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Original Article

Sesamin reduces acute hepatic injury induced by lead coupled with lipopolysaccharide

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Abstract

Background: In this study, we investigated the potential anti-inflammatory and antioxidative effects of sesamin on acute liver injury. Lead (Pb) causes oxidative damage and enhances the effects of low-dose lipopolysaccharide (LPS), inducing acute hepatic injury in rats.
Methods: Male Sprague–Dawley rats were given intraperitoneal injections of Pb acetate (5 mg/kg) and LPS (50 μg/kg) to induce liver injury, and we tested the effects of oral administration of sesamin (10 mg/kg) on liver damage. To assess the extent of acute hepatic injury in the rats, we measured the anti-inflammatory and antioxidant markers and relevant signaling pathways: serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), C-reactive protein (CRP), reactive oxygen species (ROS), tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, nitric oxide (NO), and cyclooxygenase-2 (COX-2), inducible NO synthase (iNOS) levels, mitogen-activated protein kinases (MAPKs), c-Fos, and GADD45β.
Results: Sesamin significantly decreased the serum AST, ALT, and CRP levels in the rat model. In the Pb and LPS-stressed rats, sesamin administration reduced the serum levels of TNF-α, IL-1, IL-6, NO, and ROS generation, and liver tissue expressions of c-Jun N-terminal kinase (JNK), p38 MAPK, GADD45β, COX-2, and iNOS.
Conclusion: Collectively, these results demonstrate that sesamin is associated with antioxidant and anti-inflammatory activity. The observed effect of scavenging of ROS and NO and inhibiting the production of proinflammatory cytokines may be achieved through the suppression of COX-2, iNOS, and MAPK pathways in the acute hepatic injury rats.

Keywords: cyclooxygenase-2; inducible nitric oxide synthase; kinases; mitogen-activated protein; sesamin

1. Introduction

Lead (Pb) is a persistent environment and industrial pollutant that is known to cause oxidative damage in living organisms.[1] The International Agency for Research on Cancer has upgraded Pb from a possible to a probable human carcinogen.[2] Low levels of Pb exposure may cause disorders of the circulatory, renal, and nervous systems.[3] Children are

Conflicts of interest: The authors declare that there are no conflicts of interest related to the subject matter or materials discussed in this article.

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more susceptible to Pb toxicity because enzyme inhibition and damage caused by this metal are more severe in early development.[4,5] Additionally, coexposure to Pb and lipopolysaccharide (LPS) causes severe hepatic injury in rats and mice.[6–9]

Lead synergistically increases the LPS-stimulated expression of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-Iβ.[6–9] The Pb-augmented, LPS-stimulated TNF-α directly increases liver injury in mice, although evidence suggests that it is produced outside the liver *in vivo*.[10–12] Monocytes and macrophages are the cells primarily responsible for producing excess TNF-α through protein kinase C and the p42/44 mitogen-activated protein kinase (MAPK) pathway.[12,13] Lead increases LPS-induced liver damage through protein kinase C and p42/44 MAPK modulation of TNF-α, but modulation of TNF-α does not affect nitric oxide (NO) in rats.[10,13,14]

Sesame oil is a potential anti-inflammatory agent that is commonly used as an antioxidant with sesamin and sesamolin as two major lignans. Sesamin inhibits IL-6 and TNF-α productions from microglia under LPS stimulation.[15] We have demonstrated that inhibition of LPS-induced cytokine and iNOS mRNA/protein by sesamin is mainly through its antioxidative activity and suppression of the p38 MAPK signal pathway.[15,16] P38 MAPK is thought to mediate inflammatory responses in various cell types and mice through the activation of transcription factors that induce expression of inflammatory genes.[17,18] It has been shown that treatment with sesame oil can protect mice from acute hepatic injury from Pb plus LPS toxicity, and this protection can be attributed to the inhibition of proinflammatory cytokines and NO.[19] However, the precise mechanism of this model by its active component is unclear. We hypothesized that sesamin would inhibit the inflammatory cytokines and reactive oxygen species (ROS) or reactive nitrogen species (RNS) production by suppression of certain signaling pathways in the model of acute hepatic injury. Therefore, the aim of this study was to investigate the effect and mechanism of sesamin protection from acute hepatic injury in rats induced by Pb and LPS.

