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ADVANCES IN ANTIMICROBIAL CHEMOTHERAPY

Acyclovir, Ketoconazole and the Third Generation Cephalosporins

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"The dominant factor in the emergence and spread of antibiotic-resistant microbial pathogens, whether in hospital wards or in the community, is clearly the intensive use of antibiotic agents to which resistance emerges and then spreads."

Maxwell Finland, M.D.

ACYCLOVIR

Acyclovir [ACV, acycloguanosine, 9-(2-hydroxyetroxymethyl)guanine, Zovirax®] is a new anti-herpesvirus agent that has a unique mechanism of action. Its structural formula is shown in Figure 1.

STRUCTURAL FORMULA:

(parent) (Na salt)

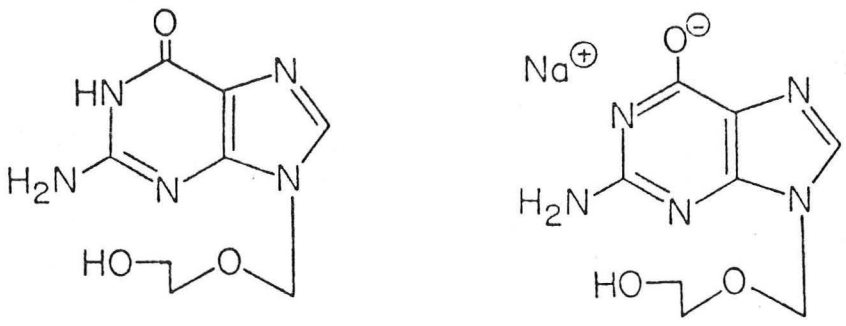


Figure 1

The sodium salt is highly soluble in water (6.24 gm/dl); the parent compound has lesser solubility (0.13 gm/dl). The solubility characteristics of the compounds have an important clinical implication. Parenteral administration involves the use of the sodium salt. When reconstituted, the salt can be administered intravenously in a small volume of fluid (water, normal saline, D5W, D5NS, etc.). Since 62-91% of the drug is excreted into the urine intact as the parent compound, crystalluria may occur if the urine volume is

insufficient. For example, the dose presently used in the treatment trial of herpes simplex encephalitis (HSE) is 10 mg/Kg at 8 hour intervals for 10 days. For a 70 Kg person, the daily dose is 2100 mg. If 62-91% of the drug is delivered intact into the urine as the parent compound, crystalluria may result if the urine volume is less than 1000 ml/day. In actuality, significant crystalluria resulting in blockage of the collecting ducts has occurred only in bone marrow recipients being given large quantities of the drug for interstitial pneumonia due to cytomegalovirus (CMV). The blockage with resulting azotemia has, to date, always been reversible with the administration of fluid. The oral and topical forms of the drug involve the use of the parent compound. The solubility of adenine arabinoside 0.06 gm/dl is less than that of the parent compound, ACV (0.13 gm/dl). The solubility characteristics of Ara-A necessitate the administration of the drug in a large quantity of vehicle, viz., 1 mg. of the drug in 2 ml of vehicle. For example, the therapy of HSE in a 70 Kg person requires the administration of Ara-A at 15 mg/Kg over a 12 hour period of 1050 mg of drug in 2100 ml of fluid. Since patients with HSE are in danger from intracranial hypertension, limiting the volume of fluid intake is a significant factor in therapy.

In man, the only significant metabolic breakdown product of ACV involves 8-14% of the compound and appears as 9-(carboxymethoxymethyl)guanine (CMMG). This metabolite represents the oxidation product of the terminal CH_2OH group of the side chain to a COOH group. With impairment of renal excretion of ACV, there is increased formation of CMMG.

MECHANISM OF ACTION

The mechanism of action of ACV is novel and enables the compound to have a high therapeutic ratio. The mechanism of action shared by another new drug,

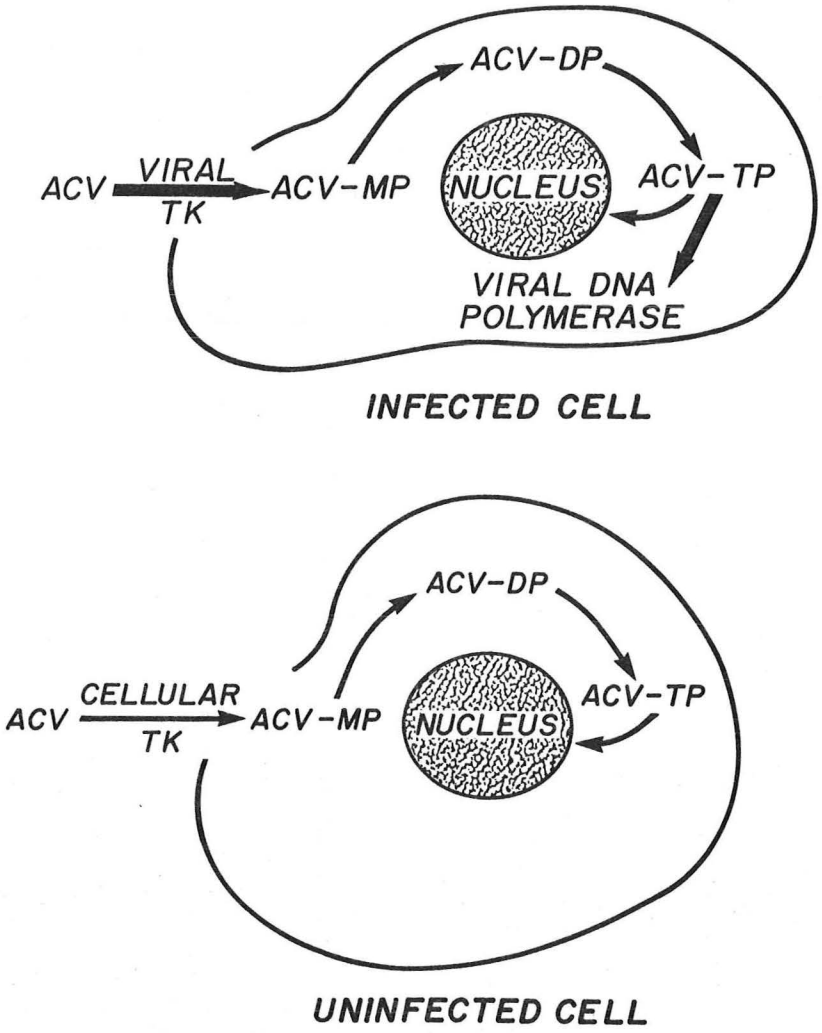

ACV-DP
ACV
VIRAL
TK
ACV-MP
NUCLEUS
ACV-TP
VIRAL DNA
POLYMERASE
INFECTED CELL
ACV-DP
ACV
CELLULAR
TK
ACV-MP
NUCLEUS
ACV-TP
UNINFECTED CELL

Figure 2

bromovinyldeoxyuridine (BVDU), is sufficiently distinctive that these compounds have been termed second generation anti-herpesvirus agents. The active form of the compound is ACV-triphosphate (ACV-TP). The formation of the monophosphate is the rate limiting step with the phosphate being added to the acyclic side chain. The viral coded enzyme, thymidine kinase (TK), converts ACV to ACV-MP. Cellular enzymes then convert ACV-MP to ACV-DP and ACV-TP. Cellular enzymes have a limited capacity to convert ACV to ACV-MP. The ACV-TP acts to inhibit viral DNA polymerase. ACV-TP is also incorporated into viral DNA where it acts as a chain terminator. ACV-TP has only a limited effect on cellular DNA polymerases. As a consequence of the action of viral TK, ACV-TP, the active form of the drug, preferentially accumulates in virus infected cells resulting in a high therapeutic index since uninfected cells have relatively small quantities of ACV-TP (Figure 2).

The quantities of viral coded enzyme, TK, are greatest for herpes simplex virus, types 1 and 2 (HSV-1 and HSV-2) and varicella-zoster virus (VZV). The amount of TK coded for by Epstein-Barr virus (EBV) is apparently limited while CMV does not code for TK. Within the HSV group of viruses, there are quantitative differences in the amount of TK formed. Although EBV probably directs little or no TK formation and the accumulation of ACV-TP in EBV-transformed cells is limited, there is an effect of EBV on viral replication since its DNA polymerase appears exquisitively sensitive to inhibition by ACV-TP. Except at very high concentrations of ACV, this drug has little effect on CMV replication. ACV has no effect on vaccinia virus, a DNA virus. If HSV is added to a vaccinia virus tissue culture system, however, vaccinia virus replication is inhibited because the HSV-TK can convert ACV to ACV-MP and then subsequently this compound can be converted to ACV-TP.

SPECTRUM OF ACTION

ACV is most active against HSV-1 followed by HSV-2, VZV and EBV. It has marginal activity against CMV. Within each virus type, there also is a spectrum of activity. The most sensitive HSV-1 isolate was inhibited by 40 times less drug than the least sensitive isolate in one study. Achieveable ACV plasma concentrations generally exceed levels required to inhibit relatively resistant isolates of VZV, HSV-1 and HSV-2 (Figure 3).

