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Differential Alterations of Endotoxin-induced Cytokine Expression and Mitogen-activated Protein Kinase Activation by Mercury in Mouse Kidney

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ABSTRACT. The present study was designed to determine the impact of mercury on endotoxin-induced inflammatory cytokine expression and corresponding signal transduction in mouse kidney. Male BALB/c mice were exposed continuously to 0, 0.3, 1.5, 7.5, or 37.5 ppm of mercury in drinking water for 14 days and at the end of the treatment period, lipopolysaccharide (LPS, 0.5 mg/kg) was injected intraperitoneally 2 h prior to euthanasia. The doses of mercury and LPS did not cause hepatotoxicity or renal toxicity as indicated by unaltered plasma alanine aminotransferase and aspartate aminotransferase levels, and terminal UTP nucleotide end-labeling assay from kidney, respectively. Mercury decreased kidney glutathione (GSH) and with LPS, it additively decreased GSH. Mercury activated p38 mitogen-activated protein kinase (MAPK) and additively increased LPS-induced p38 MAPK phosphorylation. In contrast, mercury inhibited LPS-induced activation of extracellular signal-regulated kinase (ERK) but had no effect alone. Mercury increased the gene expression of tumor necrosis factor α (TNFα) and potentiated LPS-induced TNFα expression. Mercury did not affect LPS-induced interleukin-1β (IL-1β) expression but decreased LPS-induced IL-6 expression. These results suggest that low levels of mercury might augment LPS-induced TNFα expression by altering GSH and p38 MAPK. Mercury modulates LPS-induced p38 and ERK activation, and downstream TNFα and IL-6 expression in kidney, respectively.

Keywords: Mercury, Lipopolysaccharide, Glutathione, Mitogen-activated protein kinase, Tumor necrosis factor α, Inflammation.

INTRODUCTION

Mercury is a widespread environmental contaminant and chronic exposure to low levels of mercury is common due to contamination of food and drinking water. The kidneys are the primary target organ where inorganic mercury is taken up, accumulated, and expresses toxicity. Two major intracellular thiols, glutathione and metallothionein (MT), appear to be important in regulating the renal accumulation of mercury and mercury-induced renal injury. Because of the high content of MT, the kidney especially the proximal tubule is the most vulnerable segment to the toxic effects of mercury (Zalups, 2000). It is known that immune response initiating kidney damage is caused partly by the activation of local cytokine network, as the kidney has a complete innate immune system (Tchounwou *et al.*, 2003). Acute exposure to mercuric chloride decrease sulfhydryl content, and causes renal damage by binding sulfhydryl groups (Farina *et al.*, 2003). The consequence of mercury of binding to sulfydryl groups is alteration in cell signaling. Mercury depletes cellular glutathoine (GSH) content and causes cellular and tissue damage (Shenker *et al.*, 1993; Zalups *et al.*, 1999). The GSH has an important role in the reduction of reactive oxygen species (ROS) and maintaining intracellular redox equilibrium (Gilot *et al.*, 2002). Modified redox signaling

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by lipopolysaccharide (LPS) and metals regulates mitogen-activated protein kinase (MAPK)-activation and MAPK-mediated proinflammatory cytokines such as tumor necrosis factor α (TNFα), interleukin (IL)-1β, and IL-6 (Haddad, 2002; Koropatnick and Zalups, 1997). p38 and extracellular signal-regulated kinase (ERK) mediate responses to cellular stress such as endotoxin and inflammatory signals (Lee *et al.,* 1994; Lee and Young, 1996).

We previously reported that non-cytotoxic concentrations of mercury altered macrophages response to bacterial infection (Kim *et al.,* 2002). Mercury potentiated LPS-induced TNFα expression by regulating p38 MAPK. Excess induction of TNFα causes organ injury. However, the mechanism of mercury-induced proinflammatory cytokine production, especially of TNFα, has not been well characterized in kidney.