2. Methods

2.1. Reagents

Lead acetate was purchased from Merck Co. (Darmstadt, Germany). LPS (*Escherichia coli* 0111:B4) was obtained from Sigma-Aldrich (St. Louis, MO, USA), and sesamin was provided by Joben Bio-Medical Co. (Kaohsiung, Taiwan).

2.2. Animals and drug administration

Male Sprague–Dawley rats (300–400 g) obtained from the National Laboratory Animal Center (Taipei, Taiwan) were maintained in the Animal Center of the Chinese Medical University (Taichung, Taiwan). The animal studies were performed following the guidelines of the *Guidebook for the Care and Use of Laboratory Animals* (2002) published by the Chinese Society of Animal Science in Taiwan. The rats were

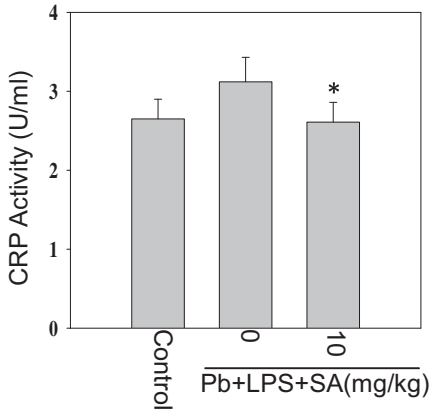

Fig. 1. The effect of sesamin on serum CRP levels in the acute hepatic injury model. Experimental rats (the PL group) were given IP injections of lead acetate (5 mg/kg) and LPS (50 μg/kg). The SA group was given oral sesamin (10 mg/rat) in addition to the IP injections. Serum CRP levels were measured after 4 hours of treatment. Data are expressed as the mean ± SD. *$p < 0.01$ as compared to the PL group. CRP = C-reactive protein; IP = intraperitoneal; LPS = lipopolysaccharide.

divided into five groups and were fasted for 12 hours prior to intraperitoneal drug administration. One control group was given saline (blank), and the experimental groups (PL) were given 5 mg/kg of Pb + 50 μg/kg of LPS. The sesamin (SA)

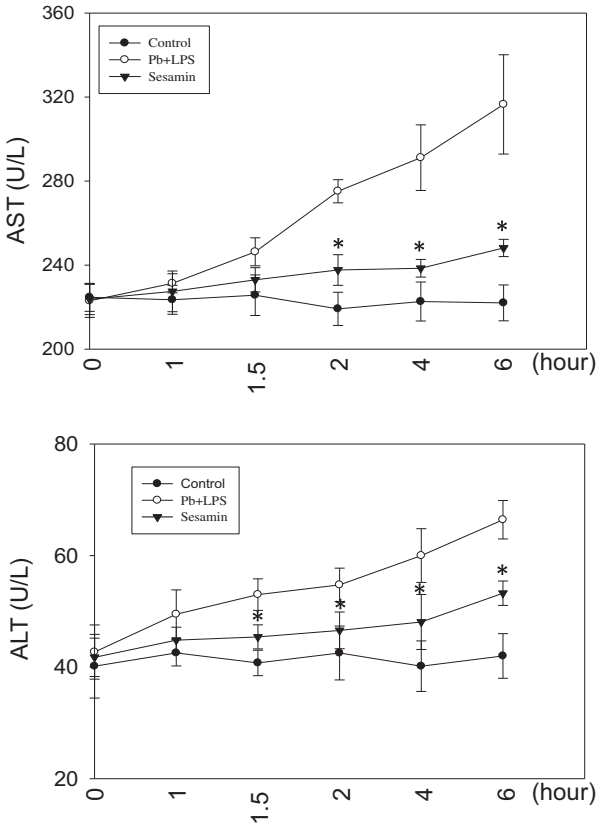

Fig. 2. Serum AST and ALT concentrations in response to Pb and LPS stress and sesamin treatment. Serum AST and ALT were determined at 0 hours, 1 hour, 1.5 hours, 2 hours, 4 hours, and 6 hours after treatment. Data are presented as the mean ± SD. *$p < 0.01$ as compared to the PL group. ALT = alanine aminotransferase; AST = aspartate aminotransferase; LPS = lipopolysaccharide; Pb = lead.