Relationship of Plasma Acyclovir Concentration in Patients Receiving a Single Intravenous Dose of 5 mg/kg to Susceptibility of Certain Herpes Viruses to the Drug

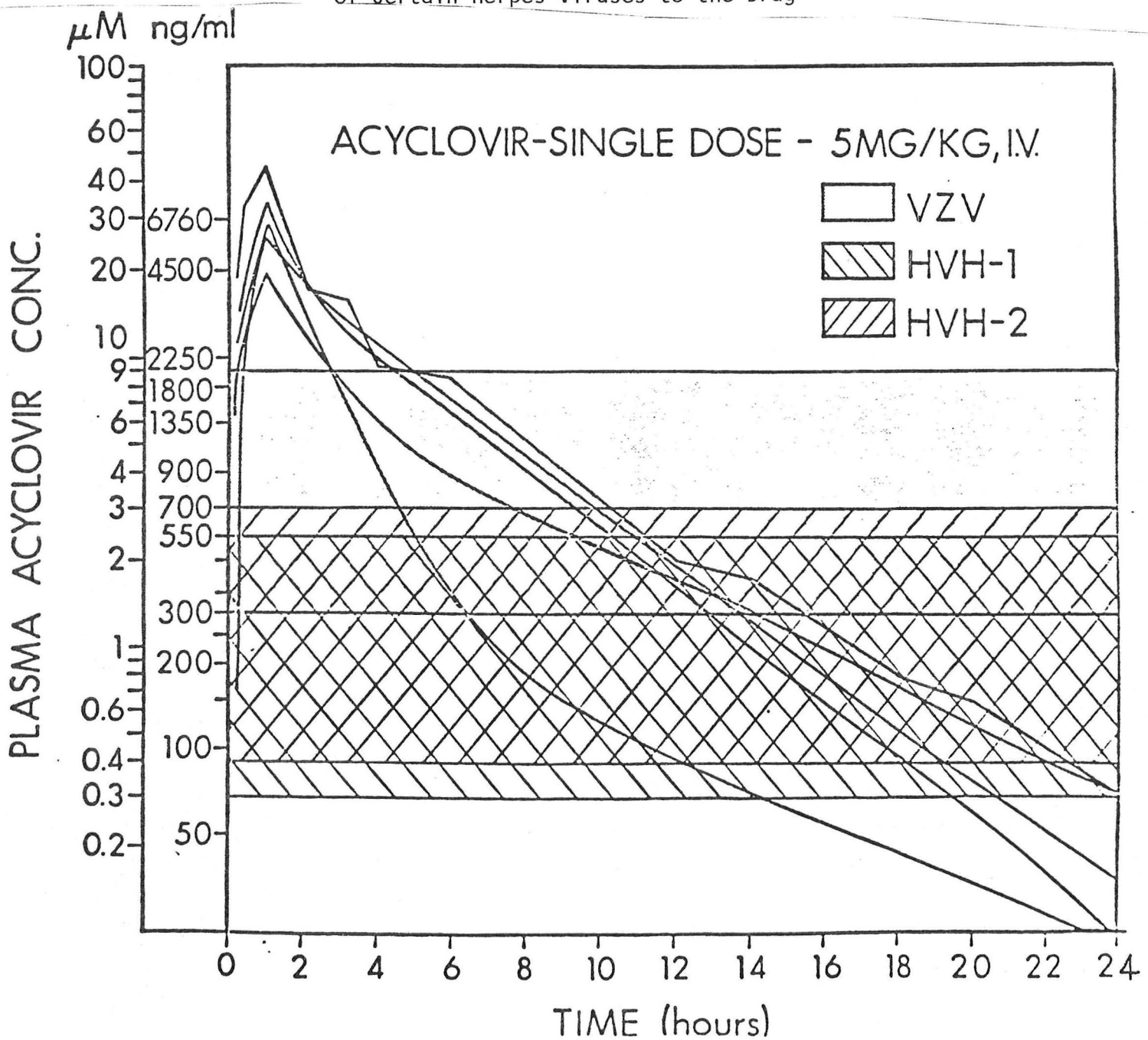


Figure 3

DEVELOPMENT OF RESISTANCE

In the laboratory, resistance to ACV can be produced by serial passage of virus isolates in the presence of sub-inhibitory concentrations of the drug. Most resistant isolates are TK^-, i.e., they do not code for the enzyme TK but use cellular TK for replication. In a few instances, the locus of resistance is related to failure to inhibit viral DNA polymerase. Recent studies have demonstrated the presence of natural resistance of certain genital isolates of HSV to ACV. In a study conducted in Seattle, Wash., 104 genital HSV isolates were tested for sensitivity to ACV by a colorimetric method in which reduction of the cytopathic effect induced by the virus was measured. The 50% inhibitory dose of the drug (ID_{50}) for each of the viruses was determined. The median ID_{50} for the 104 isolates was 0.37 µgm/ml. However, the ID_{50} for 1 isolate was 9 µgm/ml and for another 19 µgm/ml. The measurements were made before the initiation of topical therapy with ACV. Both isolates were TK^-.

There have now been 5 cases reported in which resistance to ACV has emerged during therapy. In one patient treated with topical ACV, the ID_{50} changed from 0.4 µgm/ml to 15 µgm/ml. In three bone marrow transplant recipients receiving intravenous ACV, the ID_{50} of isolates of HSV recovered at the end of therapy ranged from 5-15 µgm/ml. In a child with combined immunodeficiency caused by absence of the enzyme, adenosine deaminase and receiving prolonged oral and intravenous ACV, serial rectal HSV isolates retained ACV sensitivity, with ID_{50} values of 0.01 and 0.02 µgm/ml at the beginning and end of the treatment course. Oral HSV isolates from this child increased ID_{50} values from 0.025 to 4.25 µgm/ml. Isolates from the eye changed ID_{50} values from 1.92 to 9.43 µgm/ml.

There is reason to consider that TK^- mutants of HSV have reduced virulence. In one study in mice the LD_{50} for TK^+ HSV was 10.9 plaque forming units (PFU). In the same strain of mice, the LD_{50} for TK^- HSV was 1.86×10^4. In another study, TK^- mutants of HSV produced less local disease and diminished latent infection in ganglion cells. The authors of this study made the suggestion that such or comparable mutants might have utility as immunizing agents against HSV.

TOXICOLOGY IN ANIMALS

Topical ACV was well tolerated with no evidence of sensitization. At high dosage levels with prolonged treatment, ACV induced marrow hypoplasia and central nervous system side-effects consisting of sedation, depression and ataxia. Nephropathy due to crystal deposition in the collecting ducts was observed. At high dosage levels, inhibition of T lymphocyte proliferation and sheep red blood cell rosette formation occurred. There was no evidence of teratogenic effects nor any evidence of decreased reproduction or fertility. Given in high doses to neonatal rats, there was no evidence of retardation of CNS development. There were no carcinogenic effects. In human lymphocyte preparations, at very high ACV concentrations, minimal chromosomal breakage occurred. At doses contemplated for human use, ACV appears to be a very safe drug with a high therapeutic ratio. At higher doses, urine output must be maintained to avoid nephropathy.

HUMAN PHARMACOLOGY

For HSE in adults and HSV infection in neonates, a dose of 10 mg/Kg delivered over a 1 hour period every 8 hours for 10 days has been chosen for the clinical trials. ACV will be compared to Ara-A and Ara-AMP for HSE and

to Ara-A at an increased dosage level (25 mg/Kg) in neonatal herpes. Following infusion of ACV, there is a biexponential decline of drug concentrations. The renal clearance of ACV exceeds the creatinine clearance, implying tubular secretion but probenecid does not affect ACV plasma levels or renal excretion. It should be recalled that the median ID_{50} for 104 HSV genital isolates was 0.37 µgm/ml. Peak and trough values for ACV are given in Table 1. CSF and plasma concentrations are also compared at varying times following infusion (Table 2).

Table 1

Plasma Levels of ACV (µgm/ml)

	Peak	Trough
5 mg/Kg q 8 h	10.9	0.8
10 mg/Kg q 8 h	23.	2.5

Table 2

CSF and Plasma Levels of ACV

Time after infusion (hours)

	CSF	Plasma
2	4.2	9.3
2	4.5	9.
1.5	4.5	10.8

Since the drug is excreted by the kidneys, a modification of the dosage must be made in renal failure (Table 3). Sixty percent of the drug is removed by hemodialysis.

Table 3

Creatinine Clearance ($ml/min/1.73\ m^2$)	Interval between Doses (hours)
>50	8
25-50	12
10-25	24
0-10	24-48 and after hemodialysis

CLINICAL EFFICACY

National collaborative trials organized by the NIAID are underway comparing ACV, Ara-A and Ara-AMP in HSE and ACV and Ara-A at a higher dose level (25 mg/Kg) for neonatal HSV infection. Double-blind trials of ACV (ACV vs placebo) have been undertaken by Burroughs Wellcome Co. for extending mucocutaneous HSV disease in immunosuppressed patients and herpes zoster and varicella in the same group of patients. Studies of HSV reveal preliminary effects on viral excretion in mucocutaneous disease in immunosuppressed patients that appear quite promising. Topical treatment of extending HSV disease in the immunosuppressed with 5% ACV in a polyethylene glycol base shortens virus excretion from 9.5 days (average for placebo controls) to 2.5 days (ACV therapy).

In recurrent herpes labialis, two centers (Salt Lake City, Boston), ACV influenced viral excretion but had no effect on clinical symptomatology. A patient initiated study is underway in both centers. In the patient initiated studies, the patient is given either ACV or placebo and asked to apply ointment at the beginning of the prodrome. This type of study insures that the drug will be applied as early as possible to the lesions. In Seattle, in primary genital infection, patients receiving ACV experienced a shorter mean duration of pain, itching and viral excretion. In recurrent genital disease, ACV influenced the length of viral excretion but not clinical symptomatology. Patient initiated studies on recurrent genital HSV infections are now underway in several institutions.

Oral ACV drug studies are in progress to determine if the course of recurrent genital herpes can be affected. Intravenous ACV is being studied in primary HSV genital infections to determine if latency can be prevented. Oral and intravenous ACV is being used in a test of prophylaxis to determine if HSV and VZV infections can be prevented in severely compromised patients (bone marrow transplant recipients, cancer chemotherapy patients). A trial of ACV vs placebo is being conducted in varicella pneumonia in normal persons. This trial is underway in Kuwait where army recruits generally from rural backgrounds have a high incidence of varicella after induction into the armed services.

In HSV keratitis, ACV has been proven effective as has Ara-A, IDUR and TFT. A potential extra benefit of ACV is that it can be found in sufficient concentrations in the anterior chamber to influence the replication of HSV.

PRESENT AND FUTURE USES

Ara-A has just been shown to reduce cutaneous dissemination in herpes zoster from 24 to 8% if administered early in the disease coures (<72 hours after onset) and visceral dissemination from 16% to 2%. There was only a limited effect on post-herpetic neuralgia in the immunosuppressed patients on whom the drug was tried. In varicella in immunosuppressed patients, visceral complications were reduced from 40% to 5%.

Most authorities consider that ACV will be licensed for certain indications by the FDA within a year. Given the efficacy of Ara-A, ACV must demonstrate superior efficacy or safety. Comparative trials are underway in HSE and neonatal herpes. There has been a discussion of a potential trial between Ara-AMP, administered intramuscularly and oral ACV in the outpatient treatment of herpes zoster in the immunosuppressed. Use of ACV in herpes labialis and genital herpes awaits further confirmation of results and needs to be balanced by the consideration that ACV resistant mutants can be induced.

REFERENCES

1. Biron, K.K. and Elion, G.B. In vitro susceptibility of Varicella-Zoster virus to acyclovir. *Antimicrobial Agents & Chemotherapy*. 18:3, Sept. 1980, pp 443-447.

2. Biron, K.K. and Elion, G.B. Sensitivity of Varicella-Zoster virus in vitro to acyclovir. *Current Chemotherapy and Infectious Disease*. Proceedings of the 11th ICC and the 19th ICAAC, Vol. II, American Society for Microbiology, p. 1377 (Abstract)

3. Blyth, W.A., Harbour, D.A., Hill, T.J. Effect of acyclovir on recurrence of herpes simplex skin lesions in mice. *J. Gen. Virol.* 48, 1980, (Great Britain) pp 417-419.

