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BURN PLUS LIPOPOLYSACCHARIDE AUGMENTS ENDOPLASMIC RETICULUM STRESS AND NLRP3 INFLAMMASOME ACTIVATION AND REDUCES PGC-1 α IN LIVER

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Abstract

Extensively burned patients often suffer from sepsis (especially caused by *Pseudomonas aeruginosa*), which may prolong metabolic derangement, contribute to multiple organ failure, and increase mortality. The molecular and cellular mechanisms of such infection-related metabolic derangement and organ dysfunction are unclear. We have previously shown that severely burned patients have significant and persisting hepatic endoplasmic reticulum (ER) stress. We hypothesized that ER stress and the unfolded protein response correlate with NOD-like receptor, pyrin domain containing 3 (NLRP3) inflammasome activation in burn. These may trigger profound metabolic changes in the liver, which form the pathological basis of liver damage and liver dysfunction after burn injury. A two-hit rat model was established by a 60% total body surface area scald burn and intraperitoneal injection of *P. aeruginosa*-derived lipopolysaccharide (LPS) 3 days after burn. One day later, animals were killed, and liver tissue samples were collected for gene expression and protein analysis of NLRP3 inflammasome activation, ER stress, and glucose and lipid metabolism. Liver damage was assessed by plasma markers (alanine aminotransferase and aspartate aminotransferase) and liver immunohistochemical analysis. Our results showed that burn injury and LPS injection induced inflammasome activation in liver and augmented hepatic ER stress and liver damage. Although there was an increased metabolic demand after burn, hepatic NLRP3 inflammasome activation corresponded to inhibition of PGC-1 α (peroxisome proliferator-activated receptor γ -coactivator 1 α) and its upstream regulators protein kinase A catalyst unit, AMP-activated protein kinase α , and sirtuin-1 may provide a mechanism for the enhanced metabolic derangement after major burn injury plus sepsis. In conclusion, burn + LPS augments inflammasome activation and ER stress in liver, which in turn contribute to postburn metabolic derangement.

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Keywords

Thermal injury; sepsis; ER stress; inflammasome; metabolism

INTRODUCTION

Sepsis and associated multiorgan failure are the major causes of death in extensively burned patients who survive the initial phase of burn shock (1). Large-scale clinical studies showed that sepsis increased intensive care unit resource utilization and mortality in patients with traumatic injury. Despite the reduction in the incidence of sepsis over the last two decades, there has been no reduction in sepsis-associated mortality (2, 3). Research is thus required to understand the molecular mechanisms of the pathological changes in burn patients with sepsis and to find out the effective therapeutic targets accordingly.

Sepsis is an infection-induced systemic inflammatory response that has profound impact on metabolism, immunity, and tissue regeneration. Our previous studies have shown that the liver is the central organ for metabolism and immunity and the target organ for stress and inflammation induced parenchymal cell damage in burn (4, 5). It has also been shown in microarray analysis that there is a significant albeit temporary upregulation of the expression of hepatic genes involved in immune response and receptor activity in the animals with endotoxemia (6). Furthermore, overwhelming proinflammatory cytokines (e.g., tumor necrosis factor α , interleukin 1 β [IL-1 β], IL-6) signal septic response via membrane-associated receptors such as cytokine receptors and Toll-like receptors (7, 8). In recent years, there is a growing attention to the importance of cytosolic signaling pathways of inflammation. Among these, NOD-like receptor, pyrin domain containing 3 (NLRP3) inflammasome is of particular interest because it can be activated by a number of different stimuli, which are common in severe trauma and are termed as danger-associated molecular patterns (DAMPs) (9). Especially, our previous study showed that burn injury induces hepatic endoplasmic reticulum (ER) stress and subsequent unfolded protein responses, and this is closely correlated with postburn metabolic dysfunction and insulin resistance (10). It is thus important to know whether there is interplay between hepatic ER stress, inflammasome activation, and metabolic derangement and how these processes are linked together in severely burned patients.

