GENTAMICIN: ACTIVITY *IN VITRO* AGAINST GRAMNEGATIVE ORGANISMS AND CLINICAL EXPERIENCES IN THE TREATMENT OF URINARY TRACT INFECTIONS

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Gentamicin is a new broad-spectrum antibiotic which is produced by the fermentation of *Micromonospora purpurea*, originally found by WEINSTEIN *et al*[1,3]. Chemically, gentamicin is composed of two components closely related each other, both of which contain three primary amino groups[1,2]. 2-Desoxystreptamine was isolated after acid hydrolysis from both components. Acetylation deprived of their antibiotic properties as was in the cases of other antibiotics belong to oligosaccharide group. Formula weight of gentamicin determined by osmometric measurements was 425 ± 21[1,2]. The renal clearance of gentamicin was approximately equal to the glomerular filtration rate[4]. Single doses of 0.8 mg/kg administered intramuscularly gave average peak blood levels of 4.7 mcg/ml and average urinary levels of 240 mcg/ml at first two hour fraction[4]. With larger intramuscular doses, 86 to 100% recovery in urine was obtained during 24 hours[4]. Activity of gentamicin was very much affected by pH and salt concentration in the medium. The antibiotic was most active at an alkaline pH, and decrease of activity was noted at acid pH. The area of most significant change was around the point of neutrality. Increasing concentrations of sodium chloride in the medium caused a progressive decrease in the sensitivity of the organisms[5]. Gentamicin was found to act bactericidally at any phase of growth, and sulfate salt of gentamicin was approximately twice as active on a weight basis as its sulfonate salt[5]. Toxicity studies in the cat showed that vestibular damage (ataxia) was noted at the 50 mg/kg level on the 15 th day. Also, renal tubular necrosis was found in the rat and dog dosed with more than 40 mg/kg[4].

In vitro and clinical studies were undertaken to determine its efficacy in stubborn urinary tract infections caused by multi-resistant organisms.

MATERIALS AND METHODS All organisms tested were the strains which had been isolated from infected urine specimens in out-patient clinic and wards of Urology service at Keio University Hospital. Most of them had multiple drug resistance. In the study of cross resistance an *E. coli* and two *Klebsiella* strains which harbor R 6(R-factor carrying resistance markers of sulfa-drug, streptomycin. tetracycline, chloramphenicol, kanamycin and neomycin) were employed. The three substrains of *E. coli* K-12, which received these R-factors by the method described elsewhere, were also used in this experiment. Generous supply of Watanabe was a substrain of K-12 carrying R 6 of Lebeck[6].

All organisms were cultured in Penassy broth or on Penassay agar (Difco). Gentamicin sulfate was donated by the Shionogi Pharmaceutical Co. Ltd., Osaka, Japan. Incubation of cultures was at 37°C for 16 to 20 hours. Minimal inhibitory concentrations of gentamicin against these organisms were determined *in vitro* by the serial agar dilution method. About 10^4 cells were inoculated on each small segments of plates using a small platinum loop. The patients who received gentamicin courses were those who had been treated at clinic and wards of our service and had been suffering from complicated urinary tract infections. Most of them had some underlying conditions such as stones, tumors or fistules, and some of them had received surgeries followed by indwelled catheter treatment re-

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Tables 1 Pattern of sensitivity of urinary pathogens to gentamicin

Species	No. of strains tested	No. of strains inhibited (dug concentration in mcg/ml)							
		0.25	0.5	1.0	2.5	5.0	10	25	25<
E. coli	10	0	4	5	10	10	10	10	10
Citrobacter	3	1	3	3	3	3	3	3	3
Klebsiella	6	3	4	4	6	6	6	6	6
Cloaca	2	0	1	1	2	2	2	2	2
Proteus vulgaris	2	0	0	0	2	2	2	2	2
Morganella	3	1	2	3	3	3	3	3	3
Rettgerella	3	3	3	3	3	3	3	3	3
Providencia	2	0	1	1	1	1	1	1	2
Pseudomonas	15	3	10	10	10	13	15	15	15
Total	11	11	28	30	43	44	45	45	46

