Hepatoprotective role of ganoderma lucidum polysaccharide against BCG-induced immune liver injury in mice

Guo-Liang Zhang, Ye-Hong Wang, Wei Ni, Hui-Ling Teng, Zhi-Bin Lin

AIM: To examine the effect of ganoderma lucidum polysaccharide (GLP) on the immune liver injury induced by BCG infection, and investigate the relationship between degrees of hepatic damage and NO production in mice.

METHODS: Immune hepatic injury was markedly induced by BCG-pretreatment (125 mg·kg⁻¹, 2-week, iv) or by BCG-pretreatment plus lipopolysaccharide (LPS, 125 µg·kg⁻¹, 12-hour, iv) in mice in vivo. Hepatocellular damage induced by BCG-pretreated plus inflammatory cytokines mixture (CM), which was included TNF-α, IL-1β, IFN-γ and LPS in culture medium in vitro. Administration of GLP was performed by oral or intracutaneously with culture medium at immune stimuli simultaneity. Liver damage was determined by activity of alanine aminotransferase (ALT) in serum and in hepatocytes cultured supernatant by liver weight changes and histopathological examination. NO production in the cultured supernatant was determined by the Griess reaction. Moreover, inducible nitric oxide synthase (iNOS) protein expression was also examined by immunohistochemical method.

RESULTS: Immune hepatic injury was markedly induced by BCG or BCG plus inflammatory cytokines in BALB/c mice in vivo and in vitro. Under BCG-stimulated condition, augmentation of the liver weight and increase of the serum/ supernatant ALT level were observed, as well as granuloma formation and inflammatory cells soakage were observed by microscopic analysis within liver tissues. Moreover, NO production was also increased by BCG or CM stimuli in the culture supernatant, and a lot of iNOS positive staining was observed in BCG-stimulated hepatic sections. Application of GLP significantly mitigated hepatic tumefaction, decreased ALT enzyme release and NO production in serum/supernatant, improved the pathological changes of chronic and acute inflammation induced by BCG-stimuli in mice. Moreover, the immunohistochemical result showed that GLP inhibited iNOS protein expression in BCG-immune hepatic damage model.

CONCLUSION: The present study indicates that NO participates in immune liver injury induced by Mycobacterium bovis BCG infection. The mechanisms of protective roles by GLP for BCG-induced immune liver injury may be due to influence NO production in mice.

INTRODUCTION

Ganoderma lucidum polysaccharide (GLP) is an important pharmacological ingredient extracted from fruit bodies and mycelium of mushroom Ganoderma Lucidum (Fr.) Karst. It has been extensively documented that GLP can improve the damage induced by specific and nonspecific immunity responses. In our laboratory recently studies, it was confirmed that GLP enhanced phagocytosis of intraperitoneal macrophage, inhibited the growth of implanted Sarcoma 180 and HL-60 tumor cells in vitro. However, the regulating mechanism of GLP in the immune response remain unknown.

In the recently studies, Mycobacterium bovis bacillus Calmette-Guerin (BCG) infection has been proven to induce immune hepatic injury in rodent animal. In this pathological model, the releases of hepatic endogenous cytokines, such as TNF-α, IFN-γ and IL-1β were observed in vivo. Moreover, in our laboratory previously experiment, it has been observed that inflammatory cytokines including TNF-α and IL-1β stimulated NO production in the primary cultured rat hepatocytes in vitro, but the influence of GLP in this immune damage model and the exact function of NO production in the presence of inflammatory stimuli have not been elucidated yet. Therefore, the present study was performed to determine the effects of GLP on the BCG-stimulated immune liver injury in vitro and in vivo, to investigate the possible mechanism of the influence induced by GLP in this immune response.

MATERIALS AND METHODS

Reagents

Following reagents were purchased from Sigma Chemical Co.: collagenase (Type IV, 340 kU·g⁻¹), bovine insulin, and lipopolysaccharide (LPS, E.coli.O111:B4). Other materials were obtained from the following sources: kit for determining...
serum and culture supernatant alanine transaminase (ALT) was from Beijing Institute of Biological Products (Beijing); Mycobacterium tuberculosis Bacille Calmette-Guérin (BCG) vaccine was from the National Vaccine and Serum Institute (Beijing); human recombinant (rh) tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interferon-gamma (IFN-γ) were from Academy of Military Medical Sciences (Beijing), and Dulbecco’s modified Eagle’s medium (DMEM) from Gibco BRL; Ganoderma lucidum polysaccharide (GLP) was isolated from mycelium of Ganoderma lucidum and provided by the Department of Phytochemistry, College of Pharmacy, Beijing University. For using immunohistochemistry, iNOS polyclonal antibody (rabbit anti-mouse immunoglobulin) was purchased from Beijing Zhong-Shan Biotechnology co., LTD.