In a 25% total body surface area (TBSA) scald burn murine model, Osuka et al. (11) observed that burn injury induced NLRP3 inflammasome activation primarily in macrophages, where it played a protective role in the host response to severe injury. This is the only report available in burn injury-related inflammasome activation, but the finding of survival advantage associated with caspase 1 blockade is somehow different from more lethal models of infection or sepsis (12).

To improve our understanding of inflammasome activation in a more clinically relevant setting, we used a two-hit rat model of major burn plus intraperitoneal injection of *Pseudomonas aeruginosa*-derived lipopolysaccharide (LPS). We hypothesized that (i) severe burn injury and following stress responses produce DAMPs that signal NLRP3

inflammasome activation, and (ii) this inflammasome activation exacerbates postburn metabolic dysfunction in the liver.

MATERIALS AND METHODS

Animal model

Animal experiments were approved by the Animal Care and Use Committee of Sunnybrook Research Institute in Toronto, Ontario, Canada. The National Institutes of Health Guidelines for the Care and Use of Experimental Animals were met.

Male Sprague-Dawley rats (n = 6 per group), 275 to 300 g, were purchased from Charles Rivers Laboratory International Inc (Wilmington, Mass) and were allowed to acclimate for 1 week before we conducted the experiments. Rats were housed in the Sunnybrook Research Institute animal care facility and received a high-protein diet (Ensure, #22017C8; Abbott Laboratories, Abbott Park, Ill) and water *ad libitum* from arrival until they were killed. Ensure was administered 7 days before the study to adjust the animals to the liquid diet.

A well-established method was used to induce a full-thickness scald burn (13, 14). The treatment groups included sham, sham + LPS, burn, and burn + LPS. Animals were anesthetized (ketamine 40 mg/kg body weight and xylazine 5 mg/kg body weight, both injected intraperitoneally), the dorsum of the trunk and the abdomen were shaved, and then a 60% TBSA burn was induced by placing the animals in a mold that exposed defined areas of the skin of the back and abdomen. The mold was placed in a 98°C water bath, scalding the back for 10 s and the abdomen for 1.5 s. Full-thickness cutaneous burn was confirmed by histological section. Lactated Ringer's solution (40 mL/kg body weight) was administered intraperitoneally immediately after the burn for resuscitation. After recovering from the anesthesia, the rats were placed into separate cages. Sham animals were anesthetized and shaved but not burned.

Animals in the LPS groups (sham + LPS and burn + LPS) received intraperitoneal injection of 10 mg/kg *P. aeruginosa*-derived LPS (Sigma, St Louis, Mo) 72 h after burn. Rats were killed 24 h after the LPS injection (or 96 h after burn).

Plasma and tissue collection

Blood was collected into EDTA-containing tubes (30 µL of 0.5 M EDTA). The tubes were placed on ice temporarily for at least 30 min and centrifuged at 4°C at 1,000g for 10 min, and then the plasma supernatant was aliquoted for later analysis. Liver tissues were collected after brief portal vein perfusion with phosphate-buffered saline (20 mL) and were either immediately frozen in dry ice and then stored at -80°C for further analysis or put in 10% formalin overnight and then transfer to 70% ethanol for paraffin-embedding and tissue slide preparation for immunohistochemical analysis.

Real-time quantitative reverse transcriptase-polymerase chain reaction

Total RNA was isolated from liver tissue following manufacturer's instructions (RNeasy Mini Kit; Qiagen, Hilden, Germany), quantified using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, Del) and reverse transcribed (Applied Biosystems,

San Diego, Calif). Real-time polymerase chain reaction (PCR) was performed on cDNA with the housekeeping gene rRNA 18S. Target genes included inflammasome activation-related genes IL-1 β (*Il-1*), EGF module-containing mucin-like hormone receptor 1 (*Emr*), *Nlrp3*; ER stress marker genes CCAAT/enhancer-binding protein homologous protein (*Chop*), 78-kDa Glucose-regulated protein/binding immunoglobulin protein (*Bip*), X-box binding protein 1-spliced form (*Xbp1-s*), protein disulfide isomerase (*Pdi*), and glucose and lipid metabolic modulator genes peroxisome proliferator-activated receptor γ -coactivator 1 α (*Pgc-1 α*), glucose 6-phosphatase (*G6pase*), fatty acid synthase (*Fasn*), and salt inducible kinase 1 (*Sik 1*). The sequences of primers were as follows:

