Effects of glycine on plasma and liver tissue changes of TNF-α, ET-1 and nitric oxide contents in rats with obstructive jaundice

He-Qing Fang, Ying-Bin Liu, Hai-Jun Li, Shu-You Peng, Yu-Lian Wu, Bin Xu, Jian-Wei Wang, Jiang-Tao Li, Xin-Bao Wang

INTRODUCTION

Endotoxemia is one of the major causes that can lead to complicated pathophysiologic alterations in the process of obstructive jaundice. Current research has demonstrated that severe endotoxemia in obstructive jaundice could activate immunocompetent cells such as monocytes, macrophages and endothelial cells to produce a variety of cytokines that could contribute to an uncontrollable inflammatory cascade causing multiple organ dysfunction (MODS) or even death[1-3]. Among these cytokines, TNF-α, ET-1 and NO have been considered to be the main effectors in endotoxemia[4-10]. It is also worthy to note that glycine could provide an effective protection against endotoxemia that has been recently published in several reports[11-15]. The present study, therefore, was conducted with the aim to evaluate the effect of glycine on the plasma and liver tissue changes of TNF-α, ET-1 and nitric oxide contents in rats with obstructive jaundice.

MATERIALS AND METHODS

Animal model and experimental protocol

Ninety healthy Wistar rats of both sexes weighing 275±25 g were employed in the study. According to the experimental protocol, the animals were randomly divided into Group A in which the rats were performed a sham operation, Group B in which the rats were operated merely to ligate the common bile duct and Group C in which the rats were treated with both the ligation of common bile duct and a glycine regimen. Before operation, all animals were allowed access to standard rat chow and water ad libitum for 5 days except that the rats in Group C drank 5% glycine solution (provided by Shanghai Institute of Medical Science & Technology Ltd.) instead of water[16]. After a 12-h fasting period, the animals were weighed and anesthetized with 1% pentobarbitale sodium (30 mg/kg, ip) and their common bile duct and Group C in which the rats were treated with both the ligation of common bile duct and a glycine regimen. Before operation, all animals were allowed access to standard rat chow and water ad libitum for 5 days except that the rats in Group C drank 5% glycine solution (provided by Shanghai Institute of Biological Products Research) instead of water[12]. After a 12-h fasting period, the animals were weighed and anesthetized with 1% pentobarbitale sodium (30 mg/kg, ip) and their common bile duct was exposed and ligated to form a complete obstruction of the extrahepatic bile duct except that the common bile duct in rats of Group A was only exposed following laparotomy. When recovered for a period of 24 h, the rats were fasted before and after operation. Blood and liver tissue were sampled at the time of sacrifice on the 8th day post operation. Plasma total bilirubin, endotoxin, liver tissue were sampled at the time of sacrifice on the 8th day post operation. Plasma total bilirubin, endotoxin, levels, respectively, whereas they did not display any statistically significant difference between the former groups (P=0.417 and 0.374 respectively). Likewise, TNF-α, ET-1 and NO contents in both plasma and liver tissue were significantly increased in both bile duct-ligated and bile duct-ligated plus glycine-treated rats compared with sham-operated animals (P=0.00813, 0.00793, 0.00804, respectively) in plasma and 0.00912, 0.00981 and 0.00635 in liver tissue respectively). However, these inflammatory mediators in both plasma and liver tissue were significantly reduced in bile duct-ligated rats fed on 5% glycine solution compared with that without (P=0.00953, 0.00891, 0.00975, 0.00967 and 0.00907 in plasma and liver tissue respectively).

CONCLUSION

Reduction of TNF-α, ET-1 and NO contents in plasma and liver tissue of rats fed on glycine may be helpful to alleviate pathological lesions in obstructive jaundice.

Fang HQ, Liu YB, Li HJ, Peng SY, Wu YL, Xu B, Wang JW, Li JT, Wang XB. Effects of glycine on plasma and liver tissue changes of TNF-α, ET-1 and nitric oxide contents in rats with obstructive jaundice. World J Gastroenterol 2003; 9(10):2374-2376 http://www.wjgnet.com/1007-9327/9/2374.asp
concentration was finally expressed as Eu/ml.

Measurement of ET-1 level was performed using 1 ml of blood samples that was collected in a test tube containing 15 µl of 10 % disodium edetate and 20 µl of aprotinin, and centrifuged at 3 000xg for 10 min at 4 °C. The resulting plasma and liver homogenate were determined with ET-1 radioimmunoassay kit following the protocol of Research Institute of Radioimmunotechnology, General Hospital of PLA. Heparinised blood samples were collected in a separator tube and spun at 4 000 g for 10 min for detection of plasma bilirubin and TNF-α. To measure TNF-α, the resulting plasma and liver homogenate were analysed with a radioimmunoassay kit according to the instructions supplied by Research Institute of Radioimmunotechnology, General Hospital of PLA. Plasma bilirubin was determined with an automatic multifunction-biochemical analyzer.