Animals treatment and liver damage induction
Male BALB/c mice weighing 18-22g (6-8 weeks old), were provided by Experimental Animal Center, Beijing University. Immune hepatic injury was induced by intravenous injection of BCG (125 mg·kg⁻¹) for two weeks, or induced by LPS (125 µg·kg⁻¹) for 12 hours at BCG-pretreated 14 day later[13,19]. Control group mice were treated by same volume of phosphate buffered saline (PBS). After animals were BCG-pretreated 7 days, the group mice were treated by same volume of phosphate buffered saline (PBS). After animals were BCG-pretreated 7 days, the different concentrations (25 mg·kg⁻¹, 50 mg·kg⁻¹, 100 mg·kg⁻¹ and 200 mg·kg⁻¹, respectively) of GLP were intragastric administered once at everyday within succedent one week. At immune stimulating 2 weeks later, mice were killed by cervical dislocation, blood was collected and centrifuged at 3000 rpm for 5 min. Serum was obtained at the supernatant for mensuration enzyme level. Liver samples were removed rapidly for histopathological and immunohistochemical examination.

Hepatocyte isolation and culture
Hepatocytes were harvested from control mice or BCG-pretreated for 2 weeks mice using an in situ collagenase perfusion technique[20]. After inhalation anesthesia, the abdomen of the animals was opened and shaved, the portal vein was exposed and cannulated. Then the liver was perfused at 37 °C in situ first with a calcium-free phosphate-buffered saline solution (PBS) with 6-8 mL/min velocity of flow. This perfusion was continued for 5 min, then it was switched to 0.5 g·L⁻¹ collagenase and 10 g·L⁻¹ bovine albumin in PBS buffer for 15 min. The liver was removed and the cells were combed gently in tissue culture medium. Hepatocytes were pelleted, washed, and separated from nonparenchymal cells by differential centrifugation at 50×g. Viability of cells exceeded 85 % as determined by trypan blue exclusion. Hepatocytes were plated onto 6-well plastic tissue-culture plates (1×10⁶ cells·L⁻¹ in each well). Medium in the control consisted of DMEM with L-arginine (0.5 mmol·L⁻¹), insulin (1 mmol·L⁻¹), Heps (15mmol·L⁻¹), L-glutamine, penicillin, streptomycin, and 100 mL·L⁻¹ low-endotoxin newborn calf serum. After overnight incubation, the medium was changed with a cytokines mixture (CM) containing LPS (10 mg·L⁻¹), IL-1β (10 KU·L⁻¹), TNF-α (500 KU·L⁻¹) and IFN-γ (100 KU·L⁻¹). Other experimental conditions included addition of GLP, at the different concentrations (50 mg·L⁻¹ or 200 mg·L⁻¹), to the CM. After primary cultures were maintained for 24 h at 37 °C in 50 mL·L⁻¹ CO₂, hepatocytes or cultured supernatants were collected for nitrite and ALT activity assays[21].

Table 1

| Group                  | Liver weight (g) | ALT (U·L⁻¹) |
|------------------------|------------------|-------------|
| Control                | 0.99±0.16        | 22.03±10.99 |
| BCG (125 mg·kg⁻¹)      | 1.79±0.24        | 245.18±81.03|
| BCG (125 mg·kg⁻¹) + LPS (125 µg·kg⁻¹) | 1.84±0.14 | 285.88±23.81|
| BCG (125 mg·kg⁻¹) + GLP (25 mg·kg⁻¹) | 1.78±0.20 | 236.86±27.94|
| BCG (125 mg·kg⁻¹) + GLP (50 mg·kg⁻¹) | 1.57±0.18 | 189.81±43.99|
| BCG (125 mg·kg⁻¹) + GLP (100 mg·kg⁻¹) | 1.28±0.20 | 178.78±13.16|
| BCG (125 mg·kg⁻¹) + GLP (200 mg·kg⁻¹) | 1.41±0.43 | 208.18±27.93|