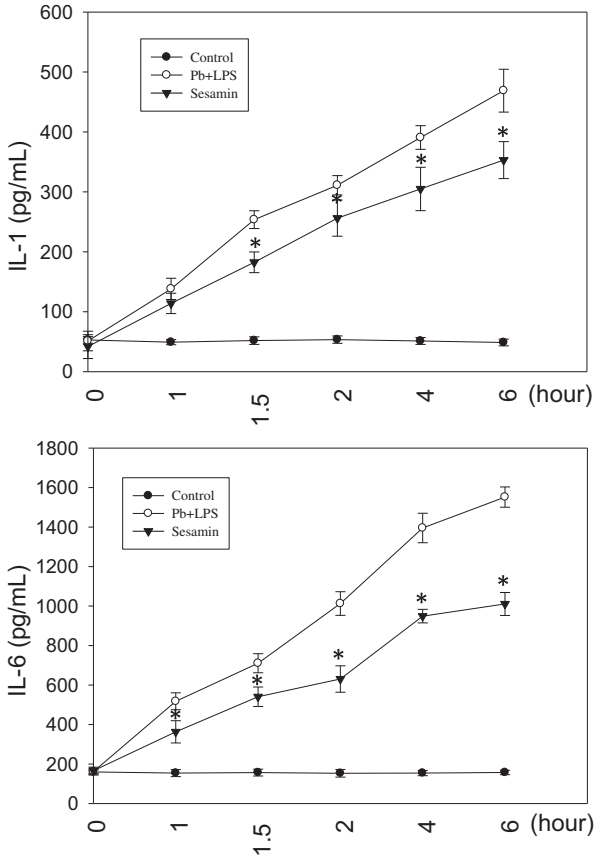

Fig. 3. Effects of sesamin on serum IL-1 and IL-6 levels under Pb plus LPS stress. Serum IL-1 and IL-6 were determined at 0 hours, 1 hour, 1.5 hours, 2 hours, 4 hours, and 6 hours after treatment. Data are presented as the mean ± SD. *$p < 0.01$ as compared to the PL group. IL = interleukin; LPS = lipopolysaccharide; Pb = lead.

group was given 10 mg/kg of sesamin by gastric gavage after injection with LPS plus Pb.

2.3. Biochemistry and histopathology analysis

Blood samples (0.8 mL) were withdrawn by cardiopuncture at 0.5 hours, 1 hour, 1.5 hours, 2 hours, 4 hours, 6 hours, 12 hours, and 24 hours after drug administration. The blood samples were collected in microtubes and centrifuged at 10,000*g* for 15 minutes at 4°C to isolate the serum. Serum cytokine levels were measured at 0 hour, 1.5 hours, 3 hours, and 6 hours after treatments. TNF-α, IL-1β, and IL-6 levels in the serum were measured using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) by measuring $A_{450\ nm}$ in a microplate reader (Model, TECAN, Salzburg, Austria). ELISA results have a detection limit of 32.5 pg/mL. Serum C-reactive protein (CRP) level was measured with a rat CRP ELISA kit (Immunology Consultants Laboratory, Portland, OR, USA). The activities of iNOS and glyceraldehyde-3-phosphate dehydrogenase in leukocytes and liver tissue were examined 6 hours after treatment. In addition, TNF-α, IL-1β, nitrite, and iNOS expression levels in hepatic tissue were assessed 6 hours after treatment as described in the following section. Liver tissues were fixed with a 10% formaldehyde solution overnight and H&E (hematoxylin and eosin) stained. Hepatic function was assessed by measuring serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) with an automatic blood analyzer (Hitachi High-Technologies, Tokyo, Japan).

2.4. Measuring TNF-α, IL-1β, and IL-6 levels in the liver tissue

Liver tissue was homogenized in deionized water (1:10; weight/volume) and centrifuged at 3000*g* for 10 minutes at 4°C. The TNF-α, IL-1β, and IL-6 levels in the tissue supernatant were determined using ELISA kits (R&D Systems). Protein concentration (pg/μg) in the liver tissue was determined using a dye-based protein assay (Bio-Rad Laboratories, Hercules, CA, USA).