Table 2 Susceptibility of strains carrying R_6 to gentamicin

Strains	Minimal inhibitory concentration (mcg/ml)
E. coli 93	0.5
Klebsiella 309	0.5
Klebsiella 313	0.5
E. coli K-12	0.5
E. coli K-12/R-93*	0.5
E. coli K-12/R-309*	0.5
E. coli K-12/R-313*	0.5
E. coli K-12-R_6**	0.5

* *E. coli* K-12 received resistance factor from the strains cited by number

** *E. coli* K-12 haboring resistance factor which carries six resistance marker found by LEBECK

Table 3(1) Results in patients treated with gentamicin

	Diagnosis	Age, Sex	Underlying coditions	Dosis mg×days
1	Chronic pyelonephritis	52 M	Stricture at P-U junction Y-V plasty	80×7
2	Chronic pyelonephritis	28 M	Stricture at P-U junction Y-V plasty	80×7
3	Chronic pyelonephritis	8m.F	Vesicoureteral reflux. Hydronephrosis(unilateral)	40×7
4	Chronic cystitis	60 M	Bladder tumor. Transurethral resection	80×7
5	Chronic cystitis	51 M	Bladder tumor. Partial resection of bladder	80×7
6	(Prophylactic use)	65 M	Bladder tumor. Transurethral resection	80×7
7	Chronic cystitis	61 M	Benign prostatic hyperplasis (B. P. H.) Suprapubic prostatectomy	80×7
8	Chronic cystitis	56 M	B. P. H. Suprapubic prostatectomy	80×7
9	Chronic cystitis	64 M	B. P. H. Suprapubic prostatectomy	80×3
10	Chronic systitis	81 M	B. P. H., T. U. R. prostate	80×7
11	(Prophylactic use)	60 M	B. P. H. Suprapubic prostatectomy	80×7
12	Chronic pyelonephritis	36 M	None	80×7
13	Chronic pyelonephritis	64 M	None	80×7
14	Chronic cystitis & chronic pyelonephritis	45 F	Ureterovaginal fistule. Radical hysterectomy	80×7
15	Chronic cystitis & wound infection	53 F	Ureterovaginal fistule. Ureteroneocystomy Vesico-abdominal fistule. Radical hysterectomy	80×7
16	Chronic cystitis	32 F	Vesico-vaginal fistule. Radical hysterectomy	160×7
17	Chronic pyelonephritis	30 F	Uretero-cutaneostomy with indwelled catheter	80×7
18	Chronic cystitis	26 M	Uretero-ileo-cystostomy. Contracted bladder (tuberculosis)	80×7
19	Chronic cystitis	4 M	Bladder rupture. Cystostomy	80×7
20	Chronic cystitis	27 M	Urethral stricture. Urethro-plasty, cystostomy	80×7
21	Chronic cystitis	22 M	Bladder calculus	160×7
22	Chronic pyelonephritis	59 M	Ureteral stone (unilateral). Ureterolithotomy Hydronephrosis	80×9
23	Chronic pyelonephritis	37 M	Episode of ureteral calcul(?)	80×7
24	Chronic cystitis & acute pyelonephritis	29 M	Urethral stricture. Bladder stone	80×7