4. Collins, P., Tisdale, S.M., and Furman, P.A. Electron microscopic study of the effect of acyclovir on the in vitro replication on herpes simplex virus. *Current Chemotherapy and Infectious Disease*. Proceedings of the 11th ICC and the 19th ICAAC, Vol. II, American Society for Microbiology, pp 1383-1385. (Abstract)

5. Collum, L.M.T., Benedict-Smith, A., and Hillary, I.B. Randomised double-blind trial of acyclovir and idoxuridine in dendritic corneal ulceration. *British J. Ophthalmology*. 64, 1980, pp 766-769.

6. Corey, L., Keeney, R.E., Remington, M.R. et al. Double blind trial of topical acyclovir in genital herpes simplex virus (HSV) infection. *20th Interscience Conference on Antimicrobial Agents and Chemotherapy*, New Orleans, La., 22-24 September, 1980, American Society for Microbiology, No. 515. (Abstract)

7. Coster, D.J., Wilhelmus, K.R., Muchaud, R., and Jones, B.R. A comparison of acyclovir and idoxuridine as treatment for ulcerative herpetic keratitis. *British J. Ophthalmology*. 64, 1980, pp 763-765.

8. Datta, A.K., Colby, B.M., Shaw, J.E. and Pagano, J.S. Acyclovir inhibition of Epstein-Barr virus replication. *Proc. Nat'l Acad. Sci. USA*. 77:9, Sept. 1980, pp 5163-5166.

9. deMiranda, P., Whitley, R.J., Blum, M.R. et al. Acyclovir kinetics after intravenous infusion. *Clin. Pharmacol. Ther.* 26:6, 1979, pp 718-728.

10. deMiranda, P., Whitley, R.J., Blum, M.R. and Keeney, R.E. Pharmacokinetics of the antiviral drug acyclovir in humans. *Current Chemotherapy and Infectious Disease*. Proceedings of the 11th ICC and the 19th ICAAC, Vol. II, American Society for Microbiology, pp 1388-1390. (Abstract)

11. Field, H.J., Darby, G., and Wildy, P. Pathogenecity for mice of acycloguanosine-resistant mutants of herpes simplex virus. *Current Chemotherapy and Infectious Disease*. Proceedings of the 11th ICC and the 19th ICAAC, Vol. II, American Society for Microbiology, pp 1392-1394. (Abstract)

References - Continued

12. Furman, P.A., Collins, P., and McGuirt, P. Effect of acyclovir on viral polypeptide synthesis in herpes simplex virus type I-infected cells. *Current Chemotherapy and Infectious Disease.* Proceedings of the 11th ICC and the 19th ICAAC, Vol. II., American Society for Microbiology, pp 1381-1383. (Abstract)

13. Fyfe, J. and Biron, K. Phosphorylation of acyclovir by a thymidine kinase induced by Varicella-Zoster virus. *Current Chemotherapy and Infectious Disease.* Proceedings of the 11th ICC and the 19th ICAAC. Vol. II. American Society for Microbiology, pp 1378-1379. (Abstract)

14. Klein, R.J., DeStefano, E., Brady, E., and Friedman-Kien, A.E. Experimental skin infection with an acyclovir resistant herpes simplex virus mutant: Response to antiviral treatment and protection against reinfection. *Arch. Virology* 65, 1980, pp 237-246.

15. Park, N-H, Pavan-Langston, D., McLean, S.L. and Albert, D.M. Therapy of experimental herpes simplex encephalitis with aciclovir in mice. *Microbial Agents & Chemotherapy* 15:6, June 1979, pp 775-779.

16. Pavan-Langston, D., Lass, J., and Campbell, R. Antiviral drops: Comparative therapy of experimental herpes simplex keratouveitis. *Arch. Ophthalmology* 97, June 1979, pp 1132-1135.

17. Pavan-Langston, D. Current trends in therapy of ocular herpes simplex: Experimental and clinical studies. *Adv. Ophthalmology* 38 (Karger,Basel 1979)pp 82-88.

18. Selby, P.J., Jameson, B., Watson, J.G. et al. Parenteral acyclovir therapy for herpesvirus infections in man. *The Lancet* Dec. 15, 1979, pp 1267-1270.

19. Spector, S.A., Hintz, M., Quinn, R.P., Keeney, R.E. and Connor, J.D. Single-dose pharmacokinetic and toxic properties of acyclovir. *Current Chemotherapy and Infectious Disease.* Proceedings of the 11th ICC and the 19th ICAAC, Vol. II, American Society for Microbiology, pp 1390-1392. (Abstract)

20. Spruance, S.L., Schnipper, L.E., Kern, E.R., Crumpacker, C.S., Wenerstrom, G., et al. Treatment of herpes simplex labialis with acyclovir. *20th Interscience Conference on Antimicrobial Agents and Chemotherapy,* New Orleans, La., 22-24 September 1980, American Society for Microbiology, No. 516. (Abstract)

21. St. Clair, M.H., Furman, P.A., Biron, K.K., and Elion, G.B. Inhibition of several virally induced DNA polymerases by the triphosphate of 9-(2-Hydroxyethoxymethyl)guanine. *Current Chemotherapy and Infectious Disease.* Proceedings of the 11th ICC and the 19th ICAAC, Vol. II, American Society for Microbiology, pp 1380-1381. (Abstract)

References - continued

22. Steele, R.W., Marmer, D.J. and Keeney, R.E. Comparative in vitro immunotoxicology of acyclovir and other antiviral agents. *Infection and Immunity* 28:3, June 1980, pp 957-962.

23. Whitley, R.J., Blum, R., DeMiranda, P. Keeney, R.E. et al. Pharmacology and clinical experience with acyclovir (ACV) in humans. *20th Interscience Conference on Antimicrobial Agents and Chemotherapy*, New Orleans, La. 22-24 September 1980, American Society for Microbiology, No. 517. (Abstract)

KETOCONAZOLE

Ketoconazole is a new, orally absorbed imidazole derivative that has a wide-spectrum antifungal effect. Its structure is shown in Figure 4. The compound is soluble in acid solutions and gastrointestinal absorption is maximal when the contents of the stomach are at an acid pH. It has been shown that absorption is best when the drug is taken with meals.

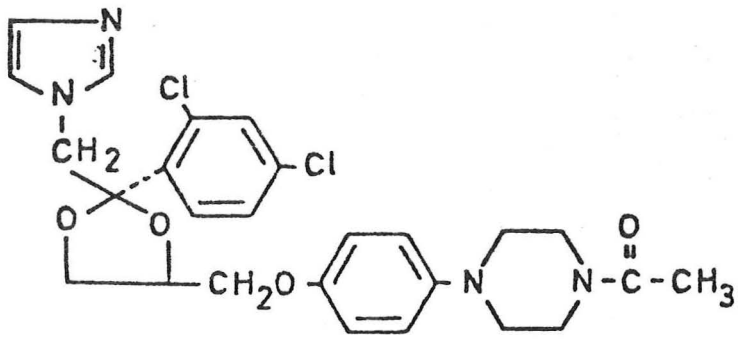


Figure 4

MECHANISM OF ACTION

The imidazoles (miconazole, clotrimazole, ketoconazole) inhibit fungal cell growth by interfering with the synthesis of fungal cell membranes. Specifically, this involves interference with the formation of ergosterol, the major sterol in the fungal membrane. This inhibition coincides with an accumulation of lanosterol-like sterols, i.e., sterols with a 14 - α - methyl group in their structure. The accumulation of C14 methylsterols suggests that miconazole and ketoconazole are potent inhibitiors of one of the metabolic steps involved in demethylation at the C14 site. At doses presently utilized, ketoconazole is fungistatic. It has been pointed out that the

utility of ketoconazole could not have been demonstrated if *in vitro* sensitivity testing had solely been performed with conventional media. In Eagle's minimum essential medium (EMEM), *Candida albicans* forms germ tubes and then pseudomycelial forms. In the presence of very low concentrations of ketoconazole, e.g., 0.05 µgm/ml, pseudomycelial formation is strikingly inhibited. If polymorphonuclear leukocytes and macrophages are added to cultures of *C. albicans* in the presence of low concentrations of ketoconazole, yeast forms predominate and phagocytosis occurs. Pseudomycelial formation effectively stops phagocytosis (Figure 5). Interference with cell membrane formation enhances the permeability of the cell envelope; the drug is then able to penetrate into the cell where it interferes with RNA and protein synthesis, impairs lipid metabolism and finally leads to cell necrosis. With fungicidal concentrations of miconazole, intracellular concentrations of hydrogen peroxide are increased leading to the degeneration of subcellular structures.

Although imidazole derivatives theoretically may affect cholesterol synthesis, this only occurs at very high concentrations of the drugs. Any potential effect is also mitigated by the fact that host cells can utilize dietary cholesterol. At therapeutic concentrations, ketoconazole has been shown not to induce hepatic microsomal enzymes that could lead to its more rapid metabolic degredation. The practical implication of this fact is that steady state levels of the drug remain constant for long periods without any necessity for increasing the dosage of the drug.

