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EFFECT OF KANAMYCIN ON THE REPRODUCTION OF ORTHOMYXOVIRUSES

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Summary. — Kanamycin sulphate at a concentration of 8 mmol/l had no effect on the protein synthesis in uninfected chick embryo cell (CEC) cultures, but caused a 2-fold decrease of virus-specific protein synthesis in CEC infected with fowl plague virus (FPV). Kanamycin at a concentration of 2 mmol/l decreased the yield of infectious FPV in one growth cycle experiments on CEC culture by 1.5 $\log_{10}$ units and when added into the agar overlay it decreased the plaque number by nearly 1 $\log_{10}$ unit. Inoculation of 10 mg of kanamycin into a chick embryo decreased the yield of virus by 1.0 $\log_{10}$. Administration of kanamycin to mice (5-10 mg for three days post infection) reduced mortality of the animals 2—3-fold. Antibiotics of the streptomycin group presumably may penetrate into orthomyxovirus-infected cells due to virus-induced impairment of leakiness of the cell membrane and inhibit both the virus protein synthesis and formation of infectious virions.

Key words: *Orthomyxovirus*; *kanamycin*; *protein synthesis*; *virus inhibitors*

Introduction

Recent investigations on a number of viruses showed that some inhibitors with molecular weights of less than 750 daltons, uncapable of penetrating into uninfected cells, can enter these cells after viral infection due to virus-induced impairment of cell membrane leakiness and affect certain stages of the synthesis of virus macromolecules (Carrasco, 1978; Contreras and Carrasco, 1979; Ghendon and Klimov, 1981).

In previous experiments on fowl plague virus (FPV) we showed (Ghendon and Klimov, 1981) that, under certain conditions after virus infection, certain inhibitors exerting no effect on the protein synthesis in uninfected cells (including the antibiotic hygromycin B of the streptomycin group) are capable of penetrating through the cell membrane and inhibit both the virus protein synthesis and the yield of infectious virus. The present investigations were aimed at studying the effects of kanamycin (an antibiotic of the streptomycin group widely used for treatment of a number of human bacterial diseases) on the synthesis of cellular and viral proteins as well as on the reproduction of influenza viruses in cell cultures, chick embryos and mice.

Materials and Methods

Viruses. Fowl plague virus (FPV) strain Weybridge (H7N7), human influenza virus A/Hong Kong/1/68 (H3N2), and variants of human influenza viruses A/Hong Kong/1/68 (H3N2) and A/Frunze/57/ (H2N2) adapted to mice by intranasal inoculation were used.

Cells, chick embryos, mice.(Primary chick embryo cell (CEC) culture, 10-day-old chick embryos and 7—10 g white mice were used.

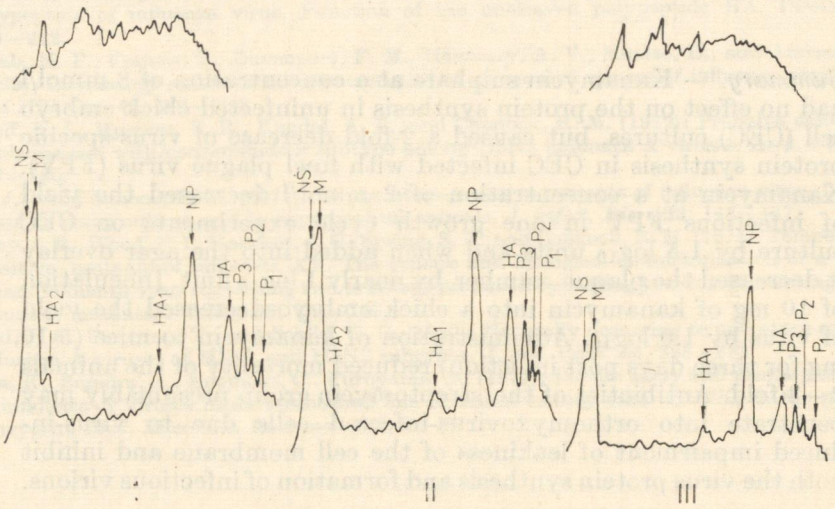

Fig. 1.

Effect of kanamycin on protein synthesis in uninfected (top row) and FPV-infected (bottom row) CEC cultures

I, II, III — Kanamycin concentrations 0 (control), 2 and 8 mmol/l respectively

^{35}S-Methionine incorporation: uninfected kanamycin-treated cells — 98% of control; infected cells: 83% (II) and 41% (III) of control (I).

