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Evaluation of Efficacy and Toxicology of Once Daily Versus Twice Daily Regimens of Amikacin in Mice

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Abstract

Five organisms were used in this study to induce septicaemia. Each organism was used to infect 2 groups of mice. One group received amikacin as a single daily dose, the other group received the same adily amount of amikacin split in two doses every 12 hours. Every day, 2 representatives of each group were sacrificed. To test for efficiency, the spleen was dissected aseptically and placed in exactly 2 ml sterile saline for bacterial count. To test for toxicity both kidneys were examined by light microscopy and serum creatinine was determined in heart blood. The 2 regimens were found to be equally effective in eradicating infection. Also both regimens showed no pathologic changes in the kidney by light microscopy, even when mice received high doses of the drug for 10 days. Serum creatinine level showed no change when the drug was given in therapeutic doses by both regimens. But when high doses were used, there was a statistically significant increase in creatinine level with the split dose regimen. Thus amikacin given as a single daily dose is as effective as the split dose, but is safer specially for patients with border-line kidney function treated for longer periods.

Introduction

AMINOGLYCOSIDES are still frequently used in the treatment of severe infections, because of the effectiveness of these drugs in rapidly and almost completely eliminating a large number of Gram-negative and Gram-postivite pathogens [1].

Amikacin, like other aminoglycosides, is bactericidal, acting directly on the 30S and 50S bacterial ribosomal subunits to inhibit protein synthesis by binding irreversibly to the bacterial ribosome. Thus, it blocks the recognition step in protein synthesis, causing the misreading of the genetic code and production of defective proteins [2].

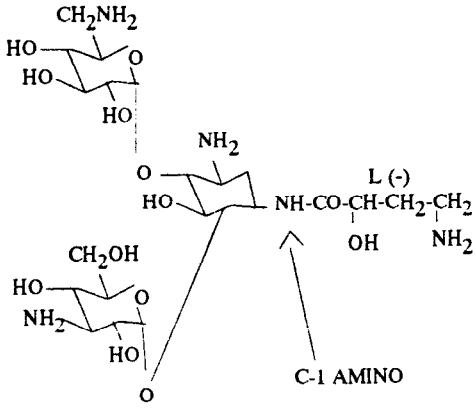

Fig. (1): Structure of amikacin.

Studies have shown that amikacin is active both in vitro and in vivo against a wide variety of Gram-negative organisms [3,4].

Inspite of the fact that on weight basis, amikacin is less active than other aminoglycosides e.g. gentamycin [3], yet it has considerably higher serum concentrations [5].

Amikacin is not metabolized and is excreted unchanged in urine by glomerular filtration with a renal excretion rate which is identical to that of creatinine clearance [6].

The most important adverse effects limiting the clinical use of aminoglycosides are nephrotoxicity and ototoxicity [7, 8].

Nephrotoxicity of amikacin affects the proximal tubular cells of the kidney sparing the glomeruli [9, 10]. The drug causes inhibition of phospholipases and sphingomyelinase resulting in phospholipiduria which is an early sign of kidney affection [11]. Since nephrotoxicity is the most important side effect of amikacin [12], its incidence can be reduced to minimum when appropriate attention is addressed to the dose of the drug and its duration of therapy. Recently, it has been postulated that aminoglycosides given once daily are as effective and less toxic than if given twice or thrice daily [13]. The aim of the present work is to investigate which is a better regimen of amikacin administration; the once daily or the twice daily regimen as regards efficacy and nephrotoxicity which might occur in both regimens.

Material and Methods

To study the efficacy and toxicology of amikacin given once daily versus twice daily, septicaemia was induced in 2 groups of mice: group A received treatment with amikacin as a single daily dose and group B as a twice daily regimen Everyday 2 representatives of each group

were sacrificed. The spleen was aseptically dissected and sent for microbiological investigation. The kidneys were also dissected and sent for pathological examination. In addition, heart blood was collected for determination of serum creatinine level.

