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EFFICACY AND PHARMACOKINETICS OF KANAMYCIN IN *PSEUDOMONAS ELUORESCENS* INFECTED *CLARIAS LAZERA* FISH

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SUMMARY

Efficacy and pharmacokinetics of kanamycin were studied in *P. fluorescens* infected *Clarias lazera* (Nile catfish). The clinical sings of *P. fluorescens* infection started after 12-24 hours post intraperitoneal inoculation. Kanamycin was injected in a single dose (20 mg/kg b.wt.) in the infected group and another apparently healthy control group. Recovery percent reached 73.33 in the infected fish gorup after 48 hours post antibiotic injection.

Kanamycin was rapidly absorbed after intraperitoneal injection and reached its highest concentration 9.87±0.00059 and 9.20±0.85 µg/ml four hours post injection in normal and infected fish groups, respectively. Kanamycin was repidly absorbed in infected fish ($\alpha = 0.2726 \pm 0.002h^{-1}$ and $t_{0.5}$ $\alpha = 2.69 \pm 0.16$ min.) than in normal fish $\alpha = 0.2042 \pm 0.009h^{-1}$ and $t_{0.5}$ $\alpha = 3.44 \pm 0.14$ min.). Kanamycin was rapidly excreted in infected fish with $t_{0.5}$ $\beta = 5.63 \pm 0.3764$ hrs than in normal fish with $t_{0.5}$ $\beta = 7.55 \pm 1988$ hrs. The interval between doses was short in infected fish 44.28 hrs than in normal fish 55.85hrs.

INTRODUCTION

Pseudomonas fluorescens in one of the common bacterial fish pathogens infecting fish (Roberts, 1978; Okaeme, 1989 and Inglis et al., 1993). *P. fluorescens* induces variety of diseased conditions in different fish species among which haemorrhagic septicaemia and fin rot are the most prevalent ones (Conroy, 1963; Roberts, 2978; Abd El-Aziz, 1988; Badran and Eissa, 1991 and Inglis et al., 1993).

Kanamycin is used successfully for treatment of Pseudomonas infections in fish (Conroy, 1963, 1963 and Meyer and Collar, 1964) at a dose of 20 mg/kg b.wt. (Van Duijn, 1973).

From this point of view, the present study was planned to follow up the pharmacokinetics of kanamycin in healthy and experimentally *P. fluorescens* infected *Clarias lazera* fish as well as its efficacy for treatment of such bacterial infection.

MATERIAL AND METHODS

Drug:

1. Kanamycin (Misr C. for Pharm. Ind. S.A.A.), obtained as vials each contains 1 g kanamycin sulphate powder.
2. Tricaine methane sulfonate (MS-222) (Sandoz), obtained as powder and is used as fish anaesthetic at a dose of 50 mg/L (Stoskopf, 1993).

Fish:

A total number of 30 apparently healthy *Clarias lazera* fish with an average body weight of 250-300g were caught in River Nile at Giza Governorate. Fish were kept in glass aquaria supplied with chlorine-free tap water at 25°C and air conductors. Fishes were acclimated for 10 days before use.

Bacteria:

A completely identified moderately virulent *P. fluorescens* isolate obtained from Animal Health Research Institute, Giza, Egypt was used for experimental purpose in the present study. The used isolate was sensitive to kanamycin.

Pharmacokinetics and efficacy of kanamycin:

1- Fish inoculation and sampling:

A total number of 30 apparently healthy *Clarias lazera* was divided into 2 groups of 15 each and kept in glass aquaria. Fish in the first group were intraperitoneally injected (I/P) with a single dose of kanamycin at a tate of 20 mg/kg b.wt. (Van Duijn, 1973) after being anaesthetized with MS-222. Blood samples were collected from each fish at 15, 30 min., 1, 2, 4, 6, 8, 10, 12 and 24 hours post injection then sera were separated. The second group was I/P injected with 0.5 ml/fish of 24 hour broth culture of *P. fluorescens* bacteria (LD_{50} : 5×10^6 bacterial cell/ml). Fish were observed for development of clinical sings indicating proegressive *P. fluorescens* infection. After developemnt of clinical sings, fish were treated with a single I/P dose of kanamycin at a rate of 20 mg/kg b.wt. Blood smaples were taken at the same previously mentioned time intervals where sera were collected for determiantion of antibiotic concentration. Treated fish were observed for the relief of clinical signs of infection and recovery. The percentages o morbidity, mortality and survival wer recorded. Dead fish were used for bacteria re-isolation. Samples from recovered fish wer bacteriologically examined

2- Microbiological assay:

Serum samples were assayed by the microbiological method using *Bacillus subtili* as standard organism (obtained from Aniam Health Research Institute, Dokki, Egypt) and antibiotic medium No. 1 (El-Nasr Pharmaceutical Chemical Co.) according to Arret et al. (1971).

