硫酸新黴素在鰻魚體內之吸收分佈與排泄

劉 朝 鑫*

A Study on the Absorption Distribution and Elimination of Neomycin Sulfate in Eels

Chaw King Liu*

Abstract

The purpose of this study was to investigate the Neomycin pattern in tissues of eels following the administration by both dipping and oral routes.

The result of tissue residue study showed that Neomycin withdrawl time in eels should spend at least five days.

Introduction

Our previous work indicated that Neomycin sulfate was highly effective in the control and treatment of Red fin disease and Edwardsiellosis of eels[1]. In the present study, a series of experiments were attempted to investigate the Neomycin patterns in tissues of eels following the administration of Neomycin by both dipping and oral route.

Materials and Methods

A. Methods of administration of Neomycin sulfate.

a). Eels:

Japanese eels (*Anguilla japonica*) about 200 g body weight were used in this study.

b). Neomycin sulfate:

Concentrated Neomix* 325 (TUCO Limited, division of Upjohn Company) was used in this study. The Neomycin concentration in this paper is expressed by potency.

c). Administration of Neomycin sulfate by dipping:

Eels were dipped into Neomycin sulfate solution at a level of 200 ppm for six hours, and then reimmersed in clean water. 48 hours later, the eels were dipped

Department of Veterinary Medicine, National Taiwan University, Taipei
國立台灣大學獸醫學系

repeatedly. Tissue samples as shown in Table 1 were obtained 1, 4, 8, 12, 16 and 24 hours following the final bath. In all experiments, ten fish were used for each sample and the water temperature was maintained at 22±1°C.

d). Single oral administration of Neomycin sulfate:

Eels received Neomycin sulfate at a single dose of 40 mg/kg b. w. by using stomach tubes. Tissue samples were taken following 1, 4, 8, 12, 16 and 24 hours after medication.

e). Repeated oral administration of Neomycin sulfate:

Neomycin sulfate was given by using stomach tubes at a dose of 40 mg/kg b.w. /day for seven days. Tissue samples were obtained daily following the final medication for a total of seven days.

B. Tests and methods of assay.

Microbiological agar diffusion assay was used to assay the concentration of Neomycin in tissues of eels.

a). Cylinders:

stainless steel cylinders with an outside diameter of 8 mm (±0.1mm), an inside diameter of 6 mm (± 0.1 mm), and a length of 10 mm (± 0.1 mm) were used.

b). Plates:

Glass petri dishes having dimensions of 20 by 100 mm were used.

c). Test organisms:

In this experiment *Staphylococcus epidermidis* ATCC 12228 was used.

d). Medium:

Culture medium was formulated as follows:

Peptone	10 g
Beef extract	5 g
Sodium chloride	2.5 g
Distilled water q.s.	1,000 ml

pH 7.0 ± 0.1 after sterilization.

Base and seed layer agars were made as follows:

Peptone	6 g
Beef extract	1.5 g
Yeast extract	3 g
Dextrose	1 g
Agar	15 g
Distilled water q. s.	1 liter

pH 8.0 to 8.1 after sterilization.

e). Preparation of test organisms:

The test organisms were transferred to the culture medium, incubated for 18 hours at 37 C. The volume of the suspension was adjusted for the diameter of inhibitory zone of concentration of 1.2 mcg/ml working standard reached 20mm

(Fig. 1). The suspension was stored under refrigeration.

f). Preparation of plates:

10 ml of base layer agar was distributed evenly in the plate and was allowed to harden on a level surface. The appropriate inoculum of the adjusted suspension was added to a sufficient quantity of the seed layer agar which had been cool to 48°C. The inoculated agar was swirled to obtain a homogeneous suspension, and 4 ml was added to each of the plates containing the 10 ml of base layer agar. After the agar had hardened, six cylinders were placed on the seed layer agar surface so that they were approximately 60 degrees on a 2.8 cm radius.

