A COMPARATIVE STUDY OF THE EFFECTIVENESS OF THE INHALATION AND THE SUBCUTANEOUS METHODS OF ADMINISTRATION OF KANAMYCIN IN THE TREATMENT AND PROPHYLAXIS OF INDUCED PNEUMONIA IN ALBINO MICE

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COMPARATIVE STUDIES ON EFFICACY OF INHALATION AND SUBCUTANEOUS ADMINISTRATIONS OF KANAMYCIN IN TREATMENT AND PROPHYLAXIS OF EXPERIMENTAL PNEUMONIA OF ALBINO MICE

I. I. Prokhorova

The paper presents experimental data on high efficacy of kanamycin inhalation in the treatment and prophylaxis of Friedlander pneumonia of albino mice. A single inhalation of kanamycin aerosols was more effective at early stages of the pathological process and in prophylaxis of pneumonia. Subcutaneous administrations of kanamycin under the same conditions were less effective.

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The aerosol forms of medicines are currently in wide use in medical practice [1-3]. Depending on the particular properties of the substance inhaled, the action of an aerosol may be predominantly local, resorptive or reflexive in character.

As is well known, antibiotics introduced into an organism are not evenly distributed among the various tissues and organs. This is due to the presence of histohematic barriers which do not offer the same degree of permeability to various different substances. At various stages of the inflammation process, the permeability of tissues is found to vary, and conditions still more unfavorable to penetration by the antibiotic may arise [4]. For that reason, enteral or parenteral administration, in the case of a number of antibiotics used in treating respiratory illnesses, does not always assure retention of the antibiotic in sufficient strength at the infection focus, even though it produces high blood concentrations. In these instances, the inhalation of the antibiotic offers the best conditions for realizing a therapeutic effect, since that effect, in any event, is only possible upon direct contact between antibiotic and microbe [11, 12].

In addition to creating high concentrations of the antibiotic, the inhalation method also prolongs the retention of the drug in the lungs and bronchial secretion. This makes possible the use of smaller dosages, and hence also the reduction of the total amount of antibiotic in the blood, so that there is less chance of side-reaction.

Kanamycin aerosol has been in clinical use since 1959. Published data confirm its therapeutic effect when administered by the inhalation method for treatment of staphylococcic pneumonia in surgical patients [7], congenital cystic fibrosis of the pancreas, and tuberculosis [9, 10].

The high stability of kanamycin [5, 6], its low degree of absorption into the blood from the respiratory organs [10], its ability to accumulate in

the lungs when inhalaled [13], its action against microorganisms resistant to other antibiotics [15]—all these raise kanamycin to a special position among remedies employed in aerosol therapy.

The object of the present study was to establish experimentally the effectiveness of kanamycin against bronchial and pulmonary infections at various stages of inflammation.

Given here are the results of a study of the relative merits of the inhalation and the subcutaneous methods of administering kanamycin in the treatment of induced pneumonia in albino mice (culture of Klebsiella pneumoniae).

Materials and Methods

Albino mice weighing 16-18 g were infected intranasally, after ether anesthesia, with two daily doses of Kl. pneumoniae 444: 1 LD_{100}, 30,000-40,000 microbes per 0.02 of bouillon culture, dilution $2 \cdot 10^{-3}$, and 50 LD_{100}, 1,500,000-2,000,000 microbes in the same amount of culture, dilution 10^{-1}.

For treatment and prophylaxis the mice received a single dose of kanamycin by one of two methods (subcutaneous, inhalation). The dose in either case was 0.4 mg/subject (25 mg/kg of body weight).

A similar, untreated control group was infected with the same doses of Kl. pneumoniae. During the three-week course of the experiment, the general condition and the death rate and survival rate of the animals were studied. The innoculation, in some of the tests, was made with use of samples from the blood and organs of mice previously infected and subsequently treated by various methods.

* * *

As a result of this research it was established that following intranasal infection of mice with a culture of Friedländer's bacillus, the infection process for some period of time remains local and focal in character. Thus, six hours following infection with a 50 LD_{100} dose, not one of the control animals showed any trace of the infecting oragnism in its blood. Twenty-four hours following infection, only 20% of the control animals did so; while 48 hours following infection, most (more than 80%) had developed bacteremia, to which the mass destruction of the control group is attributed.

It is characteristic that among all the animals which died, in both groups, blood samples taken from the heart sufficed to produce a culture of the infecting organism. Evidently, death from pneumonia is almost always accompanied by bacteremia, as other investigators have also reported [14].

No distinct changes either in the behavior or in the general condition of the control animals were observed 1 hr and 3 hrs following infection. However, 6 hours following infection with a 50 LD_{100} dose, some indistinct signs of illness had appeared: slight reduction in activity, fur tattered, but no

marked dyspnea. Dissection of the mice which perished during this time showed no distinct macroscopic changes in the lungs. However, control animals infected with 50 LD_{100} when examined 24 hours after infection revealed marked dyspnea and a worsened general condition, and upon dissection showed macroscopically evident core pneumonia as well. Finally, 48 hours following infection, surviving animals showed sharply deteriorated general condition—torpidity and reduced appetite; and total or subtotal pneumonia was found in those which finally died. Abundant serous-hemorrhagic exhudate was found on sections cut from the lungs, and in some cases this appeared also in the pleural cavity.

