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EFFECTIVENESS OF PROPHYLACTIC ADMINISTRATION OF EPSILON-AMINO CAPROIC ACID DURING INFLUENZA IN MICE

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Summary. — Epsilon-amino caproic acid (E-ACA), a proteolysis inhibitor, has been found to limit the development of various forms of experimental influenza infection in mice. In sublethal infection, the extent of influenza A virus reproduction in the lungs was reduced 2 and 10 days after a single dose of E-ACA as well as 4 weeks after a 5-day prophylactic course. This correlated with the ability of proteolysis inhibitor to stimulate early production of specific serum antibodies. The favourable effect of prophylactic administration of E-ACA was especially marked in experimental lethal influenza caused by the pathogenic influenza A0/32 (H1N1) strain; a significant protection was observed from days 3 to 14 post-infection (p.i.).

Key words: prophylaxis of virus infection; ε-aminocaproic acid; experimental influenza

Introduction

In the recent decade proteolysis inhibitors have been found to produce a marked antiinfluenza therapeutic effect (Lozitsky and Polyak, 1982; Zhdanov, 1984), inhibiting virus reproduction in tissue cultures (Lozitsky *et al.*, 1977, 1979; Ruttkayová-Nedecká and Russ, 1977; Zhirnov *et al.*, 1981), in the lungs of mice (Degtyarenko *et al.*, 1978; Zhirnov *et al.*, 1983), decreasing mortality of lethal influenza (Lozitsky *et al.*, 1979; Zhirnov *et al.*, 1981) and significantly improving the course of influenza and/or acute respiratory disease in children (Lozitsky *et al.*, 1980). It has been found that ε-aminocaproic acid hydrochloride provides some protective effect in mice even if administered before influenza virus infection (Hrušková and Jarý, 1975). These data required a more detailed study of the antiinfluenza effect of E-ACA. Tis paper deals with the prophylactic effect of the proteolysis inhibitor E-ACA in the course of different forms of experimental influenza induced various times after administration of the drug.

Materials and Methods

Viruses. Allantoic cultures of influenza virus strain A0/32 (H1N1) pathogenic for mice and A/Hong Kong/1/68 (H3N2) strain unadapted to mouse lungs have been used.

Experimental animals. The experiments have been carried out in outbred albino mice of either sex weighing 15—17 g under standard housing conditions.

Tissue culture. Allantois-on-shell culture of chorioallantoic membrane (CAM) has been prepared from 11 to 13-day-old chick embryos. The culture was maintanined in polystyrene plates (Maltseva *et al.*, 1973).

E-ACA. Official preparation manufactured in the U.S.S.R. has been used. E-ACA solution was injected subcutaneously (s.c.) to mice in 0.15 mol/l NaCl for 5 days (90 mg per mouse daily in 3 injections. Control animals were given the same vol of 0.15 mol/l NaCl. 25 days after the end of the 5-day course of E-ACA treatment, the mice were infected intranasally (i.n.) with influenza virus A/Hong Kong/1/68 in a dose of 10^4 EID_{50} in 0.05 ml or 10 LD_{50} of A0/32 virus. On days 3, 5, 7 and 10 p.i. 5 mice out of each group were exsanguinated under ether anaesthesia. The amount of infectious virus in the lung tissue was determined by titration of 10-fold serial dilutions of the 10% homogenates in CAM culture, specific haemagglutination inhibiting antibodies were tested in sera heated at 56 °C for 30 min with 2 haemagglutinating units of inhibitor-resistant variant of the corresponding virus strain.

Protective action of E-ACA on lethal influenza infection was tested as follows: 2 or 10 days after a single 15 mg dose of E-ACA in 0.1 ml of 0.15 mol/l NaCl the mice were infected i.n. with the pathogenic influenza virus strain A0/32 (H1N1) in a dose of 2 or 10 LD_{50}; alternatively, 25 days after a 5-day course of E-ACA treatment described above the animals was given the same strain at dilutions ranging from 10^{-1} to 10^{-6} using 6 animals per each dilution. Deaths were registered within 14 days p.i. The 50% tissue infective dose (TID_{50}) for determination of the amount of infectious virus in lung homogenates and the 50% lethal dose (LD_{50}) in experimental infection were calculated by Kaerbers method modified according to Ashmarin. The significance of the drug effect was determined as described by Ashmarin and Vorobiev (1962). The mean geometric titres of serum antibodies were calculated and the statistical significance of the differences was estimated (Voroshilova *et al.*, 1964).

Results and Discussion

It has been found that a single s.c. administration of E-ACA in a dose of 15 mg by 2 or 10 days prior infection lead to a 20—30% increase in the survival rate of mice infected with 2 or even 10 LD_{50} of influenza virus A0/32

Table 1. The effect of single prophylactic E-ACA administration on the development of lethal form of influenza infection in mice

Group of animals	Number of animals	E-ACA[1]	Interval betveen E-ACA administration infection (days)	Dose of AO/32 (log_{10} LD_{50})	Survival rate (%)	Protective effect of E-ACA[2] (%)
1	24	—	—	2.0	33.3	—
2	12	+	2	2.0	66.7	33.4
3	12	+	10	2.0	58.3	25.0
4	24	—	—	10.0	12.5	—
5	12	+	2	10.0	41.7	29.2
6	12	+	10	10.0	16.7	4.2

[1] Single injection of 15 mg/0.1 ml.
[2] Difference between the survival rate in experimental and control groups.