Measurement of NOx/NO3 content two ml of heparinised blood samples was incubated at 37 °C for 1 h and then centrifuged at 2 000xg for 5 min. The resulting plasma and liver homogenate were determined with a NOx/NO3 assay kit following the procedures of Research Institute of Radioimmunotechnology, General Hospital of PLA.

**Statistical analysis**

Experimental data were processed by analysis of variance and t-tests for comparison between groups. Results were expressed as mean ± SE. P<0.05 was selected as the level of significance.

**RESULTS**

Poor appetite and jaundice were observed in rats of Groups B and C on the 2nd day post operation. Animals in Group A did not exhibit obvious abnormalities. But there was no significant difference of plasma total bilirubin between rats in Groups B and C (P=0.374).

Plasma endotoxin levels in rats of Groups B and C were significantly increased compared with that in rats of Group A (P=0.00921 and 0.00841 in Groups B and C respectively as compared to Group A) were also noted in these animals at the time of sacrifice on the 8th day post operation. Animals in Group A did not exhibit obvious abnormalities. But there was no significant difference of plasma total bilirubin between rats in Groups B and C (P=0.417).

Plasma levels of TNF-α, ET-1 and NO in Group B rats were significantly elevated compared with that in Group A rats (P=0.00813, 0.00793 and 0.00671 respectively), which were significantly improved when the rats were fed on 5 % glycine solution as shown in Table 1 (P=0.00953, 0.00891 and 0.0795). The data for TNF-α, ET-1 and NO levels in liver tissue are shown in Table 2. In accordance with their plasma counterparts, significant reduction of these variables was found in bile duct-ligated rats fed on 5 % glycine solution (0.00867, 0.0697 and 0.00907).

**DISCUSSION**

Obstructive jaundice is associated with an increased incidence of postoperative complications such as infection, systemic inflammatory response, and even multiple organ failure due to metabolic and hemodynamic disorders, as well as depressed immune function. Current studies have revealed that endotoxemia was one of the major causes leading to high morbidity and mortality in patients with obstructive jaundice[11,13]. Therefore, the therapeutic strategy aimed at reducing plasma endotoxin level and interrupting its biological activities has become a focus of great concern[19].

It has been found that endotoxin could stimulate monocytes and macrophages to produce a variety of cytokines, because the elevated intracellular Ca2+ concentration in these cells was resulted from the activation of Ca2+ channel by endotoxin[11,12]. It has also been noted that glycine, a nonessential amino acid, could exert protective effects on animals in multiple morbid conditions by minimizing oxidative stress, as well as toxic eicosanoid cytokine production[11,13]. Ding et al[14,15] demonstrated that the biological effects of endotoxin could be significantly inhibited by glycine via a mechanism of blocking TNF-α production in immunocompetent cells, the latter could play a critical role in the pathogenesis of endotoxin lesions[16,7]. ET and NO, although as the intense vasoconstrictor and vasodilator respectively, were also pleiotropic factors involved in endotoxin-mediated pathological processes with endotoxin and TNF-α as their potent releasing irritants[18,19]. The present study demonstrated that severe endotoxiaemia could be observed in obstructive jaundice, and the TNF-α, ET and NO contents in plasma and liver tissue were all significantly increased in bile duct-ligated rats.

Persistent severe endotoxiaemia in obstructive jaundice could stimulate not only Kupffer cells to release TNF-α to insult directly parenchymal cells of the kidney and liver[10,18], but also other immunocompetent cells to produce excessive amount of ET-1 and NO[9] to aggravate the disturbance of splanchnic circulation leading to decreased oxygen delivery, and ultimately multiple organ dysfunction syndrome (MODS).

**Table 1** Plasma levels of bilirubin, endotoxin, TNF-α, ET-1 and NOx/NO3 in different groups of rats (x̄ ± s, n =30)

| Group | Bilirubin (µmol/ L) | Endotoxin (Eu/ ml) | TNF-α (pg/ ml) | ET-1 (pg/ ml) | NOx/NO3 (µmol/ L) |
|-------|-------------------|-------------------|----------------|---------------|------------------|
| A     | 5.80±1.65         | 5.98±1.00        | 61.37±3.08      | 88.79±7.56     | 5.51±0.44        |
| B     | 45.45±6.69ab      | 11.65±1.57b      | 352.52±20.65a   | 183.24±9.01a   | 12.06±0.62a      |
| C     | 43.18±5.53        | 11.27±1.30       | 138.63±7.07ab   | 120.68±10.99ab | 8.55±0.40ab      |

P <0.01 vs group A, P <0.01 vs group B.

