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The Isolated Perfused Liver Response to a 'Second Hit' of Portal Endotoxin during Severe Acute Pancreatitis

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Key Words

Pancreatitis, acute necrotizing · Multiple organ failure · Sepsis syndrome · Perfusion and liver · Endotoxaemia

Abstract

Background/Aim: During severe acute pancreatitis (AP), the liver may show an exaggerated response to the inflammatory products of gut injury transported in the portal vein. Our aim was to explore liver proinflammatory mediator production after a 'second hit' of portal lipopolysaccharide (LPS) during AP. ***Methods:*** Twenty-four rats underwent one of three 'first-hit' scenarios: (1) severe AP induced by intraductal glycodeoxycholic acid injection and intravenous caerulein infusion, (2) sham laparotomy, or (3) no first intervention. Eighteen hours later, all animals received a 'second hit' of portal LPS in an isolated liver perfusion system. Tumour necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6 concentrations were measured in portal and systemic serum, and in the perfusate 30 and 90 min after the 'second hit'. Neutrophil activation by the perfusate was assayed using dihydrorhodamine-123 fluorescence. ***Results:*** We observed a six-fold increase in IL-6 concentration across the liver during AP. All livers produced TNF-α after the portal LPS challenge, but this was not exaggerated by AP. No differential neutrophil activation by the perfusate was seen. ***Conclusion:*** TNF-α, IL-1β, IL-6 and neutrophil activator production by the isolated perfused liver, in response to a 'second hit' of portal LPS, does not appear to be enhanced during AP.

Introduction

The incidence of acute pancreatitis (AP) is increasing worldwide [1]. A severe attack will occur in 20% of patients, with an associated mortality of 20–50% [2]. Current guidelines suggest that patients with a predicted severe attack of AP should be managed initially in a high-dependency or intensive-care setting [3, 4]. In this environment, many patients may be exposed to a 'second hit' of endotoxin. The most important 'second hit' arises from injury and impairment of physiological intestinal barrier function, leading to lipopolysaccharide (LPS) translocation from the gut lumen and infection of peripancreatic necroses [5–9]. The direct influx of gut-derived bacterial degradation products results in concomi-

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tant endotoxin release into the portal vein and onward to the liver [5–7, 10, 11].

The liver is crucial to the development of distant organ injury in severe AP [12–18]. Hepatocytes show increased tumour necrosis factor-α (TNF-α) messenger RNA expression in the early phases of AP [19], and higher serum concentrations of TNF-α, and interleukin (IL)-1β protein, have been demonstrated on the systemic side of the liver compared to the portal vein [15]. Portocaval venous bypass performed prior to the induction of severe AP in rats prevents lung injury seen at 3 h and diminishes the severity of lung injury seen at 12 h [12, 13, 20].

It remains unclear, however, whether the major contribution of the liver to the systemic inflammatory response originates from liver injury, or whether the liver may act as an amplifier of the inflammatory processes originating in the pancreas and exhibit an exaggerated cytokine response to portal endotoxin or other proinflammatory mediators. In addition, the relative importance of the hepatic portal system in disseminating gut-derived inflammatory mediators during severe AP, compared to other pathways, such as the mesenteric lymphatic system, remains poorly understood [21].

Our aim in this work was to explore whether production and release of proinflammatory mediators by the liver, in response to a 'second hit' of portal LPS, was exaggerated by the inflammatory milieu of severe AP. We used a model of severe AP, with sham-operated and non-operated controls, followed 18 h later by a 'second hit' of portal LPS, in an in situ, isolated hepatic perfusion model.

Methods

Animals

All experiments were conducted, with ethical approval, according the Use of Animals (Scientific Procedures) Act 1986, on Department of Health, Social Services and Public Safety, Northern Ireland Project Licence No. PPL2497b. Pilot data were used to calculate group sizes to detect a difference in cytokine concentrations of two points on a logarithmic scale, with a power of $Z\beta = 0.8$, and significance set at $Z\alpha = 0.05$. Twenty-four, 16-week old male outbred Wistar rats (340–360 g) were used from our in-house breeding colony, maintained at 21°C in a light-dark cycle of 12 h and provided with chow (Trouw Nutrition, Belfast, N. Ireland) and water, ad libitum. Animals were not fasted prior to surgery.