g). Preparation of the stock solution of the working standard:

Sample of working standard was accurately weighed and dissolved in sufficient 0.1 M Potassium phosphate buffer, pH 8.0, to give a stock solution of 2,000 mcg/ml. The stock solution was further diluted with the above mentioned buffer to give a stock solution of convenient concentration and a reference point of 1.2 mcg/ml. All stock solutions and reference point solution were stored at -20 C and could be used for three months effectively.

h). Preparation of the final concentrations of the working standard:

Eel tissues were smashed with glass mortars, and mixed with stock solution of working standard and 0.1M Potassium phosphate, pH 8 to give the final concentrations of working standard as follows:

Quantity of tissues (g)	10	10	10	10	10	10	10
Quantity of 0.1 M Pot. phosphate, pH 8 (ml)	39	39	39	39	39	39	40
Concentration of stock solution of working standard (mcg/ml)	240	120	60	30	15	7.5	—
Quantity of stock solution of working standard (ml)	1	1	1	1	1	1	—
The final concentration of working standard (mcg/ml)	4.8	2.4	1.2	0.6	0.3	0.15	—

The mixture of 0.1 M potassium phosphate, pH 8, and stock solution of working standard were stored under refrigeration for two hours and centrifuged at 3,000 rpm for 30 minutes. The supernatants containing 4.8, 2.4, 1.2, 0.6, 0.3, 0.15 micrograms of Neomycin per mililiter were prepared respectively. The 1.2 mcg/ml concentration was the reference point solution of the assay.

i). Preparation of standard curve:

A total of fifteen plates, three plates for each solution, were used for the preparation of standard curve. On each of the three plates, three cylinders with the reference point solution and the other three cylinders with the concentrations under test were filled. (Fig. 2). Thus, there were 45 reference point determinations and

nine determinations for each of the other points on the curve. After the plates were incubated, the diameters of the zones of inhibition were read. The readings of the reference point concentration and the readings of the point tested for each set of three plates were averaged, and all 45 readings of the reference point concentration were also averaged. The average of the 45 readings of the reference point concentration is the correction point of the curve. The average value obtained for each point was corrected to the figure it would be. Using the concentration in microgram per mililiter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. These corrected values were plotted on 2-cycle semilog paper. The standard curve was drawn by means of the following equations:

$$H=\frac{3a+2b+c-e}{5} \qquad L=\frac{3e+2d+c-e}{5}$$

Where:

L=calculated zone diameter for the lowest concentration of the standard curve;

H=calculated zone diameter for the highest concentration of the standard curve;

c=average zone diameter of 45 readings of the reference point standard solution;

a, b, d, e=corrected average values for the other standard solutions, lowest to highest concentrations, respectively.

g). Assay procedure:

40 ml of 0.1 M Potasium phosphate buffer, pH 8.0, were added to 10g smashed tested eel tissues. The mixtures were stored under refrigeration for two hours to extract the antibiotic, and centrifuged at 3,000 rpm for 30 minutes. Three plates were used for each sample. Three cylinders on each plate were filled with the reference point standard and three cylinders were filled with the supernatant of the mixture. They were incubared at 35°C for 18 hours and then measured the diameter of each zone of inhibition. The zone readings of the standard and of the sample on the three plates were averaged. The average values of the samples were corrected by comparing the average values of reference point and correction point. The potencies corresponding to these corrected values of zone sizes were read from the curve.

Results and Discussion

Neomycin was absorbed readily in eel tissues following administration by dipping. These data are presented in Table 1. Neomycin concentration in serum reached to maximum in one hour following finish of dipping. Then the drug concentration in serum dropped gradually and was still detectable at 24 hours. Neomycin diffused throughout all the tested tissues and was found in highest concentration in the gill and kidney. However, the lowest concentration was demonstrated in liver and muscle.

Concentration of Neomycin in gill reached a peak in 4 hours and maintained in

a significant high level for more than 24 hours. From this pattern, it is suggested that Neomycin was absorbed from the gill following administration by dipping.