Table 3(2) Results in patients treated with gentamicin

Organism		Urine W.B.C.	Follow up weeks	Comments
Before	At the end			
Rettgerella	None	Diminished	20 W sterile	Cured
Pseudomonas	None	Diminished	2 W recurrence symptomeless	Improved
Pseudomonas	None	Unchanged	4 W superinfection	Indeterminate
Klebsiella & Rettgerella	None	Unchanged	4 W sterile	Improved
Pseudomonas	None	Unchanged	4 W sterile	Improved
None	None	Unchanged	4 W sterile	Successful
Rettgerella	None	Diminished	4 W sterile	Cured
Pseudomonas	*Pseudomonas* 10^5/ml<	Unchanged	—	Failure
Pseudomonas	*Pseudomonas* 10^5/ml<	Unchanged	—	Failure
Cloaca	None	Diminished	1 W superinfection symptomeless	Improved
None	None	Unchanged	2 W	Successful
Pseudomonas	None	Diminished	8 W sterile	Cured
Pseudomonas	None	Diminished	1 W sterile	Improved
Klebsiella	None	Unchanged	30 W sterile	Improved
Proteus vulg.	*Proteus vulg.* 10^5/ml<	Unchanged	—	Failure
Alkaligenes	*Alkaligenes* 10^5/ml<	Unchanged	—	Failure
Pseudomonas	*Pseudomonas* 10^5/ml<	Unchanged	—	Failure
Pseudomonas	*Pseudomonas* 10^5/ml<	Unchanged	—	Failure
Pseudomonas	*Pseudomonas* 10^5/ml<	Unchanged	—	Failure
Morganella & Klebsiella	*Flavobacterium* 10^5/ml<	Unchanged	—	Indeterminate
Rettgerella & Klebsiella	None	Unchanged	2 W recurrence	Improved
Pseudomonas	*Pseudomonas* 5×10^5/ml	Diminished	4 W sterile	Improved
Pseudomonas	None	Unchanged	2 W sterile	Improved
E. coli & hemolytic *streptococcus*	None	Diminished	2 W recurrence symptomeless	Improved

cently. The pathogens were usually the multi-resistant strains which had survived some antibiotic courses. Repeated laboratory investigations were performed to find early signs of toxicity. Follow up studies were done for one to thirty weeks after discontinuance of medication.

RESULTS AND DISCUSSIONS Minimal inhibitory concentration study was done in 46 urinary isolates. Fourty three strains were inhibited to grow by the presence of gentamicin at the concentration of 2.5 mcg per ml or less. Only two moderately resistant *Pseudomonas* strains and one highly resistant *Providencia* strain were found (Table 1). In the study of cross resistance, every strain carrying R 6 failed to grow by 0.5 mcg per ml. In another word resistance marker of kanamycin and neomycin could not express resistance against gentamicin in *E. coli* and *Klebsiella* cells. In addition, strains highly resistant to streptomycin were also sensitive to gentamicin (Table 2).

In the clinical study, simple acute urinary tract infections were excluded because they are rather self-limiting and usually can be cured by less potent drugs. We have no para- and quadriplegics in our wards, it is the reason why they were not included. The results of the treatments are summarized in Table 3.

Two patients (case 1 and 2) were suffered from infected hydronephrosis following to plastic operations on their stricture at pyelo-ureteral junction. Bacteriuria and pyuria associated with tenderness at cost-vertebral angle which had been persistent against a few antibiotic couress quickly diminished by gentamicin. One of them(case 1) has remained

abacteriuric for more than 20 weeks, his urinary offender was a *Rettgerella* strain. Another patient had recurrence of bacteriuria after 2 weeks but he was virtually asymptomatic, his pathogen was a *Pseudomonas*.

An eight month old girl (case 3) had unilateral vesico-ureteral reflux followed by hydronephrosis. Upper urinary tract infection was developed by *Pseudomonas*. This organism disappeared within 72 hours after gentamicin was started. However white cells in urine remained unchanged at the termination and in the follow up course for more than 2 weeks until a *Rettgerella* strain reinfected her urinary tract.

Two male patients had developed urinary tract infections while they were treated with indwelling catheters after transurethral resection of bladder tumor or partial resection of the bladder. Gentamicin was started several days before removal of the catheters. The organisms(*Rettgerella* and *Klebsiella* in case 4, and *Pseudomonas* in case 5) were rapidly eliminated from urinary tract. However, in case 4, urine white cells did not diminish for a few weeks even though urgency and frequency have not recurred. In case 5, high fever and CVA tenderness which developed a few days after operation also subsided quickly by gentamicin. Pyuria has been persistent for several weeks though bacteriuria did not recur. Another patient was treated with gentamicin during the postoperative course of transurethral resection of bladder tumor and prevention was successful.

