Endotoxin and fibrinogen degradation product-D have different actions on carbohydrate metabolism: role of Kupffer cells

J. Mandl[a,b,*], C. Wall[a], I. Lerant[c], A. Falus[d], R. Machovich[c], R.G. Thurman[a]

[a]*Laboratory of Hepatobiology and Toxicology, Department of Pharmacology, University of North Carolina at Chapel Hill, NC, USA*
[b]*1st Department of Biochemistry, Semmelweis University of Medicine, Budapest, Hungary*
[c]*2nd Department of Biochemistry, Semmelweis University of Medicine, Budapest, Hungary*
[d]*Department of Biology, Semmelweis University of Medicine, Budapest, Hungary*

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Abstract The effect of endotoxin-derived lipopolysaccharide (LPS) and fibrinogen degradation product D (FDPD) on oxygen consumption and glycogenolysis in the perfused rat liver was investigated. 1. Infusion of LPS (100 μg/ml) or FDPD (7 μg/ml) caused a rapid stimulation of oxygen uptake by the perfused liver of 10–12 μmol/g/h. 2. LPS also caused a transient increase in glucose and lactate release into the perfusion medium from endogenous glycogen; however, FDPD was without effect. 3. Destruction of Kupffer cells by $GdCl_3$ pretreatment blocked the effects of LPS and FDPD on oxygen uptake and glycogenolysis. Further, LPS and FDPD had no effect on oxygen consumption by isolated hepatocytes. Therefore, it is concluded that Kupffer cells are involved in the increase of hepatic oxygen consumption and carbohydrate release caused by LPS, most likely via release of PGE_2 and PGD_2. Since FDPD increased oxygen but not carbohydrate release, it is concluded that it acts via stimulating the release of mediators distinct from those released following LPS infusion.

Key words: Liver; Endotoxin; Fibrinogen degradation product; Kupffer cell; Glycogenolysis; Oxygen uptake

1. Introduction

Endotoxemia has been reported in connection with various liver diseases [1–5]. Endotoxin (LPS) is known to induce an acute phase response in the liver, accompanied by a series of short- and long-term metabolic changes [6]. It has also been shown that the addition of LPS causes a prompt but transient (1–5 min) increase of glucose production in perfused rat and mouse liver [7,8].

In several liver diseases various fibrinogen degradation products (FDPs) accumulate in the blood [9,10] and increase fibrinogen synthesis in vivo [11–13]. The mechanism of action of FDPs has not been determined; however, it has been reported that FDPs directly enhance fibrinogen synthesis in cultured rat hepatocytes [14,15]. Others have suggested tht FDPs act indirectly by stimulating the release of IL-6 from monocytes [11,16], and it is known that LPS stimulates hepatic IL-6 production [6]. In previous experiments it was demonstrated that both LPS and FDP fragment D (FDPD) increase IL-6 production by the perfused liver [17], consistent with the view that FDPD acts via mechanism involving intercellular communication.

The experiment described here were designed to test the hypothesis that Kupffer cells, which are a major source of hepatic IL-6, are involved in early metabolic changes produced by FDPD. Accordingly, the effect of LPS or FDPD on oxygen consumption and carbohydrate release from the perfused liver were compared.

2. Materials and methods

2.1. Animals

Female Sprague–Dawley rats were used in this study. Animals were given free access to food and water and maintained on a 12 h light/dark cycle. $GdCl_3$ (20 mg/kg), a specific Kupffer cell toxicant [18], or vehicle (acidic saline) was administered via tail vein injection 24 h prior to experiments. FDPD was isolated from purified human fibrinogen by gel filtration as described previously [17].

2.2. Liver perfusion

Livers were perfused via the portal vein in a non-recirculating system as described previously. Krebs–Henseleit bicarbonate buffer saturated with 95% oxygen/5% carbon dioxide was used as the perfusion medium. After approximately 20 min of perfusion, LPS (100 μg/ml) or FDP-D (7 μg/ml) was infused for times indicated in figure legends, usually 5 min. Oxygen uptake was measured in the effluent perfusate as it flowed past a Clark-type oxygen electrode. Glucose and lactate were measured in samples of effluent perfusate as described previously [19].

2.3. Isolation of rat hepatocytes

Hepatocytes were isolated from rat livers according to the method of Smedsrod [20]. Briefly, livers were perfused with 0.02% collagenase for 8 min until the tissue surrounding each lobe became detached from the parenchyma. The liver was then placed in cold Krebs–Henseleit bicarbonate buffer. Hepatocytes were dispersed by gentle agitation and separated from other cells and liver debris by centrifugation at $50 \times g$ for 2 min. Viability of hepatocytes assessed routinely by Trypan blue exclusion was above 90%. Oxygen uptake by isolated hepatocytes was measured in a closed chamber fitted with a Clark-type oxygen electrode.

