Plasma Gastrointestinal Glucagon Concentration in Dogs Administered LD_{100} *Escherichia coli*

KIMIKO ISHIDA, M.D., PH.D., LERNER B. HINSHAW, PH.D., AND G. RAINEY WILLIAMS, M.D., F.A.C.S.

*From the Department of Surgery, University of Oklahoma, Oklahoma City*

At the present time, an effective therapy for septic shock has not been established. Since the final stage of septic shock involves complicated pathophysiologic changes, the only effective way to reduce mortality is by prompt recognition and treatment. It is important to look for a useful "marker" for early diagnosis and treatment. It is known that concentrations of plasma gastrointestinal-derived glucagon are markedly elevated in endotoxic shock. It is important to take notice of gastrointestinal glucagon because the gastrointestinal tract is the source of bacteria and their toxins and is one of the "target organs" during septic shock. Decreased blood flow has been observed in the gastrointestinal tract during septic shock, compounding a potentially deleterious role of the intestine. We postulate that high plasma concentrations of gastrointestinal glucagon may be used to identify the presence of severe sepsis and that these concentrations of glucagon may be directly related to the beneficial effects of therapy. This study was concerned with these hypotheses.

**Methods**

Eighteen adult dogs, with a mean weight of 24.5 kg, were screened for microfilaria and treated for intestinal parasites. They had leukocyte concentrations less than 21,000/mm³ and hematocrit readings higher than 36 percent. They were anesthetized with pentobarbital sodium (25 mg./kg.), and intubated orally. Placing the dogs between regulated heating pads maintained rectal temperature between 37.8°C and 38.5°C. The femoral artery and vein were exposed aseptically and cannulated for monitoring pressure and heart rate, infusing saline solution, *E. coli* or drugs and sampling blood. When each dog had attained an equilibrium, LD_{100} *E. coli* (type B7[086a:K61] ATCC33985)² was infused intravenously during a 1-hour period. Each dog was monitored for 6 hours and observed for a 7-day recovery period (Fig. 1). In each group, the hematocrit was maintained at a constant value by infused saline solution. The following measurements were monitored as previously reported by Hinshaw et al.²: mean systemic arterial pressure, heart rate, hematocrit reading, blood gases, white blood cell, platelet, glucose, lactate, blood urea nitrogen, creatinine, antibiotic, steroid and bacterial concentrations, and, most important, glucagon concentrations and survival rate.

Regarding the method for glucagon determinations, 500 KIU of aprotinin (Trasylol®) and 1.2 mg. of EDTA per 1 ml. of each blood sample were mixed in an iced tube. The supernatant was subjected to cooling centrifugation at 3000 r.p.m. for 10 minutes and was preserved at — 20°C until the determination. Glucagon was determined by means of a radioimmunoassay utilizing polyethylene glycol. The nonspecific antibody K-4023 (prepared by Novo Co. Ltd.) and the specific antibody OAL-123 (prepared by Japan Immunoresearch Laboratories Co. Ltd.) were used to determine total glucagon, gastrointestinal-derived glucagon (G1) and pancreatic-derived glucagon (G) levels, respectively. G1 level was calculated by subtracting G from

Extended abstract of a paper presented at the Twenty-seventh Annual University Surgical Residents' Conference, February 5, 1985, Boston.
Reprint requests: Kimiko Ishida, M.D., Ph.D., OUHSC, VA Medical Center (151C), 921 NE 13th Street, Oklahoma City, OK 73104.
FIG. 1. All dogs were infused intravenously (i.v.) for 1 hour with $1.1 \times 10^6$ Escherichia coli per kilogram of body weight. Dogs in group 1 were given saline. In group 2 immediately after all E. coli had been infused (+65 minutes) dogs were infused intravenously with 3 mg./kg. of i.v. tobramycin for 10 minutes and with 8.25 mg./kg. of i.v. tobramycin (TOB) for 280 minutes. TOB was injected intramuscularly (i.m.) in a dose of 11.25 mg./kg. at 6, 12, 18, 24, 48, 72, and 96 hours. In group 3 dogs were infused continuously with 30 mg./kg. of i.v. methylprednisolone sodium succinate (MPSS) for 15 minutes and with 30 mg./kg. of i.v. MPSS for 330 minutes. TOB regimen was the same as group 2.

Total glucagon level: GLI = total glucagon – G. Results were analyzed using the t-test for paired or unpaired data and the Fischer exact test for survival statistics.

Results

Survival Percentages

The survival percentages in each group were zero in group 1 (the average survival time was 21 hours), 17 percent in group 2, and 83 percent in group 3 (Table 1). From the Fischer exact test, the survival results were statistically significant for group 3 compared with group 1 or 2.