Five patients (case 7, 8, 9, 10 and 11) had been treated for their benign protatic hyperplasia. Three of them received suprapubic prostatectomy. The complicating chronic infections were treated with gentamicin. In 7 th patient pathogen of chronic cystitis was a *Rettgerella* strain which quickly disappeared from his urine and no signs and symptoms of infection were found for at least 4 weeks. The eighth patient was infected by *Pseudomonas* which did not respond to gentamicin. The nineth patient complained of severe nausea and vomitting on the 3 rd day of medication and the drug was discontinued.

In case 10, a cystostomy tube wes indwelled at the time of admission and the patient was complaining of heavy urgency and marked pyuria was noticed. Urine culture revealed a *Cloaca* strain which was very sensitive to gentamicin (less than 2 mcg by disc). Response to medication was marked and rapid. A week later *Pseudomonas* reinfected through the cystotomy tube, but the patient remained symptomeless. In the 11 th case, prophylactic use of gentamicin against infection *via* a catheter was again successful.

Two chronic pyelonephritis cases (12 and 13) who had been resistant to several antibiotic courses were given gentamicin. The bacteria disappeared quickly during the course and urine white cells diminished markedly. It is not possible to state whether case 13 was cured or not since follow-up course was only one week.

In two radical hysterectomy cases, uretero-vaginal fistules were the complications. Indwelling catheters in bladder or ureter induced chronic cystitis and probably chronic pyelonephritis. In case 14 gentamicin was very effective to expective to expell the bacteria (*Klebsiella*) from her urinary tract. The patient did not receive reconstructing operation and kidney function has been gradually deteriorated. However her urine has been sterile for more than half a year (probably because of ureteral occulsion). The 15 th patient was infected by *Proteus vulgaris* right after ureterocystostomy operation. Gentamicin treatment was ineffective in this case partly because of secondary vesicocutaneous fistule and prolonged duration of indwelling catheter.

Another patient (case 16) also developed complicating cystitis followed to vesicovaginal fistule produced by radical hysterectomy. Her stubborn chronic infection had resisted against every antibiotic treatment and gentamicin was no exception. *Alkaligenes* strain infecting her urinary tract was resistant more than 30 mcg of gentamicin (paper disc method in clinical laboratory).

The seventeenth patient had developed chronic pyelonephritis because of indwelled catheter in the cutaneostomized ureter. Gentamicin treatment was started to reduce urea splitting organisms in urine as a preliminary treatment of urine acidification. Offending *Pseudomonas* strain was not reduced by medication.

The eighteenth patient was a tuberculosis case of urinary tract. He received ureteroileocystostomy. and had been suffered from stubborn *Pseudomonas* infection of his reconstructed urinary tract. Gentamicin was again ineffective.

A young male (case 19) had been treated with cystostomy tube for more than 3 months since his bladder was heavily damaged by traffic accident. *Pseudomonas* infection had developed through indwelled catheter which did not respond any antibiotics including gentamicin.

The twentieth patient had been treated indwelled Foley catheter to restore his urethral lesion. Gentamicin was used to remove infecting *Morganella* and *Klebsiella* strains. After 7 days medication, culture became negative, however white cells remained unchanged. His follow up study was very short because he came home one week later and never returned to clinic.

A 22 years old male (case 21) received gentamicin treatment to get sterile urine at the time of operation to remove his bladder stone. The pathogens were *Klebsiella* and *Rettgerella* which responded gentamicin treatment. However the operation was postponed and *Klebsiella* was found again after 2 weeks.

The twenty-second patient had been suffered from chronic infection of upper urinary tract even after uretero-lithotomy. A *Pseudomonas* strain, which had been persistently found in urine after tetracycline and kanamycin courses, was markedly reduced in number. Urine white cells also diminished and his later course was uneventful. After four weeks urine culture was sterile.

