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Toxicon, Vol. 18, pp. 309–314.
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CARDIOHEPATIC AND GROSS PATHOLOGICAL CHANGES IN RHESUS MONKEYS AFTER INTRAVENOUS INJECTION OF PURIFIED CHOLERA ENTEROTOXIN*

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(*Accepted for publication* 22 November 1979)

C. T. LIU, E. J. GALLOWAY and P. S. LOIZEAUX. Cardiohepatic and gross pathological changes in rhesus monkeys after intravenous injection of purified cholera enterotoxin. *Toxicon* **18**, 309–314, 1980.—Preliminary experiments have been conducted to determine the lethal dose, survival time and cardiohepatic responses to a single i.v. injection of purified cholera enterotoxin in rhesus monkeys. Within 15 min after enterotoxin injection (50 μg/kg), systolic, diastolic and mean blood pressures showed transient increases, followed by sustained decreases throughout the experimental period. The indices of cardiac contractility increased initially and then declined. During an 8-hr period of observation, heart rate, cardiac output and stroke volume increased and liver functions and total peripheral resistance decreased. The survival time ranged from 12·5 to 18 hr in the toxin-inoculated monkeys. Diffuse hemorrhages throughout the body, especially in the cardiac muscle were observed at necropsy. Similar pathologic changes and death were also observed in pigtail and cynomolgus monkeys following an i.v. injection of cholera enterotoxin at the same dose. African green and capuchin monkeys were resistant to i.v. cholera enterotoxin challenge; no clinical illness was shown after a high dose (200 μg/kg) of cholera enterotoxin injection.

INTRODUCTION

THE ABILITY of cholera enterotoxin to produce vomiting and diarrhea is well documented (CRAIG, 1970; FORMAL *et al.*, 1973; BEISEL, 1976). Due to rapid losses of electrolytes and water via the gastrointestinal tract, severe dehydration, renal failure and circulatory shock are produced and death may ensue (GANGAROSA *et al.*, 1960; CARPENTER *et al.*, 1966). During cholera enterotoxin-induced diarrhea, the superior mesenteric arterial blood flow decreases, indicating that the increased intestinal secretion is not directly related to excessive plasma supply (CARPENTER *et al.*, 1969). Intestinal absorption of glucose and amino acids is not altered (CARPENTER *et al.*, 1968; ROHDE and CASH, 1973; SEREBRO *et al.*, 1968*b*) and intestinal epithelium remains intact (GANGAROSA *et al.*, 1960). In fact, when glucose was added to the intestinal lumen containing cholera enterotoxin, the increased secretions of fluid and electrolytes were diminished (NORRIS *et al.*, 1969). Such an inhibitory effect on intestinal hypersecretion was enhanced by the administration of both glucose and acetazolamide (NORRIS *et al.*, 1969).

*In conducting the research described in this report, the investigators adhered to the *Guide for the Care of Laboratory Animals*, as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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In oral cholera enterotoxin-induced diarrhea, a decrease in Na^+ ; K^+-ATPase and stimulation of mucosal membrane adenyl cyclase activities increase the conversion rate of intracellular ATP to cyclic AMP (SUTHERLAND, 1972); TAUB *et al.*, 1977). The elevated cellular concentration of cyclic AMP (cAMP) is believed to be responsible for the alteration of cell membrane functions, leading to losses of water and electrolytes via the intestine (SCHAFER *et al.*, 1970; KIMBERG *et al.*, 1971; FIELD, 1971). It appears that cholera enterotoxin universally increases cAMP in tissues, including liver (GORMAN and BITENSKY, 1972; BECKMAN *et al.*, 1974), kidney (FRIEDLER *et al.*, 1975; KUROKAWA *et al.*, 1975), fat cells (HEWLETT *et al.*, 1971), brain (EPSTEIN *et al.*, 1977) and leukocytes (BOURNE *et al.*, 1973). Further cholera enterotoxin inhibits active sodium transport (FUHRMAN and FUHRMAN, 1960) and mimics the action of several hormones which alter cellular functions through increasing cellular cAMP (GRAYBILL *et al.*, 1970; LIDDLE and HARDMANN, 1971; BECKMAN *et al.*, 1974).