2.5. NO assay

Nitrite was measured as NO using the Griess test. Briefly, a serum sample was reacted with an equal volume of Griess

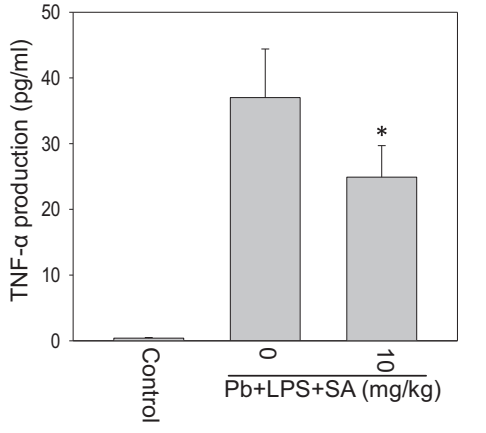

Fig. 4. The effect of sesamin on serum TNF-α level under Pb plus LPS stress. Data are expressed as the mean ± SD. *$p < 0.01$ as compared to the PL group. LPS = lipopolysaccharide; TNF-α = tumor necrosis factor-alpha; Pb = lead.

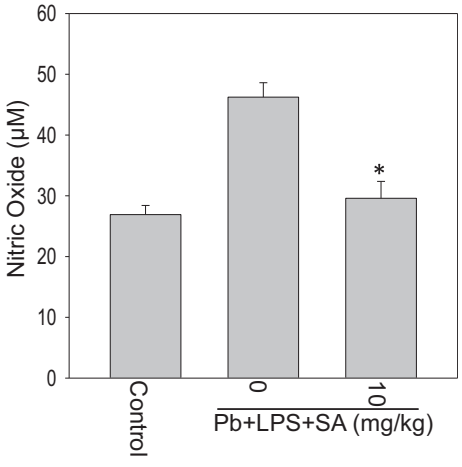

Fig. 5. Effect of sesamin on serum nitric oxide level in rats with and without Pb plus LPS stress. Data are expressed as the mean ± SD. *$p < 0.01$ as compared to the PL group. LPS = lipopolysaccharide; Pb = lead.

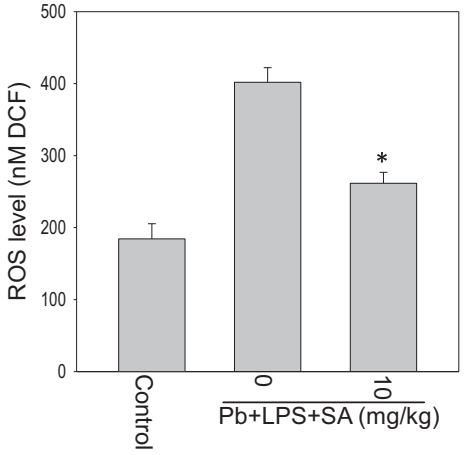

Fig. 6. The effect of sesamin on serum ROS level under Pb plus LPS stress. Data are expressed as the mean ± SD. *$p < 0.01$ as compared to the PL group. LPS = lipopolysaccharide; ROS = reactive oxygen species; Pb = lead.

reagent [0.1% naphthylethylene diamine and 1% sulfanilamide (1:1) in H_3PO_4] in 96-well plates for 10 minutes. The absorbance at 540 nm was measured in a microplate reader.

2.6. ROS generation

ROS was measured with 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA). Additionally, H_2DCF-DA was dissolved in methanol and deacetylated in serum mixed with 10 μM H_2DCF for 10 minutes in the dark. The reaction solution was plated in 96-well plates and monitored on a Fluoroskan Ascent Fluorometer (Labsystems Oy, Helsinki, Finland) using an excitation wave length of 485 nm and an emission wave length of 538 nm.