Systemic exposure to LPS results in a cascade of events involving cellular and soluble mediators of inflammation that leads to injury to organs. Endotoxin promotes the activation of immune cells which are important sensors of infections (Gordon, 1998). Human exposure to low levels of mercury or LPS is commonplace and occurs through numerous modes. The degree to that inflammatory cascades are activated during low levels of exposures may not be sufficient to cause organ injury. However, non-toxic and non-injurious doses of LPS augment toxicity of certain xenobiotic agents such as alcohol and metals (Hewett and Roth, 1993). Exposures to small amounts of LPS that do not normally evoke overt organ damage are nevertheless capable of initiating aspects of the inflammatory response. We therefore hypothesized that low doses of mercury, a known immunotoxic agent, may alter acute and low levels of LPS-mediated immune signaling in the kidney. Results of this study revealed that mercury and LPS additively decreased GSH content in kidney, accompanied by alteration of MAPK-mediated inflammatory cytokine expression. Mercury differentially modulated LPS-mediated MAPKs and inflammatory cytokine expression.

MATERIALS AND METHODS

Animal care and handling

Inbred male BALB/c mice (specific pathogen free, Harlan Inc., Indianapolis, IN, USA), 6 weeks of age with an average body weight of 20 g were procured. Mice were randomly assigned to treatment groups (four per cage) and acclimated for 1 week in the housing facility maintained at 21°C with a 12 h light/dark cycle. The mice were housed in polycarbonate shoe box-style cages lined with wood chip bedding (Betachip, Northeastern Products Corporation, Warrensberg, NY, USA) which was changed every third day. Rodent chow, Harlan Teklad 22/5 rodent chow (Harlan Teklad, Madison, WI, USA) and water were supplied *ad libitum.* Food and water consumption as well as body weight gain were recorded daily. The care and treatment of the mice were in accordance with the guidelines established by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (University of Georgia, Athens, GA, USA).

Treatment

Mercuric chloride (Sigma, St. Louis, MO, USA) was administered in the drinking water ranging 0, 0.3, 1.5, 7.5, and 37.5 ppm as mercury. Mice were continuously fed water supplemented with freshly prepared mercury solution, which was changed every other day for 14 days. At the end of the treatment period, mice were fasted overnight and 0.5 mg/kg of LPS (derived from *E. coli* serotype 026 : B6, Sigma) was intraperitoneally injected 2 h prior to euthanasia. Mice were decapitated, trunk blood was collected, and kidney was aseptically excised and weighed.

Glutathione assay

Levels of GSH in kidney were determined by measuring the total reduced GSH content in the kidney as described (Baker *et al.,* 1990). The tissue was homogenized in 5 vol of 5% of sulfosalicylic acid (SSA) to precipitate macromolecules and extract GSH from tissues and centrifuged for 15 min at 12,000 × g. The GSH was detected by the color change associated with 5,5-dithiobis-(2-nitrobenzoic acid) reduction using PowerWave$_x$ Microplate Scanning spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA) at 405 nm. The GSH concentration was calculated by GSH standard calibration and expressed as GSH equivalents per gram of kidney tissue.

Western blot analysis

Kidney samples were homogenized in lysis buffer (20 mM Tris, 137 mM NaCl, 2 mM EDTA, 10% glycerol, 1% Triton X-100, 1 mM Na_3VO_3, 100 μM DTT, 100 mM PMSF, 100 μg/ml leupeptin, 10 μg/ml aprotinin) and centrifuged for 10 min at 12,000 × g. After homogenizing, an aliquot containing 30 μg of protein was precipitated with cold acetone at 80°C for 1 h. The precipitated proteins were electrophoretically separated using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to nitrocellulose

Table 1. Primers and PCR conditions for gene expression analyzed in the kidney following exposure to mercury and LPS

	Primers		Annealing temperature (°C)	# of cycles
[a]TNFα	Sense	5'CTCTTCAAGGGACAAGGCTG3'	55	27
	Anti-sense	5'CGGACTCCGCAAAGTCTAAG3'		
IL-1β	Sense	5'GCAACTGTTCCTGAACTCA3'	55	28
	Anti-sense	5'CTCGGAGCCTGTAGTGCAG3'		
IL-6	Sense	5'TTCCATCCAGTTGCCTTCTT3'	53	32
	Anti-sense	5'CAGAATTGCCATTGCACAAC3'		
β-actin	Sense	5'ATGGATGACGATATCGCT3'	48	29
	Anti-sense	5'ATGAGGTAGTCTGTCAGGT3'		

[a]Selected by Primer3 program (Whithead Institute, Cambridge, MA).

