 and 6) of C57BL/6 wild-type mice pair-fed with regular (control) (lanes 1, 3 and 5) or ethanol-containing (lanes 2, 4 and 6) L. De Carli diets for 4 wk was measured by real-time polymerase chain reaction. HO expression in alcohol-treated mice was expressed as fold expression of that in control mice. $^bP < 0.01$.

For chronic alcohol studies, mice were pair-fed with regular or ethanol-containing Lieber De Carli liquid diets for 4 wk. HO-1 expression was significantly ($P < 0.01$) decreased (0.275 ± 0.05) in the brains of mice with chronic alcohol exposure compared to pair-fed controls (1 ± 0) (Figure 1A, lanes 1 and 2). In contrast, HO-1 expression in the liver was not altered (Figure 1A, lanes 3 and 4). However, HO-1 expression in the duodenum was significantly ($P < 0.01$) elevated (2.7 ± 1.2) with chronic alcohol exposure (Figure 1A, lanes 5 and 6). Interestingly, the expression of HO-2 was not significantly affected in the brains, liver or duodenum of mice with chronic alcohol exposure compared to control mice (Figure 1B, lanes 1-6).

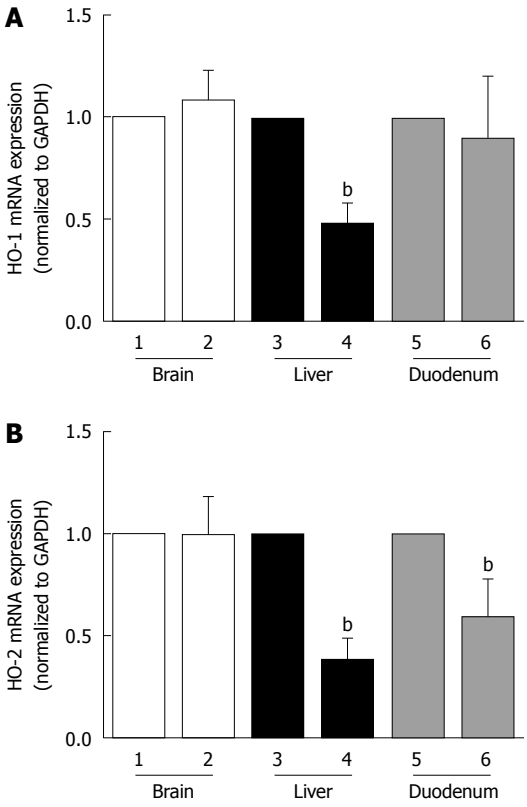

Figure 2 Effect of acute alcohol exposure on heme oxygenase expression. Heme oxygenase (HO)-1 (A) and HO-2 (B) mRNA expression in the brain (lanes 1 and 2), liver (lanes 3 and 4) and duodenum (lanes 5 and 6) of C57BL/6 wild-type mice fed with either plain water, as controls (lanes 1, 3 and 5) or 10% ethanol in the drinking water (lanes 2, 4 and 6) for 7 d was measured by real-time polymerase chain reaction. HO expression in alcohol-treated mice was expressed as fold expression of that in control mice. $^{b}P < 0.01$.

