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Quantitative relationships between in vitro antibacterial activities of cephalosporins and their *n*-octanol/water partition coefficients

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Summary

Quantitative structure/activity relationships between in vitro antibacterial activities of 7 cephalosporins in clinical use and their partition coefficients in an *n*-octanol/aqueous buffer system (pH 7.40) at 37°C have been studied. Parabolic relationships were obtained using the minimum inhibitory concentrations (MIC) against 3 Gram-positive species, and optimum partition coefficients were determined. Poor correlations were found to exist between lipophilicity and in vitro antibacterial activities against 3 Gram-negative species. The results are discussed with reference to the physicochemical and biological factors which are known to influence the susceptibilities of Gram-negative and Gram-positive cells to β-lactam antibiotics.

Introduction

Although a number of research groups have investigated the correlation of antimicrobial activities of penicillins with their partition coefficients (Biagi et al., 1970; Hansch and Deutsch, 1965; Hansch and Steward, 1964), there is little published information on structure/activity relationships between the lipophilicity of cephalosporins and their antibacterial activities. Biagi et al. (1970) investigated the influence of lipophilic character, expressed as the chromatographic R_m-value determined in a silicone oil/acetone–water system, on the in vitro activities of a number of penicillins and cephalosporins against *S. aureus*, *E. coli* and *T. pallidum*. Boyd (1982) referred to the use of *n*-octanol/water partition coefficients as one of a number of physicochemical properties of cephalosporins included in a pattern recognition study undertaken at Lilly Research Laboratories. The biological data employed included minimum inhibitory concentrations. Details of the results were not reported, but the author stated that the *n*-octanol/water partition coefficients did not correlate well with antibacterial activities.

Davies and Morris (1983) analysed the in vitro potency data of 16 penicillins and cephalosporins against a series containing 15 Gram-positive and Gram-negative strains. Two leading eigenvectors were identified in the MIC data: (a) a membrane access factor, which is related to drug hydrophobicity; and (b) a β-lactam ring-opening rate factor, which is related to β-lactam reactivity.

Abbreviations: MIC, minimum inhibitory concentration; log P, log of the partition coefficient; I, molar ionic strength; THAM, tris(hydroxymethyl)aminomethane.

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Materials and Methods

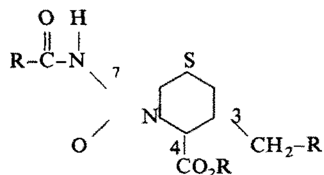
Materials

Antibiotic samples were generously supplied by pharmaceutical manufacturers (Glaxo and The Lilly Research Centre, U.K.; Smith Kline and French Labs., U.S.A.; Bristol Sermoneta, Italy).

Aqueous buffers and *n*-octanol were presaturated with each other before use. Buffer systems were phosphate-citrate (pH 2.90–5.00) and phosphate-THAM (pH 7.10–8.00), and were adjusted to ionic strength, I, of 0.2 M with potassium chloride.

TABLE 1

CEPHALOSPORIN DERIVATIVES USED IN QSAR STUDY



Compound	R	R′	R″
Cephalothin sodium	S CH_2	–O–C(=O)–CH_3	–Na
Cephaloridine	S CH_2	–$^+$N	–
Cephalexin monohydrate	–C(H)(NH_2)–	–H	–H · H_2O
Cephradine	–C(H)(NH_2)–	–H	–H
Cefazaflur sodium	CF_3 · S · CH_2	–S–C, N=N, N, N–CH_3	–Na
Cefazolin sodium	N=N, N=C, >N · CH_2	–S–C, N–N, S, C–CH_3	–Na
Cephaloglycin dihydrate	–C(H)(NH_2)–	–O–C(=O)–CH_3	–H · $2H_2O$

Experimental methods

The 7 cephalosporins used in the present study are shown in Table 1. The partition coefficients were determined using a shaker-flask method by fluorimetrically assaying the aqueous phases after partitioning at 37°C. Equilibration times were 7 h for cephaloridine and 6 h for the other compounds.

