PROSTAGLANDIN E_1 AND $F_{2\alpha}$ LEVELS DURING DEVELOPMENT OF SHOCK INDUCED BY PLAGUE TOXIN

T. D. Cherkasova, P. R. Vengrov,
V. I. Melikhov, V. P. Avrorov,
and V. A. Yurkiv

UDC 616.98:579.843.95]-06:616-001.36-008.6-022.7-092.9-07:[616-008.94:577.175.859

KEY WORDS: "murine" plague toxin; prostaglandins; toxic-infectious shock.

Prostaglandins (PG) play an important role in the pathogenesis of infectious diseases, for they take part in the development of pathophysiological reactions such as inflammation [3], fever, shock, and sepsis in animals and man [6, 7, 10]. It has now been established that bacterial lipopolysaccharides activate cyclo-oxygenase and lipoxygenase pathways of metabolism of arachidonic acid and other unsaturated fatty acids which are PG precursors [5, 11, 15]. Endotoxemia or direct application of endotoxins to tissues leads to accumulation of a wide range of compounds of this group in the blood plasma and in various organs [4, 8, 9, 12, 14].

The aim of this investigation was to study the PG content in various organs and blood plasma of rats during the development of shock induced by "murine" plague toxin, which is a lipopolysaccharide–protein complex [2].

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats weighing 150-200 g. Toxic-infectious shock was induced by injecting 0.5 ml of physiological saline containing 0.5 mg (LD_{50}) of an original preparation of toxin (fraction II according to Becker) into the caudal vein, and also by injecting 0.5 ml of the same solution, but inactivated by heating for 20 min to 100°C. Control animals were given an injection of the same volume of physiological saline into the caudal vein. All procedures were carried out under superficial ether anesthesia. The animals were autopsied 0.5, 2, and 5 h after injection of the toxin and the organs for testing were removed and frozen in liquid nitrogen. Blood was collected from the heart into a cold test tube containing 0.5 M EDTA, pH 7.4, in a volume equal to 1% of the volume of blood, and mixed. After sedimentation of the cells by centrifugation, 3 ml of ethanol was added to 1 ml of plasma. The frozen tissue was weighed and homogenized in alcohol in a homogenizer of Politron type. After extraction of the PG with ethyl acetate by the method in [13], they were determined quantitatively by radioimmunoassay, using kits from Clinical Assay (USA). All the results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the PGE_1 concentration in lung tissue was increased by 3.4 times 2 h after injection of the murine plague toxin, but fell after 5 h to near the control values. In animals with a marked picture of shock 2 h after injection of the toxin the PGE_1 concentration in the liver and small intestine was sharply increased (by 2.7 and 2 times, respectively) compared with healthy animals. The level of this PG after 5 h was the same as in the control rats. After 0.5 h the PGE_1 concentration was doubled in heart and kidney tissue, it remained at a high level 2 h after the injection, and then fell toward normal in the later stages of toxic shock. In the early stages after injection of the toxin and in the period of development of shock, the PGE_1 concentration in the spleen and blood plasma of the rats fluctuated around the control level without any significant changes, but fell sharply by 7.3 and 4 times, respectively, compared with normal 5 h after injection of the toxin.

Laboratory of the Molecular Basis of Pathogenesis of Infectious Diseases, Central Research Institute of Epidemiology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Pokrovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 3, pp. 313-315, March, 1988. Original article submitted January 20, 1987.