Assay for hepatocellular enzyme release and NO production
As a marker of hepatocytes necrosis, activity of alanine aminotransferase (ALT) was spectrophotometrically measured using a determining kit in serum and culture supernatants, at 520 nM in the presence of α-ketoglutarate, aspartate, NADH and malate dehydrogenase, as described[19]. The amount of NO production in the serum and the culture supernatants were determined as its stable oxidative product, nitrite, by an automated procedure based on the Griess reaction, as previously described[20].

Histopathological and immunohistochemical examination
Livers were removed, fixed overnight in 10 % buffered formalin, and paraffin-embedded. Six-micrometer sections were stained with hematoxylin-eosin for histological evaluation. Immunohistochemical staining for iNOS protein expression was carried out using rabbit polyclonal antibodies to iNOS on cryostat sections (five-micrometer). The sections were incubated with peroxidase-labeled rabbit anti-mouse immunoglobulin for 1 hour. After another wash in PBS, the sections were stained with AEC for several minutes to develop the color and washed in water. Each experiment was repeated two to three times with similar results. Three random sections of each liver were examined[19].

Statistics analysis
Data were presented with ±s. Statistical analysis was performed using ANOVA. Differences were judged to be statistically significant when the P value was less than 0.05.

RESULTS
Effect of Ganoderma lucidum polysaccharide (GLP) on the liver weight and the activity of serum alanine transaminase (ALT) in BCG-induced immune hepatic injury in mice in vivo
Compared with the control group, BCG-pretreatment markedly induced hepatic damage (Table 1). The augment of the liver weight and the serum ALT level were observed after BCG-administrated 2 weeks in mice (P<0.01). Furthermore, application of inflammatory lipopolysaccharides (LPS) for BCG-pretreated mice induced serum ALT activity further higher than that BCG-treated alone in mice (P<0.05), but the liver weights were not further increased than that BCG-stimulated only groups. On the other hand, under the presence of BCG stimuli conditions, administration of CLP decreased the liver weight within the range of 50 mg·kg⁻¹ (P<0.05) to 200 mg·kg⁻¹ (P<0.01), simultaneously, serum ALT release were significantly decreased by GLP treatment in a dose-dependent manner within the similar range of concentrations (P<0.05).

Effect of Ganoderma lucidum polysaccharide (GLP) on the liver weight and the activity of serum alanine transaminase (ALT) in BCG-induced immune hepatic injury in mice in vivo (Cont.)
Figure 2 Immunohistochemical examination of inducible nitric oxide synthase (iNOS) protein expression stimulated by BCG in the presence or absence of *Ganoderma lucidum* polysaccharide (GLP) in mice. (Original magnification 200×). Mice were treated with (A) control, (B) Bacille Calmette-Guérin (BCG, 125 mg·kg⁻¹, 2 weeks), (C) BCG plus lipopolysaccharides (LPS, 125 µg·kg⁻¹, 12hr), (D) BCG plus GLP (100 mg·kg⁻¹), as described in Materials and Methods.

Figure 1 Histological changes of BCG-induced immune hepatic injury in the presence or absence of *Ganoderma lucidum* polysaccharide (GLP) in mice. Hematoxylin and eosin. Mice were treated with (A) control, (B) Bacille Calmette-Guérin (BCG, 125 mg·kg⁻¹, 2 weeks), (C) BCG plus lipopolysaccharides (LPS, 125 µg·kg⁻¹, 12hr), (D) BCG plus GLP (100 mg·kg⁻¹), as described in Materials and Methods. (Original magnification 200×).
Effect of Ganoderma lucidum polysaccharide (GLP) on the pathohistological changes in BCG-stimulated hepatic tissues in mice in vivo

As shown in Figure 1, opposing with the results of control group, BCG-stimulated group were observed markedly changes of liver histologic structure (Figure 1-B), for example, infiltration within liver lobules by inflammatory cells, extensive hepatoocytes hypertrophy, nuclear narrow, and granulation and vacuolization of the hepatocyte cytoplasm were observed in the liver section. Moreover, treatment with BCG plus LPS for mice resulted in more severe histological changes including thrombosis in the central hepatic vein and hemorrhage in the liver parenchyma(Figure1-C). Granulomas formation, a marker of chronic hepatitis fibrosis' were significantly increased by BCG-stimulated hepatic tissues (Tabel 2, P<0.01). But in the presence of BCG condition, the result shown that LPS was not triggered more the granuloma forming, on the contrary, triggered more fearful hepatic tissues hemorrhage (Figure 1 B-C).