Il-1 (5'-GCACAGTTCCCCAACTGGTA-3' and 5'-ACACGGGTTCATGGTGAAG-3');

Emr (5'-GCCATAGCCACCTTCCTGTT-3' and 5'-ATAGCGCAAGCTGTCTGGTT-3');

Nlrp3 (5'-CAGACCTCCAAGACCACGACTG-3' and 5'-CATCCGCAGCCAATGAACAGAG-3');

Chop (5'-AGCGCCTGACCAGGGAGGTA-3' and 5'-GCTTGGCACTGGCGTGATGGT-3');

Bip (5'-TCGTCGCGTTTCGGGGCTAC-3' and 5'-TCATCTTGCCGGCGCTGTGG-3');

Xbp1-s (5'-GAGTCCGCAGCAGGTG-3' and 5'-CGTCAGAATCCATGGGAA-3');

Pdi (5'-CTGGTCCCCGGCCCTCCGATT-3' and 5'-ACGTCTGAGGCGGAGGCGAG-3');

Pgc-1 α (5'-AAAGGGCCAAGCAGAGAGA-3' and 5'-GTAAATCACACGGCGCTCTT-3');

G6pase (5'-CCCAGACTAGAGATCCTGACAGAAT-3');

Fasn (5'-CACAGCATTTCAGTCCTATCCACAGA-3' and 5'-CACAGCCAACCAGATGCTTCA-3'); and

Sik 1 (5'-CGATGGATGCAGGCCGACCC-3' and 5'-TGCCCAGCACCTGCTCGTTG-3').

Western blotting

Antibodies against rat phosphorylated AMP-activated protein kinase (phospho-AMPK) α and β , total AMPK α and AMPK β , phosphorylated protein kinase A catalyst unit (phospho-PKA C), sirtuin-1, caspase 3 (CASP3), and GAPDH were purchased from Cell Signaling (Danvers, Mass). Anti-GRP78/BIP and anti-peroxisome proliferator-activated receptor α (PPAR- α) antibodies were purchased from Abcam (Cambridge, Mass). Anti-NLRP3 and anti-PGC-1 α antibodies were purchased from EMD Millipore (Billerica, Mass). SuperSignal West Pico chemiluminescent substrate was purchased from Thermo Scientific Inc. (Rockford, Ill).

Approximately 40 mg of frozen liver tissue was homogenized in 150 mmol/L NaCl, 20 mmol/L Tris-HCl (pH 7.5), 1% (wt/vol) NP-40, 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L sodium orthovanadate, 1 mmol/L β -glycerol phosphate, 2.5 mmol/L sodium pyrophosphate, and 1 $\times$ complete protease inhibitor mixture (Roche Molecular Biochemicals, Indianapolis, Ind). The homogenate was centrifuged at 12,000g for 30 min at 4°C, and the pellet discarded. Western blotting was performed with 30 μ g of protein per well. Band intensities were quantified with the Image J software (National Institutes of Health, Bethesda, Md). GAPDH was used as loading control.

Blood glucose level, plasma assay, and immunohistochemical analysis for liver damage assessment

Blood glucose level was determined using blood glucose strips (Lifescan Europe, Zug, Switzerland). Liver damage was assessed by (i) quantifying plasma levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using colorimetric activity assays (BioVision, Milpitas, Calif) and (ii) immunohistochemical analysis of CASP3 (#9662; Cell Signaling) and TUNEL calorimetric assay (G7360; Promega, Madison, Wis) performed according to the product protocol.

Statistical analysis

Statistically significant differences were detected by a one-way analysis of variance with Student *t* tests. Data are presented as mean $\pm$ SD (*n* = 6 in each group). Significance was accepted at *P* < 0.05.