Since the natural route of cholera infection is oral and subsequent enterotoxic action is overwhelmingly in the intestine, little attention has been directed toward possible systemic effects if the toxin is absorbed via the intestinal mucosa. In humans, the evidence for intestinal absorption of cholera enterotixin is minimal (VAUGHAN WILLIAMS and DOHADWALLA, 1969). In animals, however, absorption has been shown in rabbits (VAUGHAN WILLIAMS and DOHADWALLA, 1967; SEREBRO *et al.*, 1968*a*) and guinea pigs (GOLDSTEIN *et al.*, 1966). When the toxin was administered i.v. to dogs (GRAYBILL *et al.*, 1970; PIERCE *et al.*, 1972), rabbits (IWERT *et al.*, 1967), rats (BAKER *et al.*, 1973) and mice (FRANKS, 1976), systematic changes were evident and included increases in hepatic cAMP, liver adenyl cyclase activity, serum alkaline phosphatase, glutamic oxaloacetic transaminase, lactic dehydrogenase and corticosterone concentrations; and decreases in plasma Na^+, liver glycogen content and blood lymphocytes and total leukocytes. The main purpose of the present study was to determine survival time, gross pathological, cardiovascular and hepatic changes, following an i.v. injection of a lethal dose of purified cholera enterotoxin in rhesus monkeys. Using the monkey model to study i.v. effects of cholera toxin, one may obtain some preliminary information concerning the cause of death during cholera toxemia, and major differences may be identified between oral and i.v. challenge routes.

MATERIALS AND METHODS

Source of cholera enterotoxin

Highly purified cholera enterotoxin was obtained from a commercial source (Schwartz-Mann, Orangeburg, NY). The following information was given in the analysis report with the toxin powder (Lot No. EZ 3399): (1) When 1 mg of cholera toxin is reconstituted to 1 ml with distilled water, this toxin (protein) suspension will contain 0·05 M Tris, 0·001 M Na_2-EDTA, 0·003 M NaN_3, and 0·2 M NaCl. The final pH of the suspension is 7·5. (2) The concentration of protein (cholera enterotoxin) was determined by extinction at 280 nm and the purity was evidenced by a single major band in disc electrophoresis. (3) The biological activity of cholera toxin was determined as 26·0 LB (limit of blueing)/μg Lowry protein. The LB dose of toxin is that amount of Permeability Factor which, when mixed with one unit of antitoxin in a total volume of 0·1 ml and injected into the clipped skin of rabbits, evokes on the average, an area of increased vascular permeability 4 mm in diameter.

Animal experimentation

Since i.v. injection of cholera enterotoxin causes death and the supply of rhesus monkeys is limited, only a few were used for preliminary observations in an attempt to obtain information which might be extrapolated to man. Three rhesus monkeys weighing 4–7 kg were used. A femoral artery and vein of each of two chair-restrained monkeys was cannulated under ketamine anesthesia. In one of the two monkeys, a 5F cardiac catheter (Mikro-tip catheter pressure transducer, Millar Instrument, Inc., Houston, Texas) was placed into the left ventricle via the left common carotid artery and aortic valve for the measurements of Vmax (maximum velocity of contractile element shortening) and dp/dt (first derivative of left ventricular pressure) as indices of cardiac contractility (ZECH *et al.*, 1974). Cardiohepatic functions according to established techniques (LIU *et al.*, 1977) were measured before and at various time intervals after a highly purified cholera

toxin (50 μg/kg) was injected into the femoral vein. A third rhesus monkey, without any surgical procedure, was kept in a cage and given a single 10 μg/kg i.v. dose of cholera toxin.

In a separate study of decreased stress, four different monkey species (n = 2–3/group) including pigtail (*Macaca nemestrina*), cynomolgus (*Macaca fascicularis*), capuchin (*Cebus apella*) and African green (*Cercopithecus aethiops*) were used. No surgery, chairing or cannulation was used on these monkeys. The i.v. dose of cholera toxin was 50 μg/kg for all species of monkeys. Since African green and capuchin monkeys were resistant to this dose of cholera toxin, a higher dose of this toxin (200 μg/kg) was given to two monkeys of each of these two species. All monkeys were observed frequently in separate cages for general changes. If death occurred, necropsies were performed within 5–10 min. The survivors were released after 6 months for use in different research projects.