On the other hand, the results of histological examination shown that GLP (100 mg·kg$^{-1}$) alleviated hepatic damage in BCG-induced acute inflammation, such as markedly decrease of infiltration within liver lobules by inflammatory cells, nuclear narrow,etc. in the observed liver section (Figure 1-D). Moreover, granulomas formation were also decreased by GLP treatment at concentration range from 100 mg·kg$^{-1}$ to 200 mg·kg$^{-1}$,(P<0.01).

Table 2 Effect of Ganoderma lucidum polysaccharide (GLP) on the granuloma formation (numbers/ microscopic view) in BCG -pretreated mice hepatic histological slides. (±s)

| Group                  | Granulomas |
|------------------------|------------|
| Control                | 0          |
| BCG (125 mg·kg$^{-1}$) | 64.67±4.97 |
| BCG (125 mg·kg$^{-1}$) + LPS (125 µg·kg$^{-1}$) | 54.40±4.93 |
| BCG (125 mg·kg$^{-1}$) + GLP (50mg·kg$^{-1}$) | 60.00±4.24 |
| BCG (125 mg·kg$^{-1}$) + GLP (100mg·kg$^{-1}$) | 4.00±4.22   |
| BCG (125 mg·kg$^{-1}$) + GLP (200mg·kg$^{-1}$) | 36.80±5.81  |

*P<0.05, **P<0.01 compared with control. **P<0.05, ***P<0.01 compared with BCG-pretreated group. n=5 microscopic views.

Effects of Ganoderma lucidum polysaccharide (GLP) on the ALT activity and NO production induced by BCG in the presence or absence of cytokines mixture (CM) in primary cultured mice hepatocytes in vitro

The result of this part of experiment shown that inflammatory cytokines increased NO production and ALT release into the supernatant in the primary cultured hepatocytes prestimulated by BCG (P<0.01, Table 3). In the absence of cytokines condition, addition of CLP only had not influence on the activity of ALT enzyme and NO production in BCG-pretreated cultured supernatant (P>0.05). Whereas, in the presence of inflammatory cytokines plus BCG prestimulus condition, ALT activity and NO production were markedly inhibited by application of GLP (P<0.01).

Table 3 Effects of Ganoderma lucidum polysaccharide (GLP) on the alanine transaminase (ALT) activity and nitrite (NO$\alpha$) production induced by BCG-pretimulating in the presence or absence of cytokines mixture (CM) in primary cultured mice hepatocytes in vitro (±s)

| Group                        | ALT (U·L$^{-1}$) | NO$\alpha$ (µmol·L$^{-1}$) |
|------------------------------|-----------------|-----------------------------|
| Control                      | 11.52±1.41      | 1.41±0.72                   |
| BCG                          | 17.87±3.14      | 3.52±1.72                   |
| BCG + GLP (50 mg·L$^{-1}$)   | 21.30±2.87      | 3.95±2.27                   |
| BCG + GLP (200 mg·L$^{-1}$)  | 18.03±2.24      | 3.24±1.08                   |
| BCG + CM + GLP (50 mg·L$^{-1}$) | 23.98±6.33   | 4.11±2.26                   |
| BCG + CM + GLP (200 mg·L$^{-1}$) | 20.61±3.74   | 3.49±1.38                   |

*P<0.05, **P<0.01 compared with BCG-pretreated group; **P<0.05, ***P<0.01 compared with BCG+CM group. n=7 mice . (3 wells for each treatment in each experiment).