Table 2. Exposure to mercury and water and food consumption

Mercury in water (ppm)	LPS	Dose of mercury (mg/kg/day)[a]	Water consumption (ml/day/group)	Food consumption (g/day/group)
0	-	0	20.9 ± 1.16	15.0 ± 0.40
37.5	-	4.91	11.6 ± 1.26*	11.8 ± 0.45
0	+	0	20.7 ± 1.33	14.9 ± 0.46
0.3	+	0.06	21.0 ± 1.38	15.2 ± 0.97
1.5	+	0.35	21.6 ± 1.32	14.5 ± 0.67
7.5	+	1.66	20.2 ± 1.45	15.8 ± 0.74
37.5	+	5.28	12.7 ± 1.04*	12.8 ± 0.45*

Mean ± SE (n=4).
[a]Calculated dose based on water consumption.
*Significantly different from the control group (no treatment) at $P<0.05$.

membrane as previously described (Kim *et al.,* 2002). The activation of p38 and ERK was assayed using (1:1000) anti-phospho-p38, anti-p38, anti-phospho-ERK, and anti-ERK antibody (Cell Signaling, Beverly, MA, USA). Immunodetection was performed using enhanced chemiluminescence (ECL) detection kit (Amersham Pharmacia, Piscataway, NJ, USA).

Semiquantitative analysis of gene expression

Total RNA was isolated from the tissue using the protocol described earlier (Johnson and Sharma, 2001). First strand complimentary DNA (cDNA) was synthesized using Superscript II reverse transcriptase enzyme (Life Technologies, Grand Island, NY, USA). Reverse-transcriptase polymerase chain reaction (RT-PCR) was used to analyze the expression of mRNA for TNFα, IL-1β, IL-6, and β-actin (internal control). The condition for reverse transcription and PCR steps was as previously reported (Kim *et al.,* 2004). The respective primer sets were chosen by Primer3 program (Whithead Institute, Cambridge, MA, USA) and are shown in Table 1. Cycle number was optimized to ensure product accumulation in an exponential increase. Amplified products were separated by electrophoresis on 2% agarose gel and documented using a Kodak DC 290 digital camera and digitized using UN-SCAN-IT software (Silk Scientific, Orem, UT, USA). Band intensities for the genes of interest were normalized to that of β-actin in the same sample.

Table 3. The effect of oral mercury supplementation and LPS injection on body weight gain, kidney weights in male BALB/c mice

Mercury in water (ppm)	LPS	Body weight gain, g (% change from initial body weight)	Kidney/body weight ratio (g/100 g)
0	-	2.35 ± 0.25 (11.3)	1.65 ± 0.02
37.5	-	0.58 ± 0.13 (2.1)*	2.05 ± 0.06*
0	+	2.52 ± 0.19 (12.2)	1.66 ± 0.03
0.3	+	1.80 ± 0.38 (7.2)	1.62 ± 0.06
1.5	+	2.32 ± 0.31 (10.6)	1.64 ± 0.07
7.5	+	2.01 ± 0.30 (9.3)	1.83 ± 0.03*
37.5	+	0.70 ± 0.29 (3.2)*	2.10 ± 0.05*

Mercury treated in drinking water for 14 days and LPS (0.5 mg/kg) was injected intraperitoneally 2 h prior to euthanasia the mice.
Mean ± SE (n=4).
*Significantly different from the control (no treatment) at $P<0.05$.

Statistical analysis

All statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC, USA). Treatment effects were analyzed using one way analysis of variance (ANOVA) followed by a post-hoc Duncan's Multiple Range test. A value of $P<0.05$ was used to

indicate significant differences.