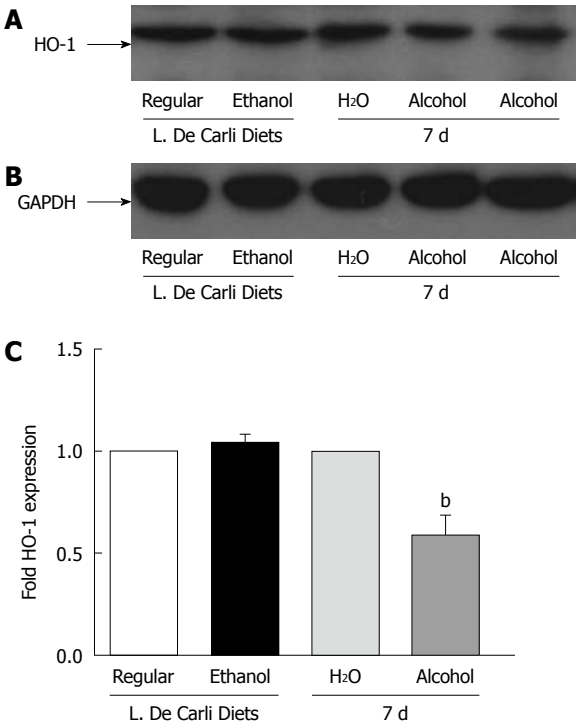

Figure 3 Effect of chronic and acute alcohol exposure on heme oxygenase-1 protein expression. Sixty micrograms of whole cell lysate proteins isolated from the livers of C57BL/6 wild-type mice either pair-fed with regular or ethanol-containing L. De Carli diets for 4 wk or fed with plain water or 10% ethanol (alcohol) for 7 d were resolved by SDS-PAGE. A: Heme oxygenase (HO)-1 expression was determined by western blotting; B: An anti-GAPDH antibody was used to confirm equal protein loading; C: Autoradiographs were scanned by a densitometer, and HO-1 expression in each sample was quantified by normalizing to GAPDH protein expression. Normalized HO-1 expression in alcohol-treated mice was expressed as fold expression of that in control mice. $^{b}P < 0.01$.

In order to study the effect of acute alcohol exposure, mice were exposed to 10% ethanol in the drinking water or plain water (control) for 7 d. Unlike chronic alcohol exposure, no significant change was observed in HO-1 expression in the brains and duodenums of mice with acute alcohol exposure (Figure 2A, lanes 1, 2, 5 and 6). On the other hand, the expression of HO-1 in the liver was significantly ($P < 0.01$) decreased (0.48 ± 0.1) by acute alcohol exposure compared to control mice fed with plain water (1 ± 0) (Figure 2A, lanes 3 and 4). Similar to HO-1, the expression of HO-2 in the liver was also significantly ($P < 0.01$) downregulated (0.39 ± 0.1) by acute alcohol exposure compared to control mice (1 ± 0) (Figure 2B, lanes 3 and 4). The expression of HO-2 in the duodenum was also significantly ($P < 0.01$) attenuated (0.6 ± 0.18) by acute alcohol exposure compared to the control mice (1 ± 0) (Figure 2B, lanes 5 and 6). Acute alcohol exposure did not affect the expression of HO-2 in the brain (Figure 2B, lanes 1 and 2). Similar to HO-1 mRNA expression, the level of HO-1 protein expression in the liver was downregulated by acute, but not chronic, alcohol exposure (Figure 3).

Alcohol also induces changes in mitochondria and the level of ROS, resulting in oxidative stress[43]. HOs are induced by oxidative stress. The transgenic mice, $Sod2^{+/-}$ were therefore included to study the role of mitochondria and $O_2^{\cdot-}$ accumulation in the regulation of HOs. $Sod2^{+/-}$ mice were fed with 10% ethanol for 7 d. HO-1 expression in the brains of $Sod2^{+/-}$ mice treated with alcohol was significantly ($P < 0.01$) decreased (0.4 ± 0.2) compared to $Sod2^{+/-}$ mice fed with plain water, as controls (1 ± 0) (Figure 4A, lanes 1 and 2). However, no significant alcohol-mediated changes were observed in HO-1 expression in the livers of $Sod2^{+/-}$ mice (Figure 4A, lanes 3 and 4). In contrast, the level of HO-1 expression in the duodenum was significantly ($P < 0.01$) increased (3.2 ± 1.16) in alcohol-treated $Sod2^{+/-}$ mice compared to control mice (1 ± 0) (Figure 4A, lanes 5 and 6). Unlike HO-1, alcohol did not cause any significant changes in HO-2 expression in the brains of $Sod2^{+/-}$ mice (Figure 4B, lanes 1 and 2). Similarly, no significant alcohol-mediated changes in HO-2 expression in the liver were observed (Figure 4B, lanes 3 and 4). Interestingly, in contrast to HO-1, the expression of HO-2 in the duodenum was attenuated (0.6 ± 0.3) in alcohol-fed $Sod2^{+/-}$ mice compared to control mice (1 ± 0) (Figure 4B, lanes 5 and 6). 8-hydroxyl-2'-deoxyguanosine (8-OHdG) is a biomarker of oxidative stress[44]. Immunostaining with an antibody that detects 8-OHdG indicated a moderate level of 8-OHdG generation in the livers of $Sod2^{+/-}$ mice compared to negative littermate control mice (Figure 5A and B).

