JOP. J Pancreas (Online) 2003; 4(5):178-183.

Functional Disturbance of Biliary Indocyanine Green Excretion in Rat Cerulein Pancreatitis Followed by Endotoxemia: Role of the Prime and the Second Attack

Akihiro Okabe, Masahiko Hirota, Yu Kimura, Keisuke Maeda, Michio Ogawa

Department of Surgery II, Kumamoto University Medical School. Kumamoto, Japan

ABSTRACT

Context Hepatic injury is considered one of the critical complications associated with acute pancreatitis. It was proposed that initial insults to the liver in the early phase of the attack have an important priming effect, and the subsequent infectious attack (e.g. infectious pancreatic necrosis, bacterial translocation episode) constitutes a second attack on the liver.

Objective To evaluate the role of priming by induction of cerulein pancreatitis and a following second attack by endotoxemia.

Main outcome measures Plasma clearance and biliary excretion of indocyanine green (a hepatophillic hydrophobic organic anion).

Design A model of acute pancreatitis in rats.

Setting Four groups of rats: untreated control, cerulein pancreatitis, endotoxemia and endotoxemia following the induction of cerulein pancreatitis (pancreatitis + endotoxemia).

Results Biliary indocyanine green excretion was significantly disturbed only in the pancreatitis + endotoxemia group. Plasma clearance (a reflection of hepatic uptake) of indocyanine green from the blood was only slightly affected in endotoxemia group.

Conclusion Biliary secretion is quite sensitive to this hepatic injury model. Both the preceding priming insult and the following second attack are important in the development of hepatic injury.

INTRODUCTION

Severe acute pancreatitis often leads to a poor prognosis. In most cases, distant organ injury is the cause of mortality in acute pancreatitis patients. Hepatic injury is one of the critical complications of acute pancreatitis. Although the liver may be one of the neglected organ-systems in acute pancreatitis, several experimental reports have demonstrated the occurrence of hepatic injury accompanying acute pancreatitis. Distortion of the hepatic microvasculature and decreased hepatic mitochondrial function have also been demonstrated in acute pancreatitis [1, 2, 3, 4]. Furthermore, Hori *et al*. reported impaired vesicular transport of lipopolysaccharide (LPS) in acute pancreatitis [5]. However, although impaired hepatic circulation and humoral toxic factors, such as cytokines, have been suggested as a cause of hepatic injury [5, 6], the underlying mechanisms are poorly understood.

Various inflammatory mediators including cytokines are produced in acute pancreatitis [7, 8, 9]. Initial insults in the early phase of the attack result in the priming of

inflammatory cells including neutrophils, lymphocytes, and macrophages [10]. These inflammatory cells are primed to respond to further stimulation. Thus, subsequent infectious complications (e.g. infected pancreatic necrosis, bacterial translocation episode) under these circumstances result in a repeated induction of cytokines and the primed inflammatory cells attack surrounding tissues. As a result, organ function is further impaired. Ogawa has described this type of organ injury as a "second attack" phenomenon [11, 12]. The "second attack" theory is relevant to models of hepatic injury. Okamura *et al*. reported hepatic injury by LPS injection after infection with *Propionibacterium acnes* (*P. acnes*/LPS treatment) [13]. Galanos *et al*. reported that administration of subtoxic doses of LPS to D-galactosamine (GalNAc)-primed mice resulted in hepatic injury (GalNAc/LPS treatment) [14] and we also observed marked hepatic injury upon administration of LPS to rats with cerulein-induced pancreatitis (pancreatitis/LPS treatment) [6]. In this report, we sought to analyze the importance of "priming" followed by a "second attack" using induction of cerulein pancreatitis followed by endotoxemia.

MATERIALS AND METHODS

Animals and Chemicals

Fifty-two male Wistar rats (230-260 g) (Kyudo Experimental Animal Center, Kumamoto, Japan) were used in this study. The animals were provided with rodent chow and water *ad libitum*. Cerulein and LPS (*Escherichia coli* 0111: B4) were purchased from the Sigma Chemical Co. (St. Louis, USA). All other reagents used were of analytic grade.

Experimental Protocol

The animals were divided into four experimental groups: i) untreated controls, ii) mild pancreatitis induced by cerulein injection (pancreatitis), iii) simple endotoxemia induced by LPS injection (endotoxemia), and iv) endotoxemia following induction of cerulein pancreatitis (pancreatitis + endotoxemia).