**Table 2** Levels of TNF-α, ET-1 and NOx/NO3 in liver tissues of different groups of rats (x̄ ± s)

| Group | Samples | TNF-α (pg/ ml) | ET-1 (pg/ ml) | NOx/NO3 (µmol/ L) |
|-------|---------|----------------|---------------|------------------|
| A     | 10      | 43.51±2.58    | 43.18±4.47    | 2.15±0.18        |
| B     | 10      | 298.46±18.74  | 124.56±11.67a | 10.53±0.87a      |
| C     | 10      | 113.45±6.67ab | 81.49±7.39ab  | 7.23±0.34ab      |

P <0.01 vs group A, P <0.01 vs group B.
Besides, NO itself is also a highly active free radical and can convert further into NO$_2^-$ and peroxynitrite. The more vigorous oxidants could result in cell injury\[9\]. Although glycine could reduce the plasma and liver tissue endotoxin level in our study, it did prevent TNF-α, ET-1 and NO from excessive production, which might be beneficial to alleviate the organ injury in obstructive jaundice. However, TNF-α, ET-1 and NO levels in both plasma and liver tissue remained elevated to some extent in rats fed on glycine as rats compared with sham-operated rats. To elucidate the mechanism underlying this phenomenon is thus the purpose of our further studies.

REFERENCES

1. Greig JD, Krukowski ZH, Matheson NA. Surgical morbidity and mortality in one hundred and twenty-nine patients with obstructive jaundice. Br J Surg 1988; 75: 216–219
2. Reynolds JV, Murchan P, Leonard N, Clarke P, Keane FB, Tanner WA. Gut barrier failure in experimental obstructive jaundice. J Surg Res 1996; 62: 11-16
3. Inan M, Sayek I, Tel BC, Sahin-Erdemli I. Role of endotoxin and nitric oxide in the pathogenesis of renal failure in obstructive jaundice. Br J Surg 1997; 84: 943-947
4. Sheen-Chen SM, Chen HS, Ho HT, Chen WJ, Sheen CC, Eng HL. Effect of bile acid replacement on endotoxin-induced tumor necrosis factor-alpha production in obstructive jaundice. World J Surg 2002; 26: 448-450
5. Heller J, Sogni P, Barriere E, Tazi KA, Chauvelot-Moachon L, Guimont MC, Bories PN, Poiré O, Moreau R, Lebrec D. Effects of lipopolysaccharide on TNF-alpha production, hepatic NOS2 activity, and hepatic toxicity in rats with cirrhosis. J Hepatol 2000; 33: 376-381
6. O’Neil S, Hunt J, Filkins J, Gamelli R. Obstructive jaundice in rats results in exaggerated hepatic production of tumor necrosis factor-alpha and systemic and tissue tumor necrosis factor-alpha levels after endotoxin. Surgery 1997; 122: 281-286
7. Kennedy JA, Lewis H, Clements WD, Kirk Sj, Campbell G, Halliday MI, Rowlands BJ. Kupffer cell blockade, tumour necrosis factor secretion and survival following endotoxin challenge in experimental biliary obstruction. Br J Surg 1999; 86: 1410-1414
8. Liu BH, Xiao N, Chen HS, Zhou JH. Dynamic alteration and interaction of endothelin-1 and nitric oxide levels in the plasma and the liver during endotoxemia. Zhonghua Chuangshang Zazhi 2003; 17: 166-168
9. Wang D, Zhu JY, Leng XS, Li S, Wang FS, Du RY. The detection of nitric oxide synthase (NOS) in liver tissue from cirrhotic patients with portal hypertension and their clinicopathological significance. Zhonghua Putong Waike Zazhi 1999; 14: 31-33
10. Sarac AM, Aktao AO, Moini H, Bilsel S, Scapa E. Role of endotoxin in obstructive jaundice. Am J Physiol 1999; 277:SPT 1: C952-C959
11. Portoles MT, Arahuetes RM, Pagani R. Intracellular calcium alterations and free radical formation evaluated by flow cytometry in endotoxin-treated rat liver Kupffer and endothelial cells. Eur J Cell Biol 1994; 65: 200-205
12. Wheeler MD, Thurman RG. Production of superoxide and TNF-alpha from alveolar macrophages is blunted by glycine. Am J Physiol 1999; 277(SPT 1): L952-L959
13. Ding JW, Andersson R, Soltesz V, Willen R, Bengmark S. Obstructive jaundice impairs reticuloendothelial function and promotes bacterial translocation in the rat. J Surg Res 2000; 92: 276-282
14. Sheen-Chen SM, Chau P, Harris HW. Obstructive jaundice alters Kupffer cell function independent of bacterial translocation. J Surg Res 1998; 80: 205-209

Edited by Zhu L and Wang XL