Research Article

AN INSIGHT FOR SCREENING OF ANTIBIOTICS FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT

The general principles of screening antibiotics for antimicrobial activity are similar to those for screening of compounds for other pharmacological effects. The system should be adapted to the specific character of the test substance and the objectives of the program. In the screening of β-lactams, standard tests, such as determination of the MICs, effects of inoculum size or activity against systemic infection in mice, should be supplemented by less conventional studies on for instance activity against dormant bacteria or, in the case of penems or carbapenems, stability in the presence of kidney and lung dehydropeptidases.

Keywords: Carbapenam, β-lactam antibiotics, Screening, Dehydropeptidases.

INTRODUCTION

No functional screening system for antibacterial activity could be established that would be applicable to antibiotics of every class and still furnish relevant results without becoming oversized and cumbersome. This brief contribution is accordingly not intended for presentation of a generally valid design for an antibacterial screening system. Introductory comments of a general nature are followed by specific recommendations based on the authors' personal experience confined to the setting up of rather unconventional tests for screening penems. Enumeration and discussion of generally known screening tests such as determination of the MIC, assessment of activity against systemic infections in mice etc, are omitted. When establishing a screening system for antibiotics, the following basic principles have to be observed:

• The selection of tests is very largely dictated by the specific properties of the particular class of antibiotics under investigation.

• The system should have a high through-put and assure a high degree of reproducibility of the results, without requiring undue time and labor.

• The system should not be regarded as a rigid and unalterable series of tests. The methods should conform to the prevailing state of the art; however, uniformity of the reference values must be ensured throughout the screening program.

** In this paper, the term 'antibiotic' is used to refer to both synthetic and semi synthetic derivatives.

The penems and carbapenems constitute a relatively new class of antibiotics. Comparatively little is therefore known about the extent to which results of an in vitro test may be extrapolated to an in vivo model and to clinical situations. Moreover, these antibiotics differ, sometimes very distinctly, from other β-lactams in their antibiotic properties. For that reason, it is likely to be advantageous if the primary screen is already designed in accordance With the principle formulated by Wold[2]: i.e. to include as many tests and obtain as many data as possible.

Stability against Dehydropeptidases

Measurement of the rate of hydrolysis of penems and carbapenems by dehydropeptidase I from the human and mouse kidney should be part of the primary screen. Owing to the high degree of extra renal metabolism in rodents[3], it is recommended that the stability of the antibiotics be examined not only in homogenates of human and mouse kidney, but also in lung homogenares. Ideally, purified enzymes should be used.

Pharmacokinetics

Knowledge is particularly lacking on the predictability of the pharmacokinetic behavior of penems and carbapenems in man on the basis of animal data, including absorption from the gastrointestinal tract, and the correlation between their pharmacokinetics and therapeutic activity. A simple bioassay for determination of the main pharmacokinetic parameters in mice would be invaluable for the interpretation of discrepancies between in vitro and in vivo results.

Measurement of the stability of the compounds in mouse or rat serum should be mandatory. Before the results of specific pharmacokinetic studies become available information on the extent of binding to both human and mouse serum protein is considered helpful in estimating the time an antibiotic is present in the body and the ability of an antibiotic to penetrate into deep compartments.

Specific characteristics of penems and carbapenems[1] (selected examples)

Recognition by renal dehydropeptidase-I

Recognition by a lung lactamase of rodents

Pharmacokinetics

Stability against β lactamases

Activity against resistant bacteria

Relative loss of activity against *Haemophilus*, *Proteus* and *Pseudomonas spp.*

Activity against nongrowing bacteria

Stability against β-Lactamases

Penems and carbapenems are generally highly resistant to almost all bacterial β-1actamases; they would appear to be readily hydrolyzed only by the enzyme of *Pseudomonas maltophilia*[4] testing for stability against this β-1actamase would increase the structure-enzyme stability relationship and might finally lead to an antibiotic with enhanced activity against the pathogen. The tests can be performed with either crude or purified enzyme.

As illustrated in Table 1, tests of stability against other β-lactamases can easily be performed by simple determination of the MIC values for isogenic pairs of strains, one of which produces the β-1actamase constitutively, while the other does not.

Activity against Resistant Bacteria

Instead of determining MICs for hundreds of resistant strains, investigations using bacterial mutants with various well-defined mechanisms of resistance can be performed to advantage. A few examples of such mutants are given in Table 2.