The pharmacokinetic deta were calculated according to the method described by Baggot (1978).

RESULTS

Efficacy of kanamycin:

Clinical signs of experimental *P. fluorescens* infection in *Clarias lazera* started 8 hours post injection. Infected fish showed dark skin discolouration, sluggish movement, weak response to external stimuli, rapid and shallow respiration. Twenty four hours post bacterial inoculation, most fish showed congestion of abdominal region (Fig. 1) and by 36 hours, morbidity reached 100% (Table 1). After 48 hours, some fish developed abdominal distention with varying degrees (Fig. 2). After development of previously mentioned clinical signs (48 hr.), figh were treated with a single I/P dose of kanamycin. Clinical signs started to relieve after 12 hr. of antibiotic injection and complete recovery of clinical signs was recorded after 48 hr of antibiotic inoculation. Mortalities and recovery percent reached 26.66 and 73.33, respectively (Table 1). Postmortem lesions of dead fish

Table 1: Results of experimental inoculation of P. fluorescens and efficacy of kanamycin in Clarias lazera. (n=15)

P.B.I.(hr)	12	24	36	** 48	60	72	84	96	108	120	Cummulative	
											No	Percent
Mortality	-	1	-	2	1	-	-	-	-	-	4	26.66
Survival	15	14	14	12	11	11	11	11	11	11	11	73.33

**: Time point at which kanamycin is injected.
P.B.I.: Post Bacterial Inoculation.

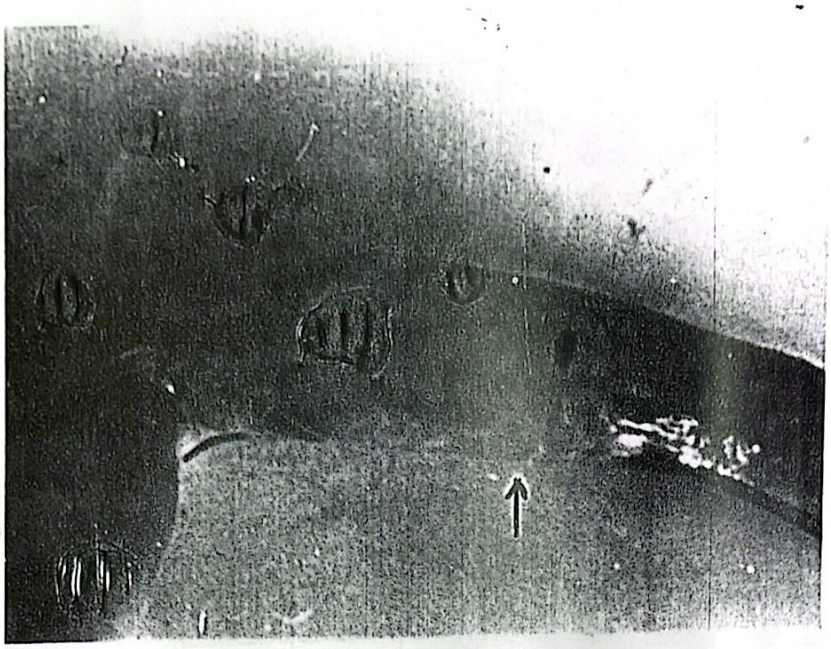

Fig. 1: Infected fish showing congestion of ventral abdominal region.

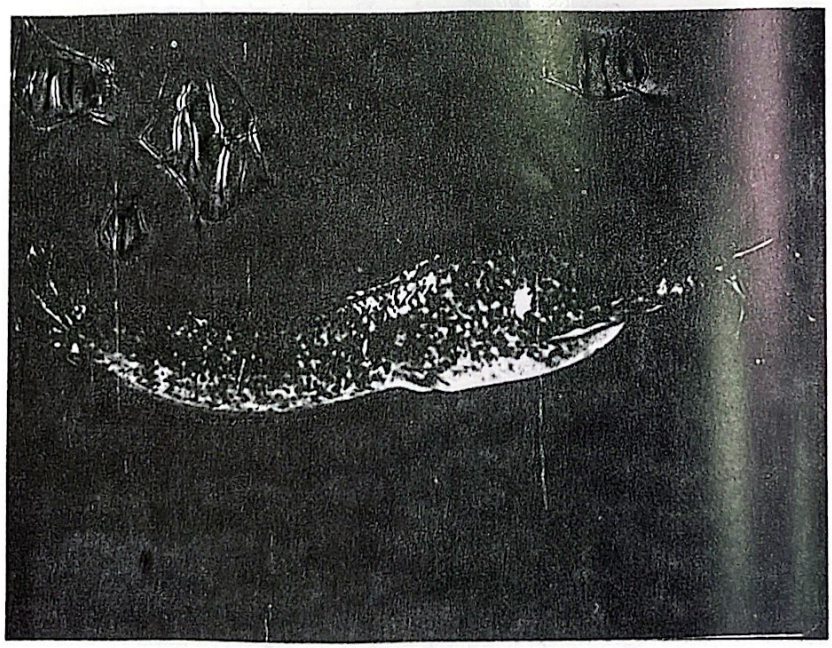

Fig. 2: Infected fish showing abdominal distention.

revealed the presence of reddish-yellow ascitic fluid inside the abdominal cavity and congestion of internal organs with variable degres. Re-isolation of *P. fluorescens* from dead fish was carried out while samples taken from recovered fish were bacteriologically free.

