Activity of FL 1060, a new β -lactam antibiotic, against urinary tract pathogens

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SYNOPSIS The effect of the new β -lactam antibiotic FL 1060 on urinary tract pathogens, of which 71 were sensitive to and 100 resistant to ampicillin, is presented here. When tested by conventional methods FL 1060 was found to be highly active against ampicillin-sensitive strains of *E. coli* but phenotypically resistant variants readily emerged, particularly when a large inoculum was used. Thirty-one per cent of ampicillin-resistant strains were found to be sensitive to less than 500 μ g FL 1060 per ml, and in general this antibiotic was more active than ampicillin against resistant strains. In a system simulating the mechanical features of the urinary bladder the activity of FL 1060 against three sensitive strains of *E. coli* was found to be greater than ampicillin in conditions of low, but not high, osmolality.

FL 1060 (6 β -[(hexahydro-1H-azepin-1-yl)-methylene-amino]-penicillanic acid) is the most active of a group of new antibiotics—the 6 β -amidinopenicillanic acids—recently described by Lund and Tybring (1972). Despite a close structural similarity to other β -lactam antibiotics, FL 1060 has different properties (Greenwood and O'Grady, 1973), notably in the nature and the spectrum of its antibacterial activity. It is much more active against Gram-negative than Gram-positive organisms (Lund and Tybring, 1972) so that an important potential therapeutic area is the treatment of urinary tract infection. This paper reports a survey of the activity *in vitro* of the new compound against a variety of urinary tract pathogens and an examination of its activity against infection in a model simulating conditions of bacterial growth in the urinary bladder.

Materials and Methods

FL 1060 powder was supplied by Leo Laboratories Ltd. Dilutions were made in sterile distilled water and kept at 4°C for not longer than four days. The bacteria studied were recent isolates from infected urines sent to the diagnostic laboratory of the hospital. Included in the survey were 71 strains sensitive to ampicillin (18 *Escherichia coli*, 20 *Proteus mirabilis*, 20 *Micrococcus* spp of Baird-Parker's subgroup 3 (Baird-Parker, 1963, 1965) and

13 *Streptococcus faecalis*) and 100 consecutively isolated strains resistant to ampicillin, identified, according to the scheme of Cowan and Steel (1965) as *E. coli* (43), *Klebsiella aerogenes* (13), *Klebsiella* spp (14), *Enterobacter* spp (4), *P. mirabilis* (8), *P. vulgaris* (1), *P. morganii* (1), *Serratia marcescens* (6), *Hafnia* spp (5), *Alcaligenes* spp (1), *Providencia* A and B (3), and *Citrobacter* sp (1). Sensitivity to ampicillin was judged from the zone of inhibition around 10 μ g discs on blood agar seeded to give subconfluent growth.

Minimum inhibitory concentrations (MIC) of FL 1060 were estimated by the agar dilution method using an automatic multipoint inoculator (Denley-Tech Ltd, Billingshurst, Sussex) and sensitivity test agar (Wellcotest, Wellcome Reagents Ltd, Beckenham, Kent). The strains were maintained on nutrient agar slopes, and tubes containing 15 ml nutrient broth were inoculated from the slopes using a straight-wire and incubated at 37°C for six hours. Two series of antibiotic-containing plates were inoculated, one with undiluted culture and the other with culture diluted 1 in 1 000. The prongs of the inoculator deposited about 5×10^5 organisms from the undiluted and about 5×10^3 organisms from the diluted culture. The MIC of FL 1060 for all the ampicillin-resistant strains and for those ampicillin-sensitive strains used for morphological observation of the effect of the antibiotic were also determined by titration in broth (Casitone pancreatic digest, Difco, 10 g; yeast extract, Difco, 5 g; K_2HPO_4 3 g;

KH₂PO₄ 1 g; glucose 5 g and NaCl 5 g per l) containing doubling dilutions of FL 1060 from 500 to 0.015 μ g per ml. One drop of a four-hour broth culture was added to each tube giving a concentration of about 10^4 to 10^5 organisms per ml.