RESULTS

The two-hit of burn injury with LPS injection induced liver damage

We observed liver damage in rats that received the two-hit treatment. There was significant elevation of the plasma activity of ALT and AST in rats receiving LPS, burn, as well as LPS + burn when compared with controls; however more so in the burn + LPS group indicating more profound parenchymal liver damage in burn + LPS group (Fig. 1, A and B).

Immunohistochemical study of liver tissue indicated robust elevation of CASP3 in LPS-only and burn + LPS group (data not shown). Western blot of CASP3 also showed significant elevation of CASP3 expression in liver tissue in LPS-only, burn-only, and burn + LPS groups (Fig. 1, C and D). A focal positive TUNEL staining was found in liver of + LPS treatment group (Fig. 1, E–H). In addition, in the LPS alone and burn + LPS groups, we observed decreased food intake (data not shown) and weight loss (Fig. 1I). Despite the reduced food intake and consequent weight loss in these two groups, the blood glucose level remained high at around the upper reference range (Fig. 1J). These data indicated that burn + LPS caused liver damage by inducing apoptosis in a subpopulation of hepatocytes, which led to increase in ALT and AST in the serum of these animals.

Burn plus LPS injection augmented hepatic NLRP3 inflammasome activation

Burn, LPS, and burn + LPS increased NLRP3 inflammasome activation in the liver. We observed significantly increased *Il-1*, *Emr*, and *Nlrp3* mRNA expression (Fig. 2, A–C) and a

significant elevation of NLRP3 protein (Fig. 2, D and E) in the liver of burn and burn + LPS groups compared with the sham.

Burn and LPS injection induced hepatic ER stress

We observed significantly increased expression of *Chop*, *Xbp1-s*, and *Pdi* genes, markers of ER stress in liver, in sham + LPS and burn + LPS groups (Fig. 3, A, C, and D). *Bip* mRNA and BIP protein levels increased significantly in the LPS, burn, and burn + LPS groups (Fig. 3, B, E, and F). Therefore, burn injury, LPS, and their combination led to hepatic ER stress.

Burn induced hypermetabolism

To determine the underlying mechanism for the differences in weight and glucose, we monitored gene expression of hepatic metabolic modulators, including *G6pase*, which is the final-step catalyst in gluconeogenesis and glycogenolysis and therefore plays a key role in the homeostatic regulation of blood glucose level (15); *Fasn*, which catalyze the synthesis of palmitate from acetyl-CoA and malonyl-CoA in the presence of NADPH and thus is fundamental to energy storage and biosynthesis of hormones and other important biological molecules; *Sik 1*, which is a serine-threonine kinase related with steroidogenesis and metabolic regulation in adipose tissue (16). We also determined the hepatic protein level of G6Pase and SCD1, which is the rate-limiting enzyme catalyzing the biosynthesis of monounsaturated fatty acids (17). In the burn group, *G6pase*, *Fasn*, and *Sik1* increased significantly compared with sham. In LPS group, *G6pase* mRNA level decreased compared with sham (Fig. 4, A–C). Protein level of G6Pase in liver tissue decreased significantly in LPS-only and burn + LPS groups (Fig. 4, D and F), whereas the level of SCD1 decreased significantly in burn-only and burn + LPS group (Fig. 4, E and G).

We were particularly interested in the gene expression and tissue abundance of PGC-1 α in the liver because it has profound impact on mitochondria energetic metabolism, glucose metabolism, and lipid metabolism (18). We observed increased gene expression of *Pgc-1 α* in all treatment groups (Fig. 5A).

Unlike burn, which downregulates sirtuin-1, LPS inhibits PKA C/AMPK α

Lipopolysaccharide alone caused a significant decrease in the protein level of spliced PGC-1 α (Fig. 5, B and D). To investigate the possible mechanisms of such PGC-1 α inhibition, we measured the activation of PKA C, sirtuin-1, and AMPK, which are upstream regulators of spliced and full-length PGC-1 α , respectively. Western blot analysis showed that LPS alone decreased phospho-AMPK α and phospho-PKA C, whereas there were no significant changes in phospho-AMPK β and PPAR- α . We found that sirtuin-1 decreased in burn and burn + LPS groups but not in the LPS-only group (data not shown) (Fig. 6).