Concentration of Neomycin in kidney rose gradually and reached a peak concentration at 24 hours following finish of dipping. This pattern may reveal that Neomycin was mainly eliminated from the kidney of eels.

Table 1. Tissue concentration of Neomycin in eels following administration by dipping

Tissues	Time after finish of dipping (hours)					
	1	4	8	12	16	24
Serum	12,00	11.00	9.25	6.25	5.25	4.25
Gill	11.25	11.50	10.25	9.25	9.00	7.50
Skin	4.50	4.25	3.50	3.00	1.65	0
Liver	3.25	2.75	2.00	2.00	0	0
Kidney	3.75	4.00	6.75	12.50	13.50	14.25
Muscle	3.25	3.00	2.50	1.50	0	0

Unit: mcg/ml or g Limit of assay: 1.5 mcg/ml or g

A single oral dose of 40 mg/kg Neomycin was given to eels by using stomach tubes, and were reimmersed in a clean water at 22±1°C. Neomycin tissue levels were detected throughout 24 hours following administration of the drug. It is interesting to note, as shown in Table 3, that Neomycin was absorbed readily from the gut of eels. It is surprisingly contrast to domestic animals in which Neomycin is poorly absorbed from the gut[2,3].

It is clear from this result that following oral administration Neomycin tissue levels were much higher than those obtained from dipping administration. But the tissue pattern was similar in both oral and dipping administrations.

A peak drug concentration in serum was detected following 12 hours of oral administration, then the concentration dropped gradually. The serum level in 8 hours was similar to that in 24 hours. This data indicates that effective Neomycin serum level persists more than 24 hours following single oral administration of the therapeutic dose.

Neomycin distributed well in each tissue following absorption from the gut. The highest drug concentration was found in the gill, while the lowest in the liver. The drug kidney pattern may also indicate that Neomycin was eliminated from the kidney.

Table 2 Tissue concentrations of Neomycin in eels following single oral administration.

Tissue	Time after single oral administration (hours)					
	1	4	8	12	16	24
Serum	21.00	21.50	31.00	47.50	42.50	32.50
Gill	115.00	117.50	102.50	80.50	62.50	41.00
Skin	16.50	17.50	15.50	7.50	7.00	6.25
Liver	5.50	3.50	2.75	1.75	1.50	0
Kidney	22.00	17.50	21.00	27.50	31.00	37.50
Muscle	7.50	10.00	11.50	8.50	6.50	5.00

Unit: mcg/ml or g. Limit of assay: 1.5 mcg/ml or g.

Neomycin was administered orally to eels at the rate of 40 m/kg/day for a 7 day medication period to study the Neomycin tissue residue pattern in eels under the water temperature of 22 ± 1°C. Table 3 showed that Neomycin in serum, gill, skin, liver and muscle of eels was not detectable following 3 days post-administration by the oral route, while the kidney took 4 days to eliminate the drug. These data show Neomycin withdrawn time in eels should spend at least 5 days.

Table 3 Neomycin tissue residue patterns in eels following repeated oral administration.

Tissue	Days after withdrawal					
	1	2	3	4	5	6
Serum	26.00	8.25	0	0	0	0
Gill	30.25	15.00	0	0	0	0
Skin	5.25	0	0	0	0	0
Liver	0	0	0	0	0	0
Kidney	27.25	13.50	1.65	0	0	0
Muscle	3.50	0	0	0	0	0

Unit: μg/ml or g, limit of assay: 1.5 μg/ml or g.