Morphological observations were made by microscopical examination of simple wet preparations.

Three strains of *Escherichia coli* found by tube titration to be highly susceptible to FL 1060 were examined in a mechanical system designed to simulate conditions of bacterial growth in the urinary bladder (Mackintosh, Watson, and O'Grady, 1973; O'Grady, Mackintosh, Greenwood, and Watson, 1973). In this system, 20 ml of a fully grown broth culture, such as occurs in overnight urine or during the day in patients in whom the bladder residual volume is increased, were incubated at 37°C in the light path of a simple photometer which provides a continuous record of the opacity of the culture. Fresh broth was constantly added to the culture by means of a metering pump at a rate of 1 ml/min equivalent to the diurnal ureteric urine flow rate. At one-hour intervals, a pump emptied the 'bladder' (simulating micturition) leaving again the 'residual volume' of 20 ml. The influence of osmolality on the effect of the antibiotic in these conditions was studied by adding sufficient FL 1060 to produce an initial concentration of 500 μ g per ml and diluting the 'bladder' culture at 1 ml per min with broth in which salt was included (osmolality: about 325 mOsm/kg) or was omitted (osmolality: about 170 mOsm/kg). In the latter case the overnight broth culture used to prime the system was also grown in broth without salt.

Results

AMPICILLIN-SENSITIVE ORGANISMS

The MICs of FL 1060 for 58 ampicillin-sensitive strains, read after 18 hr at 37°C and using the light inoculum (about 5×10^2 organisms), are shown in figure 1. Most strains of *E. coli* were inhibited by 0.125 μ g of the antibiotic/ml although one of the 18 strains was resistant to more than 64 μ g FL 1060/ml. After 48 hours' incubation, all the *E. coli* strains grew, even on the plates containing the highest concentrations of FL 1060 (64 μ g/ml), if the heavy inoculum (about 5×10^5 organisms) had been used, but not in the plates seeded with the light inoculum (about 5×10^2 organisms). Five of these strains were also titrated in broth, using an inoculum of 10^4 to 10^5 organisms/ml and incubation was continued until all the tubes showed growth. Considerable variation in behaviour was found. Some strains showed visible growth at concentrations of FL 1060 well above the MIC after 24 to 48 hours'

Fig 1 Distribution of MIC of FL1060 for ampicillin-sensitive strains of *P. mirabilis*, *E. coli*, and *Micrococcus subgroup 3*.

incubation while others did not do so until incubated for 72 hours. Despite the appearance of active regrowth, microscopical examination showed the typical asymmetrically dividing spherical forms induced by exposure to FL 1060 (Greenwood and O'Grady, 1973) even in tubes containing low concentrations of the antibiotic.

The MICs of FL 1060 for the *P. mirabilis* strains were somewhat higher than those for *E. coli* and showed a much greater scatter (fig 1). Regrowth was studied in five of these strains using broth titrations. All yielded visible growth within 28 hr at concentrations of FL 1060 exceeding 64 μ g/ml although on microscopical examination the growing cells showed typical morphological damage.

Among ampicillin-sensitive Gram-positive organisms of 20 strains of *Micrococcus subgroup 3* were all inhibited by 16 to 64 μ g/ml FL 1060, and no regrowth occurred on prolonged incubation of either plates or broth. The 13 strains of *Streptococcus faecalis* were uniformly resistant to 64 μ g/ml.