1. Induction of septicaemia [14]: Five different bacterial strains were used : E. coli (ATCC 25922), one strain of E. coli isolated from a patient with urinary tract infection, Pseudomonas aeruginosa (ATCC 27653), one strain of Ps aeruginosa also isolated from a patient with urinary tract infection, in addition to one strain of Klebsiella oxytocia isolated from a patient with septicaemia. From each strain an 18 h broth culture was made and its concentration was adjusted to that which produced death in 50% of a group of mice after 24 h when injected intraperitoneally.

2. Mice: White albino mice, each weighing 20-22 gm. wheter males or females were used.

3. Amikacin: commercially available ampoules were used. The dose given was adjusted to 15 mg/kbw every 24 h for group A, & 7.5 mg/kbw every 12 h for group B. the same ampoules were used for determination of MIC for the five strains used. Treatment with amikacin started one hour after injecting the septicaemic dose and continued for 7 days in case of infection with E. coli and El. Oxytocia and for 10 days for Ps. aeruginosa.

4. Bacteriological investigation: For each organism, 2 groups of mice (A and B) and 2 control mice were injected with the septicaemic dose on day 0. Each group consisted of 14 animals (in case of 7 days treatment) or 20 animals (when treatment was extended for 10 days). After 1 hour, treatment was started for the 2 groups and the 2 control animals were sacrificed. Everyday, 2 mice from each group (A and B) were sacrificed just before injecting amikacin in the other mice. Each sacrificed mouse was anaesthetized by thiopentone and dissected aseptically. The spleen was removed and placed in exactly 2 ml sterile saline and sent immediately to the microbiology laboratory where it was tested within 1 hour. The spleen was vigorously shaken and serial dilutions were then made in sterile saline. Fifty microns of three different dilutions were spread on the surface of agar plate (2 plates for each dilution) and incubated aerobically at 37 C for 24 hours. The colonies on each plate were counted and the number of organisms per spleen was calculated. For each count the average of the 2 mice was taken. The MIC of amikacin for each organism was determined by the tube dilution method.

5. Seum creatinine was measured according to Giamarellou et al [13].

6. Histopathological examination: both dissected kidneys were fixed in 10%

buffered formaline-saline solution for 24 hours, routinely processed and paraffin embedded. Five microns - sections were stained with hematoxylin and eosine and Masson trichrome for light microscopy. The examination included search for tubular degenerative changes, necrosis, inflammatory cellular infiltration and congestion. Each sacrificed animal was compared with its corresponding control.

7. toxicological studies: 36 uninfected mice divided into 6 groups were subjected to increasing doses of amikacin as follows: 75 mg/kbw, 150 mg/kbw and 300 mg/kbw every 24 hours for groups 1, 2 and 3 respectively and half of each dose every 12 h for groups 4, 5 and 6 respectively. The injection schedule continued for 10 days where 2 mice were sacrificed on the 4 th, 7 th and 10 th day. The kidneys were sent for pathological examination and the heart blood was analysed for serum creatinine level. The control group consisted of 3 mice; one was sacrificed on each of the 4th, 7th and 10th day.

Results

All the 5 organisms tested were sensitive to amikacin. The MIC of the drug ranged between 0.5 and 4 mg/ml as shown in Table (1). Table (2) reveals the effect of single and split dose regimens on the eradication of the organisms. The control group that received the lethal dose of organism without any subsequent treatment showed a bacterial count of about $12x10^6$ [6] organism per spleen for E, coli and Ps. aeruginosa and $9x10^6$ for Kl. oxytoca. As regards the effect of the 2 different regimens on eliminating the organisms, no significant difference was noted. Both regimens achieved rapid and almost complete eradication of the organisms (> 99.9%) after 1 day treament, except for Kl oxtyoca, where complete eradiation was reached after 2 days. The complete eradication was continued throughout all the subsequent days of treatment (7 or 10 days) without regrowth of any resistant subpopulations.

Serum creatinine level showed no change when the drug was given in therapeutic doses by both regimens. However, serum creatinine was significantly increased after 4 days. administration when the doses of 15 mg/kbw and 300 mg/kbw were given as split doses every 12 hours (Table 3).

Table (1): MIC of Amikacin for the Organisms Tested. All Strains Were Sensitive to Amikacin.