 0007-4888/88/0003-0368$12.50

TABLE 1. Changes in PGE_1 Concentration in Blood (ng/ml) and Organs (ng/g tissue) under the Influence of the Original Preparation (1) and the Thermostable Fraction (2) of Plague Toxin (M ± m)

Test object	Control (healthy animals, n = 10)	0,5 h		2 h		5 h	
		1 (n=10)	2 (n=8)	1 (n=10)	2 (n=8)	1 (n=10)	2 (n=8)
Lungs	68,7±7,1	84,9±9,5	86,7±8,9	231,5±24,2*	213,6±1,8*	66,9±6,7	75,3±8,3
Heart	43,3±4,5	89,1±9,5*	98,4±10,1*	177,9±21,1*	160,7±18,4*	44,2±6,1	54,4±6,2
Spleen	67,9±8,5	70,8±8,5	78,5±8,7	77,6±8,3	78,0±3,4	9,3±1,1*	7,9±0,9*
Liver	17,5±1,9	21,4±3,2	18,7±2,1	47,0±4,9*	40,5±5,1*	19,4±2,3	14,0±2,8
Kidneys	73,3±8,2	134,0±17,1*	124,2±20,1*	194,2±16,5*	191,7±18,3*	66,1±4,5	55,3±6,7
Intestine	8,2±0,8	9,3±1,4	10,4±2,2	17,6±2,1*	16,8±1,4*	7,6±0,7	8,5±0,9
Blood	11,1±2,5	10,8±1,6	10,1±1,4	13,5±1,8	14,8±1,8	2,7±0,2*	2,5±0,3*

Legend. Here and in Table 2: *p < 0.05 compared with control; n) number of animals.

TABLE 2. $PGF_{2\alpha}$ Concentration in Blood (ng/ml) and Organs (ng/g tissue) under the Influence of Original Preparation (1) and Thermostable Fraction (2) of Plague Toxin (M ± m)

Test object	Control (healthy animals, n = 10)	0,5 h		2 h		5 h	
		1 (n=10)	2 (n=8)	1 (n=10)	2 (n=8)	1 (n=10)	1 (n=8)
Lungs	2,6±0,3	6,3±0,1*	8,7±0,6*	7,3±0,6*	8,9±0,3*	5,6±0,4*	5,8±0,4*
Heart	1,3±0,2	1,1±0,1	1,6±0,2	1,8±0,2*	2,0±0,4	1,8±0,3	0,9±0,2
Spleen	1,8±0,2	7,1±0,7*	6,9±0,5*	2,8±0,1*	2,4±0,2	1,5±0,2	1,7±0,2
Liver	0,9±0,1	1,2±0,1	0,9±0,3	1,3±0,4	0,7±0,1	1,0±0,1	0,7±0,1
Kidneys	1,4±0,1	5,5±0,6*	7,8±1,5*	3,9±0,3*	4,8±0,2*	1,6±0,3	0,8±0,2
Intestine	1,2±0,1	1,1±0,1	0,9±0,1	2,6±0,1*	2,9±0,.*	1,3±0,1	1,0±0,2
Blood	1,4±0,1	1,2±0,1	1,7±0,2	1,7±0,3	1,6±0,2	0,6±0,1*	0,5±0,1*

The $PGF_{2\alpha}$ concentration (Table 2) in lung tissue was 2-2.8 times higher than the control value at all times of observation. The $PGF_{2\alpha}$ concentration in the tissues of the kidney and spleen was increased by 3.9 times of 0.5 h after injection of the toxin, it remained high until 2 h, and returned to the control value 5 h after injection. The $PFG_{2\alpha}$ concentration in the small intestine was doubled after 2 h and returned to the control value after 5 h. Unlike the PGE_1 concentration, the $PGF_{2\alpha}$ concentration in heart and liver tissue remained unchanged at all times of observation. The plasma $PGF_{2\alpha}$ level fluctuated around the control value without any statistically significant changes 0.5 and 2 h after injection of the murine plague toxin, and fell sharply by 2.3 times below the control level after 5 h.

The thermostable fraction of the toxin had the same effect on the PGE_1 and $PGF_{2\alpha}$ concentrations in all the organs and blood plasma tested in the course of development of toxic-infectious shock as the original preparation of toxin (Table 1 and 2).

The results are thus evidence of a marked restructuring of the cyclo-oxygenase pathway of PG biosynthesis in all organs and in the blood plasma during the development of toxic-infectious shock due to plague toxin in rats.