AMPICILLIN-RESISTANT ORGANISMS

Some strains which grew overnight in high concentrations of FL 1060 showed marked morphological damage which was apparent even on exposure to low concentrations of the drug. The results (table I)

Species	Number of Strains			
	Tested	Inhibited by FL 1060 (500 µg/ml)		
		> 500 Resistant		<500 Sensitive
		Morphological Damage in 1 µg FL 1060/ml		
		0	+	
<i>E. coli</i>	43	22	2	19
<i>K. aerogenes</i>	13	7	3	3
<i>Klebsiella</i> spp	14	10	2	2
<i>Enterobacter</i> spp	4	2	2	0
<i>P. mirabilis</i>	8	8	0	0
<i>P. vulgaris</i>	1	0	1	0
<i>P. morgani</i>	1	0	1	0
<i>S. marcescens</i>	6	5	0	1
<i>Hafnia</i> spp	5	2	0	3
<i>Alcaligenes</i>	1	1	0	0
<i>Providencia</i> spp	3	1	0	2
<i>Citrobacter</i> spp	1	0	0	1
Total	100	58	11	31

Table I Sensitivity of ampicillin-resistant coliform bacilli to FL 1060

Fig 2 Cross-resistance patterns of 31 ampicillin-resistant strains of *E. coli* and other enterobacteria having MICs FL 1060 of 500 µg/ml or less (category 3—see text).

Number of Strains

Sensitive to Ampicillin ($\mu\text{g/ml}$)	In presence of FL 1060 (500 $\mu\text{g/ml}$)		
	Grew Overnight with Morphology		Failed to Grow
	Normal	Abnormal	
> 500	58	4	19
128-500	0	7	6
32- 64	0	0	6
Total	58	11	31

Table II Comparative sensitivity of coliform bacilli to ampicillin and FL 1060

were consequently grouped into three categories: (1) 58 strains resistant to 500 $\mu\text{g/ml}$ which showed no morphological abnormality; (2) 11 strains resistant to 500 $\mu\text{g/ml}$ but showing conversion to spherical forms even at FL 1060 concentrations of 1 $\mu\text{g/ml}$ or below; (3) 31 strains sensitive to 500 or less $\mu\text{g/ml}$.

CROSS RESISTANCE

All the strains in the first category (completely resistant to 500 μg FL 1060 per ml) were also resistant to 500 μg ampicillin per ml. Of the 11 strains in

the second category, seven were partially sensitive to ampicillin of which the MIC for these strains was 128-500 μg per ml (table II). The cross-resistance patterns of the 31 strains in the third category are shown in fig 2: all 19 *Escherichia* and four of the five *Klebsiella* were inhibited by less than 64 μg FL 1060 per ml. Twelve of these 31 strains were partially sensitive to ampicillin and six (four *E. coli*, one *Klebsiella*, one *Providencia*) were sensitive to less than 128 μg ampicillin per ml. Conversely, some sensitivity to FL 1060 was found in 19 strains

Fig 3 Typical opacity trace of an ampicillin-sensitive *E. coli* strain grown in the simulated bladder system in the presence of added NaCl. FL 1060, to achieve a concentration of 500 $\mu\text{g/ml}$, added at time 0. Arrow indicates point at which the concentration of FL 1060 fell below the MIC.

Fig 4 Typical opacity trace of an ampicillin-sensitive *E. coli* strain grown in the simulated bladder system in conditions of low NaCl concentration. FL 1060, to achieve a concentration of 500 $\mu\text{g/ml}$, added at time 0. Arrow indicates point at which the concentration of FL 1060 fell below the MIC.

resistant to 500 µg ampicillin per ml. Of these, 16 (13 *E. coli*) were sensitive to less than 64 µg FL 1060 per ml. Only two strains (one Providence B, one *Klebsiella*) were more sensitive to ampicillin than FL 1060.

SIMULATED BLADDER SYSTEM

The presence or absence of NaCl in the media had no effect on the pattern of growth in the bladder model. Figures 3 and 4 show typical records of the opacity changes produced when highly sensitive strains of *E. coli* were grown in the bladder model in the presence or absence of NaCl with the addition of sufficient FL 1060 to produce an initial concentration of 500 µg/ml. In the presence of 0.5% NaCl (fig 3) a reduction in opacity in excess of that attributable solely to the dilution effect was first detectable about two hr after the addition of FL 1060 and regrowth commenced as the concentration of antibiotic fell below the MIC. In the absence of NaCl (fig 4) a sharp fall in opacity occurred earlier and regrowth took longer. The time at which regrowth was first detected varied with the strain and was as long as 15 hr with one of the three organisms tested in the absence of NaCl. The time which elapsed after the addition of antibiotic before regrowth was detectable is shown for each strain in table III.