Thirty-seven years old male patient (case 23) had also been suffering from chronic pyelonephritis. He had an episode of flank colicky pain several months ago but exact diagnosis was obscure because of negative X-ray finding. The offender was *Pseudomonas* which had been persistent after tetracycline and kanamycin treatments. Urine culture obtained 72 hours after gentamicin was started was negative. Clinical improvement including tenderness at cost-vertabral angle and backache was remarkable though pyuria did not diminish. After two weeks white cells in urine has gradually disappeared and culture was negative.

The twenty-fourth patient had urethral stricture complicated with bladder stone, and urine culture revealed *E. coli* and *α-hemolytic Streptococcus*. He suddenly developed acute pyelonephritis and gentamicin treatment was started. On seventh day urine was sterile, however white cells suggested latent infection and recurrence of bacteriuria was noticed within two weeks. Surviving pathogens in the layeres of the stone might cause the relapse.

As a whole, sterilization of urine was achieved in 62% of the cases (Table 4). With one exception gentamicin was very effective to all strains belonged to *Enterobacteriaceae*. Five of 13 strains of *Pseudomonas* were resistant to gentamicin therapy. An *Alkaligenes* strain was highly resistant to gentamicin. The cases selected for gentamicin courses were those who were expected to be difficult to get satisfactory results by antibiotics treatment only. In these complicated infections failure and recurrence are common. The layers of stones inbedding bacteria, abscess in the infected wound, incomplete drainage *etc.* are the main cause of difficulty. Thus, in the cases shown sbove recurrences were frequent and final improvement was obtained in 60% of whole cases. Superinfection was rare probably because of wide antibacterial

Table 4 Evaluation of gentamicin in the repect of change in bacterial population at the end of treatment

Species	Number of strains				
	Total	Disappeared	Diminished	Unchanged	Substituted
E. coli	1	1	0	0	0
Klebsiella	4	3	0	0	1 (*Flavobacterium*)
Cloaca	1	1	0	0	0
Proteus vulg.	1	1	0	0	0
Rettgerella	4	4	0	0	0
Morganella	1	0	0	0	1 (*Flavobacterium*)
Pseudomonas	12	6	1	5	0
Alkaligenes	1	0	0	1	0
-hemolytic *streptococcus*	1	1	0	0	0
Total	26	16 (62%)	1	7 (27%)	2

Table 5 Evaluation of gentamicin in the respect of clinical results

Diagnosis	Total No. of patients treated	Cured	Improved	Indeterminate	Failure
Chronic cystitis	11	1	4	1	5
Chronic pyelonephritis	8	2	4	1	1
Chronic cystitis & chronic pyelonephritis	1	0	1	0	0
Chronic cystitis & acute pyelonephritis	1	0	1	0	0
Chronic cystitis & wound infection	1	0	0	0	1
Total	22	3	10	2	7

spectrum of this drug. Also, in the cases of mixed infection gentamicin had remarkable effect. No serious toxic effect was found in whole series of the patient. In two cases nausea and loss of apetite were the mild expression of probable vestibular damage (case 9 and 14). Elevation of serum glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase was found in two cases (case 9 and 20). However both of them were affected by serum hepatitis and had high transaminase levels previously, and it is still obscure whether gentamicin is toxic to liver cells.

In one azotemic patient, blood urea nitrogen level elevated only in minimal degree (from 30 to 38 mg per dl) with 560 mg of gentamicin. Hematology and serum electrolytes remained in normal range.

At present gentamicin can be recommended for the treatment of complicated urinary tract infections. However, medication should be limited in selected cases to prevent development of resistant clones and to avoid possible toxic effects. Especially in patients with impaired renal function serum level of the drug should be frequently measured during the course.

CONCLUSION Gentamicin was found to have very broad spectrum of antibacterial activity which covers whole species of urinary tract offenders including *Proteus* species aud *Pseudomonas* strains. No cross resistance was found between gentamicin and other members of oligosacharide group of antibiotics. Resistance markers of kanamycin and neomycin on R factor could not express resistance against gentamicin. Clinical results were rather satisfactory. Improvement was obtained in about 60% of the complicated cases. Toxic efiects were minimal in our series. However, selection of appropriate patients for gentamicin treatment and prevention of serious toxic effects must swait further clinical experience.

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