Effect of Ganoderma lucidum polysaccharide (GLP) on the inducible nitric oxide synthase (iNOS) protein expression in BCG-stimulated mice hepatic tissues in vivo

To confirm the possible mechanism about hepatoprotective role of GLP against BCG-stimulated in mice, the correlativity between iNOS expression and immune hepatic damage were investigated. As shown in the results of immunohistochemistry, compared with control group mice, there was a lot of iNOS positive brown stained agglomerate observed in BCG-stimulated hepatic section (Figure 2 A-B). But consisted with the results of granuloma formation, there were not more the iNOS expression induced by LPS in the presence of BCG stimuli condition (Figure 2-C). On the contrary, treatment of GLP significantly inhibited iNOS protein expression under similary BCG-stimulated condition (Figure 2-D).

DISCUSSION

In the present experiment, the results shown that the administration of GLP was effective against acute and chronic hepatic inflammation induced by BCG-immunostimulant in mice. Administration of GLP significantly decreased serum or supernatant ALT level in BCG-caused acute inflammatory response in vivo and in vitro. Histological changes, such as hemorrhage and necrosis in hepatic lobules, inflammatory infiltration of lymphocytes and kupffer cells around the central vein, were simultaneously improved by the treatment of GLP. These results were consistent with that GLP showed anti-inflammatory and antioxidative activities in the previous other laboratory observed results[23]. Moreover, pathohistological examination also showed that GLP decreased the granuloma formation, which is popularly considered as the first step of fibrillar repair in the chronic inflammatory process[23-26]. This result suggested that GLP may be not only as an anti-inflammatory agent, but also may be used as an antigibrotic therapy for hepatocirrhosis.

To investigate the possible mechanisms of the hepatic protective effect of GLP in the immune-stimulated condition, we further detected NO production in primary cultured hepatocytes and iNOS protein expression in the BCG-stimulated hepatic tissues[27-30]. The results shown that GLP alone had no effect on the production of NO in the cultured hepatocytes. In the presence of BCG condition, cytokines
mixture (CM) including TNF-α, IFN-γ, and LPS, significantly increased the NO production. When combined with GLP, this effect has been remarkably reversed. At the same time point, GLP also attenuated the increase of ALT activity in inflammatory cytokines-stimulated hepatocytes in vitro. It has been recognized that NO is produced by cNOS and/or iNOS in mice liver.[31-37]. The results of immunohistochemistry showed that GLP effect on NO production is mainly through iNOS under immunological stimuli condition. The results of this study suggested that although the exact mechanism of action of GLP on such macrophage/lymphocyte properties of granulomas remain unknown, nevertheless, it might be related to NO production induced by cytokines[38-42]. Therefore, inhibition of NO production is partly the mechanisms of GLP protective effect on the immunological injured liver.

In summary, the present study indicates that NO participates in immune liver injury induced by Mycobacterium bovis BCG infection. Furthermore, the mechanisms of protective roles by GLP for BCG-induced immune liver injury in mice may be due to influence NO production. However, further study is needed to understand the exact mechanisms of the antihypertotoxic activity and the free radical scavenging activity of GLP. The clinical applicability of GLP remains to be established.