We know that PG possess a broad spectrum of regulatory action on the metabolism of organs and tissues. Changes in their concentration and in their normal proportions in the organs under the influence of murine plague toxin may be one of the causes leading to disturbance of the hemodynamics, and may also induce the development of a combination of metabolic disturbances of cell functions, leading to the development of shock in the experimental animals.

It must be emphasized that the original preparation of toxin and its thermostable fraction led to equal and parallel changes in the PG concentration in all organs and at all times of observation. It can be postulated that the activating effect on metabolism of arachidonic acid and other unsaturated fatty acids, which are PG precursors, is due to the presence of a thermostable lipopolysaccharide component in the original preparation of the toxin, that is the endotoxin of *Yersinia pestis* cells. The protein part of the "murine" toxin evidently has no marked effect on PG metabolism.

The time course of the changes in PGE_1 and $PGF_{2\alpha}$ concentrations in the blood plasma and lungs after intravenous injection of murine plague toxin and of its thermostable fraction, incidentally, differs from that observed under the influence of classical endotoxins isolated from other Gram-negative bacteria. We found no statistically significant increase in the concentration of these classes of PG in the blood plasma in the initial stages or in the stage of development of the shock reaction in animals treated with the toxin, as has been demonstrated with other endotoxins in other investigations [4, 8, 9, 12], and in the late stages the levels of these compounds fell sharply. The $PGF_{2\alpha}$ concentration in the lung tissue was higher at all times of observation, and this also is not in agreement with data obtained previously for typical endotoxins [9].

It can thus be postulated on the basis of these data that the specific nature of the metabolic effects of the endotoxin of *Y. pestis* is due to its structural differences from the lipopolysaccharides of other Gram-negative bacteria, established previously [1].

LITERATURE CITED

1. E. É. Bakhrakh, "Somatic antigens of plague bacteria and the use of isolated preparations for the detection of immunoallergic modification of the organism in plague," Author's Abstract of Dissertation for the Degree of Doctor of Medical Sciences, Saratov (1973).
2. M. N. Dzhaparidze and T. I. Damaradskaya, The Molecular Structure of Bacterial Toxins and Genetic Control of their Biosynthesis [in Russian], Moscow (1985), p. 43.
3. S. Arora, P. K. Lahiri, and R. K. Samyal, Int. Arch. Allergy, 39, 186 (1970).
4. H. Bult, A. G. Herman, J. Beetens, and P. Vercruysse, Adv. Prostaglandin Thromboxane Res., 7, 839 (1980).
5. H. Bult and A. Herman, Cardiovascular Pharmacology of the Prostaglandins, ed. by A. G. Herman et al., New York (1982), pp. 327-345.
6. R. R. Butler, W. C. Wise, P. V. Heluska, and J. A. Cook, Adv. Shock Res., 7, 133 (1982).
7. J. A. Cook, W. C. Wise, and P. V. Heluska, J. Clin. Invest., 65, 227 (1980).
8. J. R. Fletcher and P. W. Ramwell, Brit. J. Pharmacol., 64, 185 (1978).
9. J. R. Fletcher and P. W. Ramwell, Adv. Shock Res., 2, 219 (1979).
10. J. R. Fletcher, P. W. Ramwell, and R. H. Harris, Adv. Shock Res., 5, 143 (1981).
11. J. R. Fletcher, B. L. Short, L. C. Casey, et al., Adv. Prostaglandin Thromboxane Leucotriene Res., 12, 117 (1983).
12. A. G. Herman and J. R. Vane, Br. J. Pharmacol., 39, 79 (1976).
13. B. M. Jaffe and H. R. Behrman, Methods of Hormone Radioimmunoassay, ed. by B. M. Jaffe and H. R. Behrman, New York (1974), pp. 19-34.
14. A. Kahn and E. Brachet, Prostaglandins Med., 6, 23 (1981).
15. T. Lüderitz, V. Schade, and E. T. Rietschel, Eur. J. Biochem., 155, 377 (1986).