DISCUSSION

In this study, by using a two-hit rat model of burn + LPS intraperitoneal injection, we investigated the activation of NLRP3 inflammasome and ER stress in liver, their interplay, and its impact on postburn metabolism. We found that both burn and LPS induce significant NLRP3 inflammasome activation in liver, and such NLRP3 inflammasome activation

augmented liver damage and metabolic derangement in burn + LPS group. This is the first study to report NLRP3 inflammasome activation in liver tissue after burn injury.

Our previous studies have demonstrated that severe burn injury induces enormous and persisting stress response and hypermetabolism (19). The postburn stress response leads to the accumulation of unfolded and misfolded proteins in the ER and increased intracellular heat shock and chaperone proteins (20). Postburn hypermetabolism causes increased ATP production, increased oxidative phosphorylation and reactive oxygen species production in the mitochondria, and increased gene transcription (21). All of these increased intracellular biomolecules are DAMPs indicating challenging environment and have been shown to trigger NLRP3 inflammasome activation in other disease models (22, 23). Composed of NLRP, ASC, and pro-caspase 1, the NLRP3 inflammasome generally assembles in macrophages where it activates caspase 1 to ultimately produce IL-1 β , which is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis (24). Moreover, IL-1 β interferes with insulin receptor signaling (25) and may thus directly exacerbate metabolic derangement. In the current study, we confirmed such NLRP3 inflammasome activation in the liver in burn and sepsis animals by showing increased inflammasome-related gene expression (*Il-1*, *Nlrp3*) and increased NLRP3 protein. EMR is a specific marker of macrophages (26). The very similar pattern of *Emr* mRNA expression with *Il-1* and *Nlrp3* suggests macrophage involvement of liver inflammasome activation. Moreover, our observations suggest that thermal injury with or without sepsis induces NLRP3 inflammasome activation in the liver. This is consistent with our understanding that ER stress produces DAMPs and may thus contribute to the initiation of inflammasome formation (27).

We showed in the current study that ER stress and inflammasome activation (assessed by gene expression and protein analysis) shared the same pattern in the LPS, burn, and burn + LPS groups. Further research in knockout models will be important to establish whether there is a causative relationship between these two pathological processes. We noticed that there was less robust ER stress-related genes expression in burn-only group. The possible explanation might be that the rats were recovering from initial thermal injury after 96 h. Our previous study showed that the stress response after burn peaks indeed between 24 and 48 h (28). Nevertheless, we did observe augmented responses of both ER stress and inflammasome activation in burn + LPS treatment group.

Enhanced ER stress and NLRP3 inflammasome activation affected postburn metabolism. By monitoring the metabolic modulator genes' expression, liver function, and blood glucose level, we noticed increased metabolic demand from burn injury and lowered metabolic capacity by LPS treatment. Robust increase in *G6pase*, *Fasn*, and *Sik 1* in burn group postulates an increased need for gluconeogenesis and lipid metabolism, whereas decreased protein level of G6Pase in LPS-only and burn + LPS groups indicated impaired metabolic functioning attributed to LPS treatment. Significant decrease in hepatic SCD1 might be due to insulin resistance of burn and burn + LPS groups (29).

Particularly, PGC-1 α was of our research interest for its multiple, potent roles in mitochondria respiration, gluconeogenesis, lipid metabolism, and so on. PGC 1 α has two isoforms: a 113-kDa full-length isoform and a 38-kDa spliced isoform. Spliced PGC-1 α has the same functional domain with its full-length form but can more freely transport between the nucleus, mitochondria, ER, and cytosol and thus has a more powerful effect on metabolic modulation (30). A significant increase in *Pgc-1 α* mRNA expression in all three treatment groups suggested increased demand for it. However, in the LPS group, we did not observe corresponding increased protein levels of full-length PGC-1 α and actually observed significantly decreased levels of spliced PGC-1 α . This may partly account for the metabolic impairment and liver dysfunction in the burn + LPS group.