Pharmacokinetics in normal and infected fish:

Following intraperitoneal injection of kanamycin (20 mg/kg b.wt.), kanamycin was absorbed rapidly and reached its high concentration (9.87±0.0059 and 9.20±0.85 µg/ml) at 4 hours post-injection in normal and infected fish respectively (Table 2 and Figs. 3&4). Kanamycin persisted in concentration higher than the minimal bactericidal concentration 3.1 µg/ml (Conroy, 1963) over 12 hours post injection in diseased and normal fish.

Kanamycin was rapidly absorbed in infected fish with high absorption rate constant α (0.2726 h^{-1} ± 0.0222 min.) and short absorption half life $t_{0.05}$ α (2.690±0.1608h^{-1}) in infected fish than in normal fish (α = 2.2042 ± 0.00gh^{-1} and $t_{0.05}$ α 3.449±0.1410 min.).

Kanamycin was rapidly excreted in infected fish than in normal with $t_{0.5}$ (β) (5.63±0.3746 h. and 7.55±0.1988h.), respectively. The infected fish showed slight higher Cmax (8.137±0.1322) Than normal fish (7.65±0.1356 µg/ml).

Kanamycin in infected fish showed short interval between doses 44.289±0.76 hr. than normal fish 55.85±1.89 hr. (Table 3). The protein binding percent with fish serum proteins was 11.05.

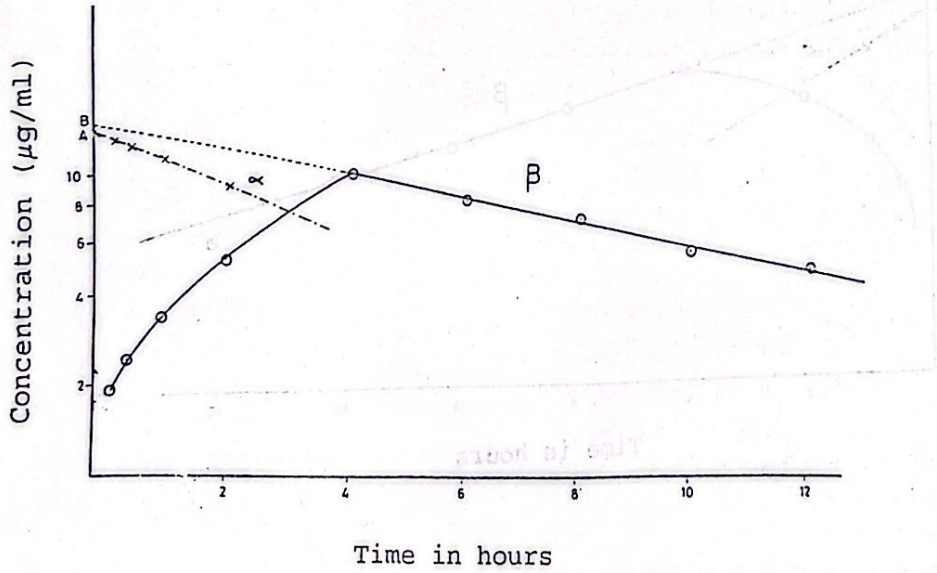

Fig. 3: Semilogarithmic graph depicting the time course of kanamycin in normal *Clarias lazera* (n=15).

Table 2: Kanamycin concentrations (ug/ml) in sera of Clarias lazera following I/P injection of 20 mg/kg b.wt. (n = 15)

Time	Normal	Infected
15 min.	1.87 + 0.033	2.92 + 0.110
30 min.	2.41 + 0.027	4.03 + 0.130
1 h.	3.59 + 0.030	5.48 + 0.100
2 h.	4.92 + 0.410	7.74 + 0.080
4 h.	9.87 + 0.050	9.21 + 0.850
6 h.	8.11 + 0.120	7.46 + 0.040
8 h.	7.02 + 0.040	5.72 + 0.030
10 h.	5.55 + 0.060	4.56 + 0.020
12 h.	4.94 + 0.030	2.87 + 0.110

Fig. 4: Semilogarithmic graph depicting the time concentration course of kanamycin in *Pseudomonas fluorescens* infected *Clarias lazera*.