In the case of the monoprotic monoionic compounds cefazolin, cephalothin and cefazaflur, the acid dissociation constants were determined potentiometrically at 37°C and I = 0.2 M. These pK_a values were combined with the values of the apparent partition coefficients measured in the pH range 2.90–5.00 to calculate the partition coefficients, P, at pH 7.40, using equations previously reported (Scherrer and Howard, 1977; Tsuji et al., 1977).

In the case of the other 4 cephalosporins, the partition coefficients were measured directly at pH 7.40. Log P values are listed in Table 2.

TABLE 2

PARTITION COEFFICIENTS OF CEPHALOSPORINS IN AN *n*-OCTANOL/AQUEOUS BUFFER SYSTEM (pH 7.40) AT 37°C

Compound	log P
Cefazolin	−4.92
Cephalothin	−4.30
Cefazaflur	−3.98
Cephaloridine	−1.52
Cephradine	−1.15
Cephalexin	−1.10
Cephaloglycin	−1.05

Biological data

The antimicrobial data used in the correlation analysis are shown in Table 3. These data were obtained from 4 publications (Actor et al., 1977; Laverdiere et al., 1978; Shadomy et al., 1977; Webber and Ott, 1977) and are in vitro MICs in μg/ml against 3 Gram-negative and 3 Gram-positive species. All strains were clinical isolates and the MIC assays were performed in the absence of added serum. The *Staphylococcus* data refer to penicillinase-producing strains.

In the QSAR analysis, MIC values are used in preference to log (1/MIC) for the reasons given by Boyd (1983).

Results

Parabolic regression analyses of minimum inhibitory concentrations and log P (at pH 7.40) of the cephalosporins are presented in the following equations. Linear analysis showed poorer correlation in every case.

TABLE 3

IN VITRO MINIMUM INHIBITORY CONCENTRATIONS (MIC) IN μg/ml OF CEPHALOSPORINS AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE SPECIES

Compound	*Escherichia coli* [a]	*Enterobacter aerogenes* [a]	*Salmonella heidelberg* [a]	*Staphylococcus aureus* [b]	*Staphylococcus epidermidis* [c]	*Streptococcus pneumoniae* [d]
Cefazolin	2	2	0.9	1.05	0.26	0.10
Cephalothin	17	5	2	0.57	0.39	0.24
Cefazaflur	0.7	0.4	0.1	0.72	0.34	0.16
Cephaloridine	5	4	4	2.29	0.20	0.08
Cephradine	20	16	14	6.14	4.17	1.32
Cephalexin	9	7	7	7.36	4.86	1.64
Cephaloglycin	2	1	1	4.56	3.82	0.59

[a] Data from Webber and Ott (1977).
[b] Data from Webber and Ott (1977), and Laverdiere et al. (1978).
[c] Data from Laverdiere et al. (1978).
[d] Data from Actor et al. (1977), and Shadomy et al. (1977).

Gram-negative species

Escherichia coli:

$$\text{MIC} = -0.332(\log P)^2 - 1.061(\log P) + 8.281$$
$t = -0.09$ $t = -0.05$
$(P = 0.925)$ $(P > 0.925)$
$n = 7$ $r = 0.190$ $s = 9.317$

Enterobacter aerogenes:

$$\text{MIC} = 0.570(\log P)^2 + 4.627(\log P) + 11.721.$$
$t = 0.24$ $t = 0.34$
$(P = 0.82)$ $(P = 0.75)$
$n = 7$ $r = 0.460$ $s = 5.825$

Salmonella heidelberg:

$$\text{MIC} = 0.537(\log P)^2 + 4.738(\log P) + 11.389$$
$t = 0.27$ $t = 0.42$
$(P = 0.80)$ $(P = 0.70)$
$n = 7$ $r = 0.598$ $s = 4.852$

Gram-positive species

Staphylococcus aureus:

$$\text{MIC} = 0.888(\log P)^2 + 6.414(\log P) + 11.575$$
$t = 1.50$ $t = 1.89$
$(P = 0.208)$ $(P = 0.13)$
$n = 7$ $r = 0.905$ $s = 1.447$
$\log P_0 = -3.61$