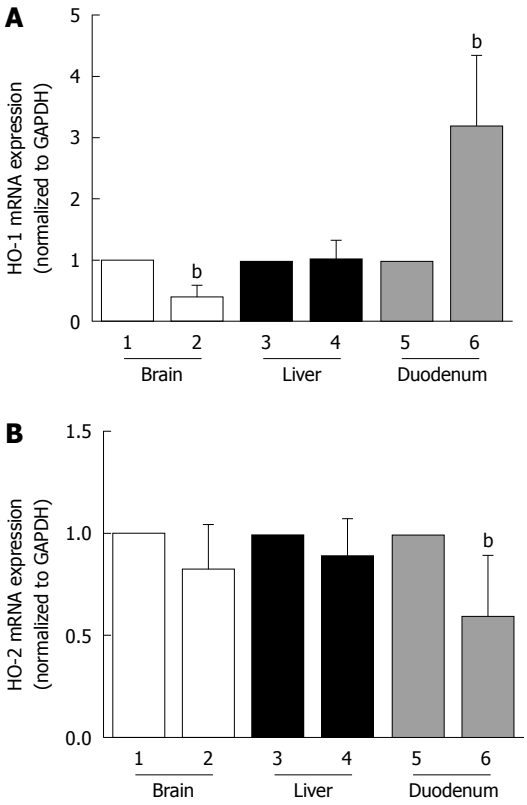

Figure 4 MnSOD knockout mice (Sod2$^{+/-}$) and heme oxygenase expression. Heme oxygenase (HO)-1 (A) and HO-2 (B) mRNA expression in the brain (lanes 1 and 2), liver (lanes 3 and 4) and duodenum (lanes 5 and 6) of Sod2$^{+/-}$ mice fed with plain water as control (lanes 1, 3 and 5) or 10% ethanol in drinking water (lanes 2, 4 and 6) for 7 d was measured by real-time polymerase chain reaction. HO expression in alcohol-treated Sod2$^{+/-}$ mice was expressed as fold expression of that in control Sod2$^{+/-}$ mice fed with plain water. $^{b}P < 0.01$.

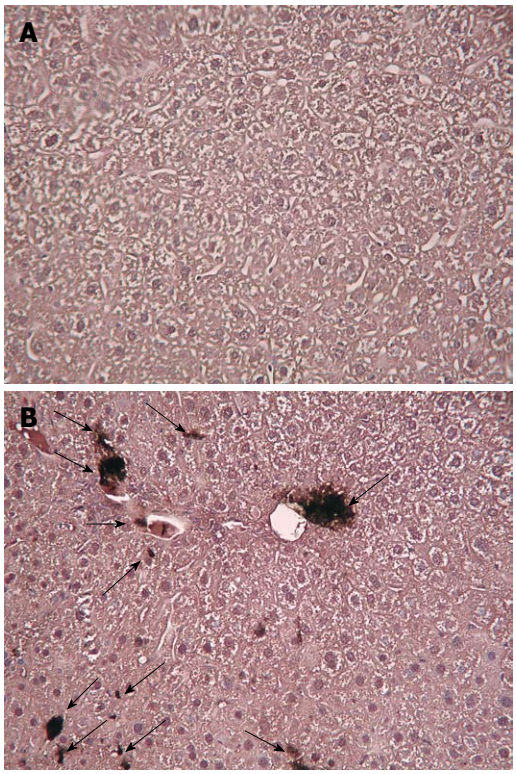

Figure 5 Oxidative stress in MnSOD knockout mice (Sod2$^{+/-}$). Immunoperoxidase staining of liver sections from negative littermate control (A) and Sod2$^{+/-}$ mice (B) by an anti-8-OHdG antibody. The arrows indicate examples of 8-OHdG-positive staining (original magnification 20 ×).

Hypoxia has been reported to alter HO expression[18,45-47]. Chronic alcohol consumption is well known to induce hypoxia in the liver[48]. We determined the effect of acute alcohol exposure on the expression of the transcription factor, HIF-1α, which is induced by hypoxia[49]. Mice exposed to 10% ethanol for 3 or 7 d displayed an increase in HIF-1α protein expression in the liver compared to control mice (Figure 6A-C). This increase was significant in mice treated with ethanol for 7 d (Figure 6C). Liver-specific ARNT heterozygous knockout mice (ARNT$^{+/-}$/Alb-Cre$^{+/-}$) were generated (see Methods). They were used to study the role of hypoxia in the regulation of HO expression by acute alcohol exposure in the liver. ARNT floxed mice served as the controls. ARNT floxed and ARNT$^{+/-}$/Alb-Cre$^{+/-}$ mice were both fed with water or 10% ethanol for 1 wk. Acute alcohol exposure significantly diminished HO-1 expression in ARNT floxed control mice (Figure 7A). Conversely, no significant difference in HO-1 expression was observed between water-fed and ethanol-fed ARNT$^{+/-}$/Alb-Cre$^{+/-}$ mice (Figure 7B).