RESULTS

To determine the subtoxic doses of mercury and LPS, we first examined the dose-dependent effects. Administration of 0.3, 1.5, 7.5, and 37.5 ppm of mercury for 2 weeks and 0.5 mg/kg of LPS for 2 h did not induce liver toxicity as indicated by plasma ALT and AST increase (data not shown). To determine whether these amounts of mercury and LPS cause renal apoptosis, we employed terminal UTP nucleotide end-labeling (TUNEL) assay from fixed kidney tissue resulted in no renal apoptosis (data not shown). Mice treated with the highest dose (37.5 ppm) of mercury for 2 weeks exhibited significantly smaller body weight gain compared to the control group. However, the relative kidney weights (organ/body weight ratio) were significantly increased in mice exposed to mercury at 7.5 and 37.5 ppm, which may have been due to a decrease in body weights.

Administration of 37.5 ppm of mercury for 2 weeks to mice decreased the renal GSH level to similar extent as 0.5 mg/kg of LPS in 2 h. Mercury further increased the LPS-induced renal GSH depletion (Fig. 1).

Treatment of mice with LPS increased phosphorylation of p38 and ERK in kidney (Fig. 2). Treatment of 37.5 ppm of mercury for 2 weeks increased the phosphorylation of p38 in mice kidney. Mercury additively increased LPS-induced p38 activation. Treatment of 37.5 ppm of mercury itself had no effect on the phosphorylation of ERK; in contrast, mercury from 1.5 to 37.5 ppm decreased LPS-induced ERK activation.

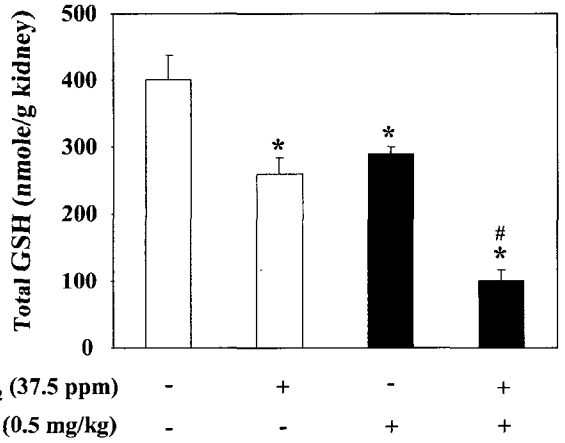

Fig. 1. Renal GSH levels in mice exposed to mercuric chloride and LPS (0.5 mg/kg, intraperitoneally). Male BALB/c mice were treated with 37.5 ppm of mercury in the drinking water for 14 days. Animals were sacrificed 2 h after LPS injection to measure renal GSH levels. Results are expressed as mean ± SE (n=4). *Significantly different from the control group at $P<0.05$. #Significantly different from the LPS alone group at $P<0.05$.