Organism	MIC (μg / ml)
E. coli (ATCC 25922)	0.5
E. coli (isolated strain)	0.5
Ps. aerugionsa (ATCC 27853)	2.0
Ps. aeruginosa (isolated strain)	4.0
Kl. oxytoca	2.0

Table (2): The Efficacy of Single and Split Dose Regimens in Treatment.

Day	Count / spleen (% of eradication)														
	E.coli (ATCC 25922)			E.coli (isolated Strain)			Ps. aeruginosa (ATCC 27853)			Ps. aeruginosa (isolated strain)			Kl. oxytoca		
	Control	Single dose	Split dose	Control	Single dose	Split dose	Control	Single dose	Split dose	Control	Single dose	Split dose	Control	Single dose	Split dose
0	$12x10^6$ (0)			$11x10^6$ (0)			$12x10^6$ (0)			$12x10^6$ (0)			$9x10^6$ (0)		
1		$6x10^3$ (> 99.9)	0 (100)		$2x10^2$ (> 99.9)	$4x10^2$ (> 99.9)		$6x10^3$ (> 99.9)	$15x10^2$ (> 99.9)		$3x10^2$ (> 99.9)	$8x10^2$ (> 99.9)		$3x10^4$ (>99.6)	$2x10^4$ (> 99.7)
2		0 (100)	0 (100)		$2x10^2$ (> 99.9)	0 (100)		$1x10^2$ (> 99.9)	0 (100)		20 (> 99.9)	20 (> 99.9)		$9x10^2$ (> 99.9)	$14x10^2$ (> 99.9)
3		0 (100)	$5x10^2$ (> 99.9)		$1x10^2$ (> 99.9)	20 (> 99.9)		$1x10^2$ (> 99.9)	0 (100)		0 (100)	0 (100)		0 (100)	0 (100)
4		0 (100)	0 (100)		$1x10^2$ (> 99.9)	40 (> 99.9)		20 (> 99.9)	0 (100)		0 (100)	0 (100)		80 (> 99.9)	0 (100)
5		0 (100)	0 (100)		0 (100)	0 (100)		20 (> 99.9)	0 (100)		0 (100)	0 (100)		$1x10^2$ (> 99.9)	0 (100)
6		0 (100)	0 (100)		0 (100)	0 (100)		0 (100)	0 (100)		0 (100)	0 (100)		0 (100)	0 (100)
7		0 (100)	0 (100)		0 (100)	0 (100)		0 (100)	0 (100)		0 (100)	0 (100)		0 (100)	0 (100)
8								0 (100)	0 (100)		0 (100)	0 (100)			
9								0 (100)	0 (100)		0 (100)	0 (100)			
10								0 (100)	0 (100)		0 (100)	0 (100)			

Over 99.9% eradication of the organism was achieved by both regimens at the same time.

Table (3): Comparison between Mean Creatinine (mg/dl) after Single and Split Dose Regimens Using Increasing Doses of Amikacin after 4 Days Administration.

Dose	Single regimen	*p*	Single regimen	*p*
15 mg / kbw	0.7175 ± 0.0414	0.800	0.7322 ± 0.0534	0.119
75 mg / kbw	0.7150± 0.0543	0.898	0.7433± 0.0413	0.193
150 mg / kbw	0.7617 ± 0.0293	0.122	0.9100 ± 0.0358	0.000*
300 mg / kbw	0.8050 ± 0.0383	0.006	1.0050 ± 0.0979	0.001*

* = Significant *p* value ($p < 0.005$).

Table (4): After 7 Days Administration.

Dose	Single regimen	*p*	Single regimen	*p*
15 mg / kbw	0.7203 ± 0.0555	0.819	0.7767 ± 0.0345	0.821
75 mg / kbw	0.7250± 0.0532	0.760	0.8517± 0.0564	0.002*
150 mg / kbw	0.7750 ± 0.0378	0.113	0.9750 ± 0.0740	0.000*
300 mg / kbw	0.8383 ± 0.0417	0.004*	1.0000 ± 0.0515	0.000*

* = Significant *p* value ($p < 0.005$).

Table (5): After 10 Days Administration.