ANTI-FUNGAL SPECTRUM

Ketoconazole has a wide spectrum of anti-fungal activity. Furthermore, its activity is also influenced by the culture medium used, e.g., inhibition

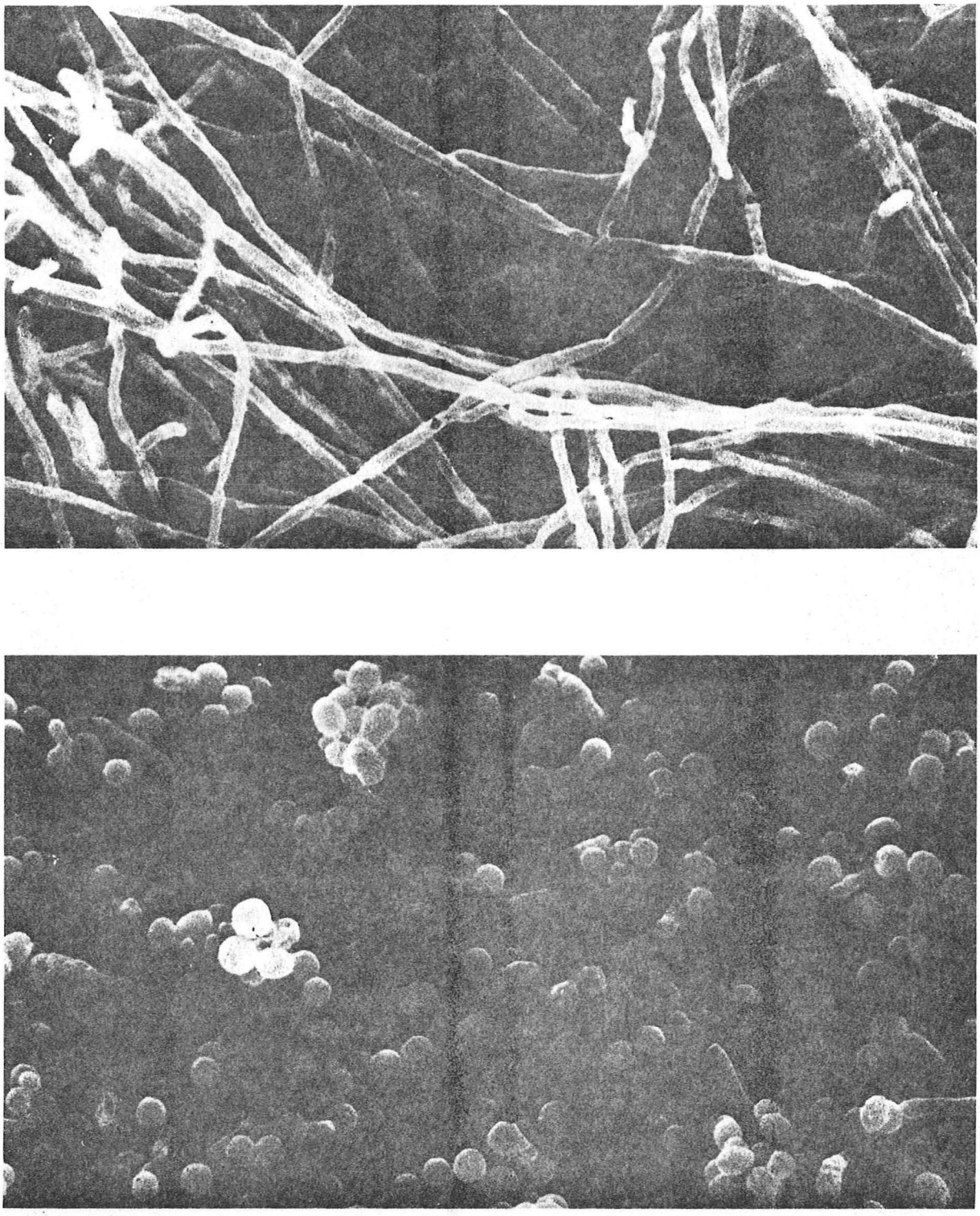


Figure 5. Scanning electron micrographs of *Candida albicans* inoculated in the yeast form and grown in a mycelium-promoting medium (Eagle's minimal essential medium) for 24 hr without ketoconazole (*top*) and with 10^{-7} M ketoconazole (0.05 μg/ml) (*bottom*). Note that ketoconazole completely prevented the outgrowth of long branching hyphae like those seen in the untreated culture (×1,075). Ref. 3.

Table 4. In vitro antifungal activities of R41,400 and miconazole against 175 isolates of human pathogenic fungi as determined by an agar dilution procedure.

Organism (no. tested)	Compound	Cumulative percentages of isolates inhibited at indicated concentration (μg/ml) of drug										Geometric mean MIC (μg/ml)
		≤0.125	0.25	0.50	1	2	4	8	16	32	≥64	
Systemic and subcutaneous pathogens												
Coccidioides immitis (10)	R41,400	20	90	100	...	...	...	...	...	...	...	0.23
	Miconazole	...	...	...	80	90	100	...	...	...	...	1.23
Histoplasma capsulatum (9)	R41,400	44	89	100	...	...	...	...	...	...	...	0.17
	Miconazole	100	...	...	...	...	...	...	...	...	...	0.08
Blastomyces dermatitidis (12)	R41,400	17	25	33	58	100	...	...	...	...	...	0.70
	Miconazole	25	42	58	92	100	...	...	...	...	...	0.37
Sporothrix schenckii (10)	R41,400	...	...	...	...	...	60	80	100	...	...	6.06
	Miconazole	...	...	...	30	100	...	...	...	...	...	1.62
Allescheria (Petriellidium) boydii (11)	R41,400	...	18	...	...	55	100	...	...	...	...	1.66
	Miconazole	...	18	...	36	91	100	...	...	...	...	1.29
Aspergillus fumigatus (14)	R41,400	...	...	...	...	...	7	79	100	...	...	8.83
	Miconazole	...	...	...	...	...	...	86	100	...	...	8.83
Dematiaceous fungi (10)*	R41,400	...	...	...	10	60	80	...	...	90	100	4.00
	Miconazole	...	...	20	40	...	80	...	...	90	100	3.25
Pathogenic and commensal yeasts												
Cryptococcus neoformans (10)	R41,400	10	70	90	...	...	...	...	...	100	...	0.44
	Miconazole	...	50	100	...	...	...	...	...	...	...	0.35
Candida albicans (10)	R41,400	...	...	...	...	...	...	20	40	60	100	27.86
	Miconazole	...	...	...	...	...	...	20	50	100	...	19.70
Candida tropicalis (5)	R41,400	20	...	...	...	...	...	...	...	40	100	13.82
	Miconazole	...	20	...	...	...	...	...	...	100	...	12.13
Candida parapsilosis (5)	R41,400	60	...	...	...	...	...	...	...	80	100	1.14
	Miconazole	...	...	...	...	60	...	80	...	100	...	4.60
Torulopsis glabrata (5)	R41,400	...	...	...	...	40	...	...	...	...	100	21.11
	Miconazole	...	...	20	...	40	...	...	60	...	100	9.19
Dermatophytic fungi												
Epidermophyton floccosum (9)	R41,400	100	...	...	...	...	...	...	...	...	...	0.06
	Miconazole	100	...	...	...	...	...	...	...	...	...	0.06
Microsporum audouinii (2)	R41,400	...	...	...	...	...	50	...	100	...	...	8.00
	Miconazole	...	...	...	...	...	100	...	...	...	...	4.00
Microsporum canis (7)	R41,400	...	...	...	...	17	50	67	100	...	...	6.35
	Miconazole	...	...	...	29	86	100	...	...	...	...	1.81
Microsporum gypseum (4)	R41,400	...	...	...	...	...	...	...	50	100	...	22.63
	Miconazole	...	...	...	...	...	50	...	75	100	...	9.51
Trichophyton mentagrophytes (8)	R41,400	...	...	12	...	...	25	100	...	...	...	5.19
	Miconazole	...	...	...	12	50	100	...	...	...	...	2.59
Trichophyton rubrum (18)	R41,400	6	...	...	17	22	...	56	78	94	100	7.70
	Miconazole	11	...	...	28	39	67	94	...	100	...	2.51
Trichophyton tonsurans (16)	R41,400†	...	...	...	25	42	67	100	...	...	...	3.72
	Miconazole	...	...	...	12	25	56	94	100	...	...	4.36

*Includes four isolates of *Phialophora* species and three isolates each of *Cladosporium* and *Fonsecaea* species.
†Only 12 isolates tested with R41,400.

of pseudomycelial formation by *C. albicans* in EMEM. Table 4 lists susceptibility testing of ketoconazole (R41,400) using an agar dilution method with casein yeast extract glucose agar. Achieveable peak serum concentrations at a 400 mg. daily dose approximate 6 µgm/ml and are usually at a 2-4 µgm/ml level with 200 mg. per day. As noted, the effect on *C. albicans* in EMEM, animal studies or in human disease would not have been predicted using this system of susceptibility testing. Not included in this table is *Paracoccidiodes brasiliensis*, the cause of paracoccioidomycosis, a fungal infection highly prevalent in Brazil and for which effective therapy has been lacking. Two strains of this organism were inhibited by 0.002 µgm/ml while another two strains were inhibited at 0.004 µgm/ml.

ANIMAL MODEL STUDIES

Ketoconazole has been studied in multiple animal model studies including coccidioidomycosis and blastomycosis in mice infected intranasally, various forms of candidal infection in differing animals, and histoplasmosis and cryptococcosis in the nude mouse model. These studies all have demonstrated the efficacy of the drug. In Levine and Cobb's murine coccidioidomycosis model, treatment was effective if begun 4, 12, and 30 days after intranasal infection (Figure 6).

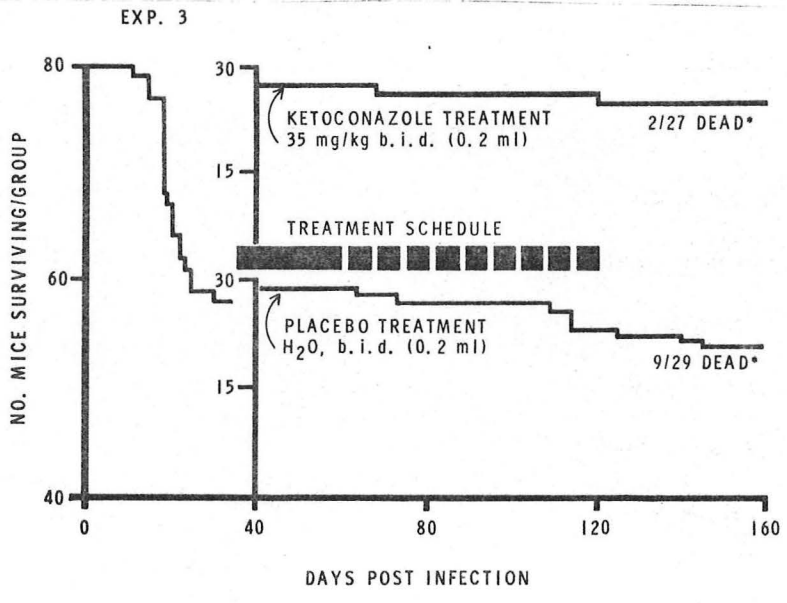


Figure 6. The influence of late treatment with ketoconazole on the survival of mice infected intranasally with 42 arthrospores of *Coccidioides immitis*. On the 30th day after infection, surviving animals were assigned to drug and placebo groups. Treatment was begun on the 35th day after infection and was continued for 21 consecutive days; thereafter, treatment was given five days per week through the 120th day after infection. Drug and placebo were administered orally. b.i.d. = twice daily. *P = <0.05 (Fisher-Irwin-Yates' exact test for fourfold tables).