中 文 摘 要

本研究以藥浴及經口投藥方法將硫酸新黴素投與鰻魚後，測定各組織中的濃度，其明瞭其吸收途徑，在各組織內的消長及排泄途徑。

從組織殘留試驗明瞭硫酸新黴素經口投藥以後的休藥期應爲 5 天。

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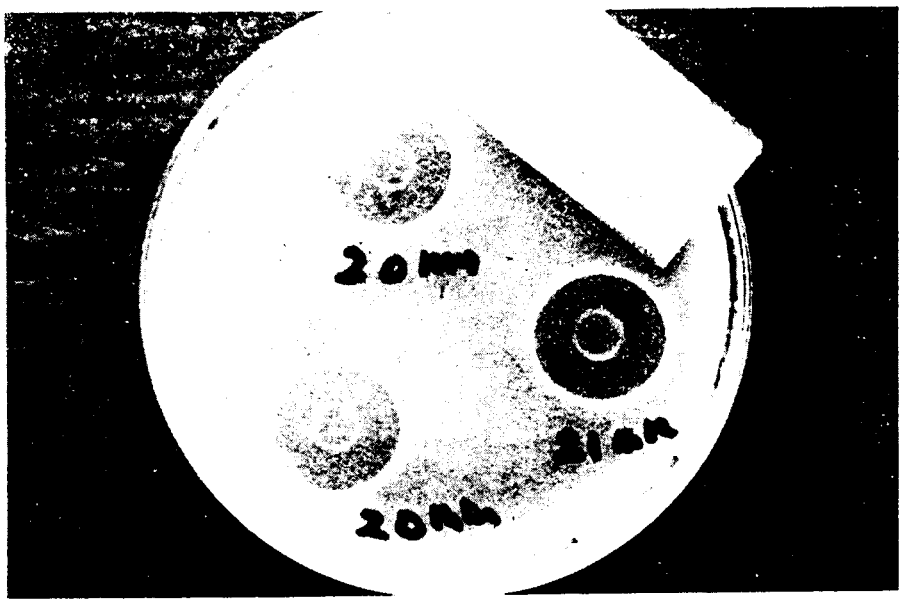

Fig. 1. Inhibitory zone of reference point of working standard of Neomycin sulfate.

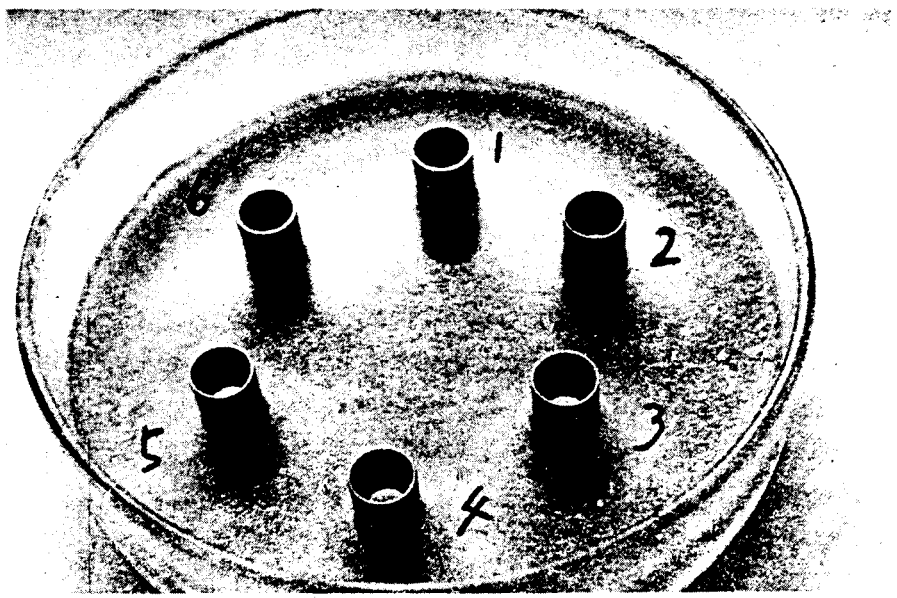

Fig. 2. Six cylinders on the seed layer agar surface. Cylinders 1, 3, 5 fill with reference point solution and the other three cylinders fill with the concentration under test.