Induction of Pancreatitis and Endotoxemia

Cerulein pancreatitis was induced by the administration of four intramuscular injections of cerulein (50 μg/kg) at 1-hour intervals [6, 7, 8, 9]. The resultant pancreatitis was mild and edematous. Endotoxemia was provoked by intraperitoneal injections of LPS (30 mg/kg). LPS was administered 6 hours after the first cerulein injection in the pancreatitis + endotoxemia group.

Measurement of ALT Activity

Plasma samples for ALT measurement were collected from 5 rats in each group via the femoral vein at 9 hours after LPS injection or 15 hours after the first cerulein administration, and stored at –80 °C until used. An assay kit for ALT activity was used for measurement (Wako, Osaka, Japan).

Plasma Clearance and Biliary Excretion of Indocyanine Green

The plasma clearance (3 rats per group) and biliary excretion of indocyanine green (ICG) (5 rats per group), a hepatophillic hydrophobic organic anion, were analyzed. Nine hours after LPS injection or 15 hours after the first cerulein administration, a bile drainage tube was inserted into the extrahepatic bile duct by laparotomy in the pancreatitis group, endotoxemia group and pancreatitis + endotoxemia group. In the untreated control group, animals were subjected to experimentation without receiving cerulein or LPS. After 30 min, when the bile flow became stable, ICG (2.4 μmole/kg) was administered via the femoral vein, and blood samples were collected for 15 minutes. Bile samples were collected every 10 minutes for 3 hours after ICG injection. The plasma and bile samples containing ICG were diluted with 0.1N NaOH after

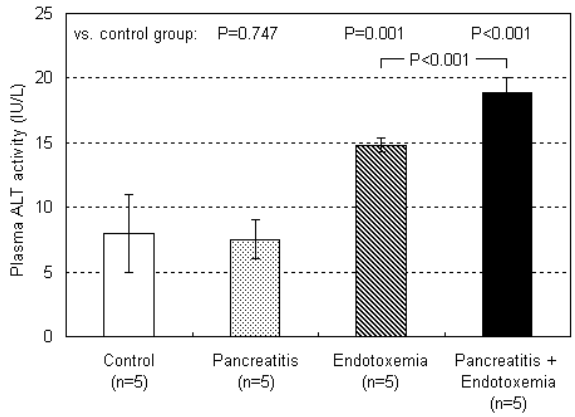

Figure 1. Plasma ALT activity.

centrifugation, and the absorbance at 805 nm was measured.

ETHICS

All animals were maintained under standard conditions according to National Institutes of Health (NIH) animal care guidelines [15].

STATISTICAL ANALYSIS

Data are reported as mean±SD. Statistically significant differences in the data were assigned according to the Student t test and distribution. A two-tailed P value less than 0.05 was regarded as significant. Statistical analyses were performed by running the SPSS/PC+ package.

RESULTS

Plasma ALT Activity

Plasma ALT activity was measured 9 hrs after LPS administration. The ALT levels significantly increased as compared to the control group after the injection of LPS (endotoxemia group; P=0.001) as well as in the pancreatitis + endotoxemia group (P<0.001). Elevation of plasma ALT activity was significantly (P<0.001) much higher in the pancreatitis + endotoxemia group than in the simple endotoxemia group (Figure 1).

Plasma Clearance of ICG

ICG transport ability was examined to assess hepatic function. Nine hours after LPS administration, the plasma clearance of injected ICG was compared among the four experimental groups. However, no significant differences were observed among the four groups, suggesting that the uptake of ICG into hepatocytes did not deteriorate in any group (Figure 2). The disappearance rate (K-value) of indocyanine green from the blood was only slightly (P=0.046) affected in endotoxemia group (Table 1).

Biliary Excretion of ICG and Bile Volume

The kinetics of ICG excretion into the bile was monitored for three hours after an intravenous injection of ICG. The excretion of ICG into the bile and the volume of the bile decreased significantly only in the pancreatitis + endotoxemia group when compared to the controls (Figures 3 and 4), while the pancreatitis and the endotoxemia groups did not show any significant difference vs. the controls. In all groups, the reduction in ICG excretion appeared to parallel the decrease in bile flow. While 70 to 80% of the ICG administered is normally

Table 1. Comparison of the hepatic function in the various experimental groups (Data are reported as percentages with the control group regarded as 100%).

	Pancreatitis	Endotoxemia	Pancreatitis+ Endotoxemia
Plasma clearance of ICG (K-value) (n=3 each group)	103±15 (P=0.762)	74±10 (P=0.046)	116±16 (P=0.225)
Bile volume (n=5 each group)	111±3 (P=0.001)	110±25 (P=0.422)	63±16 (P=0.007)
Biliary excretion of ICG in the first hour (n=5 each group)	102±10 (P=0.678)	112±38 (P=0.519)	39±22 (P=0.003)

P values vs. control group (i.e. 100%).

