REDUCTION OF BIOLOGICAL ACTIVITY OF CHOLERA VIBRIO CULTURE FILTRATES BY NEURAMINIDASE INHIBITORS

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With edema of the albino mouse paw as experimental model the action of neuraminidase inhibitors on the cholerogenic effect of cholera vibrio culture filtrates (CVCF) was studied. Addition of inhibitors to CVCF was found to depress their biological activity. Since purified neuraminidase preparations from cholera vibrios had no cholerogenic action it was postulated that the region of the cholerogen responsible for fixation on cell membranes is chemically similar to the active center of neuraminidase.

KEY WORDS: cholera vibrio culture filtrates; neuraminidase; neuraminidase inhibitors.

The very great loss of fluid and electrolytes in cholera is due to the action of an enterotoxin (cholerogen) [12]. Cholerogen can also be obtained on liquid nutrient media if certain strains of Vibrio cholerae are grown on them. In that case cell-free filtrates contains not only cholerogen, but also other biologically active substances, including a large quantity of neuraminidase [6]. The biological role of this enzyme has not been finally explained, although it is supposed that the change in permeability of the cell membranes is the result of its activity [2].

Changes in the biological activity of cholera vibrio culture filtrates (CVCF) under the influence of neuraminidase inhibitors were studied.

EXPERIMENTAL METHOD

The CVCF were obtained by culturing V. cholerae 569 B on 5% peptone water in the usual way [6]. The CVCF contained free "intestinal units" [10] and 256 "blueing doses" [11] in 1 ml and possessed neuraminidase activity to the extent of hydrolyzing 1.65 mmoles free sialic acids during incubation for 30 min at 37°C [9]*. The biological activity of the CVCF was modified by synthetic inhibitors (derivatives of arabino-octonic acid [8]), two preparations of which (A and B) were generously provided by A. Ya. Khorlin and I. M. Privalova, to whom the author is grateful. To determine the action of the inhibitor a sample of the necessary weight was dissolved in a diluted (1:4) buffered physiological solution (pH 7.2) of CVCF. The resulting solution was incubated for 30 min at 37°C and injected in a dose of 0.05 ml into the left foot pad of BALB/c mice. The same dose of CVCF but without inhibitor was injected at the same time into the right foot pad. The biological activity of the native CVCF and a mixture of it with neuraminidase inhibitors was compared with respect to the individual difference in size of the edema recorded 48 h after injection of CVCF [13]. The original method of recording the size of the edema, based on direct measurement of the limb volume, was replaced by weighing of the hind limbs amputated strictly through the ankle joint [3]. Significance of the difference was estimated by the criterion of signs [1].

*Neuraminidase activity of CVCF per se and on the addition of inhibitors was determined by I. M. Privalova.

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TABLE 1. Effect of Synthetic Neuraminidase Inhibitors on Ability of CVCF to Produce Edema of the Limbs in Albino Mice

Series of experiment	No. of group of animals	Limb studied	Agent injected: CVCF	Agent injected: inhibitor[1]	Mean weight of amputated limbs	No. of animals[2]: R	No. of animals[2]: L	P	Residual cholerogenicity (%)
I	1	Right	+	—	369	5	4	>0 05	107
		Left	+	A,5	377				
	2	Right	—	—	126	0	10	0,01	101
		Left	+	A,5	370				
II	1	Right	+	—	358	9	1	0,05	41
		Left	+	A,20	254				
	2	Right	—	—	110	0	5	0,05	31
		Left	+	A,20	283				
	3	Right	+	—	362	10	0	0,01	11
		Left	+	B,20	156				
	4	Right	—	—	100	0	5	0,05	7
		Left	+	B,20	178				
III	1	Right	+	—	370	9	0	0,01	29
		Left	+	A,40	247				
	2	Right	+	—	335	10	0	0,01	<14
		Left	+	A,80	144				
	3	Right	+	—	311	10	0	0,01	<15
		Left	+	B,40	128				

[1]Preparation of inhibitor (A and B) and its final concentration (in mg/ml)

[2]Number of mice in which weight of right limb was greater than weight of left limb (R) or vice versa (L)

EXPERIMENTAL RESULTS

The results of three series of experiments are given in Table 1 and they show that addition of the inhibitors in concentrations of 20 mg/ml and above significantly ($P \leq 0.05$) reduced the biological activity of CVCF. This was manifested by the fact that in each individual animal the weight of the left limb into which a mixture of inhibitor and CVCF was injected, as a rule, was smaller than that of the right limb, into which only the native CVCF was injected.