Dose	Single regimen	*p*	Single regimen	*p*
15 mg / kbw	0.7167 ± 0.0540	0.933	0.8683 ± 0.0491	0.000*
75 mg / kbw	0.7317± 0.0512	0.542	0.9167± 0.0550	0.000*
150 mg / kbw	0.8267 ± 0.0539	0.007	1.0950 ± 0.2195	0.000*
300 mg / kbw	0.9983 ± 0.1067	0.001*	1.4467 ± 0.3777	0.000*

* = Significant *p* value ($p < 0.005$).

After 7 days administration, the previously mentioned doses as wwell as 75 mg/kbw split regimen and 300 mg/kbw single dose regimen produced significant increase (Table 4). The 10 days drug administration produced significant increase in serum creatinine when dose of 75 and 150 mg/kbw were given as split regimens and with the dose of 300 mg/kbw administered by both regimens (Table 5).

Infection with various organisms did not significantly alter serum creatinine (Table 6).

Statistical analysis was done using 2 way analysis of variances comparing both regimens in relation to increasing the dose and duration of treatment. Serum creatinine level was significantly lower when amikacin was given in the same dose for the same duration in animals treated with sinle dose compared to the split dose (Figs. 2 and 3). In Fig. 2, increaseing the dose increased the serum creatinine (split was higher than the single). In Fig. 3, prolonging the period of treatment also increased the values were higher when the split dose is used.

Kidneys of all animals treated with doses less than 300 mg/kbw whether single or the split dose regimens, showed on light microscopic changes. Animal injected with split daily dose of 300 mg/kbw showed granularity of cytoplasm on day 4, focal tubular necrosis on day 7 and lymphocytic infiltration and 10 (Fig. 4). Those injected with an equal dose given once daily showed no changes.

Discussion

The dosage and frequency with which antibiotics should be administered has long been a source of controversy. Due to their narrow therapeutic index aminoglycosides dosing regimen is still not well established [15]. Generally aminoglycosides are administered by IM or IV routes. Amikacin produces serum peak

Table (6): Mean Serum Creatinine Values after Iniection with Various Organisms.

Organism	Mean serum creatinine	*p* alue
E. coli [ATCC. 25922)	0.7217 ± 0.0440	0.845
E. coli [Isolated strain]	0.7317± 0.050	0.536
Pseudomonas [ATCC 27853]	0.7183 ± 0.062	0.990
Pseudomonas [Isolated]	0.6983 ± 0.049	0.372
Klebsiella oxytoca	0.7233 ± 0.057	0.828

* = Significant *p* value ($p < 0.005$).

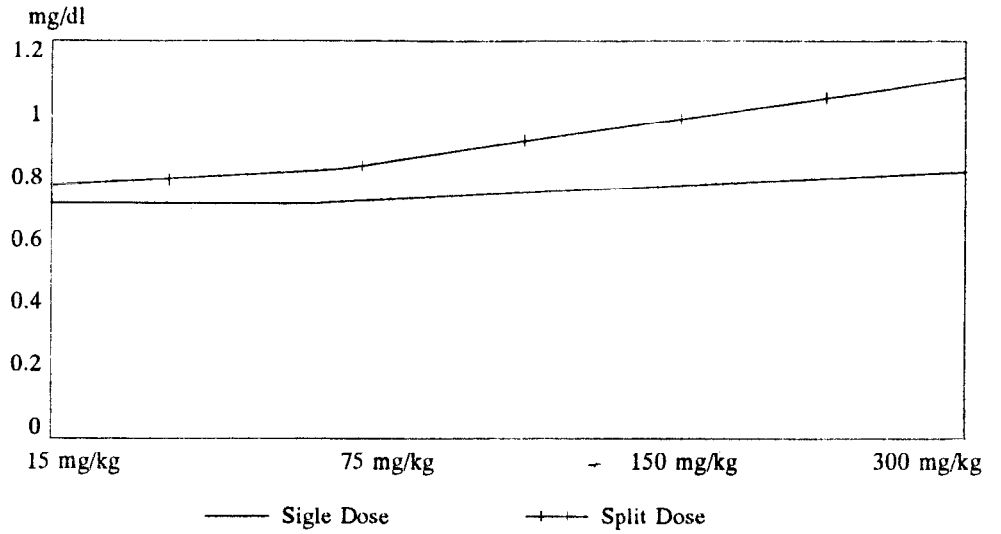

Fig. (2): Effect of increasing dose on serum creatinine:
The increase in serum creatinine levels was significantly higher when the same doses were given as split rather than as a single dose.