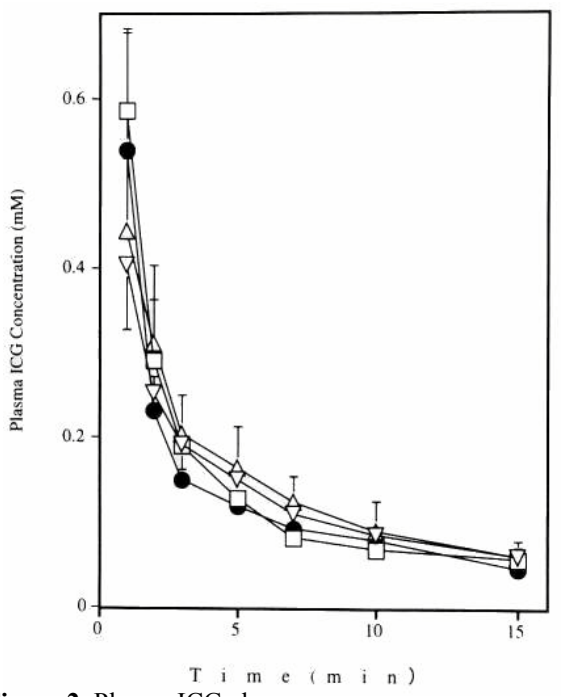

Figure 2. Plasma ICG clearance.

Inverted triangles: control group; squares: pancreatitis group; triangles: endotoxemia group; closed circles: pancreatitis + endotoxemia group.
Data represent the mean values ± SD.
* P<0.05 vs. control group.

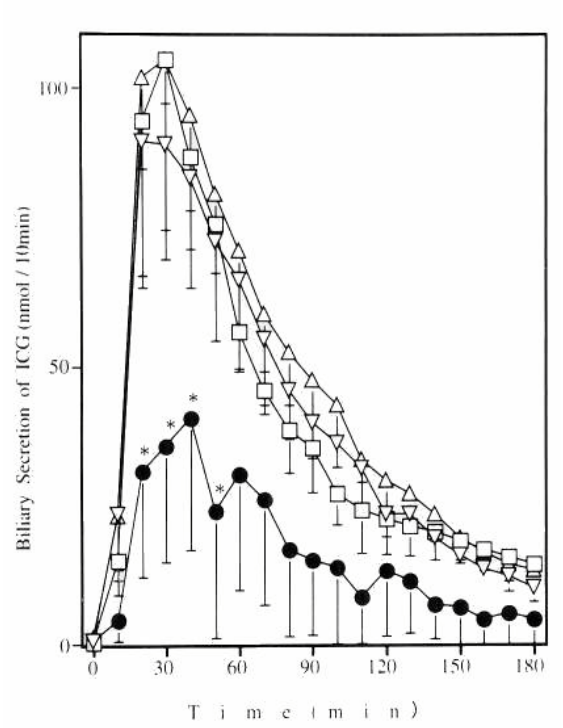

Figure 3. Biliary excretion of ICG.

Inverted triangles: control group; squares: pancreatitis group; triangles: endotoxemia group; closed circles: pancreatitis + endotoxemia group.
Data represent the mean values ± SD.
* P<0.05 vs. control group.

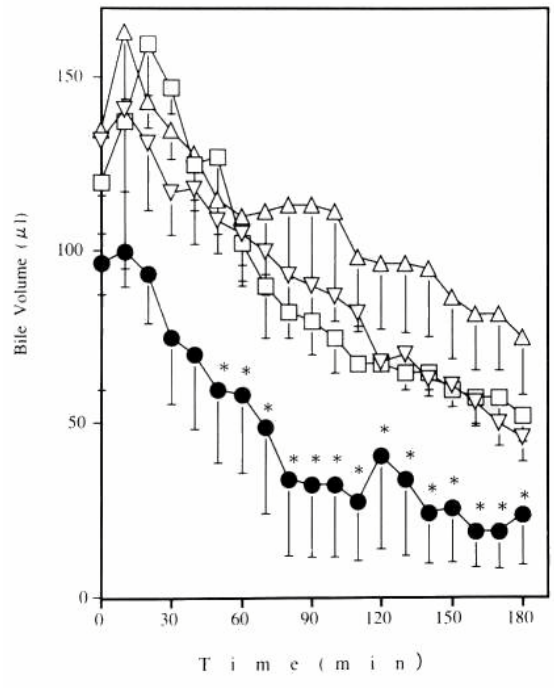

Figure 4. Bile volume.

