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COMMUNICATIONS

Cepharanthine (biscoclaurine alkaloid) treatment in endotoxic shock of suckling rats

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Abstract—Gram-negative sepsis/septic shock causes significant mortality in newborns. However, there has been no established method for newborn endotoxic shock treatment. Prostaglandins play a role in endotoxic shock. Cepharanthine is a biscoclaurine alkaloid that primarily inhibits phospholipase A_2. Therefore, the effects of cepharanthine have been studied on endotoxic shock in newborn rats. Cepharanthine decreased the 24 h mortality of endotoxic shock in a dose-related manner. At the dose of 0·2 mg kg^{-1} it effectively reduced the mortality from 90 to 21% in newborn rats. It also induced hyperglycaemia in control rats and blunted the hypoglycaemia of endotoxic shock. Cepharanthine did not suppress body weight gain nor did it delay death as seen with glucocorticoid treatment. We conclude that cepharanthine is beneficial in the treatment of newborn endotoxic shock.

The mortality of Gram-negative sepsis/septic shock continues to be high in the human newborn (Harris & Polin 1983), but there has been no established method of treatment. In this study, cepharanthine, a biscoclaurine alkaloid, was used for the treatment of newborn rat endotoxic shock. The glucoregulatory changes and mortality were monitored.

Northover & Subramanian (1962) first reported that aspirin, a prostaglandin synthesis inhibitor, reduced the mortality of endotoxic shock. Fletcher et al (1981) and Halushka et al (1983) showed that prostaglandins play a role in septic shock. However, the benefits of prostaglandin synthesis inhibitors such as phospholipase A_2 inhibitors and cyclo-oxygenase inhibitors for the treatment of septic shock have been controversial (Parrat & Sturgess 1974; Halushka et al 1983; Young et al 1984; The Veterans Administration Systematic Sepsis Cooperative Study Group 1987; Goto et al 1990).

Young et al (1984) demonstrated that dexamethasone, which also inhibits phospholipase A_2, attenuated the hypoglycaemia and lactacidaemia of endotoxic shock in suckling rats. Our previous study (Goto et al 1990) confirmed Young's observations. However, a multi-centre clinical trial of steroid therapy failed to show the beneficial effects of steroids in adult patients with sepsis/septic shock (The Veterans Administration Systematic Cooperative Study Group 1987). Indomethacin, a cyclo-oxygenase inhibitor, ameliorated the metabolic and haemodynamic deterioration of endotoxic shock in adult rats (Parratt & Sturgess 1974; Halushka et al 1983). Indomethacin also blunted the hypoglycaemia and decreased the mortality of endotoxic shock in newborn rats (Goto et al 1990). However, Young et al (1984) reported that indomethacin damaged the liver and brain in endotoxic suckling rats.

Cepharanthine was isolated from *Stephania cepharantha* Hayata (Menispermaceae) in 1934 (Kondo et al 1934), and is used clinically as an antidote for snake (Ancistrodon) venom. Recently cepharanthine was found to inhibit phospholipase A_2 and stabilize the plasma membrane (Miyahara et al 1978); we hypothesized that it may be effective for the treatment of newborn endotoxic shock. If proved to be effective, it would be a more desirable drug for the treatment of circulatory shock than cyclo-oxygenase inhibitors such as indomethacin or aspirin, because no serious side effects in pharmacological doses have been reported.

Glucose dyshomeostasis is a common sign of septic shock (Filkins 1978; Wolfe & Burke 1978; Zeller et al 1988). There is a close relationship between plasma glucose level and the prognosis of newborn rat endotoxic shock (Fitzgerald et al 1988). Therefore, plasma glucose and lactate were monitored in 10-day-old rats after endotoxin injection, and the effects of cepharanthine on glucose metabolism were studied. The body weight changes as a parameter of metabolism, and mortality rates were observed.