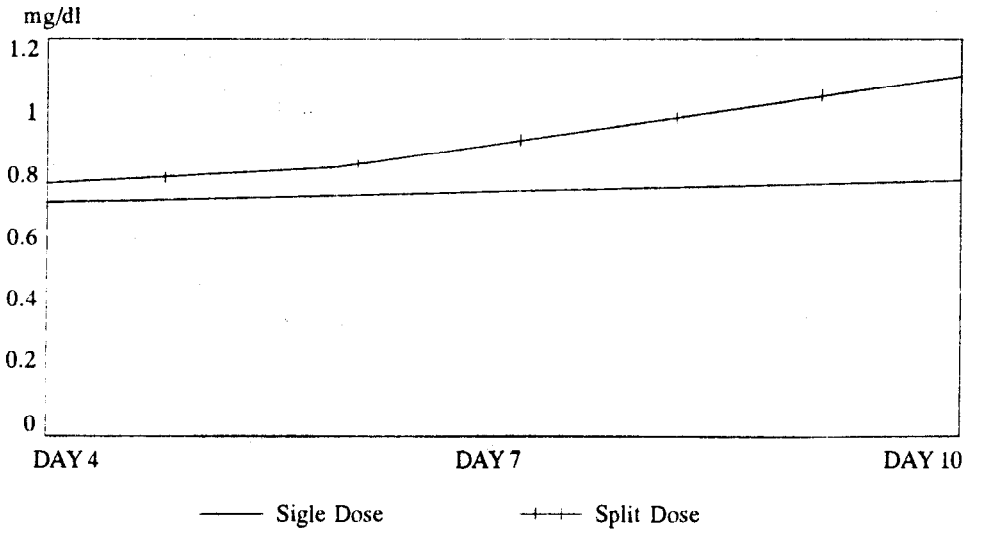

Fig. (3): Efect of increasing number of days of drug administration on serum creatinine:
The increase in the serum creatinine levels was significantly higher after the same duration of treatment when given as a split dose rather than as a single dose.

Fig. (4): Section of mouse kindney showing focal lymphocytic infiltrationn and congestion (H & E X 100).

concentration, after a rapid distribution phase of the drug approximately 30-40 minutes after IV administration, a dose of 7.5 mg/kbw of amikacin produces a peak of serum concentration of 25-30 μg/ml and a trough conc. of 8 μg/ml **[2]**. A dose of 15 mg/kbw produces peak serum conc. of 40 μg/ml and a trough conc. of < 5 μg/ml **[16]**. While nephrotoxicity appears to be related to consistently elevated trough concentraion **[17]**, the rate at which aminoglycosides kill bacteria is related to their peak concentration **[18]**.

In fact the bacteriological efficiency of an antiobiotic is directly proportionat to the MIC. Higher values of this ratio are considered more effective **[19]**.

The MIC of amikacin for the five strains of organisms used in this study ranged between 0.5 and 4 μg/ml, i.e. they were all highly sensitive. This might explain in our study, why there was more than 99.9% eradication of the invading organism 24h after the start of treatment, by both the single and split dose regimens. The difference in eradication by both regi-

mens was statistically insignificant which proves that amikacin given in a single daily dose is as effective as the split dose regimen.

The higher serum peak concentration reached after a high single dose causes more rapid and efficient killing of the organism. This initial rapid killing may be followed by regrowth of some resistant subpopulations in vitro [20]. But this is checked by two things first the greater post-antibiotic effect (PAE) which is more constant and more effective after exposing the organism to a high dose of the antibiotic whether in vivo or in vitro [19]. Second, in vivo, the immune system of the host can eliminate such few numbers of organisms if present [21]. Thus a once daily dosing is more bactericidal immediately after administration and is more effective in preventing bacterial regrowth despite the long period of subinhibitory drug concentration before administering the second dose [19].

The present work demonstrated an elevation in serum creatinine level with the split dose regimen which was very obvious with higher doses, especially when given for long periods. The single dose did not cause any elevation in serum creatinine except with very high dose (300 mg/kbw) especially when given over a period of 10 days.