Staphylococcus epidermidis:

$$\text{MIC} = 0.781(\log P)^2 + 5.405(\log P) + 8.736$$
$t = 1.39$ $t = 1.69$
$(P = 0.24)$ $(P = 0.17)$
$n = 7$ $r = 0.855$ $s = 1.368$
$\log P_0 = -3.46$

Streptococcus pneumoniae:

$$\text{MIC} = 0.174(\log P)^2 + 1.234(\log P) + 2.165$$
$t = 0.78$ $t = 0.97$
$(P = 0.48)$ $(P = 0.39)$
$n = 7$ $r = 0.717$ $s = 0.545$
$\log P_0 = -3.55$

Discussion

Gram-positive and Gram-negative species

The mode of action of β-lactam antibiotics is well established. They act by interfering with the biosynthesis of the peptidoglycan component of bacterial cell walls by reacting with two categories of enzyme involved: transpeptidase and carboxypeptidase (Richmond, 1978).

In addition to the peptidoglycan structure, Gram-negative organisms possess an outer cell-wall membrane which has an organised structure and is composed of protein, lipoprotein, phospholipid and lipopolysaccharide (Nikaido, 1979). β-Lactam antibiotics penetrate this membrane almost exclusively through water-filled porins, and the rates of penetration have been shown to be linearly related to the *n*-octanol: water partition coefficients of the unionised forms of mono-anionic cephalosporins. Decreased hydrophobicity improves penetration rate. Positively-ionised groups on the molecule promote, and negative groups retard, penetration (Nikaido et al., 1983). However, penetration times as measured by $t_{1/2}$ (the time taken, after application of antibiotic solution to a cell suspension, for intracellular concentration to reach half the extracellular concentration) are expressed in seconds or fractions of seconds and are very short in comparison with normal drug exposure times. Therefore, penetration rates might not affect antibacterial efficacy, and in fact pairs of antibiotics with similar $t_{1/2}$-values are found which do not have similar potencies against Gram-negative organisms (Nakaido, 1981). An examination of Table 2 of this communication shows that in general low MIC values against Gram-negative species are not associated with the rapidly penetrating zwitterionic compounds cephaloridine, cephradine, cephalexin and cephaloglycin.

Theoretically, the ability of a β-lactam antibiotic to elicit a biological response may be influenced by any or all of at least 3 characteristics:

(a) ability to penetrate to the target enzyme; (b) resistance to inactivation by β-lactamase enzymes; and (c) chemical reactivity at the receptor site (Davies and Morris, 1983; Richmond, 1978). The factors which predominate in determining the effectiveness of cephalosporins in inhibiting the growth of Gram-negative bacteria are apparently not the same as those for Gram-positive bacteria. The evidence for this is that: (a) MICs against many Gram-negative species correlate well with those against other Gram-negative species, and similarly for Gram-positive species, but MIC data for Gram-negative species do not correlate significantly with the data for Gram-positive species (Davies and Morris, 1983); and (b) the quantum mechanical indices (these indices correlate well with each other) which reflect the reactivity of the electrophilic β-lactam structure correlate well with the in vitro antibacterial activities of cephalosporins against Gram-negative, but not Gram-positive, cells (Boyd, 1982). Thus, β-lactamase resistance and cell penetration seem to be relatively unimportant in Gram-negative species (Boyd, 1982).

Useful QSAR indices are more elusive in the case of Gram-positive organisms. Boyd (1982) quotes the results of a physicochemical pattern recognition study using two series of experimental cephalosporins and activity against *S. aureus* (penicillinase-producing) and *S. heidelberg*. The study produced relationships with relatively poor correlation coefficients in the case of *S. aureus* ($n = 8$ and 11, $r = 0.31–0.78$) for various parameters. It is stated in the report on that study that log P (octanol : water, pH 3 and pH 7) did not correlate well with antibacterial activity (expressed as MIC and growth inhibition coefficient R) against either species. Interestingly, the R-values are related to MIC with low values of the correlation coefficient ($r^2 < 0.5$).