Inverted triangles: control group; squares: pancreatitis group; triangles: endotoxemia group; closed circles: pancreatitis + endotoxemia group.
Data represent the mean values ± SD.
* P<0.05 vs. control group.

excreted into bile within one hour, the excretory level of ICG in the pancreatitis + endotoxemia group fell markedly to 28% (Figure 5).

Data of the kinetics of ICG excretion into bile, expressed as percentage values vs. control groups, are reported in Table 1. These results demonstrated that both the excretion of ICG into bile canaliculi and the bile volume itself decreased in the pancreatitis + endotoxemia group vs. the controls (P=0.003

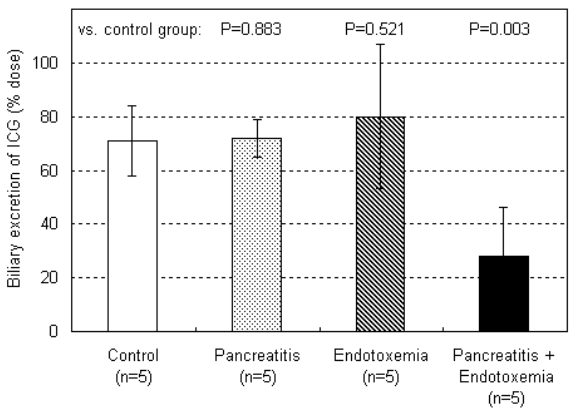

Figure 5. Biliary excretion of ICG during the first hour (Data are reported as % of the administered doses).

and P=0.007, respectively). In addition, an increase of the bile volume (P=0.001) should be noted in the pancreatitis group.

DISCUSSION

We demonstrated that, in addition to the elevation of plasma ALT levels, biliary ICG excretion was significantly affected in the pancreatitis + endotoxemia group. However, plasma clearance (reflecting hepatic uptake) of ICG from the blood was not affected. These results suggest that: i) biliary secretion might be the most sensitive function affected by hepatic insults, and ii) both the preceding priming effect and the following second attack are important in the development of hepatic injury.

We have emphasized the importance of a second infectious attack following the primed condition, as an exaggerating factor [11, 12]. In cerulein pancreatitis, the mRNA induction of cytokines occurred locally in the pancreas [16, 17], although systemic cytokinemia was not provoked. Simple endotoxemia induced moderate systemic cytokinemia. On the other hand, plasma cytokine concentrations dramatically increased in the pancreatitis + endotoxemia group [8, 9, 18]. These results suggest that, when primed by the induction of cerulein pancreatitis, the stimulation of cytokine production by subsequent endotoxemia as a second attack might be augmented. Under such conditions, the second attack episode induces primed neutrophils to attack distant organs in which the neutrophils have accumulated [10]. Ogawa has described this type of organ injury as a second attack phenomenon [11, 12]. Ogawa considers the term “second attack” to be very important for the prevention of organ dysfunction, because physicians and surgeons can do nothing about the first attack or initial insult, but they can prevent or reduce the second attack. Concerning the first attack, acute pancreatitis is already present before the patient visits a medical institution. However, the second attack can be avoided or reduced. Therefore, the important procedure against organ dysfunction is to pay close attention to any second attack, thus avoiding or reducing it [11, 12].

Hepatic transport of organic anions including ICG may depend on microcirculation in the liver. However, no significant decrease in the hepatic blood flow was observed in our experimental system (data not shown). Furthermore, the plasma clearance of ICG, which is also dependent on the hepatic blood flow, was not disturbed in any of the experimental groups, suggesting that the hepatic blood flow was normal. Alternatively, humoral mediators such as cytokines may directly or indirectly impair the hepatic transport of organic anions. As mentioned above, we have reported an increased production of several cytokines in the pancreatitis + endotoxemia model [8, 9, 18]. Histologically, findings of cholestasis were not observed in the pancreatitis + endotoxemia group (data not shown). Together, these results suggest a possible role for humoral mediators in hepatic functional disturbance in this model.

