Activity of Gentamicin Against *Pseudomonas* and Hospital Staphylococci

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Gentamicin is a new basic antibiotic complex isolated from an actinomycete, *Micromonospora purpurea*, in the Research Laboratories of the Schering Corporation, New Jersey. It consists of two components, referred to as gentamicin C₁ and gentamicin C₂, which are closely related in structure and have a similar antibacterial spectrum. Both substances contain a 2-deoxystreptamine component and are chemically related to the streptomycin group of antibiotics (Rosset et al., 1963). Gentamicin is supplied as the sulphate, which is a white powder readily soluble in water.

Gentamicin has been shown to be bactericidal *in vitro* against a wide range of Gram-positive and Gram-negative bacteria, including hospital drug-resistant strains of *Staphylococcus aureus* and coliform bacilli, and has outstanding activity against *Pseudomonas pyocyanea* (see White, 1963; Weinstein et al., 1963; Rubenis et al., 1963; Rabinovich et al., 1963). Early clinical trials showed that the antibiotic is effective in the treatment of infections of the urinary tract due to many Gram-negative bacilli, including *Ps. pyocyanea* (Bulger et al., 1963; Jao and Jackson, 1963; Brayton and Loria, 1964; Klein et al., 1964), but if the blood level rises to about 15 µg./ml. or more there is a danger of severe vestibular damage (Jao and Jackson, 1964). Stone et al. (1964, 1965) reported the successful treatment of severe burns with *Ps. pyocyanea* septicaemia, both systemic treatment and local gentamicin ointment being used.

The present investigation was undertaken in order to compare the activity of gentamicin with that of other streptomycin-like antibiotics against strains of *Staph. aureus* and coliform bacilli isolated in a British hospital.

Materials and Methods

Bacteriostatic Tests.—These were carried out by serial dilution in nutrient agar. The inoculum was made with a standard 1-mm. loop from an overnight broth culture; except where otherwise stated the culture was first diluted 1 in 500.

Serum-binding.—The effect of serum on bacteriostatic activity was tested by adding 0.5 ml. of serial dilutions of gentamicin in water to 4.5 ml. of pooled human serum, giving a final concentration of 90% serum. The results were compared with those obtained when 0.5 ml. of the gentamicin solutions was added to 4.5 ml. of nutrient broth.

Bactericidal Tests.—Bactericidal activity was tested by a modification of the cellophane transfer technique (Chabbert, 1957; Chabbert and Patte, 1960), the antibiotic being incorporated in the medium in known concentrations. Control tambours were used to check carry-over of antibiotic: this was found to be substantial and similar to that of neomycin (Chabbert and Waterworth, 1965).

Development of Resistance.—Representative strains of *Staph. aureus* were serially passaged on ditch-plates containing gentamicin or one of the streptomycin group of antibiotics and tested for the development of resistance and cross-resistance.

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Results

General Spectrum of Activity

The bacteriostatic activity of gentamicin was compared with that of kanamycin against a range of Gram-positive cocci and Gram-negative bacilli. The results are given in Table I, from which it will be seen that both antibiotics have a similar pattern of activity, except against some strains of *Ps. pyocyanea* and *Staph. aureus*.

TABLE I.—Sensitivity of Common Pathogenic Bacteria to Gentamicin and Kanamycin

	No. of Strains	Minimum Inhibitory Concentration (µg./ml.)	
		Gentamicin	Kanamycin
<i>E. coli</i>	15	1-4	2-8
<i>Proteus mirabilis</i>	9	2-8	2-8
" <i>vulgaris</i>	10	1-4	2-8
" <i>morganii</i>	9	1-4	2-8
" <i>retzii</i>	9	1-8	2-4
<i>Klebsiella aerogenes</i>	7	1-2	1-4
other spp.	7	0.06-1	0.12-2
<i>Salmonella</i> spp.	9	0.25-1	1-4
<i>Shigella</i> spp.	9	1-2	4-8
<i>Ps. pyocyanea</i>	25	1-8	8-128
<i>Str. pyogenes</i>	14	16	64-256
" <i>pneumoniae</i>	16	16-32	64-128
" <i>faecalis</i>	15	8-16	8-32
<i>Staph. aureus</i> (1)	105	0.12-1	0.5-2
" " (2)	58	0.12-4	64- > 512

Effect of Size of Inoculum.—With six strains of *Staph. aureus* and one strain each of *E. coli*, *Klebsiella*, and *Ps. pyocyanea* tests were carried out with two different-sized inocula varying by a thousandfold. The minimum inhibitory concentration of gentamicin was increased four to eight times in the case of staphylococci and only twofold with the Gram-negative bacilli.