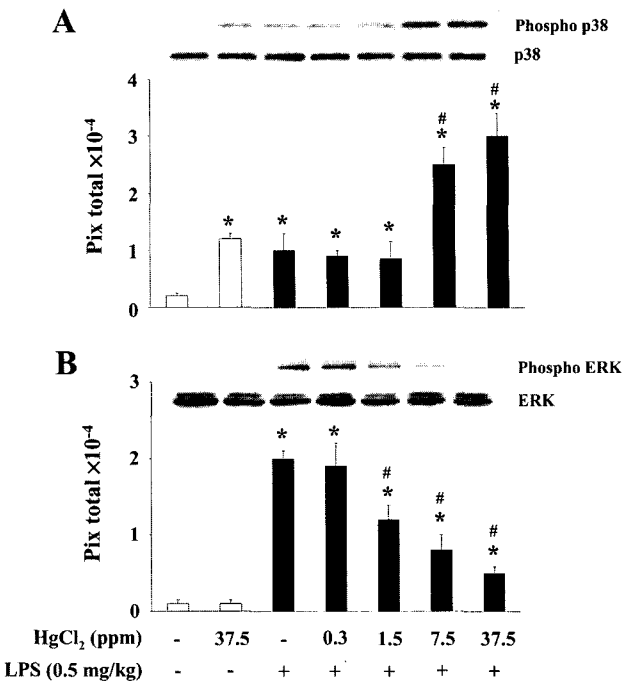

Fig. 2. Renal p38 and ERK MAPK activation in mice exposed to mercuric chloride (drinking water) and LPS (0.5 mg/kg, intraperitoneally). Male BALB/c mice were treated with 0.3, 1.5, 7.5, 37.5 ppm of mercury in the drinking water for 14 days. Animals were sacrificed 2 h after LPS injection. Protein (30 μg) was analyzed by 12% SDS-PAGE, and p38 (A) and ERK (B) MAPK was visualized by western blot analysis. Results are expressed as mean ± SE (n=4). *Significantly different form the control group at $P<0.05$. #Significantly different from the LPS alone group at $P<0.05$. Inserts, representative western blots in the same order.

LPS increased the mRNA expression of all three types of proinflammatory cytokines, i.e., TNFα, IL-1β, and IL-6 (Fig. 3). Mercury at 37.5 ppm increased TNFα expression. Mercury at 7.5 and 37.5 ppm with LPS additively increased TNFα expression. Mercury at 37.5 ppm did not alter IL-6 expression but mercury at 7.5 and 37.5 ppm decreased LPS-induced IL-6 expression. Mercury had no effect on IL-1β expression.

DISCUSSION

Our hypothesis in this study was that the low levels of mercury exposure exacerbate the cellular signaling in kidney elicited by a subtoxic dose of LPS. Results from our study demonstrated that oral exposure to mercury altered inflammatory cytokine expression during endotoxin treatment. The outcome of exposure to large doses of LPS is an inflammatory response in the host