Strain	Time (hr) before Detectable Regrowth at Osmolality (mOsm/kg) of	
	170	325
<i>E. coli</i> 1	9	6
<i>E. coli</i> 2	11	9
<i>E. coli</i> 3	15	13

Table III Time after addition of antibiotic before detectable growth

Discussion

As judged by conventional titrations FL 1060 shows outstanding activity against ampicillin-sensitive strains of *E. coli*. All but one of 18 ampicillin-sensitive strains were inhibited by 0.25 µg or less FL 1060 per ml—an activity at least ten times that of ampicillin in similar conditions. However, as we have previously reported (Greenwood and O'Grady, 1973) continued incubation revealed in all cases the presence of a small fraction of the population which was able to continue growth despite morphological evidence of surface damage.

Ampicillin-sensitive *P. mirabilis* strains showed a much wider distribution of sensitivities than *E. coli* and regrowth occurred much sooner. The two factors

are probably related since early regrowth masks the true endpoint. The tendency to regrow quickly may result from the related resistance of *P. mirabilis* strains to osmotic lysis (Greenwood and O'Grady, 1972) and is also in keeping with the hypothesis that phenotypically resistant cells which grow in the presence of FL 1060 have a low internal osmolality (Greenwood and O'Grady, 1973). Only six (4 *E. coli*) of the 100 ampicillin-resistant strains showed the exceptional sensitivity to FL 1060 seen in ampicillin-sensitive strains. Of the rest 31 showed partial sensitivity to FL 1060 (MIC 1 to 500 µg/ml) and many of these also showed some degree of sensitivity to ampicillin; nevertheless FL 1060 was nearly always the more active against these strains (fig 2). In 19 cases (13 *E. coli*) organisms resistant to 500 µg ampicillin per ml were inhibited by less than 64 µg FL 1060 per ml after overnight incubation.

In the system designed to simulate the mechanical features of the urinary bladder considerable strain variation was found among highly sensitive *Escherichia* corresponding with the differences previously demonstrated when the same strains were exposed in a static system (Greenwood and O'Grady, 1973). A single pulse of FL 1060 was found to suppress the growth of those sensitive strains of *E. coli* from a very high inoculum for six to 13 hours in the presence of 0.5% NaCl (osmolality of medium about 325 mOsm/kg) and nine to 15 hours in the absence of NaCl (osmolality of medium 170 mOsm/kg). The osmolality of 325 mOsm/kg falls within the normal range for human urine; that of 170 mOsm/kg is easily achieved by increasing fluid intake. At the higher osmolality, such strains exposed to a 500 µg per ml dose of ampicillin grow within about 10 hours (O'Grady *et al*, 1973) so that in these circumstances the performance of the two agents is similar despite the higher activity of FL 1060 in conventional titration. However, the effect of osmolality on performance is greater with FL 1060 than with ampicillin (Greenwood and O'Grady, 1973, and unpublished observations) so that in the conditions of lower osmolality the higher activity of FL 1060 became more evident.

The therapeutic significance of phenotypic variants which, although remaining morphologically reactive to FL 1060, are no longer inhibited by the antibiotic (Greenwood and O'Grady, 1973) has not yet been established. It may be that such damaged cells will succumb readily to natural defence mechanisms. In addition, their survival in the presence of the drug probably depends on their having an internal osmolality less than that of the growth medium and it is for this reason that lowering the osmolality of the medium markedly enhances the effect of FL 1060. It may be, therefore, that the benefits of

combining antibiotic therapy with diuresis in the treatment of urinary tract infection which have previously been argued on other grounds (Cattell, Sardeson, Sutcliffe, and O'Grady, 1968) will prove to be of particular importance with this agent.

We are indebted to Leo Laboratories for a grant towards the cost of this work.

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