Protein synthesis. Cells were infected with FPV at a multiplicity of 30—50 PFU/cell; adsorption lasted 30 min at room temperature. The infected cells were then incubated for 1 hr at 36° C, after which the medium was replaced by methionine-free one and kanamycin (kanamycin sulphate, molecular weight 582, manufactured by the "Sintez" enterprise, Kurghan, U.S.S.R.) was added and incubation continued for 4 hr at 36°C. Then 0.74 MBq ^{35}S-methionine per sample (^{35}S--methionine, Radiochemical Centre, Amersham, specific activity 48 TBq/mmol) was added and incubation continued for 30 min at 36°C. In control experiments, FPV-infected cells were treated and incubated under similar conditions without kanamycin. In experiments, on uninfected cells, the conditions of treatment and incubation were similar to those with infected cells. Finally, the cell monolayers were collected in a solubilizing solution (5 mol/l urea, 1% sodium dodecyl sulphate, 0.1% β-mercaptoethanol), boiled for 3 min and analysed by electrophoresis in 25% polyacrylamide gels (PAGE) using a buffer system described by Laemmli (1970). Autoradiography was carried out according to Russel and Skehel (1972). Densitometry of the autoradiographs was performed ou a Joyce Loebl Chromoscan 200.

Virus reproduction. CEC cultures were infected with FPV at a multiplicity of 1-3 PFU/cell. After 30 min of adsorption at room temperature, the cells were washed 5 times in buffered saline and medium 199 was added; immediately thereafter one sample (sample 0) was frozen and all others were incubated for 1 hr at 36° C. Thereafter kanamycin was added and after another 15 hr of incubation the cells were once frozen and thawed and assayed for virus by the plaque

Table 1. Effect of kanamycin on FPV reproduction in CEC cultures

Reproduction during one growth cycle* Conditions of experiment	Virus yield (PFU/ml)	Kanamycin concentration	Plaque formation under agar overlay** No. of plaques per bottle after inoculation of 200 PFU	20 PFU	2 PFU	Inhibition of plaque formation
Time 0	2×10^2	0		24; 23;	3; 2; 1;	
			> 100	16; 16;	1; 0; 0	
No kanamycin	8×10			15; 12		
Kanamycin 1 mmol/l	9×10^6					
Kanamycin 2 mmol/l	1×10^6	1 mmol/l	78; 70;	12; 10;	0; 0; 0;	52%
			66; 62;	8; 8;	0; 0; 0	
			58; 51	6; 3		
		2 mmol/l	32; 26;	3; 3; 2;	0; 0; 0;	87%
			22; 20;	2; 0; 0	0; 0; 0	
			16; 11			

* Cells were infected with FPV (1—3 PFU/cell), incubated at 36 °C and supplied with inhibitor; after 15 hr of incubation, the virus titres were determined. Time 0 — sample frozen immediately after inoculation.

** FPV was diluted to the required PFU content and inoculated in monolayer CEC cultures (6 bottles per each virus dilution and kanamycin dose). Cells were overlaid with kanamycin-containing agar overlay and after 3 days of incubation the plaques were counted.

Table 2. Effect of kanamycin on A/Hong Kong/1/68 influenza virus reproduction in chick embryos

Conditions of the experiment	Yield of the virus	
	$\log_{10}$ EID_{50}/0.1 ml	$\log_2$ HA units per 0.5 ml
Without kanamycin	8.0	7
10 mg kanamycin simultaneously with virus	7.0	5
20 mg kanamycin simultaneously with virus	6.5	5
10 mg kanamycin 24 hr before virus + 10 mg kanamycin simultaneously with virus	7.0	5

method in CEC cultures. In another experimental variant, monolayer CEC cultures were infected with FPV (2-200 PFU of virus per mono layer). After 30 min of adsorption at room temperature the monolayers were overlaid with agar overlay without or with kanamycin in various concentrations (see Table 1). After 3 days of incubation at 36°C, the plaques were counted. In experiments on chick embryos, 10-day-old embryonated eggs were infected with A/Hong Kong/1/69 influenza virus (10^3 EID_{50} per embryo). Doses and time of addition of kanamycin are shown in Table 2. After 48 hr of incubation, the allantoic fluids were removed and assayed for infectivity in chick embryos and haemagglutinating activity.

Pathogenicity for mice. White mice weighing 7—10 g were inoculated intranasally with mouse-adapted influenza virus variants. In experiments on the A/Frunze/57/ strain the inoculated dose of 10^4 EID_{50} per mouse caused death of nearly 90% of the mice. In experiments on the A/ /Hong Kong/1/68 strain, a dose of 10^2—10^3 EID_{50} was used; it caused death of 60—70% of the animals. Kanamycin was injected intramuscularly at intervals and in doses given in Table 3. Forty mice were used in each variant of the experiment; they were observed for 10 days.

Results

Effect of kanamycin on protein synthesis and FPV reproduction in CEC cultures

Fig. 1 shows that kanamycin at a concentration of 8 mmol/l practically did not inhibit the protein synthesis in uninfected CEC culture. At the same time kanamycin at a concentration of 2 mmol/l decreased virus-specific protein synthesis in FPV-infected cells by 17% (in other experiments — by 16—22%), and 8 mmol/l kanamycin lowered virus protein synthesis by nearly 60% (in other experiments — by 54—62%).