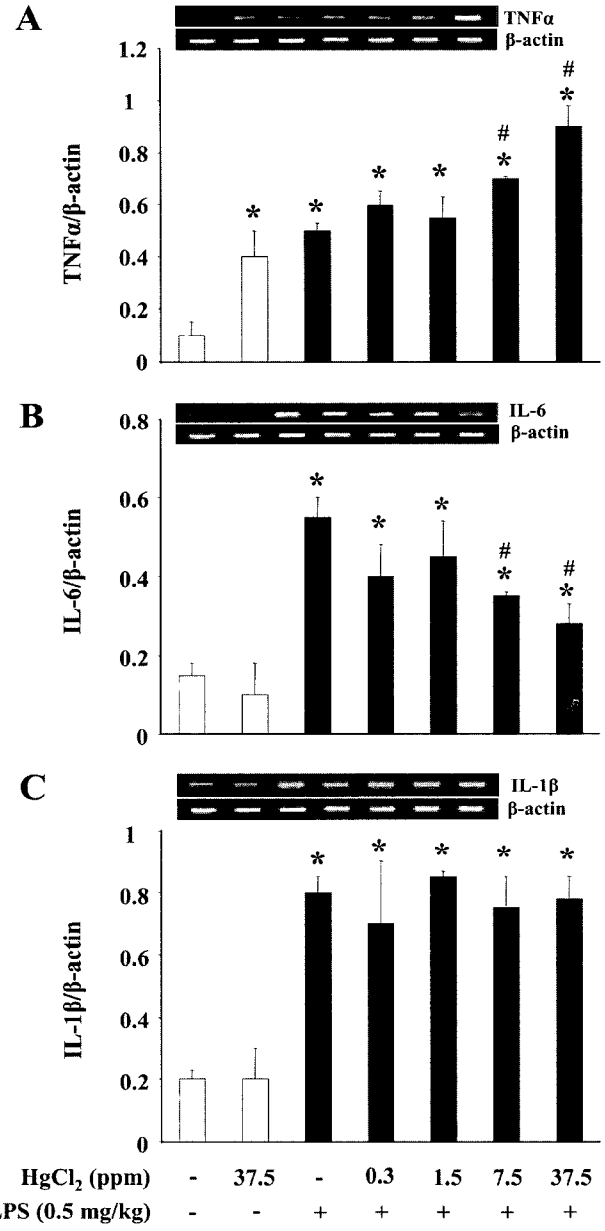

Fig. 3. Renal TNFα, IL-6, and IL-1β mRNA in mice exposed to mercuric chloride (drinking water) and LPS (0.5 mg/kg, intraperitoneally). Male BALB/c mice were treated with 0.3, 1.5, 7.5, 37.5 ppm of mercury in the drinking water for 14 days. Animals were sacrificed 2 h after LPS injection. Extraction and analysis of mRNA performed as described in Materials and methods. TNFα (A), IL-6 (B), and IL-1β (C) mRNA levels were quantified by reverse-transcriptase polymerase chain reaction and normalized against â-actin. Results are expressed as mean ± SE (n=4). *Significantly different than the control group at $P<0.05$. #Significantly different from the LPS alone group at $P<0.05$. Inserts, representative gel picture in the same order.

that results in self-destruction, which is a chain of inflammatory events initiating the elaboration of a cascade of secondary mediators that amplify the response to the initial insult (Ghosh *et al.,* 1993). The doses of mercury and LPS used did not show liver or kidney injury in short term treatment. However, highest dose of mercury treatment increased the relative kidney weight compare to the control. These data suggest that the dose of mercury we used did not cause liver or kidney injury in short term treatment but may cause an initiation of inflammation.

Mercury has been known to decrease cellular GSH content (Shenker *et al.,* 1993). However, contradictory data have been reported using in vivo experiments; mercury increased GSH content in kidney suggesting that the antioxidant potential was enhanced (Hussain *et al.,* 1999), mercury decreased GSH content and GPx activity in kidney (Goering *et al.,* 2002). In our present data, treatment of mercury decreased GSH content in kidney. We do not know the reason for the difference at this time; however, it may be because of the differences of experimental environment and susceptibility of animal species employed. GSH, an antioxidant and buffering agent of oxidative stress, is responsible for the diverse properties including regulation of the activation of redox-sensitive transcription factors such as MAPKs (Haddad, 2002; Ueda *et al.,* 2002). The MAPKs p38 and ERK are thought to be necessary for optimal cytokine gene expression in LPS-stimulated cell and tissue, and the MAPK pathways play a critical role in the inflammatory response. The stress-induced MAPK, p38, is one of the most important members of the family in control of inflammatory responses. ERK is thought to have an important role in proliferation, transformation, and differentiation (Arbabi and Maier, 2002). Numerous reports show that MAPKs regulate transcription of inflammatory cytokines, although the regulation of MAPKs on cytokine expression is controversial (Arbabi and Maier, 2002; Dong *et al.,* 2002). Endotoxin activates both p38 and ERK, but the end points of signaling cascade are different. It has been reported that while p38 plays an essential role in the NO synthesis, ERK plays a minor role. While p38 promotes induction of IL-12, ERK suppresses LPS-mediated IL-12 transcription in mouse macrophages (Feng *et al.,* 1999). Activation of ERK is not essential for LPS-induced NO and IL-1β production (Chen and Wang, 1999; Watters *et al.,* 2002). It may imply that p38 rather than ERK is important in the control of inflammatory responses. Our results showed that mercury activated p38 MAPK and with LPS additively activated LPS-induced p38 MAPK (Fig. 2). Mercury alone did not alter the activation of ERK; instead, mercury decreased LPS-induced ERK phosphorylation. These results strongly suggest that

p38 but not ERK is responsible for the augmentation of mercury and LPS-mediated proinflammatory cytokine expression. Our data show that mercury increased TNFá expression, and with LPS additively increased TNFα mRNA (Fig. 3A). These data are consistent with our previous report (Kim *et al.*, 2002) that showed the effect of mercury on LPS-induced p38 activation and downstream TNFα transcription in macrophage cells. Our data may suggest that p38 and TNFα are involved in the major pathway to mercury-induced inflammatory response in kidney.