We previously reported that post-burn ER stress induced hepatic apoptosis contributing to liver damage (31, 32). In the current study, immunohistochemical analysis showed that LPS may augment such postburn liver damage (Fig. 1, E–H).

Our results support previously reported findings of Osuka et al. (11) that macrophages actively involve NLRP3 inflammasome activation. Moreover, our observation and findings may reasonably explain the controversy of survival advantage of inflammasome activation and its detrimental effects on clinical outcome: burn- and LPS-induced inflammasome activation has more profound impact on metabolic impairment and liver dysfunction than its protective effect of cytokines balancing (12). Considering the difference of severity of injury between the two studies (60% TBSA burn with second hit of LPS vs. 25% TBSA burn), we may also postulate the different modes of inflammasome activation upon a different intensity of stimuli (i.e., being protective upon mild injury and being detrimental upon severe injury).

We did not observe significant elevation of blood glucose level in this animal model. However, considering the unwellness of the animals in the treatment groups, which included weight loss (Fig. 1I) and decreased food intake (data not shown), blood glucose level of greater than 7 mmol/L was still quite noticeable, which might still be indicative for postburn hypermetabolism.

To investigate whether LPS, ER stress, and/or inflammasome activation directly or indirectly interfere with PGC-1 α expression and function, we examined the upstream modulators of PGC-1 α . Three regulatory pathways were studied. The first is PKA C/PGC-1 α interaction. It has been shown that activation of PKA can significantly increase the nuclear content of spliced PGC-1 α , and thus, PKA modulates PGC-1 α -dependent signaling (33). We found significantly decreased phospho-PKA C indicating the inhibition of PKA activity in the LPS group, which was consistent with the decreased level of spliced PGC-1 α . Therefore, we infer that PKA C inhibition at least partly contributes to attenuation of spliced PGC-1 α in burn + LPS treatment group.

The second regulatory pathway is AMPK. AMPK may activate PGC-1 α through phosphorylation of specific serine and threonine residues (34). By Western blotting analysis of AMPK system in this two-hit model, we found that there was a significant decrease in phospho-AMPK α in the burn + LPS group. There were no significant changes in the level of

phospho-AMPK β and total AMPK α and AMPK β . Because AMPK α is the catalyst unit, whereas AMPK β is regulatory, our result suggests that AMPK activity is directly inhibited at its catalyst unit in burn + LPS treatment group. The consistency of attenuation of PGC-1 α and AMPK α activity may also indicate the possibility of involvement of AMPK α in PGC-1 α regulation.

The third upstream regulatory molecule of PGC-1 α is sirtuin-1, which activates PGC-1 α through NAD⁺-dependent deacetylation. In this way, sirtuin-1 links metabolic perturbation with cellular transcriptional output. We observed inhibition of sirtuin-1 in burn and burn + LPS treatment group but not LPS-only group. Based on this observation, we may reasonably postulate that the inhibition of PGC-1 α in burn + LPS treatment group is the overlay of burn-induced sirtuin-1 inhibition and PKA C/AMPK α signaling blockage mainly induced by LPS. Further confirmative research is needed to establish such causative relationship between PGC-1 α inhibition and liver dysfunction and damage.

Western blotting analysis of PPAR- α did not show significant changes among different treatment groups. It is thus unlikely that PPAR- α in liver would respond to the changes in burn or LPS-induced stress or inflammasome activation. Because PGC-1 α is usually regarded as the coactivator of PPAR- α (35), our result may indicate that PGC-1 α plays a regulatory role in PPAR- α transcriptional activity.

Based on this study, we summarize that there is inflammasome activation in the liver after burn and LPS administration. Second, NLRP3 inflammasome activation contributes to the post-burn ER stress response, and the two pathological processes exacerbate metabolic dysfunction in the liver. Finally, PGC-1 α most likely plays an important role in the hypermetabolic response after burn and may be regulated by PKA C, AMPK α , and sirtuin-1 signaling pathways. Future studies will investigate whether PGC-1 α , PKA C, AMPK α , and sirtuin-1 may represent potential therapeutic targets for the treatment of postburn ER stress, inflammasome activation, and subsequent metabolic dysfunction.