In a special experiment on 50 albino mice a linear relationship was found between the weight of the limbs and the logarithm of the injected dose of CVCF both when the other limb remained intact and when a constant dose of CVCF was injected into the other limb (Fig. 1). Admittedly, a relationship of this type was observed only when the weight of the limbs was increased by the action of CVCF to not less than 150-170 mg. Because of this rule it was possible by simple transformations of the linear regression equation to obtain an equation for determining the residual biological activity of the CVCF (X) in percent:

$$\log X = 2 - 0{,}301\,\frac{P_{\text{standard}} - P_{\text{expt}}}{K},$$

where $P_{standard}$ is the mean weight of the limbs after injection of native CVCF and P_{expt} the mean weight of the limb after addition of the inhibitor to the CVCF; K the change in weight of the limb in response to a twofold change in the CVCF concentration (the coefficient of linear regression was 68 mg for injection of CVCF into both limbs and 48 mg for its injection into one limb). Despite the considerable decrease in biological activity of the CVCF, it was not completely inhibited in the doses tested.

Synthetic neuraminidase inhibitors thus considerably reduce the biological activity of CVCF. However, this effect is evidently not simply the result of inhibition of neuraminidase activity by CVCF, for in vitro a concentration of inhibitor of only 5 mg/ml would be sufficient for this purpose, i.e., a concentration

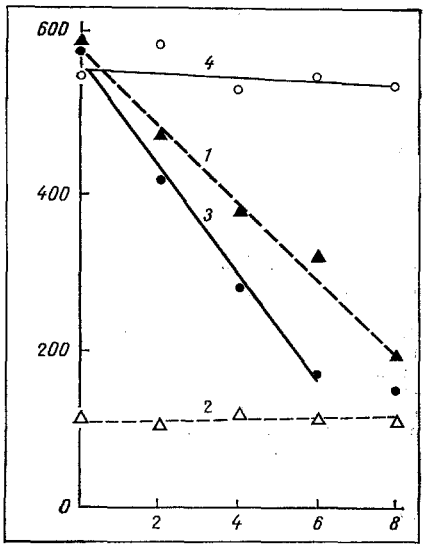

Fig. 1. Size of edema of mouse limb as a function of dose of CVCF injected: 1) different doses of CVCF injected into right limb; 2) nothing injected into left limb; 3) different doses of CVCF injected into right limb; 4) constant dose of CVCF injected into left limb. Abscissa, dose of CVCF ($-\log_2$ of dilutions); ordinate, weight of edema fluid (in mg).

which for practical purposes did not change the biological activity of the CVCF. Furthermore, highly purified commercial preparations of cholera vibrio neuraminidase, generously provided by I. M. Privalova and Yu. V. Ezepchuk, caused an increase in the weight of the limbs of only 10-12 mg. All these results agree with data in the literature [14] in showing that the cholerogenic action of CVCF is not due to neuraminidase activity.

The experimental results indicate the ability of neuraminidase inhibitors to create a biologically inactive complex with cholerogen. The mechanism of inhibition of the biological action of CVCF is not yet clear. Inactivation of the cholerogen possibly is not connected with any special feature of the chemical structure of the inhibitors whereby they can block the active center of neuraminidase [8]. However, the possibility of their specific action cannot be ruled out, if stereochemical similarity between the structure of the active center of neuraminidase and the region of cholerogen responsible for fixation of the latter to cell membranes is accepted. Such an assumption is not contrary to data in the literature. For instance, cholera vibrio neuraminidase is known to be adsorbed on erythrocytes [2]. Purified cholerogen and its natural toxoid, cholerogenoid, possess the same property [15]. Moreover, it has been postulated that the fixation of cholerogen and cholerogenoid on cell membranes takes place through binding with gangliosides [15], substances with sialic acids in their composition [7]. A similar mechanism of adsorption has been demonstrated for tetanus toxin [5]. The possibility cannot be ruled out that purified cholerogen also possesses neuraminidase activity, as has been shown for the vascular permeability factor of Corynebacterium diphtheriae [4].

The experimental results and an analysis of the literature thus suggest a possible chemical similarity in structure of the region of cholerogen responsible for its fixation on cell membranes and the active center of neuraminidase.

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