Another important outcome of this study is the reduction of LPS-induced ERK and IL-6 expression by mercury. It has been reported that ERK regulates IL-6 signaling in hepatocytes (Nguyen and Gao, 1999). Additionally, we previously reported that inhibition of ERK using specific ERK inhibitor decreases LPS-induced IL-6 expression in macrophages (Kim *et al.*, 2004). The dose-responses of mercury between ERK activation and IL-6 expression from our data may imply that ERK regulates IL-6 transcription in mouse kidney. The effect of mercury on both TNFα and IL-6 suggests that in addition to increasing cytotoxic cytokine TNFα, mercury decreased LPS-induced ERK and downstream IL-6, which may have a protective property on LPS-induced renal damage. The exact reason for the unresponsiveness of mercury to IL-1β is unknown, although IL-1β is also an important proinflammatory cytokine. A lack of change of IL-1β expression by mercury in kidney is similar to our previous report showing no effect of mercury on LPS-induced IL-1β in macrophage cells (Kim *et al.*, 2002).

In this present report, we show that mercury differentially altered inflammatory cytokine expression by differential modulation of MAPKs in kidney. Our data suggest that increase of oxidative stress by depletion of GSH, concomitant activation of p38 MAPK, and downstream TNFá expression, is the major pathway of mercury-induced exacerbation of LPS-mediated inflammatory signaling. We, for the first time, show the involvement of p38 MAPK on mercury-induced TNFá expression in kidney. It should be emphasized that the effects reported here were not clear whether direct or indirect effects of mercury. The kinetics of cytokine expression and confirmative data using specific MAPKs inhibitors are needed. More studies are required for further understanding of regulation of MAPKs on inflammatory cytokine production along with mercury contamination.

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REFERENCES

Arbabi, S. and Maier, R.V. (2002): Mitogen-activated protein kinases. *Crit. Care. Med.*, **30**, S74-S79.

Baker, M.A., Cerniglia, G.J. and Zaman, A. (1990): Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples. *Anal. Biochem.*, **190**, 360-365.

Chen, C.C. and Wang, J.K. (1999): p38 but not p44/42 mitogen-activated protein kinase is required for nitric oxide synthase induction mediated by lipopolysaccharide in RAW 264.7 macrophages. *Mol. Pharmacol.*, **55**, 481-488.

Dong, C., Davis, R.J. and Flavell, R.A. (2002): MAP kinases in the immune response. *Annu. Rev. Immunol.*, **20**, 55-72.

Farina, M., Brandao, R., de Lara, F.S., Pagliosa, L.B., Soares, F.A., Souza, D.O. and Rocha, J.B. (2003): Profile of nonprotein thiols, lipid peroxidation and delta-aminolevulinate dehydratase activity in mouse kidney and liver in response to acute exposure to mercuric chloride and sodium selenite. *Toxicology*, **184**, 179-187.

Feng, G.J., Goodridge, H.S., Harnett, M.M., Wei, X.Q., Nikolaev, A.V., Higson, A.P. and Liew, F.Y. (1999): Extracellular signal-related kinase (ERK) and p38 mitogen-activated protein (MAP) kinases differentially regulate the lipopolysaccharide-mediated induction of inducible nitric oxide synthase and IL-12 in macrophages: Leishmania phosphoglycans subvert macrophage IL-12 production by targeting ERK MAP kinase. *J. Immunol.*, **163**, 6403-6412.

Ghosh, S., Latimer, R.D., Gray, B.M., Harwood, R.J. and Oduro, A. (1993): Endotoxin-induced organ injury. *Crit. Care. Med.*, **21**, S19-24.

Gilot, D., Loyer, P., Corlu, A., Glaise, D., Lagadic-Gossmann, D., Atfi, A., Morel, F., Ichijo, H. and Guguen-Guillouzo, C. (2002): Liver protection from apoptosis requires both blockage of initiator caspase activities and inhibition of ASK1/JNK pathway via glutathione S-transferase regulation. *J. Biol. Chem.*, **277**, 49220-49229.

Goering, P.L., Morgan, D.L. and Ali, S.F. (2002): Effects of mercury vapor inhalation on reactive oxygen species and antioxidant enzymes in rat brain and kidney are minimal. *J. Appl. Toxicol.*, **22**, 167-172.

Gordon, S. (1998): The role of the macrophage in immune regulation. *Res. Immunol.*, **149**, 685-688.

Haddad, J.J. (2002): The involvement of L-gamma-glutamyl-L-cysteinyl-glycine (glutathione/GSH) in the mechanism of redox signaling mediating MAPK(p38)-dependent regulation of pro-inflammatory cytokine production. *Biochem. Pharmacol.*, **63**, 305-320.