DISCUSSION

HOs play an important role in the protection against inflammation, oxidative stress and tissue damage. Alcohol has been shown to induce the expression of ALAS-1, the rate limiting enzyme in heme synthesis in mitochondria[34,35,50]. However, the effect of alcohol on HO-1 and HO-2 is still unclear. The reports in the literature are inconclusive[34,35]. We therefore studied the effect of both acute and chronic alcohol exposure on HO expression in different organs, which are known to be affected by alcohol metabolism. Alcohol is also well known to cause hypoxia, alter mitochondrial function and induce changes in the steady state levels of ROS[43]. The antioxidant enzyme Sod catalyzes the conversion of $O_2^{\cdot-}$ into H_2O_2[51]. There are different forms of Sod and Sod2 (MnSOD) is located in the mitochondrial matrix[36]. Homozygous Sod2 knockout mice display a neonatal lethal phenotype but heterozygous mice are viable and fertile[36]. We therefore used heterozygous Sod2 mice in these studies. ARNT, which is the β subunit (HIF-1β) of the multimeric HIF-1 transcriptional complex, is essential for hypoxic gene regulation[52,53]. Liver-specific ARNT knockout mice were used to study the regulation of HOs by alcohol-induced hypoxia.

We did not observe any changes in the expression of either HO-1 or HO-2 in the livers of mice with chronic alcohol exposure. In contrast, Okuno *et al*[35] have reported an increase in HO-1 expression in rats fed with ethanol-containing L. De Carli liquid diets for 5 wk. Similar to our findings, Zheng *et al*[34] did not observe any changes in HO-1 expression in rats fed with ethanol-

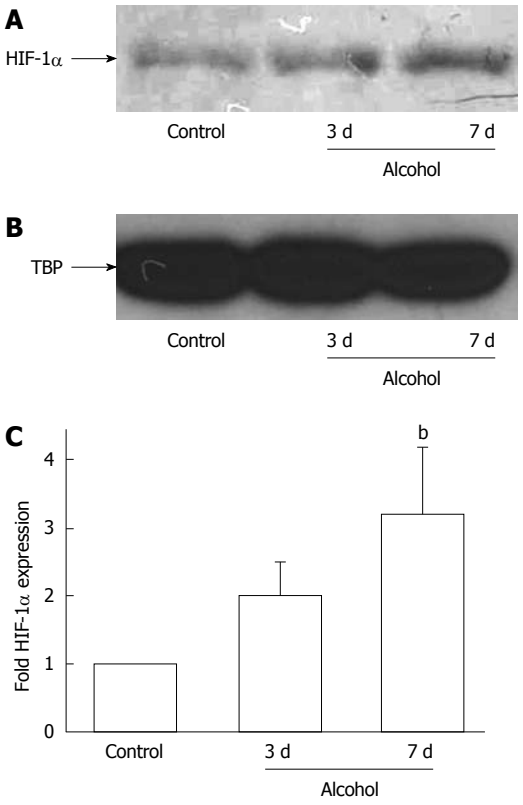

Figure 6 Alcohol and hypoxia inducible factor-1α. Twenty micrograms of nuclear proteins isolated from the livers of C57BL/6 wild-type mice fed with plain water (control) or 10% ethanol for 3 or 7 d were resolved by SDS-PAGE. A: Hypoxia inducible factor (HIF)-1α expression was determined by western blotting; B: An anti-TATA-binding protein (TBP) antibody was used to confirm equal protein loading; C: Autoradiographs were scanned by a densitometer, and HIF-1α expression in each sample was quantified by normalizing to TBP protein expression. Normalized HIF-1α expression in alcohol-treated mice was expressed as fold expression of that in water-fed control mice. $^{b}P < 0.01$.