TABLE 1

RESULTS OF KANAMYCIN TREATMENT OF ALBINO MICE INFECTED INTRANASALLY WITH Kl. Pneumoniae (1 and 50 LD_{100})

Time of administration of antibiotic following infection (hrs.)	Method	Infecting dose			
		1 LD_{100}		50 LD_{100}	
		Mortality to 9th day	Survival after 3rd week	Mortality to 9th day	Survival after 3rd week
1	Inhal.	0	100	0	95
	Subcut.	65	30	86.7	13.3
3	Inhal.	5	85	6.7	76.7
	Subcut.	76.6	6.6	73.3	20.0
6	Inhal.	-	-	30.6	44.4
	Subcut.	-	-	83.3	11.1
24	Inhal.	15	70	70.7	24.4
	Subcut.	65	15	83.3	13.9
Control		90	0	99.2	0

With doses of 1 LD_{100}, the pathologic process developed in the same sequence but somewhat more slowly.

Results of treatment of pneumonia by inhalation of the aerosol and by subcutaneous administration are shown in Table 1 above. The data are based on 204 tests, in each of which 10-15 mice were used. It is clear from these data that in the case of infection with a $1LD_{100}$ dose, a single treatment with kanamycin in aerosol form sufficed to preserve 70-100% of the animals from death 1-24 hours following infection. In parallel tests, a single subcutaneous administration preserved the lives of only 30% of the animals for so brief a period as 1 hour, and only 10-15% of them beyond that time.

In addition, kanamycin was quite effective against large doses of the infecting agent (50 LD_{100}); here the drug was administered 1-3 hours following infection. Later administration was correspondingly less effective. Treatment by subcutaneous injection in the case of 50 LD_{100} was very ineffective, regardless of the time lapse. It is noteworth that the survival rate for inhalation treatment was higher than for parallel subcutaneous treatment with kanamycin.

TABLE 2

RESULTS OF KANAMYCIN PROPHYLACTIC TREATMENT
OF ALBINO MICE INFECTED INTRANASALLY
WITH A CULTURE OF Kl. pneumoniae

Time of administration of antibiotic before infection (hrs.)	Method	Survival with 1 LD_{100} (%)	Survival with 50 LD_{100} (%)
1	Inhalation Subcutaneous	95 40	100 20
2	Inhalation Subcutaneous	95 15	75 0
24	Inhalation Subcutaneous	63.3 15	7.7 -
Control		0	0

Among the treated animals, the highest mortality occurred during the first nine days of any given test, and this was true for both dosages (actually, beginning with the third day for 50 LD and with the 4th-5th day for 1 LD). Mortality during this period was marked by a certain degree of mass death among the infected animals resulting from acute bronchopulmonary and septic infection. At later times, usually, only individuals succumbed, and in these cases there was found a predominance of the septic component over the pulmonary; asthenia, emaciation, frequent enlargement of the spleen, and sometimes abscesses in the tissues of the mediastinum, were also found. These

conditions were accompanied by reduction of the pulmonary inflammatory processes, and by restoration of breathing. The low effectiveness of inhalation therapy when applied during the later stages of the illness is evidently associated with the development of sepsis and the inflammatory process in the respiratory organs, which would reduce pulmonary ventilation and thereby also reduce the dose of antibiotic received by the animals during inhalation.

Treatment of pneumonia by a single subcutaneous administration of kanamycin is little effective precisely because of the poor penetration of the antibiotic into the lungs [13, 15, 16]. It is for this reason that a single dose does not eliminate the focus of infection in the lungs, but only retards the process of sepsis. This fact was confirmed by control cultures of mouse lung tissue grown in Petri dishes (the test animals had been treated six hours following infection with 50 LD_{100}). Before treatment of two groups of 15 each, the blood of all the animals was found to be sterile. Forty-eight hours following infection of a group treated with the inhalation method, only two mice were able to supply 0.01 ml samples which were sufficient to start two colonies of Kl. pneumoniae each; by contrast, from the group of mice treated subcutaneously, bacteremia had developed in four animals, and from these samples of the same size were sufficient to start 7 colonies each. Of the 12 control animals, during the same period, the blood had remained sterile in only 2, and that of all the others showed a massive development of the microbe.

Six days following the infection, further cultures were started from the blood of survivors. All 11 animals which had been treated with the inhalation method showed sterile blood. Of the 7 which had received subcutaneous treatment with kanamycin, three had sterile blood, but in the remaining four cases, 0.01 ml of blood sufficed to produce massive growths of Kl. pneumoniae from a start of 77 initial colonies.

The results of prophylactic treatment given at various times previous to infection are shown in Table 2 above; these data support the conclusion that the use of kanamycin inhalation for prophylaxis is fairly effective 1 and 3 hours before infection with either dose, and 24 hours before for 1 LD_{100}, whereas subcutaneous injection is effective only during the first hour before infection. Obviously, the amount of kanamycin within the respiratory organs following a single inhalation is insufficient to suppress the growth of the infecting agent; this is the cause of the lower effectiveness of aerosol prophylaxis during this period.

Conclusions

1. Inhalation of kanamycin aerosol has an active local effect on the infection process associated with induced pneumonia in white mice (infecting agent Kl. pneumoniae); it prevents the development of sepsis. The use of this aerosol is particularly effective in early treatment.

2. Use of kanamycin aerosol is less effective in the later stages of pneumonia, this being associated with advance of the septic process and with

reduction in the inhalation dose, which results in lowered pulmonary ventilation.

3. Prophylaxis with kanamycin aerosol is highly effective when applied 1-3 hours before infection; less so when applied 24 hours before infection.

4. A single subcutaneous administration of kanamycin (either for treatment or for prophylaxis of pneumonia) somewhat retards development of the process of sepsis, as shown by the infected and the control groups, but has little effect on survival rate (in other words, it does not liquidate the pulmonary focus of infection).

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