Hewett, J.A. and Roth, R.A. (1993): Hepatic and extrahepatic pathobiology of bacterial lipopolysaccharides. *Pharmacol. Rev.*, **45**, 382-411.

Hussain, S., Atkinson, A., Thompson, S.J. and Khan, A.T. (1999): Accumulation of mercury and its effect on antioxidant enzymes in brain, liver, and kidneys of mice. *J. Envi-*

ron. Sci. Health B, **34**, 645-660.

Johnson, V.J. and Sharma, R.P. (2001): Gender-dependent immunosuppression following subacute exposure to fumonisin B1. *Int. Immunopharmacol.*, **1**, 2023-2034.

Kim, S.H., Johnson, V.J. and Sharma, R.P. (2002). Mercury inhibits nitric oxide production but activates proinflammatory cytokine expression in murine macrophage: differential modulation of NF-kappaB and p38 MAPK signaling pathways. *Nitric. Oxide.*, **7**, 67-74.

Kim, S.H., Kim, J. and Sharma, R.P. (2004). Inhibition of p38 and ERK MAP kinases blocks endotoxin-induced nitric oxide production and differentially modulates cytokine expression. *Pharmacol. Res.*, **49**, 433-439.

Koropatnick, J. and Zalups, R.K. (1997): Effect of non-toxic mercury, zinc or cadmium pretreatment on the capacity of human monocytes to undergo lipopolysaccharide-induced activation. *Br. J. Pharmacol.*, **120**, 797-806.

Lee, J.C., Laydon, J.T., McDonnell, P.C., Gallagher, T.F., Kumar, S., Green, D., McNulty, D., Blumenthal, M.J., Heys, J.R., Landvatter, S.W. and *et al.* (1994): A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature*, **372**, 739-746.

Lee, J.C. and Young, P.R. (1996): Role of CSB/p38/RK stress response kinase in LPS and cytokine signaling mechanisms. *J. Leukoc. Biol.*, **59**, 152-157.

Nguyen, V.A. and Gao, B. (1999): Cross-talk between alpha(1B)-adrenergic receptor (alpha(1B)AR) and interleukin-6 (IL-6) signaling pathways. Activation of alpha(1b)AR inhibits il-6-activated STAT3 in hepatic cells by a p42/44 mitogen-activated protein kinase-dependent mechanism. *J. Biol. Chem.*, **274**, 35492-35498.

Shenker, B.J., Mayro, J.S., Rooney, C., Vitale, L. and Shapiro, I.M. (1993): Immunotoxic effects of mercuric compounds on human lymphocytes and monocytes. IV. Alterations in cellular glutathione content. *Immunopharmacol. Immunotoxicol.*, **15**, 273-290.

Tchounwou, P.B., Ayensu, W.K., Ninashvili, N. and Sutton, D. (2003): Environmental exposure to mercury and its toxicopathologic implications for public health. *Environ. Toxicol.*, **18**, 149-175.

Ueda, S., Masutani, H., Nakamura, H., Tanaka, T., Ueno, M. and Yodoi, J. (2002): Redox control of cell death. *Antioxid. Redox. Signal.*, **4**, 405-414.

Watters, J.J., Sommer, J.A., Pfeiffer, Z.A., Prabhu, U., Guerra, A.N. and Bertics, P.J. (2002): A differential role for the mitogen-activated protein kinases in lipopolysaccharide signaling: the MEK/ERK pathway is not essential for nitric oxide and interleukin 1beta production. *J. Biol. Chem.*, **277**, 9077-9087.

Zalups, R.K. (2000): Molecular interactions with mercury in the kidney. *Pharmacol. Rev.*, **52**, 113-143.

Zalups, R.K., Barfuss, D.W. and Lash, L.H. (1999): Disposition of inorganic mercury following biliary obstruction and chemically induced glutathione depletion: dispositional changes one hour after the intravenous administration of mercuric chloride. *Toxicol. Appl. Pharmacol.*, **154**, 135-144.