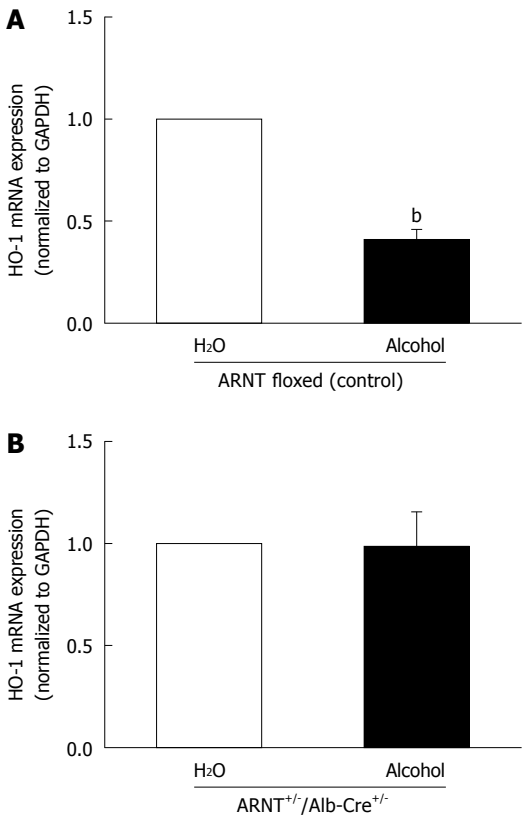

Figure 7 Heme oxygenase expression in ARNT (hypoxia inducible factor-1β) knockout mice. Heme oxygenase (HO)-1 mRNA expression in the livers of (A) ARNT floxed control mice and (B) liver-specific ARNT heterozygous knockout mice (ARNT$^{+/-}$/Alb-Cre$^{+/-}$), fed with plain water or 10% ethanol in drinking water for 7 d was measured by real-time polymerase chain reaction. HO expression in alcohol-treated mice was expressed as fold expression of that in mice fed with plain water. $^{b}P < 0.01$.

containing Lieber De Carli diets for 10 wk. The reasons for these discrepancies are unclear but the differences in experimental design may have played a role. Interestingly, acute alcohol exposure significantly attenuated the expression of both HO-1 and HO-2 in the liver (Figures 1 and 2). These findings show for the first time that acute, but not chronic alcohol exposure can alter the expression of HOs in the liver. Chronic alcohol exposure is known to induce oxidative stress in the liver[43]. We have also reported that acute alcohol exposure can similarly elevate ROS levels and induce oxidative stress in the liver *in vivo*[38]. However, the acute alcohol-induced decrease in HO-1 and HO-2 expression in the liver is probably not due to oxidative stress. This is supported by our findings showing no changes in HO expression in the livers of Sod2$^{+/-}$ mice with acute alcohol exposure. Of note, Sod2$^{+/-}$ mice express only about 50% of MnSOD activity and therefore accumulate $O_2^{\cdot-}$ in mitochondria, and display oxidative stress in several organs[36]. Accordingly, the livers of Sod2$^{+/-}$ mice exhibited 8-OHdG generation, which is indicative of oxidative stress (Figure 5A)[44]. The 8-OHdG-positive areas in the liver of Sod2$^{+/-}$ mice were significant because they were not detected in the livers of control littermate mice (Figure 5A and B).

Hypoxia has been shown to reduce the expression of HOs in human liver cell lines[18]. Chronic alcohol exposure is also known to induce hypoxia in the liver[48]. Here, we also showed the induction of the hypoxia-inducible transcription factor HIF-1α in the livers of mice with acute alcohol exposure (Figure 6). Hypoxia could therefore be a potential mechanism by which acute alcohol suppresses HO expression in the liver. Our findings with liver-specific ARNT knockout mice confirm that acute alcohol-induced hypoxia is involved in the regulation of liver HO expression (Figure 7). Our findings with liver-specific ARNT knockout mice also indicated that the absence of HIF-1β expression on one allele is sufficient to abolish the effect of alcohol on liver HO expression. These findings are significant because ARNT floxed control mice, which express HIF-1β on both alleles, displayed a significant decrease in liver HO expression following alcohol exposure (Figure 7A).

HO-1 has been shown to be expressed in Kupffer cells of the rat liver[54]. Activation of Kupffer cells by endotoxin and the release of proinflammatory cytokines, particularly tumor necrosis factor (TNF) α, from Kupffer cells play a crucial role in the onset of alcoholic liver injury. Accordingly, HO-1 has been postulated to attenuate TNFα production, and the induction of HO-1 protects

the liver from alcohol-induced inflammatory processes[29,30]. HO-2, on the other hand, has been shown to be expressed in the parenchymal cells of the liver[54]. The release of CO, which originates from heme catabolism, from parenchymal cells into the extrasinusoidal space relaxes the microvascular tone[54]. The decrease in HO-2 expression may therefore contribute to hypoxia observed with acute alcohol exposure (Figure 2B).