Effect of pH.—The activity of gentamicin against a single strain of *Staph. aureus* was tested in broth at different pHs ranging from 6.0 to 8.5. The results, given in Table II, show that within this range activity increases with pH, though the difference is smaller than those reported by Garrod (1959) for other members of the streptomycin/neomycin group.

TABLE II.—Effect of pH on Activity of Gentamicin Against *Staph. Aureus*

pH	6.0	6.5	7.0	7.5	8.0	8.5
Minimum inhibitory concentration (µg./ml.)	1.0	0.5	0.25	0.12	0.06	0.06

Serum Binding.—Comparative bacteriostatic tests carried out in nutrient broth or 90% pooled human serum with a single strain of *Staph. aureus* showed that the serum caused an eightfold decrease in activity.

Activity against *Ps. pyocyanea*

The 25 strains of *Ps. pyocyanea* had been recently isolated from infections in the hospital. Full analysis of their sensitivity to gentamicin, kanamycin, and streptomycin is given in Table III. With streptomycin the strains fell clearly into two groups—one highly resistant and the other moderately

resistant—and though with kanamycin minimum inhibitory concentrations covered a wide range the lowest figure was 8 $\mu\text{g./ml.}$ With gentamicin, on the other hand, all the strains were inhibited by 8 $\mu\text{g./ml.}$ or less, only one strain was not inhibited by 4 $\mu\text{g./ml.}$, and the majority were inhibited by 1–2 $\mu\text{g./ml.}$

TABLE III.—Activity Against *Ps. Pyocyanea* (25 strains)

	No. of Strains with Minimum Inhibitory Concentration ($\mu\text{g./ml.}$) of :							
	256	128	64	32	16	8	4	2
Gentamicin ..	—	—	—	—	—	1	4	11
Kanamycin ..	—	6	12	3	1	3	—	—
Streptomycin ..	7	—	—	—	15	3	—	—

Bactericidal Tests.—Two strains of *Ps. pyocyanea* were tested by the cellophane transfer technique. In bacteriostatic tests the minimum inhibitory concentrations of gentamicin for the two strains were 1 and 4 $\mu\text{g./ml.}$, and in the bactericidal test sterile results were obtained with concentrations of 8 and 16 $\mu\text{g./ml.}$ respectively.

Development of Resistance.—Of four strains of *Ps. pyocyanea* passaged daily on ditch-plates containing gentamicin two showed an eightfold and two a 32-fold increase in resistance after 20 transfers.

Antistaphylococcal Activity

The strains of *Staph. aureus* had also been recently isolated from hospital patients and were divided into two groups on the basis of their sensitivity to neomycin and kanamycin. Further analysis is given in Table IV. It will be seen that the 102 neomycin- and kanamycin-sensitive strains were about four times more sensitive to gentamicin, and the 57 kanamycin-resistant strains, which were also resistant to neomycin, showed little or no decrease in sensitivity to gentamicin. The kanamycin-resistant staphylococci were all resistant to benzylpenicillin, streptomycin, and tetracycline and some also to erythromycin.

TABLE IV.—Activity Against *Staph. Aureus*

	No. of Strains with Minimum Inhibitory Concentration ($\mu\text{g./ml.}$) of :											
	256 +	128	64	32	16	8	4	2	1	0.5	0.25	0.12
Group 1. Neomycin- and Kanamycin-sensitive (102 Strains)												
Gentamicin ..	—	—	—	—	—	—	—	—	1	9	73	19
Kanamycin ..	—	—	—	—	—	—	—	—	11	20	—	—
Bacitracin ..	—	—	—	—	1	3	15	54	17	1	11	—
Group 2. Neomycin- and Kanamycin-resistant (57 Strains)												
Gentamicin ..	—	—	—	—	—	—	—	—	1	1	2	5
Kanamycin ..	47	9	—	1	—	—	—	—	—	—	—	—
Neomycin ..	2	4	6	12	28	5	—	—	—	—	—	—
Bacitracin*	—	—	9	18	13	—	2	1	1	—	—	—

* Only 44 strains tested.