REFERENCES

1 Bao XF, Liu CQ, Fang J, Li XY. Structural and immunological studies of a major polysaccharide from spores of Ganoderma lucidum (Fr.) Karst. Carbohydr Res 2001;332:67-74
2 Cheung WM, Hui WS, Chu PW, Chiu SW, Ip NY. Ganoderma lucidum activates MAP kinases and induces the neuronal differentiation of rat pheochromocytoma PC12 cells. FLETS Lett 2000; 486:291-296
3 Ma L, Lin ZB. Effects of Ganoderma polysaccharides on IL-2 production by mouse splenocytes in vitro. J Biomed 1991;23:412-417
4 Lei LS, Lin ZB. Effect of Ganoderma polysaccharides on T cell subpopulations and production of interleukin 2 in mixed lymphocyte response. Aeta Pharmaceutica Sinica 1992, 27:331-335.
5 Zhang QH, Lin ZB. The antitumor activity of Ganoderma lucidum (Curt.Fr.) P. Karst. (Ling Zhi) (Aphyllophoromy ceaeidae) polysaccharides is related to tumor necrosis factor-a and interferon. J Biol M ed uum 1999;1:207-215
6 Ouyang EC, Wu CH, Walton C, Promrat K, Wu GY. Transplantation of human hepatocytes into tolerized genetically immunocompetent rats. World J Gastroenterol 2001;7:324-330
7 Guo SP, Wang WL, Zhai YQ, Zhao YL. Expression of nuclear factor-kb in hepatocellular carcinoma and its relation with the X protein of hepatitis B virus. World J Gastroenterol 2001;7:340-344
8 You J, Zhuang L, Tang BZ, Yang WB, Ding SY, Li W, Wu RX, Zhang HL, Zhang YM, Yan SM, Zhang L. A randomized controlled clinical trial on the treatment of Thymosin-a 1 versus interferon-a in patients with hepatitis B. World J Gastroenterol 2001;7:411-414
9 Li XW, Ding YQ, Cai JJ, Yang SQ, An LB, Qiao DF. Studies on mechanism of Sialy Lewis-X antigen in liver metastases of human colorectal carcinoma. World J Gastroenterol 2001;7:425-430
10 Liu BH, Chen HS, Zhou JH, Xiao N. Effects of endotoxin on endothelin receptor in hepatic and intestinal tissues after endotoxemia in rats. World J Gastroenterol 2000;6:286-300
11 Cheng JL, Tong WB, Liu BL, Zhang Y, Yan Z, Feng BF. Hepatitis C virus in human B lymphocytes transformed by Epstein-Barr virus in vitro by in situ reverse transcription-polymerase chain reaction. World J Gastroenterol 2001;7:370-375
12 Zhong L, You J, Tang BZ, Ding SY, Yan KH, Peng D, Zhang YM, Zhang L. Preliminary results of Thymosin-a 1 versus interferon-a treatment in patients with HbeAg negative and serum HBV DNA positive chronic hepatitis B. World J Gastroenterol 2001;7:407-410
13 Carpenter E, Fray L, Gormley E. Antigen-specific lymphocytes enhance nitric oxide production in Mycobacterium bovis BCG-infected bovine macrophages. Immunol Cell Biol 1998; 76:363-366.
14 Yang GS, Liu GT. Role of nitric oxide in immunological liver damage in mice. Biochem Pharmacol 1995; 49:1277-1281
15 Bai XY, Jia XH, Cheng LZ, Gu YD. Influence of IFN-2b and BCG on the release of TNF and IL-1 by Kupffer cells in rats with hepaboma. World J Gastroenterol 2001;7:419-421
16 Erb KJ, Kirman J, Delahunt B, Chen WX, Gros GL. IL-4, IL-5 and IL-10 are not required for the control of M. Bovis-BCG infection in mice. Immunol Cell Biol 1998; 76:41-46
17 Ugaz EMA, Pinheiro SR, Guerra JL, Palermo-Neto J. Effects of prenatal diazepam treatment on Mycobacterium bovis-induced infection in hamsters. Immunopharmacology 1999; 40:209-217
18 Zhang GL, Lin ZB. Effects of cytokines on the endotoxin stimulated nitric oxide production in the primary cultured rat hepatocytes. Beijing Yi Ke Xue Yu Xuebao 1998;30:180-182
19 Zhang GL, Lin ZB, Zhang B. Effects of selective inducible nitric oxide synthase inhibitor on immunological hepatic injury in rat. Zhanghua Yi Xue Zhai 1998;7:540-543.
20 Zhang GL, Lin ZB. Dinoprostone potentiates cytokines and lipopolysaccharides to induce nitric oxide production in cultured rat hepatocytes. Acta Pharmacol Sinica 1999; 20: 262-266
21 Zhang GL, Wang YH, Teng HL, Lin ZB. Effects of aminoguanidine on the nitric oxide production induced by inflammatory cytokines and endotoxin in cultured rat hepatocytes. World J Gastroenterol 2001;7:331-334