2.7. Western blotting

Rat liver cell-line (clone-9 cells) or liver tissues were homogenized in ice-cold lysis buffer (1:10, weight/volume) containing 20 mmol/L 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (pH 7.2), 1% Triton X-100, 10% glycerol, 1 mM PMSF (phenylmethylsulfonyl fluoride), 10 μg/mL leupeptin, and 10 μg/mL aprotinin. This solution was centrifuged at 10,000*g* for 30 minutes at 4°C. Thereafter, 50 μg of protein was run on an 8% or 10% sodium dodecyl sulfate–polyacrylamide gel and transferred onto nitrocellulose membranes (NEN Life Sciences, Boston, MA, USA) at 1.2 A for 3 hours. The membranes were blocked in 5% milk in Tris buffer saline with Tween-20. The membrane was then incubated with polyclonal rabbit iNOS antibody (BD Biosciences, San Diego, CA, USA) and diluted 1:1000 in blocking buffer. Membranes were incubated with secondary anti-rabbit IgG conjugated to alkaline phosphatase (1:3000; Jackson ImmunoResearch, Philadelphia, PA, USA) and detected with alkaline phosphatase substrate solution (5-bromo-4-chloro-3-

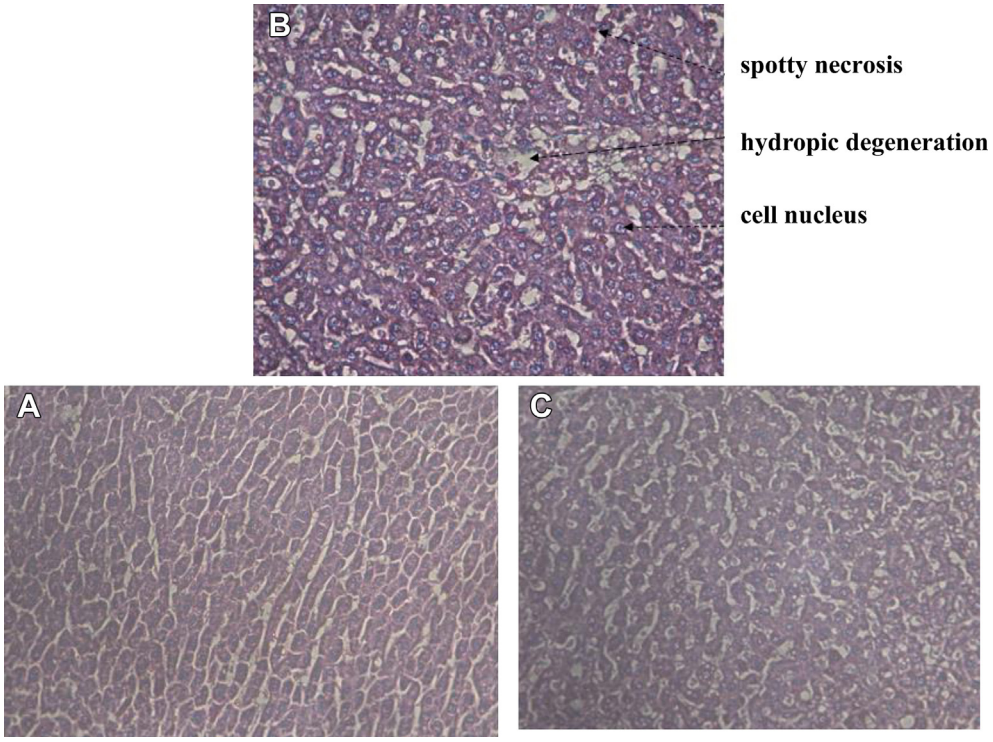

Fig. 7. Protective effect of sesamin on Pb plus lipopolysaccharide (LPS)-induced hepatic injury. Histopathology of liver slices from rats from: (A) the no treatment control group; (B) the PL group, given 5 mg/kg Pb and 50 μg/g LPS; and (C) the SA group, PL treatment supplemented with 10 mg sesame/rat for 6 hours after treatment. The photographs show liver sections at 400× magnification. Spotty necrosis and hydropic degeneration was more severe in PL than in control or SA groups. Pb = lead; PL group = experimental group; SA group = sesamin group.

indolyl-phosphate/nitroblue tetrazolium; Kirkegaard & Perry, Baltimore, MD, USA).

2.8. Statistical analysis

All data are expressed as mean ± SD. For single variable comparisons, Student *t* test was used. For multiple variable comparisons, data were analyzed with one-way analysis of variance using Dunnett's test. A *p* value of <0.05 or $p < 0.01$ was considered significant.