Materials and methods

Pregnant Sprague-Dawley rats (Harlan Co., WI) were purchased on the 17th day of gestation. Rat chow (Allied Mills Inc, IL) and water were freely available. The rats were born in our animal care facility, and were housed with their dams until the experiments were performed. Ten-day-old rats were transferred to an infant incubator (Model C-86 Air-Shields Inc. PA) 4 h before the experiments. Endotoxic shock was induced by an injection of 0·1 mg kg^{-1} of *Salmonella enteritidis* lipopolysaccharide (LPS; Difco Lab Co., MI). This dose corresponds to the LD90 at 24 h in our laboratory (Zeller et al 1988). All drugs were administered intraperitoneally.

Study 1: dose-related effects of cepharanthine. Cepharanthine (Kakenseiyaku Co., Tokyo) was dissolved in pyrogen free saline and administered immediately after the LPS injection. To determine the optimal dose of cepharanthine for the treatment of endotoxic shock, the newborn rats were divided into 7 groups and received injections as follows: Group 1 (n = 8), 0·1 mg kg^{-1} of cepharanthine + 0·1 mg kg^{-1} of LPS; Group 2 (n = 10), 0·1 mg kg^{-1} of cepharanthine; Group 3 (n = 10), 0·15 mg kg^{-1} of cepharanthine + 0·1 mg kg^{-1} of LPS; Group 4 (n = 21), 0·2 mg kg^{-1} of cepharanthine + 0·1 mg kg^{-1} of LPS; Group 5 (n = 8), 0·2 mg kg^{-1} of cepharanthine; Group 6 (n = 9), 0·25 mg kg^{-1} of cepharanthine + 0·1 mg kg^{-1} of LPS; Group 7 (n = 7), 0·3 mg kg^{-1} of cepharanthine + 0·1 mg kg^{-1} of LPS; and Group 8 (n = 6), 0·3 mg kg^{-1} of cepharanthine. After injections, the rats were kept in an incubator, and 24 h mortality was observed.

Study 2: plasma glucose, insulin and lactate responses. Rats were divided into 4 groups and received injections as follows: Group A (saline): 0·2 mL of 0·9% NaCl (saline); Group B (Ceph): 0·2 mg kg^{-1} of cepharanthine; Group C (LPS): 0·1 mg kg^{-1} of LPS; and Group D (LPS + Ceph): 0·2 mg kg^{-1} of cepharanthine + 0·1

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mg kg^{-1} of LPS. During the experiment the rats were kept in the incubator.

Blood was collected after decapitation 0, 2, 4 or 24 h after injections. Plasma glucose and lactate concentrations were determined using glucose and lactate analysers (Yellow Springs Inc., OH). Insulin was determined using radioimmunoassay kits (Cambridge, MA).

Study 3: mortality and body weight. The rats were divided into 4 groups and received injections as follows: Group A (saline): 0·2 mL of saline; Group B (Ceph): 0·2 mg kg^{-1} of cepharanthine; Group C (LPS): 0·1 mg kg^{-1} of LPS; and Group D (LPS + Ceph): 0·2 mg kg^{-1} of cepharanthine + 0·1 mg kg^{-1} of LPS. Body weights were measured and mortality was observed daily. The rats were kept in the incubator for the first 24 h, then survivors were returned to their dams.

Statistical analysis. Plasma glucose, insulin and lactate concentrations, and body weight were expressed as mean ± s.e.m. and analysed with ANOVA. The chi-square test was used for the comparison of the mortalities.