In the study of Graybill et.al. of histoplasmosis and cryptococcosis in athymic mice (nu/nu) and their heterozygous counterparts with thymuses (nu/+), ketoconazole and amphotericin were compared alone and together. In nu/nu mice with histoplasmosis after intravenous challenge, mortality was most delayed after AMB + KTZ therapy. All animals eventually succumbed but mortality was delayed over controls with KTZ being more effective than AMB. In nu/nu mice with cryptococcosis after intraperitoneal challenge, all animals eventually died but the combination of AMB + KTZ significantly delayed therapy over controls. Since ketoconazole is fungistatic, mortality could be predicted in nu/nu mice once the drug had been discontinued. Mortality curves for nu/+ mice with histoplasmosis and cryptococcosis are shown in Figures 7 and 8. The authors concluded that in this model system amphotericin B and ketoconazole are not antagonistic and that the combination of the two drugs may result in a modest benefit. Previous studies had suggested an antagonism between miconazole and amphotericin B. No human studies testing the combination of drugs has been reported. Randomized clinical trials of ketoconazole vs. amphotericin B in deep mycotic infections in humans have also not been reported.

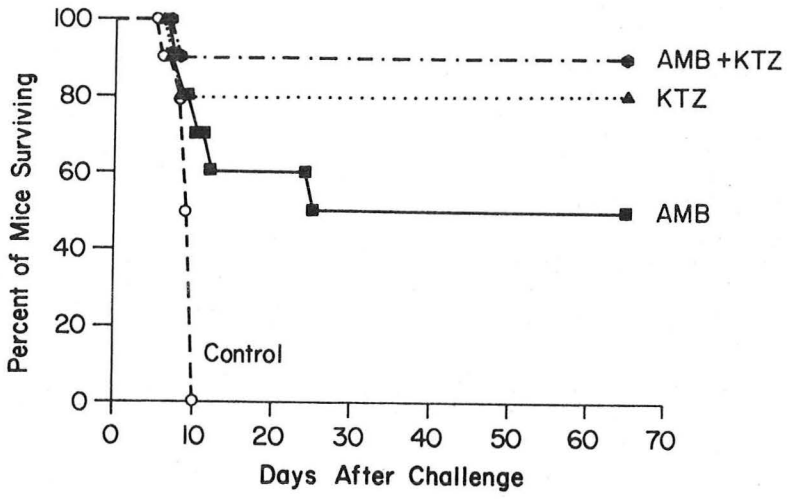


Figure 7. Survival of *nu/+* mice after iv challenge with 5×10^7 *Histoplasma capsulatum* organisms and treatment with ketoconazole (KTZ), amphotericin B (AMB), both (AMB + KTZ), or neither (control). There were 10 mice per group.

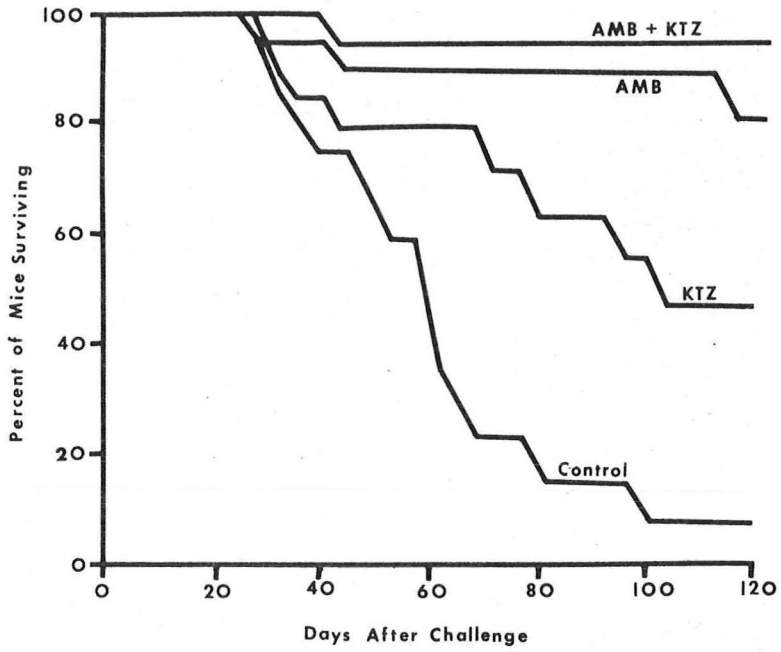
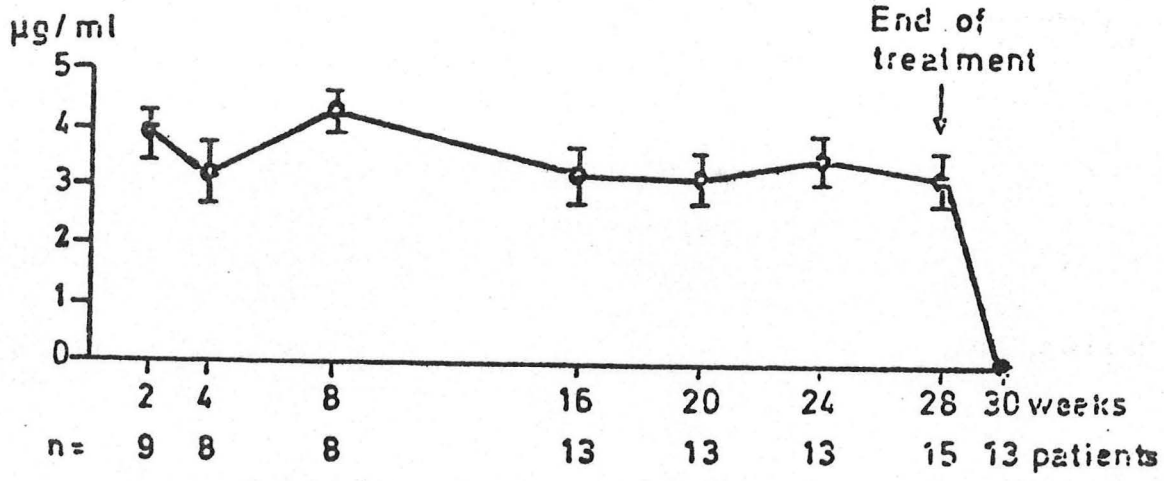


Figure 8. Survival of *nu/+* mice challenged ip with 10^6 cfu of *Cryptococcus neoformans* and treated with no drug, with amphotericin B (AMB), with ketoconazole (KTZ), or with both drugs. There were 18–20 mice per group.

When combined with 5-fluorocytosine, results did not differ *in vivo* or *in vitro* from results achieved with ketoconazole alone.

FIGURE 9. Plasma levels of ketoconazole obtained by 200 mg once daily in long-term treatment.



PHARMACOLOGY

Ketoconazole is absorbed in the stomach at an acid pH. It is extensively metabolized by the liver but at the concentrations achieved does not induce hepatic microsomal enzymes which might degrade it more rapidly. Accumulation does not occur and peak serum levels at a 200 mg. dose remain constant during the treatment course (Figure 9). The excretion of ketoconazole -^{3}H has been studied in 3 volunteers. After 4 days, about 70% of the dose had been excreted: about 57% in feces and 13% in urine. Unchanged drug accounted for 20 to 65% of the fecal radioactivity, as estimated by radio -HPLC, and for 2 to 4% of the urinary radioactivity. Thus, no alteration of the dosage of the drug need be made with renal insufficiency. The urine concentration may be a limiting factor in treating fungal urinary tract infections. If a 400 mg. dose is given, using the figures given above and assuming a urine volume of 1500 ml/day, it can be estimated that the urine concentration of the drug will only approximate 1-2 µgm/ml.

Penetration of ketoconazole into the CSF has been presumed to be limited. In one patient, however, with candidal meningitis after 30 days of therapy with 400 mg. per day of the drug, CSF ketoconazole levels were 2.1, 1.9 and 2.2 µgm/ml, 4, 6 and 8 hours after administration of the drug.

TOXICOLOGY

In animals (rats) dosed with high quantities of the drug, changes were observed in the liver, kidneys, adrenals and ovaries. At these dosage levels, liver enzyme induction occurred, resulting in increased estrogen metabolism. The drug produced both embryotoxic and teratogenic effects. In dogs, the

target organ was the liver. Alkaline phosphatase and SGOT levels increased and at 80 mg/kg after 2 to 4 weeks of dosing, clinical icterus developed.

In man, nausea and vomiting are seen but are minimized when the drug is taken with meals. SGOT elevations occurred in 4 patients out of a total of 12 receiving ketoconazole for chronic mucocutaneous candidiasis. In three patients, the SGOT elevation was transient disappearing with further therapy. In one patient treated for 6 months, SGOT, SGPT, 5'-nucleotidase and alkaline phosphatase levels showed "marked" elevations while the serum bilirubin remained normal. A reduction in ketoconazole dose was associated with improvements in the enzyme levels. A liver biopsy showed mild hepatitis; tests for evidence of infection with hepatitis A and B viruses were negative. A recent report showed that 3/40 men being treated with ketoconazole developed gynecomastia.

At present, ketoconazole is considered a safe drug for human use. Nausea and vomiting usually respond to giving the drug with meals. Hepatic toxicity may evidently be seen as well as a low incidence of gynecomastia. The drug should be avoided in women that are pregnant or in whom pregnancy has not been excluded. The drug has been licensed by the FDA and scheduled for release August 3, 1981.

CLINICAL EFFICACY

In considering human studies, various criteria for evaluating results need definition. The drug is fungistatic. In chronic mucocutaneous candidiasis, for example, cure may not be possible since the patient will almost certainly relapse when therapy is withdrawn.

Paracoccidioidomycosis. *In vitro* studies demonstrated the marked susceptibility of this fungus to ketoconazole. Of 13 patients treated with ketoconazole for 3 to 12 months at a dosage of 200 mg. per day, all patients were reported to have improved markedly, with healing of pulmonary, mucosal and skin lesions. Mycologic tests corroborated the clinical improvement. Five patients completed a year of therapy and were free of symptoms when therapy was discontinued (Table 10).

Table 10. Results of one year of ketoconazole therapy for five patients with paracoccidioidomycosis. Ref. 25

Patient no.	Clinical response	Mycologic studies			Total dose of ketoconazole (g)	Adverse effects	Follow-up after therapy (month)	
		Cultures	Decreased antibody titer*	Paracoccidioidin skin-test conversion			Well	Died, other cause
1	Marked: chest X rays cleared	Negative	Yes	Yes	60.4	None	Yes (10)	. . .
2	Marked: mucosal lesions and chest X rays cleared	Negative	Yes	Yes	58.0	Slight nausea	Yes (8)	. . .
3	Marked: skin lesions and chest X rays cleared	Negative	NA†	Yes	58.4	None	Yes (8)	. . .
4	Marked: chest X rays cleared	Negative	Yes	. . .‡	75.6§	None	Yes (4)	. . .
5	Moderate: partial clearing of chest X rays	Negative	Yes	Yes	73.2§	None	. . .	Yes (1)‖

* Includes CF and immunodiffusion tests with *Paracoccidioides brasiliensis* antigen. A decrease in titer was defined as a twofold or greater decrease or a diminished number of bands.