No histopathological changes were observed in both kidneys by light microscopy except at the dose of 300 mg/kbw given as a split dose every 12 hours. This might be explained by the following; 1. rapid elimination of necrosed cells in urine, thus escaping detection. 2. the high rate of regeneration of rat renal cortical cells. 3. focal distribution of injury that might escape detection. 4. enzymatic injury at submicroscopic level [9, 11, 22]. Our work is thus in agreement with the work of Tulkens et al [23] who found that kidneys of animals that received therapeutic doses of amikacin or short treatment could not be distinguished from controls by histopathological evaluation.

The resistance of mice kidneys compared to human kidneys may be attributed to low serum half life of amikacin in mice (30 minutes) compared to humans (150 minutes) [11]. Therefore, the dose which produces nephrotoxicity in mice is about 5-10 times the dose which can cause the same alterations in humans [22]. It also might explain the need to increase the dose in our work until a detectable change could be observed histopathologically as well as a significant increase in serum creatinine and therefore, 1/5 the used doses might produce the same changes in humans.

Nephrotoxicity, as evidenced by elevated serum creatinine and by histopathological changes, was not observed in the single dose regimens even in high doses and for prolonged periods of treatment. It

was only detected in split dose regimens. This is in accordance with the human studies of Giamarellou et al [13], Marik et al [16] and Tulkens [24]. This might be explained by the fact that amikacin produces saturable kinetics at low serum levels and a linear pattern at high serum concentrations and, hence, lower cortical concentrations [25]. Also, in 1991, De Broe et al [26] in their human study gave a further support to this explanation when they found that the cortical concentration of amikacin was influenced by the dosage schedule and that the cortical uptake of amikacin was saturable at low serum concentrations.

Therefore, it is concluded from the present study that using amikacin in the conventional twice daily dosing regimen especially in patients with border-line kidney function should be confined to the least effective dose in the shortest period of time. On the other hand amikacin could be used safely at higher doses and for longer durations if given as a single dose every 24 hours.

References

1. LIETMAN, P. S.: Aminoglycosides and spectinomycin: aminocyclitols. In: Principles and Practice of Infectious Diseases (Mandell GL, Douglas RG and Bennet JE. Eds), pp 192-206. John Wiley, New York, 1985.

2. RISTUCCIA, A. M. and CUNHA, B. A.: An overview of amikacin. Therapeutic Drug Monitoring, 7: 12-25., 1985.

3. YOUNG, L. S. and HEWITT, W. L.: Activity of five aminoglycoside antibiotic in vitro against Gram-negative bacilli and Staphylococcus aureus. Antimicrob. Agents Chemother., 4 : 617-625, 1973.

4. KEANE, C. T.; ENGLISH, L. F. and WISE, R.: *Providencia stuartii* infections. Lancet, 1: 1045, 1975.

5. CLARK, J. T.; LIBKE, G. D. and REGAMER, C.: Comparative pharmacokinetics of amikacin and kanamycin. Cli. Pharmacol. Ther, 15: 610-616, 1974.

6. WALKER, J. M. WISE, R. and MITCHARD, M.: The pharmacokinetics of amikacin and gentamycin in volunteers. A comparison of individual differences. Chemother., 94: 203-214, 1979.

7. MEYER, R. D.: LEWIS, R. P. and CARMALT, E. D.: Amikacin therapy for serious Gram negative bacillary infection. Ann. Intern Med., 83: 790-800, 1974.

8. LAU, W. K., YOUNG, L. S. and BLACK, R. E.: Comparative efficacy and toxicity of amikacin versus gentamycin carbeniscillin in leukopenic patients. Am. J. Med, 62: 959-966, 1977.

9. NIEMINEN, L.; KASAWEN, A. and KANGAS, L.: Gentamycin in the rat. Experientia, 34: 1135-1136, 1978.

10. SAIRIO, E.; KASAMEN, A. and KANGES, L.: The nephrotoxicity and renal accumulation of amikacin, tobramycin and gentamycin in rats, rabbits and guinea pigs. Exp. Path., 15: 370-375, 1978.