3. Results

Studies show that antioxidants ameliorate acute hepatic injury in various animal models,[20–22] and that sesame oil protects mice from acute hepatic injury through the inhibition of cytokines and NO production.[19] These results show that sesamin, an active component of sesame oil, relieved acute hepatic injury in rats under Pb + LPS stress. Sesamin reduced serum CRP, ALT, and AST levels in rats after an injection with Pb + LPS (Figs. 1 and 2). Serum levels of liver enzymes such as ALT and AST reflect liver function and hepatocyte integrity.[23] Levels of ALT and AST increased with Pb + LPS-induced liver injury but were lower with sesamin treatment, suggesting that sesamin may protect rats from Pb + LPS liver injury (Fig. 2; $p < 0.05$ and $p < 0.05$ vs. the PL group). Our results showed that sesamin could protect from liver injury by attenuating the increased serum IL-1, IL-6 (Fig. 3; $p < 0.0001$) and TNF-α and nitrite (Figs. 4 and 5; $p < 0.005$ vs. the PL group) in the PL-induced rats. These results are consistent with the previous finding that reduction of TNF-α, IL-1, and NO has a protective effect against Pb + LPS-induced liver injury in animals.[10,13,14]

ROS and RNS are necessary for normal physiological functions but also contribute to liver injury.[24] We found that sesamin was able to scavenge 25–44% of PL-induced serum ROS and nitrite (Figs. 5 and 6; $p < 0.005$ and $p < 0.005$ vs. the PL group, respectively). Because ROS and RNS signals can trigger the intrinsic apoptosis pathway,[25] sesamin might reduce the apoptosis of hepatocytes under Pb plus LPS stress by scavenging these free radicals. Cell atrophy, irregular arrangement with degeneration, and spotty necrosis were observed in the liver section of the PL group (Fig. 7B), but not in the control group (Fig. 7A). The SA group (Fig. 7C) showed