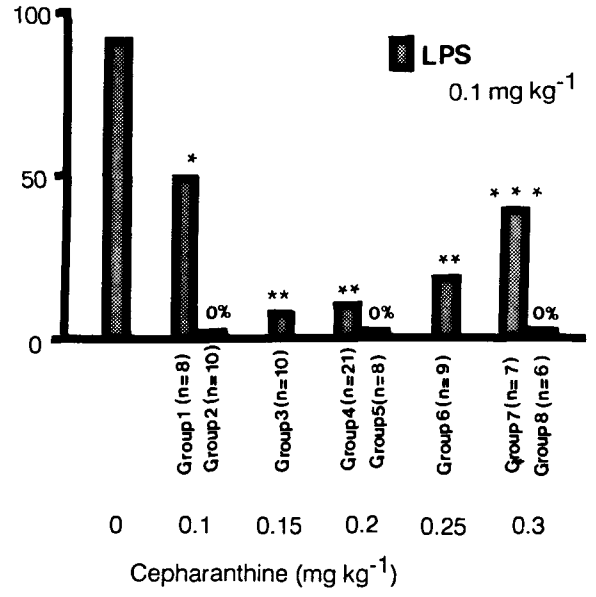

FIG. 1. LPS at the dose of 0·1 mg kg^{-1} was the LD90 at 24 h. *P < 0·05 compared with LPS alone. **P < 0·001 compared with LPS alone. ***P < 0·01 compared with LPS alone.

Results and discussion

Study 1 (Fig. 1) showed that cepharanthine at the dose between 0·1 and 0·3 mg kg^{-1} decreased the 24 h mortality of endotoxic shock in 10-day-old rats in a dose-dependent manner. Cepharanthine was most effective at the dose of 0·15–0·2 mg kg^{-1}, decreasing the mortality from 90 to 8%. Cepharanthine alone at doses between 0·1 and 0·3 mg kg^{-1} was not lethal.

Study 2 (Table 1) showed that LPS induced hyperglycaemia (P < 0·01) by 2 h in Group C (LPS). Cepharanthine did not alter LPS-induced hyperglycemia in Group D (LPS + Ceph). Prostaglandin synthesis inhibitors augmented insulin-mediated peripheral glucose uptake and lowered plasma glucose (Miller et al 1983). Although cepharanthine is a phospholipase A_2 inhibitor, cepharanthine induced hyperglycaemia in Group B (Ceph). Therefore, the mechanism of cepharanthine-induced hyperglycaemia remains unclear.

LPS induced hypoglycaemia (P < 0·01) following hyperglycaemia by 4 h in Group C (LPS). The hypoglycaemia during endotoxic shock was due to decreased gluconeogenesis and increased glucose utilization (Wolfe & Burke 1978; Bagby et al 1986; Kuttner et al 1986). In adult endotoxic shock, hyperinsulinaemia was postulated to cause the hypoglycaemia (Yelich et al 1987). However, hyperinsulinaemia is not induced in our present study (Table 1). In the newborn, blood glucose is primarily regulated by substrate (Kliegman et al 1980). Insulin may not be the most important hormone in glucoregulation of the newborn during endotoxic shock. Plasma glucose concentration correlates with the outcome of newborn endotoxic shock (Fitzgerald et al 1988). When 10 day old rats lost their righting reflex during endotoxic shock, they had severe hypoglycaemia, lactacidaemia and died (Fitzgerald et al 1988). Indomethacin, a cyclooxygenase inhibitor, ameliorated the hypoglycaemia in newborn endotoxic shock (Goto et al 1990), and decreased lethality (Miller et al 1985; Goto et al 1990). Dexamethasone, a phospholipase A_2 inhibitor, ameliorated the hypoglycaemia and decreased lethality in newborn endotoxic shock (Young et al 1984; Goto et al 1990). Cepharanthine also blunted the hypoglycaemia.

Table 1. Plasma glucose, insulin and lactate levels.