† Results of serologic tests were not available for evaluation.

‡ Patient was skin-test positive before treatment.

§ Patient received 200 mg of ketoconazole per day for the total period of treatment, whereas the other patients received 100 mg per day for the last six months.

‖ This patient had tuberculosis.

Coccidioidomycosis. Ketoconazole has been shown to be effective in the treatment of pulmonary and disseminated coccidioidomycosis by 3 groups of investigators, each treating more than 10 patients. The authors all reported clinical successes in the majority of cases but also failure of

therapy in come cases (Tables 11 and 12). (see next page)

Table 11. Results of ketoconazole therapy and sites of infection with *Coccidioides immitis.* Ref. 4

	No. of patients with infection at indicated site					
Result*	Bone	Lung	Soft tissue/skin	Meninges	Urinary tract	Liver
Responses	3	5	12	1	2	1
Failures	0	0	0	2	0	0
No change	5	4	1	0	1	0
Could not be evaluated	2†	1	1	2	0	0
Total	10	10	14	5	3	1

* If a patient had multiple sites of involvement, each site was evaluated separately. Total represents number of individuals studied as of November 26, 1979. Seventeen of the 32 patients received 400 mg of ketoconazole per day.
† Patients died of unrelated causes.

Causes for the failures of therapy were often not apparent. It has been noted that ketoconazole may have limited CSF penetration. Dosages of 200 mg/day were successful in some patients while others required 400 mg/day. The optimal duration of therapy had to be individualized depending on the response of the patient.

Histoplasmosis. Pulmonary and disseminated histoplasmosis has been treated successfully with ketoconazole but the experience is more limited than with coccidioidomycosis.

Chronic mucocutaneous candidiasis. In a double blind study, 12 patients were treated either with ketoconazole or a placebo. All six recipients of ketoconazole has remission of symptoms and virtually complete regression of mucosal, skin and nail lesions, whereas only two of the six had even temporary mucosal clearing, and none had improvement of skin or nail disease. The six patients receiving placebo in the controlled trial were then treated with ketoconazole in an open trial, and all responded favorably.

Table 12. Therapeutic responses of 15 patients with coccidioidomycosis to ketoconazole. Ref. 16

Disease, patient no.	Subjectively improved "state of well-being"*	Reduction in size of extra-pulmonary lesions	Improvement in roentgenographic abnormalities	Reduction of ≥50% in initially elevated ESR	Two-tube or greater decline in CF titer	Became culture negative	Duration of therapy (months)	Possible adverse reactions†
Pulmonary								
2	Yes	NA	Yes	Normal ESR‡	No	Yes	2§	None
3	No	NA	Yes	No	No	Yes	7	None
4	No	NA	No	No	No	No	9‖	Nausea, vomiting, pruritus, impotence, anemia
5	Yes	NA	Yes	No	No	Yes	11	None
6	No	NA	Yes	No	No	Yes	11	None
7	No	NA	Yes	Normal ESR‡	No	Yes	7	None
8	No	D	No	No	No	No	2‖	None
9	No	NA	No	No	No	No	5	None
10	No	NA	Yes	No	No	Yes	5§	Rash
Disseminated, non-meningeal								
11	Yes	No	No	No	No	No	4‖	Pruritus, arthralgias, headache
12	Yes	?	No	No	No	No	9	Headache
13	Yes	Yes	Yes	Yes	Yes	Yes	7§	None
14	Yes	Yes	NA	Yes	No	No	12	Dizziness, hyperesthesia
15	Yes	Yes	NA	No	No	Yes	3#	None
16	Yes	Yes	Yes	No	Yes	Yes	10**	None

NOTE. ESR = erythrocyte sedimentation rate; NA = not applicable; D = disseminated during therapy (see text); question mark indicates equivocal findings.

* State of well-being cannot be quantitated and may represent a placebo effect of ketoconazole or a general medical response.

† No adverse reactions noted in patients 1, 17, and 18.

‡ Normal ESR refers to pretherapy values.

§ Completed therapy, improved.

‖ Drug discontinued because of clinical failure.

\# Too early to determine decline in ESR or CF titer.

** Died of small-cell carcinoma of lung; no evidence of active coccidioidomycosis.

Superficial mycoses. Superficial mycotic infections have been treated with keotconazole. In a double-blind study of patients treated with either ketoconazole or griseofulvin, 24/29 (83%) of patients treated with ketoconazole and 6/19 (32%) of patients treated with griseofulvin were rated as cured.

Overall Summary of Therapeutic Results. Table 13 lists the overall response of patients with mycoses to miconazole therapy.

Table 13. Overall response of patients with mycoses to treatment with ketoconazole. Ref. 33

Diagnosis	Total no. of patients	Percentage of patients			
		With no response	Moderately improved	Markedly improved*	Cured*
Superficial mycoses					
Pityriasis versicolor	99	0	3	3	94
Dermatomycoses due to yeasts	51	0	6	14	80
Dermatomycoses due to dermatophytes	178	3	8	15	74
Mycosis of hair and scalp	20	15	10	55	20
Onychomycosis	21	5	19	19	57
Oral thrush	45	7	2	7	84
Chronic mucocutaneous candidosis	30	3	17	40	40
Deep mycoses†					
Paracoccidioidomycosis	50	2	4	10	84
Histoplasmosis	20	10	10	15	65
Coccidioidomycosis	54	19	44	22	15
Chromomycosis	12	25	33	25	17
Systemic candidosis	7	29	0	43	28
Aspergillosis	6	33	17	33	17
Aspergilloma	7	43	43	14	0
Mycetoma	11	55	36	9	0

* Patients in these two categories were considered to be good clinical responders; therefore, the sum of the percentages in these two categories is the total percentage of good responders.

Ketoconazole has also led to reduce numbers of *Candida albicans* being shed in feces and sputum of patients with cancer. These patients were treated with 200 mg. of drug per day. Although vaginal candidiasis has been treated successfully with ketoconazole, it has not been licensed for that use because it should not be administered to women unless pregnancy can be excluded. The drug has been licensed for the treatment of chronic mucotaneous candidiasis,

oral thrush, candiduria, paracoccidoidomycosis, coccidioidomycosis, chromomycosis and histoplasmosis. Further studies on the safety of the drug need to be performed before it can be used in superficial mycotic infections although its efficacy may be greater than griseofulvin in the treatment of some of these disorders. Resistant isolates occurring during therapy have not been reported although resistant isolates (before or with no therapy) have been documented.

References

1. Borelli, D. Treatment of pityriasis versicolor with ketoconazole. *Reviews of Infectious Diseases.* 2:4, 1980, pp 592-595.

2. Borelli, D., Bran, J.L., et al. Ketoconazole, an oral antifungal: laboratory and clinical assessment of imidazole drugs. *Postgrad. Med. J.* 55, 1979, pp 657-661.

3. Borgers, M. Mechanisms of action of antifungal drugs with special references to the imidazole derivatives. *Rev. Infect. Dis.* 2:4, 1980, pp 520-534.

4. Brass, C, Galgiani, J.N. et al. Therapy of disseminated or pulmonary coccidioidomycosis with ketoconazole. *Rev. Infect. Dis.* 2:4, 1980, pp 656-660.

5. Cucé, L.C., Wroclawski, E.L. & Sampaio, S.A.P. Treatment of paracoccidioidomycosis, candidiasis, chromomycosis, lobomycosis, and mycetoma with ketoconazole. *Internat. J. Dermatology.* 19:405-408, 1980.

6. Cucé, L.C., Wroclawski, E.L. & Sampaio, S.A.P. Treatment of paracoccidioidomycosis, candidosis, chromomycosis, lobomycosis, and mycetoma with ketoconazole: A brief summary. *Rev. Infect. Dis.* 2(4): 1980, p 650.

7. DeFelice, R., Johnson, D.G. & Galgiani, J.N. Gynecomastia with ketoconazole. *Antimicrobial Agents & Chemotherapy.* 19(6): June 1981, pp 1073-1074.

8. Dixon, D., Shadomy, S., et al. Comparison of the in vitro antifungal activities of miconazole and a new imidazole, R41,400. *J. Infect. Dis.* 138, 1978, pp 245-248.

9. Dismukes, W.E., Bennett, J.E. et al. Criteria for evaluation of therapeutic response to antifungal drugs. *Rev. Infect. Dis.* 2(4):, 1980, pp 535-545.

10. Drouhet, E. & Dupont, B. Chronic mucocutaneous candidosis and other superficial and systemic mycoses successfully treated with ketoconazole. *Rev. Infect. Dis.* 2(4), 1980, pp 606-619.

11. Fibbe, W.E., Van Der Meer, J.W.M., Thompson, J. & Mouton, R.P. CSF concentrations of ketoconazole. (Correspondence).

12. Fregoso-Dueñas, F. Ketoconazole in vulvovaginal candidosis. *Rev. Infect. Dis.* 2(4): 1980, pp 620-623.

13. Galimerti, R., Negroni, R., et al. The activity of ketoconazole in the treatment of onychomycosis. *Rev. Infect. Dis.* 2(4): 1980, pp 596-598.

14. Graybill, J.R., Herndon, J.H., Jr. et al. Ketoconazole treatment of chronic mucotaneous candidiasis. *Arch. Dermatol.* 116, Oct. 1980, pp 1137-1141.

References-cont'd.

15. Graybill, J.R., Williams, D.M. et al. Combination therapy of experimental histoplasmosis and cryptococcosis with amphotericin B and ketoconazole. *Rev. Infect. Dis.* 2(4): 1980, pp 551-558.

16. Graybill, J.R., Lundberg, D. et al. Treatment of coccidioidomycosis with ketoconazole: clinical and laboratory studies of 18 patients. *Rev. Infect. Dis.* 2(4): 1980, pp 661-673.

17. Hanifin, J.M. Ketoconazole--an oral antifungal with activity against superficial and deep mycoses. *Am. Acad. Dermatol.* 2(6): 1980, pp 537-539.

18. Harvey, R.P., Isenberg, R.A., Stevens, D.A. Molecular modifications of imidazole compounds: studies of activity and synergy in vitro and pharmacology and therapy of blastomycosis in a mouse model. *Rev. Infect. Dis.* 2(4): 1980, pp 559-569.

19. Hay, R.J., Wells, R.S. et al. Treatment of chronic mucocutaneous candidosis with ketoconazole: A study of 12 cases. *Rev. Infect. Dis.* 2(4): 1980, pp 600-605.