Our findings therefore strongly suggest that the inhibition of HO-1 and HO-2 expression in the liver by acute alcohol may be involved in the activation of Kupffer cells, the release of proinflammatory cytokines, and hypoxia, all of which are well known to prime the liver for the onset of alcoholic liver injury. Furthermore, our findings showing that chronic alcohol exposure does not alter liver HO-1 and HO-2 expression also suggest that the changes occurring in the later stages of alcoholic liver disease progression may not involve HOs or heme catabolism by-products.

Besides the liver, alcohol also induces inflammation and oxidative stress in the brain[55,56]. In contrast to the liver, the brains of wild-type mice with chronic alcohol exposure exhibited a significant decrease in HO-1 expression, which was not observed in mice with acute alcohol exposure. Brain expresses high amounts of HO-2 but acute or chronic alcohol exposure did not alter brain HO-2 expression. However, HO-1 has been reported to protect hippocampal neurons from ethanol-induced neurotoxicity[57]. Furthermore, the astrocyte vulnerability in the brain induced by the deletion of HO-2 has been shown to be rescued by adenoviral delivery of HO-1[58]. Our findings therefore suggest that the inhibition of HO-1 expression may be one of the mechanisms by which chronic alcohol exposure induces injury in the brain. Interestingly, similar to wild-type mice with chronic alcohol exposure, we have also observed a significant decrease in brain HO-1, but not HO-2 expression in $Sod2^{+/-}$ mice with acute alcohol exposure. Based on these correlations, it is possible that the increase in ROS originating from mitochondria may be responsible for the inhibition of HO-1 expression in the brains of mice with chronic alcohol exposure.

Alcohol can alter intestinal permeability, releasing endotoxins into the circulation and also reduce intestinal motility[59,60]. Heme metabolism is also involved in intestinal defense mechanisms and motility[61]. Interestingly, wild-type mice with chronic alcohol exposure and $Sod2^{+/-}$ mice with acute alcohol exposure displayed a significant increase in HO-1 expression in the duodenum. In contrast, duodenal HO-2 expression was decreased in both wild-type and $Sod2^{+/-}$ mice with acute alcohol exposure. These findings therefore suggest that the expression of HO-1 and HO-2 in the duodenum is regulated, in an opposite manner, by alcohol-induced oxidative stress. The increase in HO-1 expression may be part of the anti-inflammatory defense mechanisms against alcohol injury. HO-2 is expressed mainly in the myenteric plexus of the gut and the interstitial cells of Cajal, thus, the decrease in HO-2 expression may play a role in the alcohol-mediated decrease in intestinal motility.

Collectively, our data suggest that acute and chronic alcohol exposure regulates HO-1 and HO-2 expression differently in the brain, liver and duodenum. Mitochondria and oxidative stress may be involved in chronic alcohol-induced changes in HO-1 expression in the brain and duodenum, but not in the liver. On the other hand, the changes in liver HO expression are mainly regulated by acute alcohol exposure. Hypoxia induced by acute alcohol exposure might be one of the underlying mechanisms in this process. These findings may further our understanding of the mechanisms involved in alcohol-induced injury in different tissues *in vivo*.

COMMENTS

Background

Excessive alcohol consumption impairs the function of multiple organs including the liver, intestine and brain. The precise mechanisms of alcohol-induced tissue injury are unclear. Endotoxin, inflammatory cytokines and oxidative stress have been shown to be involved. Patients with alcoholic liver disease frequently display evidence of iron overload. Alcohol also modulates heme metabolism. It activates aminolevulinic acid synthase-1, the rate-limiting enzyme in heme synthesis in mitochondria. Heme oxygenases (HOs) catalyze the oxidative degradation of heme. The effect of alcohol on HOs is not well understood. The reports in the literature are contradictory and the studies have been mainly performed on the liver. This study investigated the role of both acute and chronic alcohol exposure in the regulation of HOs in the liver, duodenum and brain. The involvement of alcohol-mediated oxidative stress and hypoxia was also studied.