	Time (h)			
	0	2	4	24
Group A (saline)				
glucose (mg dL^{-1})°	94 ± 2	94 ± 5	91 ± 1	73 ± 6
insulin (μ units mL^{-1})	24 ± 1	28 ± 4	28 ± 2	24 ± 4
lactate (mM)	1·29 ± 0·05	1·30 ± 0·09	1·33 ± 0·03	1·35 ± 0·12
Group B (Ceph)				
glucose (mg dL^{-1})°		120 ±	90 ± 3	93 ± 2
insulin (μ units mL^{-1})°°		29 ± 3	31 ± 2	—
lactate (mM)°		1·00 ± 0·04	1·26 ± 0·07	1·27 ± 0·05
Group C (LPS)				
glucose (mg dL^{-1})°		112 ± 4	52 ± 3	73 ± 2
insulin (μ units mL^{-1})°°		22 ± 1	30 ± 2	21 ± 2
lactate (mM)°		1·99 ± 0·07	3·27 ± 0·22	2·26 ± 0·29
Group D (LPS + Ceph)				
glucose (mg dL^{-1})°		117 ± 7	98 ± 10*	86 ± 5
insulin (μ units mL^{-1})°°		22 ± 2	23 ± 1	17 ± 3
lactate (mM)°		1·05 ± 0·13	2·59 ± 0·14*	1·76 ± 0·14

°n = 10 to 16. °°n = 8–14. *P < 0·001 compared with Group C.

Table 2. Cumulative mortality and body weight.

		Time (days)		
		1	2	3
Group A (saline) (n = 30)	Mortality	0%	%	0%
	Weight (g)	28·6 ± 0·6	26·0 ± 0·5	27·8 ± 0·5
Group B (Ceph) (n = 10)	Mortality	0%	0%	0%
	Weight (g)	27·4 ± 0·6	25·5 ± 0·5	26·1 ± 1·2
Group C (LPS) (n = 80)	Mortality	90%	90%	90%
	Weight (g)	31·0 ± 0·4	28·7 ± 0·6	28·6 ± 0·7
Group D (LPS + Ceph) (n = 29)	Mortality	21%*	21%*	21%*
	Weight (g)	26·6 ± 0·3	25·1 ± 0·2	25·1 ± 0·4

*$P < 0·01$ compared with Group C.

LPS increased the level of plasma lactate over 4 h ($P < 0·01$) in Group C (LPS) (Table 1). Cepharanthine attenuated the lactacidaemia ($P < 0·001$) in Group D (LPS + Ceph). Indomethacin and dexamethasone attenuated lactacidaemia in endotoxic shock (Goto et al 1990). Therefore, the mechanism of beneficial effects of cepharanthine may be due to the maintenance of plasma glucose levels and the attenuated lactacidaemia.

Study 3 (Table 2) showed that 90% mortality occurred by 24 h after LPS injection in Group C (LPS) but there was no additional mortality between 24 and 72 h. In Group 4 (LPS + Ceph), cepharanthine decreased the mortality to 21% ($P < 0·01$ vs Group C) by 24 h with no additional mortality by 72 h. In Group A (saline) and Group B (Ceph), body weight decreased in the first 24 h and body weight gain started on day two. In Group C (LPS), the body weight gain was not observed by day two.

Our previous study (Goto et al 1990) showed that although dexamethasone decreased the 24 h mortality, dexamethasone suppressed body weight gain and delayed lethality after LPS injection. We have speculated that such adverse effects of dexamethasone may be due to persistent catabolism. However, cepharanthine did not show these adverse effects. Cepharanthine-induced hyperglycaemia was returned to normal by 4 h (Table 1) and cepharanthine did not suppress body weight gain (Table 2). The mechanism for the different effects on glucose metabolism between dexamethasone and cepharanthine remains unclear.

Indomethacin decreased the 24 h mortality (Goto et al 1990). Although indomethacin did not cause delayed lethality, the 24 h mortality was still high (32%) (Goto et al 1990). Therefore, cepharanthine appears to be more beneficial than indomethacin or dexamethasone.

Cepharanthine has been used in the treatment of various diseases in Japan, including snake (Ancistrodon) bite, disseminated intravascular coagulation, and radiation-induced lipid peroxidation. The drug's effects are based on its membrane stabilizing effect, and its inhibitory effect on phospholipase A_2 (Nakatsuka & Nakazawa 1982; Koga et al 1988). Adverse effects of cepharanthine at doses between 0·1 and 0·2 mg kg^{-1} have not been reported. Our results showed that cepharanthine may also be effective in the treatment of highly lethal endotoxic shock in newborns.

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