22 Lee JM, Kwon H, Jeong H, Lee JW, Lee SY, Baek SJ, Surh YJ. Inhibition of lipid peroxidation and oxidative DNA damage by Ganoderma lucidum. Phytother Res 2001;15: 245-249
23 Nie QH, Cheng YQ, Xie YM, Cao YZ. Inhibiting effect of anti-323eigenol oligonucleotides phosphorothioate on gene expression of TIMP-1 in rat liver fibrosis. World J Gastroenterol 2001;7:363-369
24 Huang YQ, Xiao SD, Mo JZ, Zhang DZ. Effects of nitric oxide synthesis inhibitor in long term treatment on hypertensive rats. World J Gastroenterol 2001;7:486.
25 Feng ZJ, Feng LY, Sun ZM, Song M, Yao XX. Expression of nitric oxide synthase protein and gene in the splanchnic organs of liver cirrhosis and portal hypertensive rats. World J Gastroenterol 2000;6 (Suppl 3):31
26 Vernia S, Beaune P, Coloma J, Lopez-Garcia PM. Differential sensitivity of rat hepatocyte CYP isoforms to self-generated nitric oxide. FEBS 2001;488: 59-63
27 Wang JH, Redmond HP, Wu QD, Boucher-Hayes D. Nitric oxide mediates hepatocyte injury. Am J Physiol 1998; 275: G1117-G1126
28 Alexander B. The role of nitric oxide in hepatic metabolism. Nutrition 1998; 14: 311
29 Kaibori M, Sakitani K, Oda M, Kamiyama Y, Masu Y, Nishizawa M, Ito S, Okumura T. Immunosuppressant FK506 inhibits inducible nitric oxide synthase gene expression at a step of NF-kb activation in rat hepatocytes. J Hepatol 1999;30: 1138-1145
30 McCaffery DM, Maddox JS, Swan MG, Kubes P. Inducible nitric oxide synthase plays a critical role in resolving intestinal inflammation. Gastroenterology 1997;112:1022-1027
31 Moriyama A, Tabata T, Unoki H, Abe S, Masumoto A, Otsuki M. Plasma nitrite/ nitrate concentrations as a tumor marker for hepatocellular carcinoma. Clinica Chimica Acta 2000; 296: 181-191
32 Har A, Miltani N, Aidi T. Inhibitory effect of nitric oxide on the induction of cytochrome P450 3A4 mRNA by 1,
25-Dihydroxyvitamin D3 in Caco-2 cells. Free Rad Res 2000; 33: 279-285
33 Yu J, Guo F, Ebert MPA, Malfertheiner P. Expression of inducible nitric oxide synthase in human gastric cancer. World J Gastroenterol 1995; 11: 430-431
34 Ji XL, Shen MS, Yin T. Liver inflammatory pseudotumor or parasitic granuloma? World J Gastroenterol 2000; 6: 458-460
35 Heneka MT, Loschmann PA, Gleichmann M, Weller M, Schulz JB, Wullner U, Klockgether T. Induction of nitric oxide synthase and nitric oxide-mediated apoptosis in neuronal PC12 cells after stimulation with tumor necrosis factor-α/lipopolysaccharide. J Neurochem 1998; 71: 88-94
36 Liu SH, Tzeng HP, Kuo ML, Lin-Shiau SY. Inhibition of inducible nitric oxide synthase by β-lapachone in rat alveolar macrophages and aorta. Br J Pharmacol 1999; 126: 746-750
37 Vos TA, Gouw AS, Klok PA, Havinga R, Goor H, Huitema S, Roelofsen H, Kuipers F, Jansen P, Moshage H. Differential effects of nitric oxide synthase inhibitors on endotoxin-induced liver damage in rats. Gastroenterology 1997; 113: 1323-1333
38 Tzeng E, Billiar TR, Williams DL, Li J, Lizonova A, Kovacs I, Kim YM, Pa P. Adenovirus-mediated inducible nitric oxide synthase gene transfer inhibits hepatocyte apoptosis. Surgery 1998; 124: 278-283
39 Nomura T, Ohtsuki M, Matsui S, Sumi-Ichinose C, Nomura H, Hagino Y. Nitric oxide donor NOR3 inhibits ketogenesis from oleate in isolated rat hepatocytes by a cyclic GMP-independent mechanism. Pharmacol Toxicol 1998; 82: 40-46
40 Imagawa J, Yellon DM, Baxter GF. Pharmacological evidence that inducible nitric oxide synthase is a mediator of delayed preconditioning. Br J Pharmacol 1999; 126: 701-708
41 Tuncan B, Uludag O, Altug S, Abacloglu N. Effects of nitric oxide synthase inhibition in lipopolysaccharide-induced sepsis in mice. Pharmacol Res 1998; 38: 405-411
42 Ohmori H, Egusa H, Ueura N, Matsumoto Y, Kanayama N, Hikida M. Selective augmenting effects of nitric oxide on antigen-specific IgE response in mice. Immunopharmacology 2000; 46: 55-63

Edited by Pang LH