Received May 24th, 2003 - Accepted July 14th, 2003

Keywords Bile; Endotoxemia; Indocyanine Green; Infection; Lipopolysaccharides; Organic Anion Transporters; Pancreatitis, Acute Necrotizing

Abbreviations ICG: indocyanine green; LPS: lipopolysaccharide

Correspondence
Masahiko Hirota
Department of Surgery II
Kumamoto University Medical School
1-1-1 Honjo
Kumamoto-city
Kumamoto 860-8556
Japan
Fax: +81-96-371.4378
Phone: +81-96-373.5212
E-mail: mhirota@kaiju.medic.kumamoto-u.ac.jp

References

1. Delaney C, McEntee G, Cottell D, McGeeney K, Fitzpatrick JM. The effect of cerulein induced pancreatitis on the hepatic microcirculation. Br J Surg 1990; 77:294-6. [PMID 1691034]

2. Kitamura O, Ozawa K, Honjo I. Alterations of liver metabolism associated with experimental acute pancreatitis. Am J Surg 1973; 126:379-82. [PMID 4353976]

3. Coticchia JM, Lessler MA, Carey LC, Gower WR, Mayer AD, McMahon MJ. Peritoneal fluid in human acute pancreatitis blocks hepatic mitochondrial respiration. Surgery 1986; 100:850-6. [PMID 3775654]

4. Dlugosz J, Pawlicka E, Gabryelewicz A. Lysosomal-mitochondrial interrelationships in the damages to the liver in acute experimental pancreatitis in dogs. Int J Pancreatol 1988; 3:343-56. [PMID 3049848]

5. Hori, Y., Takeyama, Y., Ueda, T., Nishikawa, J., Yamamoto, M., and Saitoh, T. Impaired transport of lipopolysaccharide across the hepatocytes in rats with cerulein-induced experimental pancreatitis. Pancreas 1998; 16:148-53. [PMID 9510137]

6. Sameshima H, Ikei S, Mori K, Yamaguchi Y, Egami H, Misumi M, et al. The role of tumor necrosis factor-alpha in aggravation of cerulein-induced pancreatitis in rats. Int J Pancreatol 1993; 14:107-15. [PMID 8283075]

7. Hirota M, Nozawa F, Okabe A, Shibata M, Beppu T, Shimada S, et al. Relationship between plasma cytokine concentration and multiple organ failure in patients with acute pancreatitis. Pancreas 2000; 21:141-6. [PMID 10975707]

8. Okabe A, Hirota M, Nozawa F, Shibata M, Nakano S, Ogawa M. Altered cytokine response in rat acute pancreatitis complicated with endotoxemia. Pancreas 2001; 22:32-9. [PMID 11138968]

9. Sugita H, Yamaguchi Y, Ikei S, Yamada S, Ogawa M. Enhanced expression of cytokine-induced neutrophil chemoattractant (CINC) by bronchoalveolar macrophages in cerulein-induced pancreatitis rats. Dig Dis Sci 1997; 42:154-60. [PMID 9009132]

10. Kimura Y, Hirota M, Okabe A, Inoue K, Kuwata K, Ohmuraya M, Ogawa M. Dynamic aspects of granulocyte activation in rat severe acute pancreatitis. Pancreas 2003; 27:127-32. [PMID 12883260]

11. Ogawa M. Acute pancreatitis and cytokines: 'second attack' by septic complication leads to organ failure. Pancreas 1998; 16:312-5. [PMID 9548672]

12. Ogawa M. Mechanisms of the development of organ failure following surgical insult: the 'second attack' theory. Clin Intensive Care 1996; 7:34-8.

13. Okamura H, Nagata K, Komatsu T, Tanimoto T, Nukata Y, Tanabe F, et al. A novel costimulatory factor for gamma interferon induction found in the livers of mice causes endotoxic shock. Infect Immun 1995; 63:3966-72. [PMID 7558306]

14. Galanos C, Freudenberg MA, Reutter W. Galactosamine-induced sensitization to the lethal effects of endotoxin. Proc Natl Acad Sci USA 1979; 76:5939-43. [PMID 293694]

15. National Institutes of Health, Public Health Service. Guide for the care and use of laboratory animals. NIH publication No. 86-23. Bethesda, NIH, 1985.

16. Gukovskaya AS, Gukovsky I, Zaninovic V, Song M, Sandoval D, Gukovsky S, Pandol SJ. Pancreatic acinar cells produce, release, and respond to tumor necrosis factor-□. J Clin Invest 1997; 100:1853-62. [PMID 9312187]

17. Fink GW, Norman JG. Specific changes in the pancreatic expression of the interleukin 1 family of genes during experimental acute pancreatitis. Cytokine 1997; 9:1023-7. [PMID 9417814]

18. Sugita H, Yamaguchi Y, Ikei S, Ogawa M. Effect of propentfylline on tumor necrosis factor-α and cytokine-induced neutrophil chemoattractant production in rats with cerulein-induced pancreatitis and endotoxemia. Pancreas 1997; 14:267-75. [PMID 9094157]