The strains were also tested for their sensitivity to bacitracin, since, following the widespread use in hospitals of creams and spray containing a mixture of bacitracin and neomycin, staphylococci with an increased resistance to both these antibiotics are beginning to emerge (see Rountree and Beard, 1965). It will be seen from Table IV that both the neomycin-sensitive and the neomycin-resistant strains showed a very wide range of sensitivity, but most of the neomycin-resistant strains showed a moderate increase in resistance to bacitracin. There was no direct relationship between the degree of resistance to neomycin and to bacitracin.

Bactericidal Tests.—A strain of *Staph. aureus*, the growth of which was inhibited by 0.25 $\mu\text{g./ml.}$, was sterilized on tambours by a concentration of 2 $\mu\text{g.}$ of gentamicin per ml., which was the lowest concentration tested.

Development of Resistance.—Six strains of *Staph. aureus*, of which three were naturally resistant to neomycin, were passaged 20 times on gentamicin ditch-plates. One of the neomycin-resistant strains was originally inhibited only by 2 $\mu\text{g.}$ gentamicin per ml. and showed a fourfold increase in resistance: the other five strains, originally inhibited by 0.25 or 0.5 $\mu\text{g./ml.}$, all showed 8- or 16-fold increases. All six strains became more resistant to kanamycin. A further six strains of neomycin-sensitive *Staph. aureus* were passaged 20 times on ditch-plates containing neomycin and kanamycin. Transfer on kanamycin rendered two strains highly resistant (512- and 128-fold increases), and these strains showed 64- or 128-fold increases in resistance to gentamicin and neomycin and 16- or 32-fold to streptomycin. Of the other four strains two showed fourfold and two 32-fold increases in resistance to kanamycin and similar but slightly smaller increases to gentamicin and neomycin: the former two strains remained sensitive to streptomycin and the latter two had become eight times more resistant. Habituation to neomycin produced only from 2- to 16-fold increases in resistance and similar but smaller increases to gentamicin, kanamycin, and streptomycin.

Discussion

Any drug possessing a high degree of activity against *Ps. pyocyanea* must be considered to be a valuable addition to our armoury, and the present work confirms that of others showing this organism to be uniformly sensitive to gentamicin regardless of resistance to kanamycin or streptomycin. Despite the toxicity of gentamicin, the clinical reports already cited, particularly those of Stone *et al.* (1964, 1965), amply justify its use as local treatment or even combined with systemic treatment in severe infections. Though *Ps. pyocyanea* can be rendered resistant to gentamicin *in vitro*, Stone *et al.* (1964) saw no increase in resistance *in vivo* even after five months' continuous treatment with this drug.

While severe staphylococcal infection can usually be successfully overcome with methicillin or other penicillinase-resistant penicillins, there are cogent objections to the use of these drugs for the treatment of nasal carriers, and neomycin or framycetin, often combined with another drug, has been widely used for this purpose. The threat of methicillin-resistance has receded in recent years, but in this hospital, at least, its gradual disappearance has been accompanied by a steady increase in neomycin-resistance (Barber, 1966).

Of 57 strains referred to in Table IV only two showed even a slight increase in resistance to gentamicin. Cross-resistance between members of this group is interesting: Klein *et al.* (1964) suggest that it may be unidirectional between kanamycin and gentamicin, because organisms trained to the latter become resistant also to the former. It seems more probable that different mechanisms are involved in naturally occurring and artificially produced resistance, as strains habituated to kanamycin *in vitro* also become resistant to gentamicin.

Though there is always some cross-resistance between neomycin and kanamycin, many of the strains tested were much less resistant to the former. It seems probable that these result from treatment with kanamycin, as recently three strains have been isolated after treatment with neomycin which all showed equal resistance to both drugs. In view of the quite low resistance to neomycin sometimes seen kanamycin should be used for diffusion sensitivity tests.

Rountree and Beard (1965) found that neomycin-resistant staphylococci spread only after they had become resistant to bacitracin, and it is now known that resistance to this drug became common in this country at least two years ago (Lancet, 1965), and may therefore have contributed to the spread of neomycin-resistance here. Of 47 strains of neomycin-resistant staphylococci tested against bacitracin only seven were sensitive. Of these, four were from isolated infections (three

being those referred to above) and three were from wards in which neomycin-resistant infections already existed or subsequently occurred.

