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The manner of the inflammation-boosting effect caused by acute hyperglycemia secondary to overfeeding and the effects of insulin therapy in a rat model of sepsis

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ABSTRACT

Background: The aim of the study was to investigate both the inflammation-boosting effect and the metabolic stress induced by acute hyperglycemia secondary to overfeeding with excessive glucose infusion and the effects of insulin therapy on those events in a rat model of sepsis.

Materials and methods: Sprague–Dawley rats underwent cecal ligation and puncture (CLP) or sham operation. Preestablished continuous intravenous glucose infusion was initiated immediately after surgery. First, rats with CLP-inducing sepsis were divided into three groups on the basis of the target blood glucose (BG) levels: high glucose (HG) group (overfed, >300 mg/dL), moderate glucose group (moderate hyperglycemia, 200–300 mg/dL), and no glucose group (100–150 mg/dL). The sham group received the same glucose infusion as that of the HG group. BG and plasma interleukin (IL) 6 levels were monitored over time. All rats were sacrificed 9 h after surgery to evaluate lung histology and measure hepatic total glutathione and malondialdehyde contents. Based on the results, the high glucose and insulin (HI) group was added to septic groups as a model of insulin therapy, in which insulin with the same HG dose as that in the HG group was administered to maintain moderate hyperglycemia.

Results: BG level in all groups remained in the preestablished target range throughout the experiment. Plasma IL-6 level in all septic groups increased in a time-dependent manner, whereas that in the sham group with moderate hyperglycemia hardly increased. Nine hours after CLP, plasma IL-6 level in the HG group rose to 7407.5 ± 1987.3 pg/mL, which was three times higher than that in the other septic groups. There was no significant difference among moderate glucose, no glucose, and HI groups, in which BG level remained constant at <300 mg/dL. The HG group showed the worst consequences of lung injury and oxidative stress in the liver, which were completely stable in HI group.

Conclusions: Acute severe hyperglycemia in critical illness might excessively boost the existing systemic inflammatory response in a threshold-based manner. Insulin therapy under overfeeding could strongly inhibit such a boosting effect and oxidative stress in the liver.

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1. Introduction

Since the Leuven studies, glycemic control during critical illness has been considered essential and effective in improving prognosis [1–3]. Sepsis is a representative critical illness, and many therapeutic guidelines, as typified by the *Surviving Sepsis Campaign*, stress the importance of glycemic control as a treatment for the causes of sepsis [4]. There is growing evidence of the deleterious effects of acute hyperglycemia, one of which is the promotion of the inflammatory cascade [5,6]. Previous studies have shown that acute hyperglycemia is associated with rapidly increasing concentrations of circulating cytokines such as interleukin (IL) 6 in both nonstressed and critically ill patients [5–8]. Likewise, *in vivo* studies in animals have shown that acute hyperglycemia enhances cytokine production and oxidative response within hours under both nonstressed and stressful conditions induced by endotoxin [9,10]; however, these studies offer few suggestions regarding the extent to which acute hyperglycemia during critical illness amplifies the existing systemic inflammatory response. Thus, there has been no investigation in either the clinical setting or the *in vivo* experiments to answer this important question. These considerations prompted us to clarify three key questions as follows. The first key question is whether acute hyperglycemia actually promotes excessive systemic inflammation in severe infection. If so, further detailed questions arise: (1) How much is the systematic inflammatory response boosted? (2) How much time is required before the onset of this phenomenon? and (3) What is the manner of the systemic inflammatory response boost, that is, proportionate to blood glucose (BG) levels or threshold based? The second key question is whether insulin therapy can inhibit such an inflammation-boosting effect caused by acute hyperglycemia. The third key question is whether insulin therapy also improves metabolic stress associated with acute hyperglycemia induced by overfeeding, such as the oxidative stress [9,11]. To examine these issues, we designed a rat model of acute hyperglycemia obtained by adjusting intravenous glucose loading under septic conditions induced by cecal ligation and puncture (CLP). CLP is widely used as a standard model to induce sepsis in laboratory animals [12,13]. This rat model is suitable to test the deleterious effects of acute hyperglycemia, mimicking the common clinical condition of overfeeding with excessive glucose infusion under septic conditions in clinical settings.

2. Materials and methods

2.1. Animals

Thirty-five male, seven-wk-old Sprague–Dawley rats (Nippon Clea, Tokyo, Japan), weighing approximately 300 g, were used in the experiment. The animals were maintained at 21°C under 12-h light-to-dark cycles and allowed free access to water and standard chow for 3–5 d. The experimental protocols were carried out in a humane manner after receiving approval from the Institutional Animal Experiment Committee of the University of Tsukuba and in accordance with the Regulations for Animal Experiments of our university and the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology.

2.2. Operative procedure

Before surgery, all rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (Somnopentyl; Kyoritsu Seiyaku Co, Tokyo, Japan) at a dose of 40 mg/kg body weight. Under aseptic conditions, the following was performed: (1) left carotid artery catheterization: the arterial catheter was a polyethylene tube of 0.58 mm inner diameter and 0.965 mm outer diameter (Becton, Dickinson and Co, Franklin Lakes, NJ); (2) right jugular vein catheterization in a central venous position: the tip of the central venous catheter was a silicone tube of 0.5 mm inner diameter and 1.0 mm outer diameter (Fuji Systems Co, Tokyo, Japan), and the rest of the catheter was a plastic tube of the same diameter (Imamura Co, Chiba, Japan). The distal ends of each catheter were tunneled subcutaneously and exited in the cephalad portion of the interscapular area. The catheters were fixed to the skin using a harness attached to a swivel assembly; and (3) after catheterization, CLP was performed. After a 3-cm midline incision, the cecum was exposed and ligated with a 3-0 silk suture below the ileocecal valve. The cecum was then punctured through both sides with an 18-gauge needle. After a small amount of feces had extruded from the punctured site, the cecum was placed back into the peritoneum. Sham-operated animals received catheterization and simple laparotomy but not CLP. The abdominal wall and skin were closed with 2-0 synthetic absorbable sutures (Vicryl; Ethicon, Tokyo, Japan). To ensure technical uniformity, all procedures including CLP were performed by only one surgeon (lead author of this article). After these procedures, the rats were maintained in individual metabolic cages and not allowed access to food or water. Immediately after surgery, an arterial catheter was infused with normal saline containing 0.05 U/mL of heparin at a rate of 0.1 mL/h using infusion pumps (SP-115; JMS Co, Ltd, Tokyo, Japan) for catheter maintenance.

2.3. First experimental design

Rats were divided into two main groups: groups under septic conditions induced by CLP and the sham group without sepsis. Moreover, the groups under septic conditions were divided into three groups on the basis of the target BG levels: the high glucose (HG) group ($n = 7$), in which intravenous infusion of HG at approximately 40 mg/kg body weight/min, equivalent to 228 kcal/kg body weight/d, led to severe hyperglycemia (BG level, >300 mg/dL); moderate glucose (MG) group ($n = 7$), in which intravenous MG infusion of approximately 25 mg/kg body weight per minute, equivalent to 148 kcal/kg of body weight per day, led to moderate hyperglycemia (moderate hyperglycemia, 200–300 mg/dL); and no glucose (NG) group ($n = 7$), in which normal saline was administered as a non-glucose solution allowed glycemic control (BG level, <150 mg/dL), recommended by the second edition (2008) of