20. Janssen Pharmaceutica, Inc. Nizoral ™(ketoconazole) Investigator's Brochure (New Brunswick, N.J. 08903), April 1980, pp 1-86.

21. Jones, H.E., Simpson, J.G. & Artis, W.M. Oral ketoconazole. *Arch. Dermatol.* 117: Mar. 1981, pp 129-134.

22. Kirkpatrick, C.H., Peterson, E.A. & Alling, D.W. Treatment of chronic mucocutaneous candidosis with ketoconazole: preliminary results of a controlled, double-blind clinical trial. *Rev. Infect. Dis.* 2(4): 1980, pp 559.

23. Legendre, R. & Steltz, M. A multi-center, double-blind comparison of ketoconazole and griseofulvin in the treatment of infections due to dermatophytes. *Rev. Infect. Dis.* 2(4): 1980, pp 586-591.

24. Levine, H.B. & Cobb, J.M. Ketoconazole in early and late murine coccidioidomycosis. *Rev. Infect. Dis.* 2(4): 1980, pp 546-550.

25. Negroni, R., Robles, A.M., et al. Ketoconazole in the treatment of paracoccidioidomycosis and histoplasmosis. *Rev. Infect. Dis.* 2(4): 1980, pp 643-649.

26. Odds, F.C., Milne, L.J.R., et al. The activity *in vitro* and *in vivo* of a new imidazole antifungal, ketoconazole. *J. Antimicrob. Chemother.* 6: 1980, pp 97-104.

27. Petersen, E.A., Alling, D.W., & Kirkpatrick, C.H. Treatment of chronic mucocutaneous candidiasis with ketoconazole. *Annals of Internal Med.* 93(6): 1980, pp 791-795.

References - cont'd

28. Restrepo, A., Stevens, D.A. & Gomez, I. Ketoconazole: a new drug for the treatment of paracoccidioidomycosis. *Rev. Infect. Dis.* 2(4): 1980, pp 633-642.

29. Robertson, M.H., Hanifin, J.M. & Parker, F. Oral therapy with ketoconazole for determatophyte infections unresponsive to griseofulvin. *Rev. Infect. Dis.* 2(4): 1980, pp 578-581.

30. Rosenblatt, H.M., Byrne, W., et al. Successful treatment of chronic mucocutaneous candidiasis with ketoconazole. *J. Pediatrics* 97(4): Oct. 1980, pp 657-660.

31. Samelson, L.E., Lerner, S.A. et al. Relapse of *Candida parapsilosis* endocarditis after long-term suppression with flucytosine: retreatment with valve replacement and ketoconazole. *Ann. Internal Med.* 93(6) Dec. 1980, pp 838-839.

32. Sarosi, G.A., Armstrong, D. et al. Treatment of fungal disease. American Thoracic Society, Medical Section of the American Lung Association. *Am. Rev. Respir. Dis.* 120: 1979, pp 1393-1397.

33. Symoens, J., Moens, M., Dom, J. et al. An evaluation of two years of clinical experience with ketoconazole. *Rev. Infect. Dis.* 2(4): 1980, pp 688-691.

34. Thienpont, D., Van Cutsem, J., et al. Ketoconazole--a new broad spectrum orally active antimycotic. *Specialia* 15(5): 1979, pp 606-607.

35. Thienpont, D., Van Custem, J., & Borgers, M. Ketoconazole in experimental candidosis. *Rev. Infect. Dis.* 2(4): 1980, pp 570-577.

36. Utz, J.P. Chemotherapy for the systemic mycoses: the preclude to ketoconazole. *Rev. Infect. Dis.* 2(4): 1980, 625-632.

37. Van Den Bossche, H., Willemsens, G., et al. In vitro and in vivo effects of the antimycotic drug ketoconazole on sterol synthesis. *Antimicrob. Agents & Chemother.* 17(6): June 1980, pp 922-928.

38. Welsh, O. & Rodriguez, M. Treatment of dermatocycoses with ketoconazole. *Rev. Infect. Dis.* 2(4) 1980, pp 582-585.

39. Welsh, O., Gonzalez, J.G. et al. Therapeutic evaluation of ketoconazole in patients with coccidioidomycosis. *Rev. Infect. Dis.* 2(4): 1980, pp 651-655.

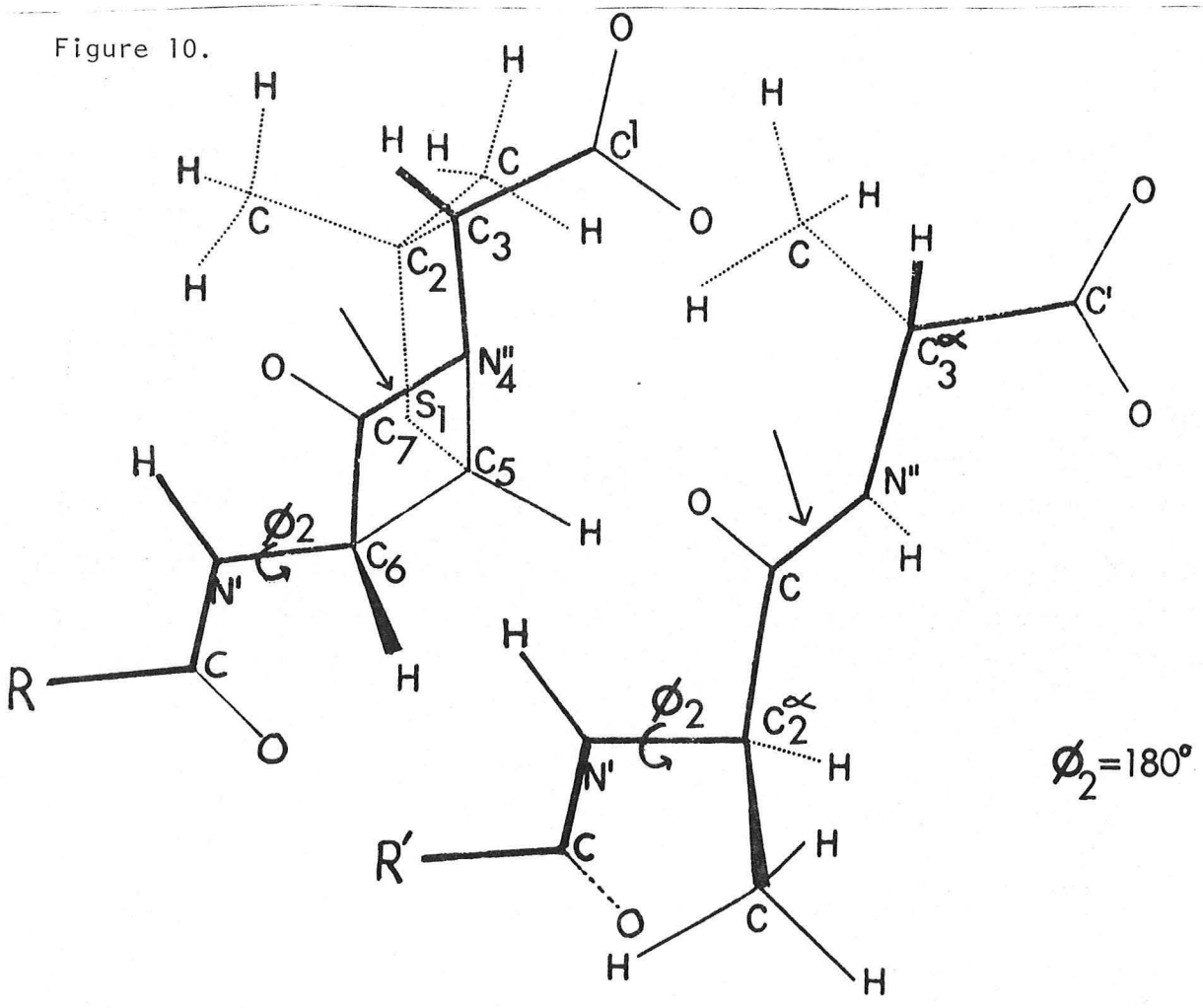
THE THIRD GENERATION CEPHALOSPORINS.

The cephalosporin antibiotics were discovered in 1945 by Giuseppi Brotzu who isolated the fungus *Cephalosporium acremonium* near a sewage outlet. He has reasoned, knowing the story of penicillin and its therapeutic properties, that antibiosis must be involved in the purification of the sewage effluent. The story extends to today; to the third generation cephalosporins, compounds with a broadened antimicrobial spectrum that hold great promise for their utility in clinical medicine.

MECHANISM OF ACTION.

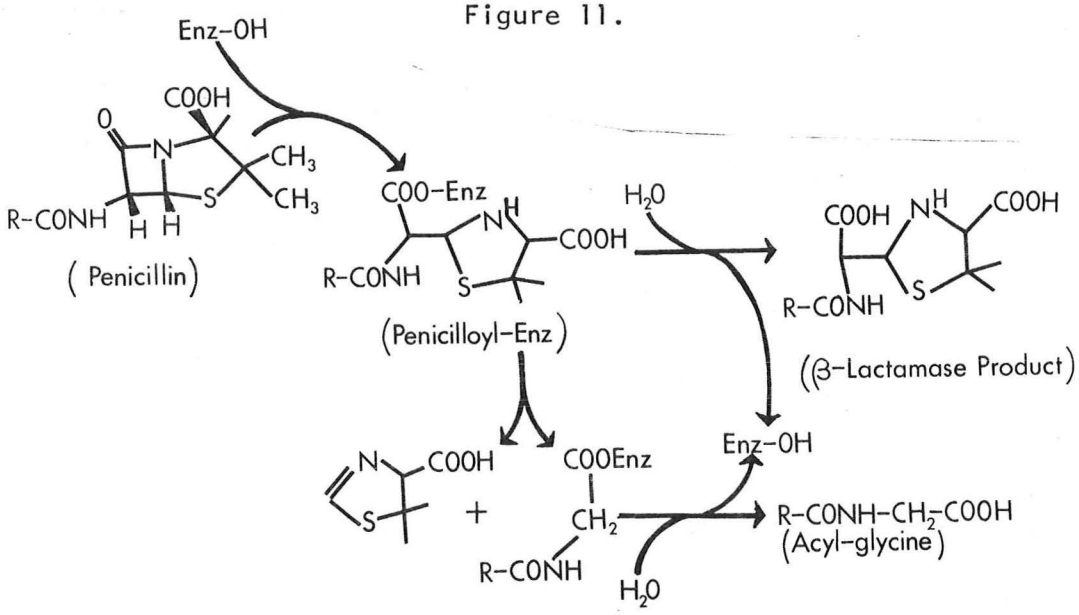
Penicillin and cephalosporin antibiotics have similar mechanisms of action. They both require the integrity of a β-lactam ring and are seen as structural analogues of acyl-D-alanyl-D-alanine (Figure 10). Enzymes involved

Figure 10.



in cell wall or peptidoglycan synthesis by forming peptide cross-links accept these antibiotics into their active sites and become acylated (Figure 11).