11. TULKENS, P. M. and LAURENTI, G.: Mechanisms of aminoglycoside induced nephrotoxicity : a review of experimental studies in animals with gentamycin and four other aminoglycosides (amikacin, tobramycin, netilmycin and dibekacin) at low doses. Chempother. suppl., 5 : 60-71, 1984.

12. WHELTON, A.: Clinical strategies for the reduction of aminoglycoside nephrotoxicity and ototoxicity. Chemother., 5 : 52-59, 1984.

13. GIAMARELLOW, H.; YIALLOUROS, K. and PETRIKKOS, G.: Comparative kinetics and efficacy of amikacin administered once or twice daily in the treatment of systemic Gram negative infections. J. Antimicrob. Chemother., 27 Suppl, C: 73-79, 1991.

14. CRAIG, W. A.; LEGGETT, J.; TORSUKA, K. and VOGELMAN, B.: Key pharmacokinetics parameters of antibiotic efficacy in experimental animal infections. J. Drug Develop., 1 Suppl, 3: 7-15. 1988.

15. SIEGENTHALER, W. E.; BONETTI, A. and LUTHY, R.: Aminoglycoside antibiotics in infectious diseases: an overview. Am. J. Med., 80, supp 6 B : 2-14, 1986.

16. MARIK, P. E.; HAVLIK, I.; MONTEEAGUDO, F. S. E. and LIPMAN. J.: The pharmacokinetics of amikacin in critically ill adult and paediatric patients: comparison of once versus twice daily dosing regimens. J. Antimicrob. Chemother., 27 Suppl, C, 81-89, 1991.

17. GIAMARELLOU, H.; METZIKOFF, C.; PAPACHRISTOPHOROU, S.; DONTAS, A.S. and DAIKOS, G. K.: Prospective comparative evaluation of gentamycin V.S., gentamcin plus cephalothin in the production of nephrotoxicity in man . J. Antimicrob. Chemother., 5: 581-586.

18. MOORE, R. D.; LEITMAN, P. S. and SMITH, C. R.: Importance of the ratio of peak concentration to minimal inhibitory concentration. J. Infec. Dis., 155: 93-99, 1987.

19. BLASER, J.: Efficacy of once and thrice daily dosing of aminoglycosides in in-vitro models of infection. J. Antimicrob. Chemother., Suppl, C: 21-28, 1991.

20. DUDLEY, M. N. and ZINNER, S. H.: Single daily dosing of amikacin in an in-vitro model. J. Antimicrob. Chemother., 27 Suppl, C : 15-19, 1991.

21. BEAUSCIRE, G.; LEROY, O.; BEUSCART, C. KARP, P. and CAILLAUX, M.: Clinical and bacteriological efficacy and practical aspects of amikacin given once daily for severe

infections. J. Antimicrob. Chemother , 27 suppl, C: 91-103., 1991.

22. TOUBEAU, G.; LAURENT, G.; CARLIER, M. B.; ABID, S.; MALDAGUE, P. and TULKENS, P.: Tissue repair in rat kidney cortex after short treatment with aminoglycosides at low doses. Lab. Invest., 54 No 4: 385-393, 1986.

23. TULKENS, P.; CARLIER, M. B.; MORIN, J. P.; BEAUWENS. B. and VAN HOOF, F.: Lysosomal toxicity of gentamycin in rat kidney comparisons with amikaycin and tobramycin. Research supported by the Belgian Fund of Medical Research Organization, 3: 4516-4791, 1989.

24. TULKENS, P.: Pharmacokinetics and toxicological evaluation of once daily regimen versus conventional schedules of netilmycin and amikacin. J. Antimicrob. Chemother., 27 Suppl, C.: 49-61, 1991.

25. DeBROE, M. E.; GIULIANO, R. A. and VERPOOTEN, G. A.: Choice of drug and dosage regimen. Two important risk factors of aminoglycosides nephrotoxicity. Am. J. Med., 80 Suppl 6B:115-118, 1986.

26. De BROE, M. E.; VERBIST, L. anv VERPOOTEN, G. A.: Influence of dosage schedule on renal cortical accumulation of amikacin and tobramycin in man. J. Antimicrob. Chemother., 27 Suppl, C: 41-47.