The results of our study, however, indicate that hydrophobicity is an important physicochemical property of clinical cephalosporins in their in vitro antibacterial activity (expressed as MIC) against Gram-positive bacteria, and *Staphylococcus* species in particular. Log P (at pH 7.40) and MIC values against Gram-negative cells are not found to be significantly interrelated.

The results may not mean that cell wall penetrability is a dominant factor in determining activity against Gram-positive species, however, as the peptidoglycan wall structure in these cells does not apparently limit access by antibiotics, though the cytoplasmic membrane may do so (Richmond, 1978). In general, other biological properties besides passive cell penetration may be related to hydrophobicity, as has been shown for instance in studies on β-lactam antibiotics and interaction with haptens (Bird, 1975) and serum protein binding (Bird and Marshall, 1967).

Hydrophobicity is not likely to correlate significantly with the chemical reactivity of β-lactams as electrophiles at the enzyme receptors. Since, as discussed above, the latter property seems to be the most important characteristic of cephalosporins in determining their activity against Gram-negative microorganisms, the poor Gram-negative MIC/log P correlations obtained in our study are not unexpected.

It may also be significant in the context of Gram-negative cells that Nikaido et al. (Nikaido, 1981; Nikaido et al., 1983) have approached the problem of variable ionisation patterns among cephalosporins by using the partition coefficients of unionised forms, even when these values had to be calculated, or the unionised molecules were hypothetical. They dealt separately with the effect of charged groups on the molecules. The fact that porin permeability (in wild type *E. coli* at least) and P (unionised) were shown to be linearly related suggests that the latter property is the dominant hydrophobic factor in outer membrane penetration, if not MIC. This appears to be the case despite the fact that all cephalosporins exist predominantly in an ionised form at pH 6, the pH at which the experiments of Nikaido et al. were conducted. The fractions are roughly one unionised molecule in every 3500 for monoanionic compounds like cefazaflur ($pK_a = 2.45$, this research), and one in every 10,000 for dipolar compounds like cephalexin (Streng, 1978). Cephaloridine does not exist in an unionised form.

Our approach to the problem of different ionisation patterns has been to use partition coefficients at physiological pH, obtained by extrapolation if necessary. Our log P data include directly

measured partition coefficients for the dipolar compounds cephradine, cephalexin and cephaloglycin at pH 7.40, and this may represent the transfer of anions into the *n*-octanol phase (Purich et al., 1973).

Optimum partition coefficients

Log P_0 is defined as the log of the partition coefficient corresponding to the minimum value of MIC in a parabolic relationship. For *S. aureus,* our analysis yields: log $P_0 = -3.61$. Cefazaflur, with a measured log $P = -3.98$, is closer to the optimum hydrophobicity than any of the other cephalosporins in our series.

Statistical note

The amount of variance "explained" (r^2) by the log P (at pH 7.40) data in our study are: *S. aureus,* 82%; *S. epidermidis,* 73%; *Streptococcus pneumoniae,* 51%.

The unreliability of biological data in assays of the MIC type may mean that QSAR results are less certain than the statistics suggest. MIC data averaged for many species has been suggested as a means of reducing this variance (Boyd, 1982; Boyd et al., 1980).

Conclusion

The critical role of lipophilicity in some biological properties of β-lactam antibiotics has been established by QSAR studies, e.g. gastrointestinal absorption (Yoshimura and Kakeya, 1983), serum protein binding (Bird and Marshall, 1967) and biliary excretion (Ryrfeldt, 1971). The results of our research indicate that hydrophobicity may be a significant physicochemical property of cephalosporins in determining MICs against Gram-positive microorganisms.

References

Actor, P., Guarini, J.R., Uri, J., Bartus, H.F., Zajac, I. and Weisbach, J.A., In vitro studies with cefazaflur and other parenteral cephalosporins. *J. Antibiot.,* 30 (1977) 730–735.

Biagi, G.L., Guerra, M.C., Barbaro, A.M. and Gamba, M.F., Influence of lipophilic character on the antibacterial activity of cephalosporins and penicillins. *J. Med. Chem.,* 13 (1970) 511–516.

Bird, A.E., Hansch analysis of interaction of haptens with benzylpenicilloyl antibodies. *J. Pharm. Sci.,* 64 (1975) 1671–1674.