RESULTS AND DISCUSSION

Cardiovascular responses to i.v. cholera enterotoxin

Results are presented in Table 1. Within 8 hr after i.v. cholera enterotoxin injection, heart rate, cardiac output and stroke volume increased. Indices of cardiac contractility, including

TABLE 1. EFFECTS OF I.V. CHOLERA ENTEROTOXIN (50 μg/kg) ON CARDIAC FUNCTIONS OF RHESUS MONKEYS

			Time after enterotoxin (hr)									
Variable*	Monkey No.	Base-line	0·25	0·50	1	2	3	4	5	6	7	8
Heart rate (beat/min)	A	180	216	240	252	276	252	240	240	240	240	240
	B	156	204	228	228	240	228	228	216	216	—†	—
Cardiac output (ml/min/kg)	A	287	274	375	338	482	422	289	579	377	210	351
	B	170	254	283	360	349	283	347	365	601	—	—
Stroke volume (ml/beat/kg)	A	1·59	1·26	1·56	1·34	1·74	1·87	1·19	2·41	1·57	0·87	1·46
	B	1·09	1·24	1·24	1·57	1·45	1·24	1·52	1·68	2·78	—	—
Peak LVP (mmHg)	A	125	110	100	100	110	100	100	95	93	100	90
Peak dp/dt (mmHg/sec)	A	5630	6050	5200	5200	5420	4010	3291	2650	2061	3092	2650
Vmax (muscle length/sec)	A	1·72	2·39	2·63	4·54	2·32	1·54	1·15	0·42	0·56	0·55	0·54
Vce at 40 DIP (muscle length/sec)	A	2·17	3·12	2·70	3·12	2·04	1·69	1·38	0·71	0·82	1·03	0·91

*LVP = left ventricular pressure; dp/dt = first derivative of left ventricular pressure; Vmax = maximum velocity of contractile element shortening; Vce at 40 DIP = velocity contractile element at 40 mmHg of developed isovolumic pressure.

†Obstructed catheter.

TABLE 2. EFFECTS OF I.V. CHOLERA ENTEROTOXIN (50 μg/kg) ON BLOOD PRESSURE, TOTAL PERIPHERAL RESISTANCE, AND HEPATIC FUNCTIONS IN RHESUS MONKEYS

				Time after enterotoxin (hr)									
Variable		Monkey No.	Base-line	0·25	0·5	1	2	3	4	5	6	7	8
Blood pressure (mmHg)	Systolic	A	120	135	110	120	115	110	85	95	110	105	105
		B	110	130	105	105	75	105	95	80	95	—	—
	Diastolic	A	75	70	55	40	65	35	40	40	20	25	20
		B	75	95	75	90	50	50	35	35	35	—	—
	Pulse	A	45	65	55	80	50	75	45	55	90	80	85
		B	35	35	30	15	25	55	60	45	60	—	—
	Mean	A	90	92	73	67	82	60	55	58	50	52	48
		B	87	107	85	95	58	68	55	50	55	—	—
Total peripheral resistance (dyne · sec/cm³)		A	5178	5532	3207	3267	2802	2095	3164	1650	2185	4007	2258
		B	9036	7441	5311	4661	2935	4248	2801	2423	1617	—	—
Half-life of cardiogreen dye (min		A	3·64	3·64	3·64	3·85	3·85	4·07	4·33	4·33	4·62	4·95	4·95
		B	4·33	3·85	4·95	4·95	6·30	6·30	6·30	6·93	6·93	—	—
Rate of dye disappearance (% min)		A	20	20	20	19	19	18	17	17	16	15	15
		B	17	19	15	15	11	11	11	10	10	—	—

peak dp/dt, Vmax, and Vce at 40 mmHg of developed isovolumic pressure in the left ventricle increased within 1 hr after toxin injection, then decreased gradually throughout the experimental period. Systolic, diastolic and mean blood pressure showed transient increases within 15 min after i.v. inoculation of enterotoxin followed by a general decrease throughout the remainder of the experimental period (Table 2). The transient elevation in blood pressure may be caused by sympathetic stimulation or adrenalin release from the adrenal medulla. (GRAYBILL *et al.*, 1960; LIDDLE and HARDMAN, 1971; BECKMAN *et al.* 1974). It appears that the heart was more responsive to the enterotoxin-induced sympathetic stimulation than the blood vessels, which showed a decrease in total peripheral resistance. It is possible that cholera enterotoxin-induced cAMP acts locally on the blood vessels and produces vasodilation (ØYE, 1975).