Research frontiers

Alcohol causes hypoxia, oxidative stress and inflammation in various organs. HOs exert cytoprotective and anti-inflammatory effects. Activation of Kupffer cells by endotoxin and the release of proinflammatory cytokines, particularly tumor necrosis factor α, from Kupffer cells play a crucial role in the onset of alcoholic liver injury. Induction of HOs has been shown to prevent alcohol-induced inflammation in the liver. They are also believed to play a protective role in fatty liver. Alcohol reduces the motility and increases permeability in the gastrointestinal tract. The activation of Kupffer cells by alcohol is mainly caused by endotoxins derived from the gastrointestinal tract. Heme metabolism is involved in intestinal defense mechanisms and motility. Interestingly, our findings demonstrate that acute alcohol exposure inhibits HO expression in the liver and duodenum. The impairment of heme metabolism may therefore be involved in the crosstalk between the liver and intestine in alcoholic liver disease.

Innovations and breakthroughs

This study is one of the first in the literature to compare the role of both acute and chronic alcohol exposure on HO expression in the liver, duodenum and brain, which is affected by alcohol. Taken together, our findings demonstrate that acute and chronic alcohol exposure regulates HO expression in a tissue-specific manner. Chronic alcohol exposure altered HO expression in the brain and duodenum, but not in the liver. In contrast, acute alcohol exposure inhibited HO expression in the liver and duodenum, but not in the brain. Our findings also suggest that hypoxia may be involved in the inhibition of HOs by acute alcohol exposure in the liver. The inhibition of HOs and their cytoprotective, anti-inflammatory action may be one of the initiating factors of alcohol-mediated tissue injury.

Applications

Understanding the mechanisms underlying the interaction of heme metabolism and alcohol is clinically relevant and may help with the development of new therapeutic and diagnostic agents for alcohol-related organ damage.

Peer review

This paper is technically sound and well-written. It is also a well-motivated investigation.

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Events Calendar 2011

January 19-20,
BioBusiness
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January 27-28
Predictive Human Toxicity and ADME/Tox Studies 2011
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January 29-February 2
LabAutomation 2011
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February 1-2
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February 6-8
5th Drug Discovery for Neurodegeneration
San Diego, United States

February 7-10
3rd International Conference and Exhibition on Drug Discovery and Therapy
Dubai, United Arab Emirates

February 13-16
Natural Products Conference 2011
Sharm el Sheikh, Egypt

February 14-17
Therapeutic Approaches to Neurodegeneration - Age Modifiers, Proteostasis, and Stem Cells
Nassau, Bahamas

February 16-19
Electrochemistry Conference 2011
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February 21-23
World Antibody Drug Conjugate Summit Frankfurt, Germany

February 22-24
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February 22-25
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International Conference on Bioscience, Biotechnology, and Biochemistry
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February 26-28
2011 International Conference on Bioscience, Biochemistry and Bioinformatics
Sentaosa, Singapore

March 4
Discussion Workshop: Perfecting the ELISPOT - a time for answers
London, United Kingdom

March 4-11
Inorganic Reaction Mechanisms
Gordon Research Conferences
Galveston, United States

March 7-8
Fragments 2011 - Third RSC-BMCS Fragment-based Drug Discovery meeting
Stevenage, United Kingdom

March 9-13
10th International Conference on Alzheimers and Parkinsons Diseases
Barcelona, Spain

March 13-18
Pittcon 2011
Atlanta, United States

March 17-20
EMBO|EMBL Symposia: Seeing is Believing - Imaging the Processes of Life
Heidelberg, Germany

March 20-22
The molecular biology of inflammatory bowel diseases
Durham, United Kingdom

March 21-23
World Congress on Biotechnology
Hyderabad, India

March 23-25
BIT's 4th Annual Protein and Peptide Conference
Beijing, China

March 25-27
2011 3rd International Conference on Bioinformatics and Biomedical Technology 3rd round call for paper
Sanya, China

March 27-April 2
EMBO Practical Course - Methods in Chemical Biology
Heidelberg, Germany

April 6-8
Faraday Discussion 150: Frontiers in Spectroscopy
Basel, United States

April 6-8
Membrane Proteins: Structure and Function
Oxford, United Kingdom

April 11-12
7th SCI-RSC symposium on Proteinase Inhibitor Design
Basel, United States

April 11-14
First EuCheMS Inorganic Chemistry Conference (EICC-1)
Manchester, United Kingdom

April 18-19
Analysis of free radicals, radical modifications and redox signalling
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2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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Write as mean ± SD or mean ± SE.

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