3. Results

Livers were perfused for 15–20 min with Krebs-Henseleit bicarbonate buffer before infusion of LPS or FDPD. Oxygen concentration was monitored continuously, and lactate and glucose were measured in samples of effluent perfusate. Both LPS and FDPD increased hepatic oxygen uptake 10 to 12 μmol/g/h (Table 1). LPS also caused a transient increase of carbohydrate release; however, FDPD had no effect (Fig. 1). Similar experiments were performed in livers where Kupffer cells were depleted by treatment of animals with $GdCl_3$ prevented both the increase in oxygen consumption by LPS and FDPD (Table 1) and the increase in carbohydrate release by LPS (Fig. 1). Neither LPS nor FDPD affected oxygen consumption of isolated hepatocytes (data not shown).

*Corresponding author. Semmelweis University of Medicine, 1st Department of Biochemistry, PO Box 260, H-1444 Budapest 8, Hungary. Fax: (36) (1) 266 2615.

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Table 1
Effect of LPS and FDPD on oxygen uptake in the perfused rat liver

Treatment	Δ Oxygen uptake (μmol/g/h)	
	Control	$GdCl_3$
Lipopolysaccharide	9.9 ± 3.6	1.7 ± 1.3
Fibrinogen degradation product-D	12.0 ± 4.7	0.7 ± 0.7

Livers from animals treated with $GdCl_3$ or vehicle were perfused to establish basal rates of oxygen uptake. Subsequently, LPS (100 μg/ml) or FDPD (7 μg/ml) was infused. Values represent the increase above basal levels and are mean ± S.D., $n = 4$.

4. Discussion

In this study, it was demonstrated that LPS and FDPD stimulated oxygen uptake very rapidly in the perfused liver (Table 1). Two findings support a role for Kupffer cell involvement in the observed metabolic changes. First, destruction of Kupffer cells by $GdCl_3$ blocked increases in oxygen uptake and carbohydrate release by the perfused liver following LPS or FDPD infusion. Second, neither LPS nor FDPD affected oxygen consumption in isolated hepatocytes. Kupffer cells are known to produce PGD_2/E_2 upon addition of LPS [7]. Further, eicosaniods are involved in the transient increase of carbohydrate release due to increased glycogenolysis by LPS [7,8]. Moreover, the kinetics of te effects of LPS on glycogenolysis are quite similar to the transient increase of carbohydrate output and oxygen uptake caused by thrombin in the perfused rat liver, which correlated closely with increased prostaglandin production [21]. Further, PGE_2 stimulates O_2 uptake in isolate hepatocytes [22]. However, FDPD did not increase carbohydrate release (Fig. 1) in spite of the fact that it stimulated oxygen uptake (Table 1). Thus, both LPS and FDPD have common as well as different metabolic effects in the liver. One possible explanation for the differing effects of LPS and FDPD on carbohydrate release is that an alternate profile of mediators may be released from Kupffer cells. Further studies are needed to identify the intercellular messengers secreted by non-parenchymal liver cells in response to FDPD.

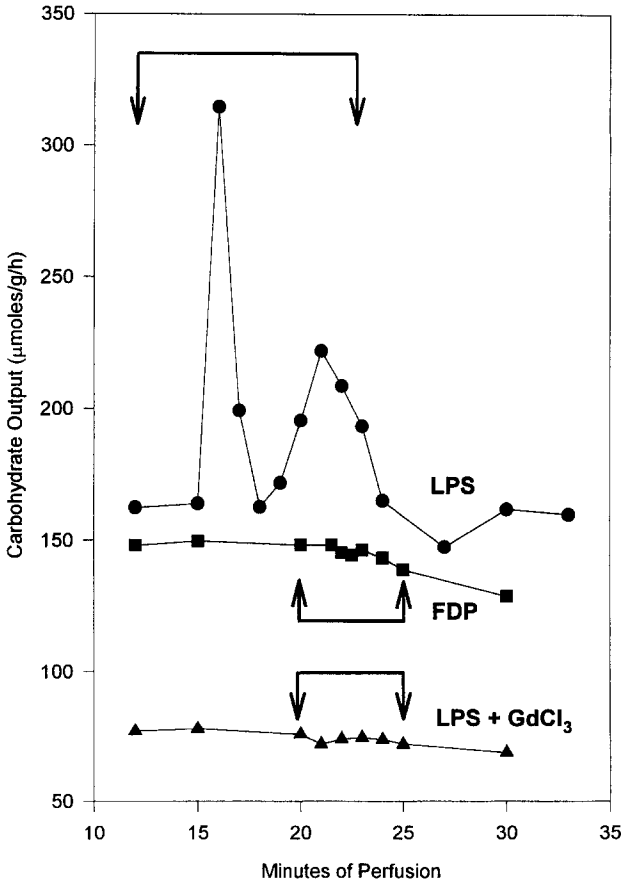

Fig. 1. Effect of LPS and FDPD on carbohydrate output by the perfused rat liver. Livers from control and $GdCl_3$ pretreated animals were perfused as described in section 2. Glucose and lactate were measured enzymatically in samples of effluent perfusate before and after infusion of LPS or FDPD. Arrows indicate periods of infusion.

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