Treatment of nasal carriers of these organisms presents a problem. Almost all are resistant also to penicillin, streptomycin, tetracycline, and erythromycin, there are obvious objections to the use of methicillin or chloramphenicol, fucidin is irritant as a nasal cream, and a small trial of vancomycin produced uncomfortable side-effects. White (1964) treated 20 nasal carriers with an ointment containing 1 or 3 mg. of gentamicin per gramme and found total suppression of staphylococci while treatment lasted, but a large proportion relapsed after it ceased. The use of gentamicin as a spray may well be more successful and fully justifies clinical trial.

Summary

The antibacterial activity of gentamicin has been examined and compared with that of related antibiotics.

It has two promising properties: (1) consistently high activity against *Ps. pyocyanea*, exceeding that of either streptomycin or kanamycin; and (2) unimpaired activity against staphylococci resistant to neomycin and kanamycin—the majority of these strains (recent clinical isolates) were also resistant to bacitracin.

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Allopurinol* and Acute Uric Acid Nephropathy

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Acute uric acid nephropathy is a rare complication of leukaemia, the reticuloses, and other malignant diseases. The renal tubules are blocked by precipitated uric acid formed by the nucleoprotein breakdown due to treatment with cytotoxic drugs. It has to be distinguished from post-renal obstruction caused by the deposition of uric acid crystals. The nephropathy usually responds to the induction of a vigorous water diuresis and alkalinization of the urine.

Allopurinol (4-hydroxypyrazolo (3,4-d)pyrimidine) is a structural isomer of the purine base hypoxanthine. It reduces uric acid production by inhibiting xanthine oxidase and has been used successfully in gout to control the hyperuricaemia and to promote the mobilization of tophi (Rundles, Wyngaarden, Hitchings, Elion, and Silberman, 1963; Klinenberg, Goldfinger, Miller, and Seegmiller, 1963; Yü and Gutman, 1964; Hall, Holloway, and Scott, 1964; Klinenberg, Goldfinger, and Seegmiller, 1965).

The present paper describes two cases of acute uric acid nephropathy, with observations on the therapeutic use of allopurinol in this condition.

Case 1

A man aged 66 with chronic myeloid leukaemia presented with excessive haemorrhage after the excision of nasal polypi in August 1963. At that time the spleen was enlarged and palpable 9 cm. below the costal margin, and the main haematological findings were: haemoglobin 12.7 g./100 ml.; total leucocyte count 99,000/c.mm.

(promyelocytes 5%, myelocytes 2%, metamyelocytes 45%, segmented neutrophil polymorphs 34%); platelets 292,000/c.mm. The spleen was irradiated and the leucocyte count fell to 37,600/c.mm. He relapsed in January 1964 and was given busulphan (2 mg. daily) with temporary improvement. In August the leucocyte count rose to 66,000/c.mm. ("blasts" 58%) and mercaptopurine 150 mg. daily was given with good response. The drug was stopped and he remained well until November, when the leucocyte count rose again to 190,000/c.mm (blasts 35%) and mercaptopurine was restarted.

Bilateral renal colic developed acutely on 25 December and was associated with dysuria, increased frequency of micturition, and nycturia. However, no calculi were passed and there was no overt haematuria. When admitted to hospital on 1 January 1965 (day 1 of this study, see Fig. 1) he was in considerable pain but not dehydrated, the spleen was enlarged and palpable 8 cm. below the left costal margin, and the blood-pressure was 115/60 mm. Hg. Uric acid crystals and red blood cells were present in the urinary centrifuged deposit, and the peripheral blood contained 102,000 leucocytes per c.mm. (blasts 60%). In spite of a positive fluid balance and the maintenance of a normal blood-pressure the volume of urine decreased from 1,300 ml. on day 2 to 85 ml. on day 10; during this period hyperuricaemia (51 mg./100 ml. on day 10) and azotaemia (blood urea 330 mg./100 ml. on day 10) progressively increased (see Fig. 1). No renal calculi were visible on a plain x-ray film of the abdomen. Retrograde ureteric catheterization was unsuccessful because of extensive inflammatory changes which obscured the ureteric orifices. A diuresis occurred after the administration of 20% mannitol intravenously (300 ml. given in one hour on days 11, 12, and 13). The urine volume reached its maximum (5,485 ml./24 hr.) on day 15. The urine was kept alkaline by intravenous injection of 100–150 mEq of sodium bicarbonate daily, the dose being regulated by measuring the pH of each specimen immediately it was passed. Hypokalaemia, hypo-

* Allopurinol is at present available for clinical trial purposes only.

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