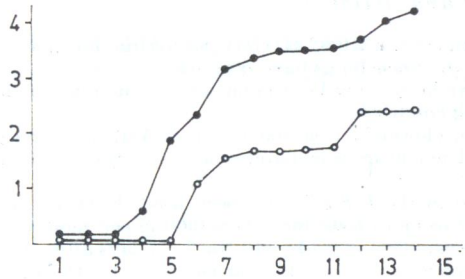

Fig. 1.
Morbidity of E-ACA-pretreated and untreated mice in influenza virus infection Abscissa: time after infection (days); ordinate: LD_{50} (log_{10}). ●——● LD_{50} in control; ○——○ LD_{50} in the group of mice treated with E-ACA 25 days prior to infection. Animals were treated for 5 days.

(Table 1). This, as well as the results reported by Hrušková and Jarý (1975), promoted us to a detailed study of the favourable prophylactic effect of E-ACA on different forms of experimental influenza infection.

The dynamics of deaths of the animals infected with the adapted influenza virus strains A0/32 (Fig. 1) was compared with the time course of virus reproduction in the lungs and formation of humoral immunity in mice infected with this virus as well as with the nonadapted virus A/Hong Kong/1/68 (Table 2) after E-ACA treatment.

The pathogenic influenza virus A0/32 reproduced in the lung tissue more intensively than the A/Hong Kong/1/68 virus. The strain A0/32 elicited a certain immunosuppressive effect within 7 days, since production of antibodies in animals who survived by that time was much lower than upon

Table 2. Prophylactic effect of E-ACA on the development of infection and immunity in mice infected by influenza viruses

Influenza virus strain	E-ACA[1]	Virus amount in the lungs (log_{10} TID_{50} (M ± SD)				Inverse values of mean geometric titres of antibodies			
		Observation days							
		3rd	5th	7th	10th	3rd	5th	7th	10th
A/Hong Kong/1/68	—	2.46 ±0.2	2.5 ±0.17	1.94 ±0.23	0	0	0	42.0	64.0
	+	2.85 ±0.14	2.82 ±0.26	0.82* ±0.06	0	1.6	8.0	56.0	64.0
A0/32	—	2.82 ±0.18	4.82 ±0.24	2.0 ±0.34	n.t.	0	0	16.0	n.t.
	+	3.38 ±0.23	3.75* ±0.22	1.3 ±0.09	n.t.	0	0	16.0	n.t.

[1] The animals were infected 25 days after a 5-day course of E-ACA with a daily dose of 90 mg.
Note: The asterisked values indicate the significant differences (at $P \leq 0.05$). n.t. — not tested.

infection with the A/Hong Kong/1/68 virus (Table 2). Pretreatment by E-ACA provided an earlier stimulation of humoral immunity in mice infected with the pathogenic virus A/Hong Kong/1/68: antibodies were detected earlier — on days 3 to 5 p.i. No such stimulation caused by the virulent virus A0/32 has been observed.

The effect of E-ACA on the dynamics of virus accumulation was studied in lungs by various forms of influenza infection. On day 3 p.i. with the either influenza virus strain, the difference between the amount of infectious virus in the lung tissue of control mice and those treated by E-ACA was statistically insignificant (Table 2). On days 5 to 7 p.i. with A0/32 strain and on day 7 p.i. with A/Hong Kong/1/68 strain there was a statistically significant decrease of infectious virus reproduction in the lungs of animals pretreated with E-ACA. After 10 days the virus could be no longer isolated from the lungs of either of experimental or control animals. The comparison of the data obtained allowed to reveal a distinct correlation between the E-ACA-induced stimulation of a more rapid appearance of antibodies in the blood starting from day 3 of the development of sublethal form of influenza infection, and the decrease with time of the amount of infectious influenza virus A/Hong Kong/1/68 in the mouse lungs.

Fig. 1 shows the results of the study of prophylactic protective effect of E-ACA in lethal experimental influenza infection. It has been found that among E-ACA-pretreated animals death occurred two days later than in the control group and thereafter a statistically reliable protective effect was observed ($p < 0.01$). By the end of the observation period (after 14 days) the differences of LD_{50} between the control and experimental groups were 1.9 $\log_{10}$.

As it has been previously shown, the therapeutic effect of proteolysis inhibitors is related to both their direct effect on virus reproduction in sensitive tissues and the pathogenetically significant regulation of proteolysis system of the organism (Parusov *et al.*, 1978; Lozitsky and Polyak, 1982). The direct effect of proteolysis inhibitors on the posttranslational processing of viral polypeptides has been also demonstrated (Zhirnov *et al.*, 1981).

The mechanism of the prophylactic effect is not yet clear. It is known that E-ACA is rapidly eliminated from the organism and, therefore, it seems that its direct effect on the infection process induced long after (4 weeks) its administration can be ruled out. It can be suggested that the administration of E-ACA causes the activation of some mechanisms of general antiviral protection. The revealed correlation between the decrease of the virus reproduction in the lung tissue of E-ACA-treated mice and the stimulation of the synthesis of specific immunoglobulins in these mice seems to be an evidence for the delayed results of the effect upon the immune system response. This is also supported by the data on immunomodulating effects of proteolysis inhibitors (Hammond *et al.*, 1978; Matsumoto *et al.*, 1981). Therefore, further research should deal with detailed analysis of the duration of the effect of proteolysis inhibitors on the functional activity of immune system cells.

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