Study Design

The study design is depicted in figure 1. At 14:00 h on day 0, animals were randomised to one of three 'first-hit' scenarios: (1) induction of severe AP, (2) sham laparotomy, or (3) no first intervention. AP and sham groups were then resuscitated overnight with saline. At 09:00 h the next morning, all animals received a portal venous injection of LPS in an isolated hepatic perfusion system, to simulate a 'second hit'. The perfusate was sampled 30 and 90 min after this 'second hit' to determine concentrations of TNF-α, IL-1β and IL-6, and the ability of the perfusate to trigger the neutrophil respiratory burst.

Experimental Procedures

We induced severe AP by combining glycodeoxycholic acid (GDOC) delivered directly into the pancreatic duct, with an intravenous infusion of caerulein, based on the method of Schmidt et al. [22]. This model generates a controllable, systemic inflammatory response to AP by superimposing pancreatic hyperstimulation on the bile acid-mediated ductal injury. Briefly, under general anaesthetic (intraperitoneal ketamine 2.5 mg/kg and xylazine 1.2 mg/kg), carotid artery and internal jugular vein cannulae were placed to allow serial blood sampling, and infusion of fluids and drugs. Through a midline laparotomy, the pancreatic duct was cannulated and a pressure-controlled retrograde infusion of 10 m*M* GDOC (Sigma, UK) was given at 8.4 ml/kg/h for 10 min. Caerulein 5 mg/kg/h (Sigma, UK), diluted in normal saline, was given as a continuous intravenous infusion at 4 ml/kg/h for 6 h. Rats were resuscitated with 4 ml/kg/h normal saline overnight, connected to a humane tether system to allow free movement of the unrestrained conscious animal. Sham-operated animals underwent cannula placement, laparotomy, and fluid resuscitation. Analgesia, in the form of buprenorphine (Schering Plough, UK) 20 μg/kg i.v. bolus injection, was given every 6–12 h during the development of pancreatitis.

To investigate the effect of a 'second hit' of portal LPS, we used an isolated liver perfusion system, based on that described by Hems and Krebs [23]. At a second laparotomy, 18 h after induction of pancreatitis or sham laparotomy, the portal and suprahepatic inferior vena cava were cannulated, blood sampled, and the liver was then perfused, orthogradely, with non-recirculating, continuously oxygenated Krebs-Henseleit buffer supplemented with 2.0 m*M* $CaCl_2$, maintained at pH 7.4, 37.2°C, at a constant pressure of 12 mm Hg. To simulate a 'second hit' of portal LPS, a bolus of 10 μg/kg *Eschericia coli* 0111:B4 LPS (Sigma, UK) was added to the influent perfusate during the first 10 min of established perfusion.

Assessment of Severe AP

Arterial acid-base status (AVL analyser, Roche Ltd, UK), haematocrit (Hawksley microcentrifuge, Sussex, UK), and serum biochemistry, including amylase, were measured (DrySlide™ Analyser, InVitros Diagnostics, UK). Pancreas tissue was fixed in formalin, embedded in paraffin wax (FFPE), stained with haematoxylin and eosin (HE), and assessed by an experienced pathologist blinded to the randomisation. We measured portal and systemic venous serum TNF-α, IL-1β and IL-6 concentrations using internally controlled, rat-specific, commercial ELISA kits with a sensitivity of 6.25 pg/ml (Quantikine™, R&D Systems, Inc., USA).

Perfusate Cytokine Response to a 'Second Hit'

Perfusate was collected for 10 min at 30 and 90 min after the 'second-hit' bolus of LPS, and centrifuged at 500 *g* for 5 min. We measured TNF-α, IL-1β and IL-6 concentrations in the effluent perfusate supernatant by ELISA, as described above.