Bird, A.E. and Marshall, A.C., Correlation of serum binding of penicillins with partition coefficients. *Biochem. Pharmacol.,* 16 (1967) 2275–2290.

Boyd, D.B., Theoretical and physicochemical studies on β-lactam antibiotics. In Morin, R.B. and Gorman, M. (Eds.), *Chemistry and Biology of β-Lactam Antibiotics, Vol. 1,* Academic Press, London, 1982, pp. 437–545.

Boyd, D.B., Substituent effects in cephalosporins as assessed by molecular orbital calculations, nuclear magnetic resonance, and kinetics. *J. Med. Chem.,* 26 (1983) 1010–1013.

Boyd, D.B., Herron, D.K., Lunn, W.H.W. and Spitzer, W.A., Parabolic relationships between antibacterial activity of cephalosporins and β-lactam reactivity predicted from molecular orbital calculations. *J. Am. Chem. Soc.,* 102 (1980) 1812–1814.

Davies, R.H. and Morris, T.R., β-Lactam ring stability and antibacterial potency: a novel eigenvalue problem. *Int. J. Quantum Chem.,* xxiii (1983) 1385–1405.

Hansch, C. and Deutsch, E.W., The structure–activity relationship in penicillins. *J. Med. Chem.,* 8 (1965) 705–706.

Hansch, C. and Steward, A.R., The use of substituent constants in the analysis of the structure–activity relationship in penicillin derivatives. *J. Med. Chem.,* 7 (1964) 691–694.

Laverdiere, M., Welter, D. and Sabath, L.D., Use of a heavy inoculum in the in vitro evaluation of the anti-staphylococcal activity of 19 cephalosporins. *Antimicrob. Agents Chemother.,* 13 (1978) 669–675.

Nikaido, H., Permeability of the outer membrane of bacteria. *Angewandte Chemie Int. Ed. Engl.,* 18 (1979) 337–350.

Nikaido, H., Outer membrane permeability of bacteria: resistance and accessibility of targets. In Salton, M. and Shockman, G.D. (Eds.), *β-Lactam Antibiotics,* Academic Press, London, 1981, pp. 249–260.

Nakaido, H., Rosenberg, E.Y. and Foulds, J., Porin channels in *Escherichia coli*: studies with β-Lactams in intact cells. *J. Bacteriol.,* 153 (1983) 232–240.

Purich, E.D., Colaizzi, J.L. and Poust, R.I., pH–Partition behaviour of amino acid-like β-lactam antibiotics. *J. Pharm. Sci.,* 62 (1973) 545–549.

Richmond, M.H., Factors influencing antibacterial action of β-lactam antibiotics. *J. Antimicrob. Chemother.,* 4 (Suppl. B) (1978) 1–14.

Ryrfeldt, A., Biliary excretion of penicillins in the rat. *J. Pharm. Pharmacol.,* 23 (1971) 463–464.

Scherrer, R.A. and Howard, S.M., Use of distribution coefficients in quantitative structure–activity relationships. *J. Med. Chem.,* 20 (1977) 53–58.

Shadomy, S., Wagner, G. and Carver, M., In vitro activities of five oral cephalosporins against aerobic pathogenic bacteria. *Antimicrob. Agents Chemother.,* 12 (1977) 609–613.

Streng, W.H., Microionization constants of commercial cephalosporins. *J. Pharm. Sci.,* 67 (1978) 666–669.

Tsuji, A., Kubo, O., Miyamoto, E. and Yamana, T., Physicochemical properties of β-lactam antibiotics: oil–water distribution. *J. Pharm. Sci.,* 66 (1977) 1675–1679.

Webber, J.A. and Ott, J.L., Structure–activity relationships in the cephalosporins II: Recent developments. In Perlman, D. (Ed.), *Structure–Activity Relationships among the Semisynthetic Antibiotics,* Academic Press, London, 1977, pp. 161–168.

Yoshimura, Y. and Kakeya, N., Structure–gastrointestinal absorption relationship of penicillins. *Int. J. Pharm.,* 17 (1983) 47–57.