Mean Systemic Arterial Pressure and Heart Rate

Mean systemic arterial pressure of group 1 and 2 decreased throughout the observation period (Fig. 2). Mean heart rates were similarly elevated.

Plasma GI

Mean plasma GI group 1 and group 3, at 1 hour. Not significant at 48 hours.

Plasma Gl

Hyperglycemic dogs given elevated at higher lethality. Intestinal glycodynamic increase glucagon c.
Mean systemic arterial pressure (mm Hg) and heart rate (beats/min.) in dogs administered LD$_{100}$ E. coli and treated with tobramycin with and without methylprednisolone sodium succinate (N = 6). * p < .05, paired comparison with zero time (control) value. ** p < .05, unpaired comparison between groups 2 and 3. TOB = tobramycin. MPSS = methylprednisolone sodium succinate.

Period (Fig. 2). Pressures of group 3 at 5 and 6 hours were the highest of the three groups. Mean heart rates of the three groups increased similarly. There were significant increases in heart rates in group 1 after 2 hours.

Plasma Glucose Concentration

Mean plasma glucose concentrations of group 1 and 2 decreased at 6 hours (Fig. 3). In group 3, the glucose concentration increased at 1 hour and decreased at 24 hours, but was not significantly changed from control after 48 hours.

Plasma Glucagon Concentration

Hyperglucagonemia was observed in all dogs given LD$_{100}$ E. coli but was markedly elevated at 6 hours in groups 1 and 2, in which higher lethality was observed (Fig. 4). Gastro-intestinal glucagon concentrations were markedly increased compared with pancreatic glucagon concentrations after E. coli infusion. Concentrations of gastrointestinal glucagon were 3417 pg./ml. in group 1, 5167 pg./ml. in group 2 and 1081 pg./ml. in group 3 by 6 hours. Methylprednisolone sodium succinate (MPSS) infusion was begun 15 minutes after the onset of E. coli infusion. MPSS infusion therapy prevented the early large rise of glucagon from 0 to 6 hours. Following cessation of MPSS infusion at 6 hours, glucagon values rose until 18 hours. From 18 hours to 7 days glucagon concentrations steadily fell and were associated with increased survival rates.

Discussion

The remarkable increases in plasma pancreatic glucagon concentrations have also been reported in endotoxic shock. In the present study, increases in plasma gastrointestinal glucagon concentrations were more readily induced by E. coli than those of pancreatic glucagon. The increases were less in the group receiving corticosteroid/antibiotic infu-
Fig. 3. Alterations in blood glucose concentrations (mg./dl.) in dogs administered LD_{50} E. coli and treated with tobramycin with and without methylprednisolone sodium succinate (N = 6). ★ p < .05, ★★ p < .025, paired comparison with zero time (control) value. ☆ p < .05, unpaired comparison between groups 2 and 3. TOB = tobramycin. MPSS = methylprednisolone sodium succinate.

Fig. 4. The concentrations of total glucagon, pancreatic glucagon and gastrointestinal glucagon (pg./ml.) in dogs administered LD_{50} E. coli and treated with tobramycin with and without methylprednisolone sodium succinate (N = 5). ★ p < .05, ★★ p < .01, paired comparison with zero time (control) value. ☆ p < .05, unpaired comparison between group 2 and 3. TOB = tobramycin, MPSS = methylprednisolone sodium succinate.
sions. Progressive decreases in gastrointestinal glucagon concentrations after 18 hours were associated with high survival rates. Therefore, monitoring plasma gastrointestinal glucagon concentrations during the recovery period from shock may be useful in determining the severity and probability of recovery from sepsis.

The Mechanism(s) for the Release of Glucagon and Its Role in Shock

Since blood flow to the gastrointestinal region decreases as the result of shock, the mucosal cell presumably suffers from ischemia, which may have precipitated the release of its glucagon. On the other hand, the increase in gastrointestinal glucagon concentration during shock may have resulted in the subsequent reversal of the damage to the gastrointestinal mucosal cell in the surviving dogs. Mason and Hess have suggested that glucagon may serve as an important mediator of both the reported myocardial dysfunction and glucose dyshomeostasis of endotoxic shock. It is known that glucagon acts as an inotropic agent. The ultimate role of glucagon in shock has not been fully established. It is postulated that plasma gastrointestinal glucagon is released after E. coli infusion because of damage to the mucosal cells, but it also serves as an essential factor for restoration of mucosal cell morphology and function during the recovery phase of shock.

As a result of the present findings, we suggest that marked increases in plasma gastrointestinal glucagon concentrations may be useful in recognizing the presence and degree of severe sepsis, and the subsequent progressive decreases in concentration are directly associated with the probability of survival.

References