Hepatic responses to i.v. cholera enterotoxin

The rate of hepatic excretion of cardiogreen dye in monkeys given i.v. cholera enterotoxin decreased from baseline clearance rates (Table 2). This phenomenon was also shown by the prolonged half-life of the injected dye. Since the liver is the only organ capable of removing the i.v. injected cardiogreen, the casuses for increased dye retention in the

TABLE 3. EFFECTS OF I.V. CHOLERA ENTEROTOXIN ON SURVIVAL TIME, PATHOLOGICAL CHANGES AND GENERAL OBSERVATIONS IN FIVE SPECIES OF MONKEYS

Species	Dose (μg/kg)	Survival time (hr)	Major pathological changes	General observations
M. mulatta	50	12·5*	1. Generalized icterus	1. Anorexia
(Rhesus)	50	18·0*	2. Petechial hemorrhage	2. Dyspnea
	10	120	of myocardium	3. Coughing
			3. Pericardial effusion	4. Epistaxis
			4. Hepatic enlargement	5. Gingival hemorrhage
M. nemestrina	50	142	1. Severe hemorrhage of	1. Flushed face
(Pigtail)	50	113	lung	
			2. Petechial hemorrhage of heart and lung	2. Decreased activity
			3. Pericardial effusion	3. Anorexia
				4. Few stools
				5. Epistaxis
M. fascicularis	50	104	1. Severe hemorrhage of	1. Anorexia
(Cynomolgus)	50	15	lung	
	50	92	2. Ascites, severe	2. Decreased activity
			3. Petechial hemorrhage of heart and necrosis of papillary muscles (left ventricle)	3. Tremors
			4. Hydrothorax, moderate	4. Few stools
			5. Congested adrenal gland	
C. aethiops	50	Indef.		
(African green)	50	Indef.		
	200	Indef.		
	200	100	1. Hemorrhage of lung	1. Anorexia
			2. Necrosis of entire left ventricle of heart	2. Few stools
			3. Peritonitis and ascites	3. Foaming at mouth
				4. Tremors
C. apella	50	Indef.	No necropsy performed	Appeared normal
(Capuchin)	50	Indef.		
	200	Indef.		
	200	Indef.		

*Chaired. All other monkeys were caged.

circulation may be attributed to decreased hepatic blood flow, impaired cellular functions of the liver, or both.

General observations and survival time

General observations during 2 weeks and survival times of chaired and caged monkeys receiving i.v. cholera enterotoxin are presented in Table 3. Vomiting or diarrhea was not observed. We postulate that cholera enterotoxin could induce death as a result of excessively increased cellular concentrations of cAMP through activation of adenyl cyclase in cell membranes. Capillary permeability is thus increased leading to death from decreased plasma and blood volumes (FINKELSTEIN *et al.*, 1966; DALLDORF *et al.*, 1969). The obvious pathological changes seen in intoxicated monkeys were epistaxis and diffuse hemorrhage, particularly in heart muscle. Decreases in cardiac contractility or cardiac depression may also play a major role leading to death. These pathologic changes were not only observed in the rhesus monkey, but also in other species including pigtail and cynomolgus monkeys. The rhesus and pigtail monkeys appeared susceptible to i.v. cholera toxin. However, African green and capuchin monkeys were resistant to i.v. doses of cholera toxin up to 200 μg/kg. Although the mechanism for species differentiation to cholera enterotoxin i.v. inoculation is not known, genetic differences in susceptibility for this toxin may play a role (LANGE and HOLMGREN, 1978).

Acknowledgements—The authors thank Drs. P. E. Ross and V. ROBBINS for performing necropsies, Dr. T. MCCABE for surgical operations, and Mr. R. SANDERS for technical assistance.

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