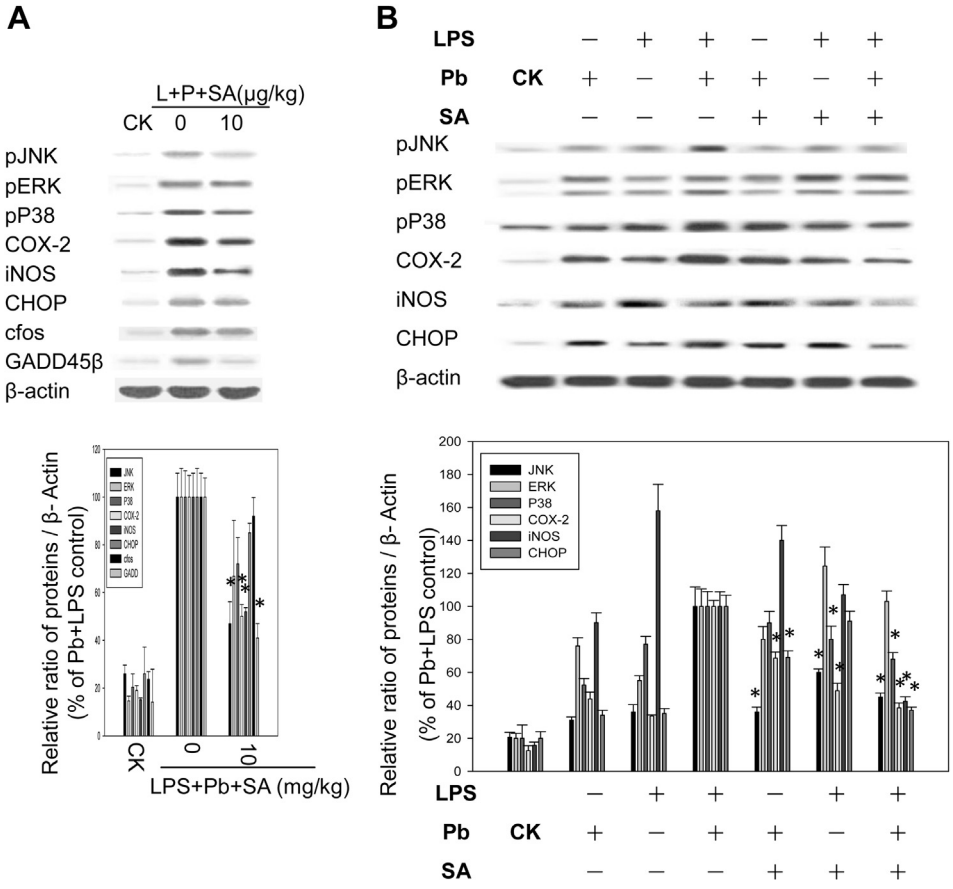

Fig. 8. Effect of sesamin on Pb plus LPS-induced signaling pathways. The activation of phospho-MAPKs, COX-2, iNOS, CHOP, c-FOS, and GADD45β expressions were assayed in Sprague-Dawley rats and clone-9 cells, respectively, *in vivo* and *in vitro*. Livers were obtained from rats treated or not treated with 10 mg sesamin/rat and injected with 5 mg/kg Pb and 50 μg/kg LPS for 6 hours (A), and Clone-9 cells were treated with 1 μg/mL LPS plus 100 μg/mL Pb and 50 μM sesamin for 10 minutes (B). Data are expressed as the mean ± SD. *$p < 0.01$ as compared to the PL group of rats or cells. COX-2 = cyclooxygenase-2; iNOS = inducible NO synthase; LPS = lipopolysaccharide; MAPKs = mitogen-activated protein kinases.

less cell degeneration and spotty necrosis than the PL group in 200 microscopic fields of the liver section.

The effects of sesamin on PL-induced signaling pathways were further examined by Western blot assay (Fig. 8A). Sesamin reduced expression of the following proteins: JNK (53 ± 9%), ERK (36 ± 6%), p38 (28 ± 7%) MAPKs, COX-2 (50 ± 6%), iNOS (48 ± 6%), CHOP (15 ± 4%), c-FOS (8 ± 3%), and GADD45β (59 ± 6%), respectively, to the PL-induced SD rats ($P < 0.01$). Similarly, sesamin also suppressed Pb + LPS-induced phospho-MAPKs, COX-2, iNOS, CHOP, c-FOS, and GADD45β expression in clone-9 cells (Fig. 8B). However, c-FOS was not affected by sesamin in clone-9 cells (data not shown).

4. Discussion

The Pb + LPS model is a relevant animal model for cytokine-associated hepatic injury.[26,27] Several studies have shown that TNF-α is a critical regulator of hepatocyte physiology in a variety of pathophysiological conditions,[28] such as viral hepatitis,[29] fulminant hepatic failure,[30] and alcoholic hepatitis.[31] In the present study, sesamin was found to lower PL-induced TNF-α production in both serum and liver tissue (data not shown). The sesamin-induced reduction of TNF-α could be due to either a decreased production or an increased clearance of TNF-α, and future studies will provide additional clarification. A previous study showed that NO induces TNF-α production in Pb + LPS-treated rats.[8] In the present study, the reduced TNF-α, IL-1β, IL-6, and NO levels in sesamin-treated model rats suggested that sesamin could be used for protection from acute hepatic injury.

Sesamin is the major lignan from sesame seeds that has antioxidative and anti-inflammatory effects.[15,16] An NO inhibitor protects against Pb + LPS-treated liver dysfunction by blocking TNF-α expression.[8] Therefore, sesamin could be a useful addition to an NO inhibitor for reducing acute hepatic toxicity in Pb LPS-treated mice. Because sesamin can protect against LPS- and oxidative-stressed injuries,[15,32] it might also reduce ROS generation in this model. The result showed that the protective effect of sesamin on acute hepatic injury could be attributed to an inhibition of ROS and RNS through suppressing the PL-induced JNK, ERK, p38 MAPKs, COX-2, iNOS, CHOP, and GADD45β signaling pathways. Recently, T-5224, a selective inhibitor of c-Fos/activator protein (AP)-1, has been shown to protect against LPS-induced liver injury by reducing the serum levels of TNF-α, HMGB1, ALT/AST, liver levels of MIP-1α and MCP-1, and overall lethality.[33] It has already been proposed that CHOP and GADD45β expression are increased during acute liver damage.[34,35] However, c-FOS was not affected by sesamin in clone-9 cells.

In conclusion, the presented data show that sesamin effectively ameliorates Pb and LPS-induced acute hepatic injury by inhibition of proinflammatory cytokines and NO. The inhibition of acute hepatic injury was mainly through the suppression of several signaling pathways such as JNK and p38 MAPKs, COX-2, iNOS, CHOP, and GADD45β.

Acknowledgments

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