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Fig. 1. Enzymatic indicators and histological evidence of liver damage, weight loss, and changes in blood glucose level in the two-hit rat model of burn and sepsis

A and B, Plasma level of ALT and AST was determined by calorimetric enzyme activity assay. The reference ranges are 17.5 to 30.2 U/L for ALT and 45.7 to 80.8 U/L for AST. C and D, Representative Western blotting and densitometry of caspase 3 (normalized by loading control of GAPDH) in liver tissue. E–H, Immunohistochemical analysis of TUNEL staining of liver tissue slides (magnification $\times 400$). Arrows with and without tails indicated positive and negative staining of TUNEL, respectively. I, Weight gain/loss presented is in percentage of pre-experimental weight. J, The level of blood glucose elevated to slightly above reference range (4–6 mmol/L) in burn-only group and burn + LPS group (compared with sham, $P > 0.05$). For A, B, D, I and J, data presented are mean $\pm$ SD ($n = 6$ in each group), * $P < 0.05$, ** $P < 0.01$ vs. sham.

Fig. 2. Two-hit model of burn + LPS injection augments inflammasome formation in rat liver Interleukin 1 (A), EMR (B), and NLRP3 (C) mRNA expressions in the liver of animals of the sham, sham + LPS, burn, and burn + LPS treatment groups (n = 6 in each group) were measured by quantitative reverse transcriptase (RT)-PCR. In A, B, and C, bars showed relative expression of each gene against the 18S house keeping gene. Protein level of NLRP3 in liver was determined by Western blot (D) and densitometry analysis (normalized by loading control of GAPDH) (E). Data presented are mean $\pm$ SD, * P < 0.05, ** P < 0.01 vs. sham.

Fig. 3. Burn and LPS induced hepatic ER stress

A–D, Endoplasmic reticulum stress marker genes expression (*Chop*, *Bip*, *Xbp1-s*, and *Pdi*) in the liver of animals of the sham, sham + LPS, burn, and burn + LPS treatment groups were measured by quantitative RT-PCR. Bars showed relative expression of each gene against the 18S housekeeping gene. E and F, Western blot and densitometry analysis of BIP (normalized by loading control of GAPDH) in liver tissue. Data shown are mean $\pm$ SD (n = 6 in each group), * P < 0.05, ** P < 0.01 vs. sham.

Fig. 4. Burn and LPS induced changes in gene expression of metabolic modulators in liver
A–C, Hepatic metabolic modulator genes expressions (*G6Pase*, *Fasn*, and *Sik1*) in the liver of animals in sham, sham + LPS, burn, and burn + LPS treatment groups were measured by quantitative RT-PCR. Bars showed relative expression of each gene against the 18S housekeeping gene. D and E, Representative Western blot of G6Pase and SCD1 in liver tissue. F and G, Densitometry analysis of the Western blot was normalized by loading control of GAPDH. Data presented are mean $\pm$ SD (n = 6 in each group), * P < 0.05, ** P < 0.01 vs. sham.

Fig. 5. Burn and LPS induced changes in PGC 1α in liver: from gene expression to tissue content of the protein

A, mRNA level of *Pgc-1α* in liver was measured by quantitative RT-PCR with 18S housekeeping gene as the reference. B, Representative Western blot of PGC-1α in liver tissue. PGC 1α has two isoforms: 113-kDa full-length form and 38-kDa spliced form. C and D, Densitometry analysis of the Western blot was normalized by loading control of GAPDH. Data presented are mean $\pm$ SD (n = 6 in each group), * P < 0.05, ** P < 0.01 vs. sham.

Fig. 6. Lipopolysaccharide reduced PGC-1α by inhibiting its upstream regulators

A, Representative Western blot of phosphor-PKA C, phosphor-AMPKα and phosphor-AMPKβ, and total AMPKα and AMPKβ, as well as PPAR-α. B–G, Densitometry analysis of the Western blot was normalized by loading control of GAPDH. Data presented are mean $\pm$ SD (n = 6 in each group), * P < 0.05 vs. sham.