Assay of infectious virus showed (Table 1) that kanamycin at a concentration of 1 mmol/l inhibited the yield of virus during one reproduction cycle by nearly 1 $\log_{10}$ unit; virus reproduction was inhibited by approximately 1.5 $\log_{10}$ units by kanamycin used at a concentration of 2 mmol/l. When added into the agar overlay, kanamycin at concentrations of 1 and 2 mmol/l inhibited plaque formation by 52 and 87%, respectively. These experiments were repeated three times with similar results.

Effect of kanamycin on influenza virus reproduction in chick embryos

Administration of kanamycin into the allantoic cavities of chick embryos (10 mg per embryo) simultaneously with virus decreased the yield of in-

Table 3. Effect of kanamycin on influenza virus pathogenicity for mice

Virus	Conditions of experiment	Mortality*	Protection index
A/Frunze/57	Without kanamycin	37/40*	
	5 mg kanamycin on days 1, 2 and 3 post infection	22/40	1.6
A/Hong Kong/1/68	Without kanamycin	25/40	
	10 mg kanamycin on days 1, 2 and 3 post infection	8/40	3.0

* Numerator: No. of dead mice; denominator: No. of mice tested.

fectious virus by 1 $\log_{10}$ unit. On increasing the kanamycin dose to 20 mg per embryo, the yield of infectious virus was decreased by 1.5 $\log_{10}$ units. Higher kanamycin doses were toxic, causing death of a number of embryos. After administration of kanamycin into chick embryos 24 hr before virus the inhibitory effect was not enhanced as compared with simultaneous administration of the inhibitor and virus. Table 2 presents the results of one of three experiments; the other results were similar.

Effect of kanamycin on influenza virus pathogenicity for mice

Under the given experimental conditions, administration of kanamycin significantly reduced the mortality of mice by 2—3-fold (Table 3). These experiments were repeated three times with similar results (protection factor ranged from 1.6 to 3.5). In uninfected control mice, the kanamycin doses used (5 and 10 mg per mouse) caused no death, but higher doses (20 mg and more) proved to be toxic for mice.

Discussion

A number of inhibitors uncapable of penetrating into eukaryotic cells can enter virus-infected cells due to altered leakiness of infected cell membranes (Carrasco, 1978). Such results were obtained with encephalomyocarditis, Mengo, Semliki forest and SV40 viruses (Contreras and Carrasco, 1979).

In our experiments on an orthomyxovirus (FPV) certain inhibitors, in particular hygromycin B, having no effect on protein synthesis in uninfected cells, inhibited virus-specific protein synthesis and lowered the yield of infectious virus (Ghendon and Klimov, 1981).

In the present investigations another antibiotic — kanamycin (belonging, like hygromycin B, to the streptomycin group and also uncapable of affecting protein synthesis in uninfected CEC culture) — was shown to inhibit considerably virus-specific proteins synthesis in FPV-infected CEC, decrease the

yield of infectious virus in CEC and chick embryos and reduce mortality of mice infected with influenza virus. The present data suggest that the ability of hygromycin B to inhibit the protein synthesis and formation of infectious influenza virus particles (Ghendon and Klimov, 1981) is not a unique feature of this antibiotic, but a characteristic also of other antibiotics of the streptomycin group.

Our preliminary experiments showed that penicillin at certain concentrations, though having no effect on the protein synthesis in uninfected cells, was also capable of inhibiting the protein synthesis and the yield of infectious FPV in CEC cultures. But the inhibitory activity of penicillin was lower than that of antibiotics of the streptomycin group.

As mentioned above, Carrasco (1978) and Contreras and Carrasco (1979) demonstrated that many inhibitors with a molecular weight of less than 750 daltons cannot enter uninfected eukaryotic cells but may penetrate into cells after viral infection, the latter causing alterations of cell membrane leakiness. Kanamycin sulphate with a molecular weight of about 600 daltons does not inhibit protein synthesis in eukaryotic cells (Vazquez, 1974). According to our results, this preparation had no effect on the protein synthesis in uninfected cells, but was capable of penetrating into orthomyxovirus-infected cells and inhibiting virus protein synthesis as well as formation of infectious virions.

Kanamycin sulphate has become widely used for treatment of a number of bacterial human diseases. Although the dose used in our experiments is considerably higher than the permissible single dose of kanamycin for man, it cannot be excluded that a certain favourable effect of antibiotics on influenza infection, reported in a number of cases by physicians, may be the result of the antibiotic's action not only on secondary bacterial infection, but also on the intracellular reproduction of influenza virus.

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