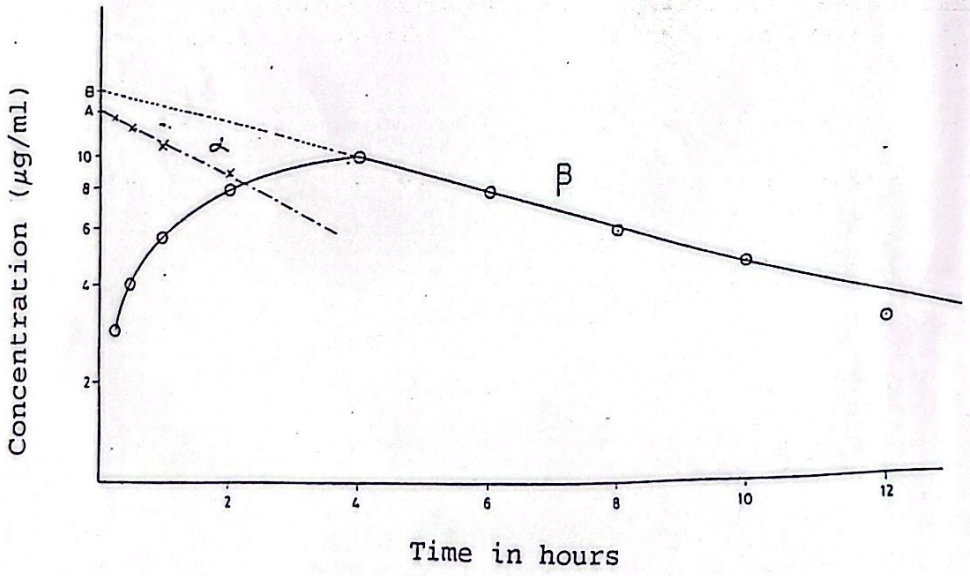

Table 3: Pharmacokinetic parameters of kanamycin in sera of *Clarias lazera* following I/P injection of 20 mg/kg b.wt. (n = 15).

Parameter	Unit	Normal	Infected
A	µg/ml	13.75±0.37	14.02±0.49
α	h^{-1}	0.2042±0.0090	0.2726±0.0222
$t_{0.5}\ \alpha$	min.	3.45±0.1410	2.69±0.1608
B	µg/ml	14.92±0.39	16.26±0.1266
β	h^{-1}	0.0924±0.0022	0.1266±0.005
$t_{0.5}\ \beta$	h,	7.55±0.1988	5.63±0.3746
t_{max} Calc.	h.	7.11±0.1724	5.38±0.1239
t_{max} Obs.	h.	4.00±0.0	4.00±0.0
C_{max} Calc.	µg/ml	7.65±0.1356	8.14±0.1322
C_{max} Obs.	µg/ml	9.87±0.06	9.21±0.85
Interval between doses	h.	55.58±1.89	44.29±0.76

DISCUSSION

Kanamycin sulphate showed high efficacy in treatment of *Pseudomonas fluorescens* infection in *Clarias lazera* following a single I/P injection of 20 mg/kg b.wt. This is due to the high antibacterial activity of kanamycin and its low minimal bactericidal concentration (3.1 μg/ml). The same results were also recorded by Conroy (1973) and Meyer and Collar (1964) in different fish species.

Results of intraperitoneal injection of kanamycin in healthy and *P. fluorescens* infected fish showed higher blood concentration in diseased fish than normal. The higher absorption rate in infected fish ($0.2726\pm0.222h^{-1}$) than in normal (0.2041 ± 0.009) is dur to high penetration power of the drug from the inflamed membranes of congested organs (Inglis et al., 1993). Taasugi et al. (1968), Pennington (1975) and Baggot (1980) stated that inflammation leads to increase the capacity of drugs to penetrate membrances. These results are also supported by the short $t_{0.5}\alpha$ (2.690±0.160 min.) in infected fish than $t_{0.5}\alpha$ (3.45±0.1410 min.) in normal fish.

The obtained results also showed higher elimination rate constant 0.1266±0.005 in infected fish than in normal ($0.0924\pm0.0022\ h^{-1}$) which is also associated with short elimination half life time $t_{0.5}\beta$ (5.63±0.3746 h) in diseased fish than in normal $t_{0.5}\beta$ (7.55±0.0.1988 h) and this may be due to high elimination of drug from th inflamed kidneys as a result of septicaemia caused by *P. fluorescens* (Inglis et al., 1993). The high elimination rate and short elimination half life time were associaed with shorter interval between doses in infected fish (44.29 hrs.) than in normal (55.85±1.89 hrs.).

From the obtained results, it is concluded that th pharmacokinetics of drug should be studied i diseased fish to obtain the optimal dosage regim for successful treatment.

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