Mode of Action[3]

Some companies actively pursuing β-lactam programs include tests for affinity to penicillin binding proteins (PBPs) in the screen to obtain basic information on the mode of action of the compounds. However, these studies are more appropriately performed at a more advanced stage of screening. On the other hand, it could conceivably be advantageous to test penems or carbapenems for affinity to a particular PBP. There would seem to be a fairly good correlation between the affinity of β-1actams to PBP 7 and their capacity to lyse non growing *Escherichia coli*[14] (Table 3). This test would be easier to perform than direct investigations on the activity of an antibiotic against nongrowing or slowly growing organisms, which require highly specialized know-how and equipment (chemostats).

Activity against Slowly Growing Bacteria

There remains little doubt that bacteria which have grown in vivo in infected animals or humans differ considerably in many respects from those grown *in-vitro*, for instance in their slower rate of multiplication[15, 16].

If the know-how is available, tests can be established to determine the activity of antibiotics against bacteria multiplying at various generation times. For instance, a screen can be run to test the sensitivity of glucose-limited cultures of *Escherichia coli* grown at different multiplication rates, as shown in Table 4. If activity is detectable, different bacteria cultured under different limitations, e.g. amino acids, phosphate, magnesium or iron, can be used in more advanced tests.

Table 1: Beta lactamase stability of carbapenam and ceftrioxone expressed as MIC ratio for isogenic pairs of strains one strain constitutively producing β-1actamase, the other not[15]

Pair of strains[a]	Betalactamase Type (5)	MIC ratio[b] carbapenem	MIC ratio[b] Ceftrioxone
Enterobacter cloacae 908R/908S (6)	Ia	1.5	2048
Escherichia coli 255/255-L-7 (7)	Ib	0.25	64
Proteus vulgaris GN76C/GN76C-1-2 (7)	Ic	0.5	2048
Pseudomonas aeruginosa 18SI-I/18S-5	Id	2	>256
Proteus mirabilis N29/N29-5 (7)	IIb	0.5	1
Klebsiella pneumonia G N691692-1 (7)	IIIa	0.25	1
Enterobacter cloacae 53/X(8)/206	IVa	ND	ND
Escherichia coli MI1410RGN238/ML1410 (7)	Va	0.5	1
Pseudomonas aeruginosa PA0303R 151/PA0303 (9)	Vb	0.25	2
Pseudomonas aeruginosa PA0303RIP-641PA0303 (9)	V c, d	0.25	1
Pseudomonas aeruginosa PA0303 Rms-149/PA0303 (9)	Vd	0.25	1
Staphylococcus aureus 2999/2999pa		0.5	0.5

Table 2: Activity of carbapenem and ceftriaxone against strain pairs with various mechanisms of resistance[15]

Strain pair [a]	Resistance Mechanism	MIC ratio[b] carbapenem	MIC ratio[b] Ceftrioxone
Escherichia coli D21/D21f2 (10)	**Permeability** smooth/deep rough	0.5	2
Escherichia coli CS483/W3110(11)	porin Ic/porins Ia+Ib	0.5	8
Escherichia coli JE5509/5990 (12)	**Targets** PBP4+/PBP4-	0.25	8
Escherichia coli JE5509/5683 (12)	PBPIa, 4, 5, 6+/PBPIa, 4, 5, 6-	2	64
Pseudomonasaeruginosa PCCM/PAO503 (13)	**Intrinsic resistance** Over expression of PBP6+/PBP6-	0.5	32

[a]Reference for the strains in brackets; [b]MIC of constitutively producing strain to MIC of deficient strain; ND = not determined.

Table 3: Lytic activity of selected β-1actams against nongrowing *Escherichia coli* and affinity to the penicillin binding protein 7 (PBP 7)[15]

Antibiotic	Affinity to PBP7	Lysis of nongrowing bacteria by 10×MIC
Cefsulodin	-	-
Penem CGP 30779	-	-
Penem CGP 31608	+	+

Table 4: Activity of two β-1actams against *Escherichia coli* grown in a chemostat under conditions of glucose limitation[15]

Antibiotic	Generation time (hr)	Log_{10} CFU reduction/hr	
		1xMIC	10xMIC
Ceftazidime	7 3.2 1.4	ND 0.04 ND	0.09 0.05 0.1
Penem 31608	7 3.5 1.5	0.05 0.09 0.2	0.35 0.46 0.56

CONCLUSION

The results obtained during this study indicate that antibacterial compound produced more potent activity against various bacterial strains like *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumoni, B. subtilis* and *Staphylococcus aureus* and showing MIC at a very low effective dose. This investigation reveals the importance of various antibacterial agents in the evaluation of antibacterial activity.

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