Figure 11.



Further steps in the reaction involve deacylation in which the acyl group is transferred to water or to another receptor. The general model for the reaction can be stated as follows:

E (enzyme) + S (substrate) → ES → EP → E + P (product)

The degree of enzymatic inhibition depends on the rate of formation of ES and EP and the reversibility of the reaction.

It has now been established that each bacterial species contains multiple enzymes that are inhibited by the penicillins or cephalosporins. Other proteins not involved in enzymatic activities can also bind these antibiotics. Collectively, these enzymes and proteins are termed penicillin binding proteins (PBP) (Table 14 - next page). Different penicillin and cephalosporin derivatives bind to these PBPs with varying avidity. Different morphological forms result depending on which enzymatic activities are primarily inhibited. For

Table 14 Summary of available data on penicillin-binding proteins (PBP) of *Escherichia coli* K12. Ref. 6

PBP	Mol wt	No. of molecules per cell	Map site (min)	Symbol	Relative affinity for					Proposed function
					Pen G	Mec	Kex	Lori	Cefox	
1A	95	230	73.5	*pon* A	+	0	+	++	++	Peripheral cell wall extension, transpeptidase
1Bs	90	230	3.3	*mrc, pon* B	+	0	+	+	+	
2	66	20	14.4	*rod* A	+	+	0	0	0	Rod shape maintenance
3	60	50	1.8	*fts* I	+	0	++	+	+	Septum formation
4	44	110	68	*dac* B	+	0	+	...	...	D, D-carboxypeptidase 1B, 1C
5	42	1,800	13.7	*dac* A	++	0	0	...	...	D, D-carboxypeptidase 1A
6	40	570			++	0	0	...	...	

example, mecillinam preferentially binds to PBP 2 of *E. coli*, an enzyme that performs a transpeptidase function and is responsible for maintenance of a rod shape. Mecillinam causes the formation of enlarged rounded forms with loss of viability but only slow lysis. The concept of PBPs explains how synergy might occur if another antibiotic such as cefoxitin is added. Cefoxitin binds to PBPs 1A, 1Bs and 3. The combination of mecillinam and cefoxitin thusly inhibits 4 enzymatic activities and synergy with this antibiotic combination has been demonstrated *in vitro* against multiple Gram negative bacilli.

Autolysins are naturally occurring enzymes that are engaged in the continuing normal process of cell wall breakdown. The bactericidal effects of penicillins and cephalosporins are dependent on continuing autolysis. Tolerant organisms are defined as organisms that have stopped cell growth but remain viable in the presence of the antibiotic because lysis has not occurred. Naturally occurring inhibitors of autolysins occur. It has been postulated that penicillins and cephalosporins may permit the continuing occurrence of autolysis by allowing inhibitors to be lost from the bacterial cell by leakage through walls made porous by inhibition of cross-linkage.

Two types of antimicrobial resistance to penicillins and cephalosporin compounds have been discovered. The first and least common is exemplified by methicillin resistance in *S. aureus*. Resistance is chromosomally mediated and is related to the fact that the cell wall is impermeable to the antibiotic. Methicillin cannot cross the cell wall and hence it cannot reach the PBPs which are located on the cytoplasmic membrane. Inhibition of peptidoglycan synthesis cannot occur. Resistance of this type extends to other penicillinase resistant semisynthetic penicillins (PRSP) and also to the cephalosporins. Treatment of a methicillin resistant *S. aureus* infections usually requires an antibiotic such as vancomycin. Although increasing in its frequency, methicillin resistance in *S. aureus* has been slow in its development considering that PRSPs were first introduced into clinical medicine in the early 1960's.

The second type of resistance is much more common and relates to the fact that enzymes that degrade penicillins and cephalosporins are present in the periplasmic space (between the cell wall and cytoplasmic membrane). These enzymes are β-lactamases and the end product reaction results in hydrolysis of the β-lactam ring. There are multiple β-lactamase enzymes, coded chromosomally and by plasmids. The enzymes vary in structure and substrate specificity by bacterial species. The recent advances in the utilization of penicillins and cephalosporins relate to structural modifications of these antibiotics so that the β-lactam ring is protected from hydrolysis by these enzymes. (Table 15 - next page). The efficacy of penicillins and cephalosporins, in summary, *in vitro* relates to the capacity of these antibiotics to cross the cell wall, evade β-lactamase destruction and their avidity for PBPs involved in the synthesis of the cell wall.

Table 15 A comparison of the stabilities of cephaloridine (CER), cefoperazone (CPZ), cefazolin (CEZ), cephalothin (CET), cephalexin (CEX), cefamandole (CMD), and penicillin G (PC-G) against beta-lactamases. Ref. 9

Enzyme Source	Type of Beta-Lactamase	Specific Activity†	Relative Rate of Hydrolysis*						
			CER	CPZ	CEZ	CET	CEX	CMD	PC-G
E. coli GN5482	CSase	0.24	100	< 0.04	135	691	55.5	< 0.04	28.7
P. aeruginosa GN918	CSase	0.24	100	0.04	160	480	62.9	0.04	24.8
P. vulgaria GN76	CSase	0.40	100	7.00	375	204	52.0	276	21.0
E. cloacae GN7471	CSase	3.68	100	0.80	50	402	54.0	1.70	83.1
C. freundii GN346	CSase	3.27	100	0.01	120	127	81.1	68.9	7.0
P. morganii GN5406	CSase	0.60	100	< 0.04	73.5	242	31.0	< 0.04	121
E. coli W3630 Rms212+	PCase Type I	2.10	18.2	12.4	7.2	7.3	< 1.3	20.5	100
E. coli W3630 Rms 213+	PCase Type II	0.23	18.8	< 3.9	4.6	9.2	< 2.6	11.0	100
E. coli W3630 Rte 16+	PCase Type III	0.29	68.0	7.1	18.0	9.8	1.6	12.9	100
P. aeruginosa ML4259 Rms 139+	PCase Type IV	0.66	8.6	< 0.4	< 0.05	< 0.5	< 0.6	< 0.4	100
K. pneumoniae GN69	PCase	0.97	15.1	13.7	2.7	2.8	< 0.5	1.7	100

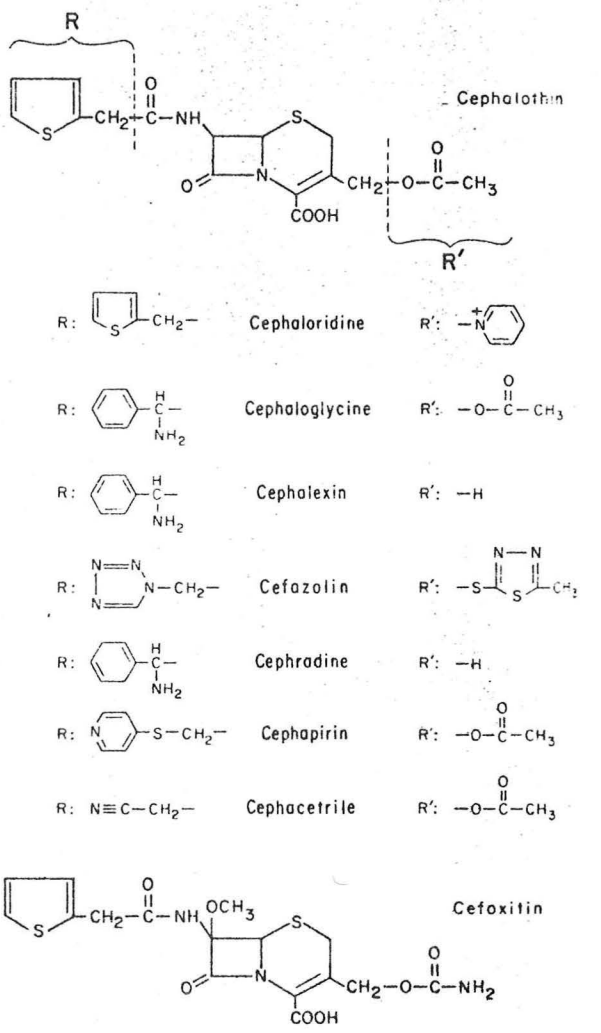
* Hydrolysis of each substrate by PCase and CSase is expressed as relative rate of hydrolysis, assuming an absolute rate of PC-G and CER hydrolysis as 100, respectively.

† Units per milligram of protein.

THE CEPHALOSPORINS; FIRST, SECOND AND THIRD GENERATIONS.

The cephalosporin nucleus differs from the penicillin nucleus in having a 6 member ring structure instead of a 5 member ring structure attached to the β-lactam portion of the molecule. The first generation cephalosporins depicted in Figure 12 [next page] (cephalothin, cephaloridine, cephaloglycine, cephalexin, cefazolin, cephradine, cephapirin and cephacetrile) differ from the second generation cephalosporin, cefoxitin, in that the latter has a 7-α-methoxy group in the nucleus protecting the β-lactam ring.

Figure 12.



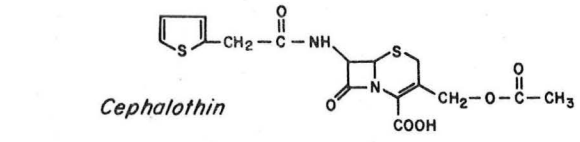
Structure of Cephalosporin-Like Antibiotics, Including the Cephamycin Antibiotic Cefoxitin.

In general, the first generation cephalosporins have more similarities than differences. 1). The antimicrobial susceptibilities of the different compounds are essentially equivalent. In sensitivity testing in the laboratory, a single disc (cephalothin) can be used to determine the susceptibilities of different organisms to all of the compounds. Cefaclor, an oral first generation drug does, however, possess activity against β-lactamase + and - strains of *H. influenzae*. Cephalothin is the most stable drug to staphylococcal β-lactamases but this difference most probably is insufficient to separate it clinically from cephapirin and cefazolin, both of which have been used successfully in serious staphylococcal infections. 2). With the exception of the Enterococcus, the development of resistance to the cephalosporins over time has not been marked. Obvious exceptions occur, e.g., multi-drug resistant *K. pneumoniae*. 3). The drugs do not penetrate the meninges in an active form. 4). With the exception of cephaloridine, the compounds are remarkably safe with the chief side-effects being allergic in nature. Cephaloridine is nephrotoxic

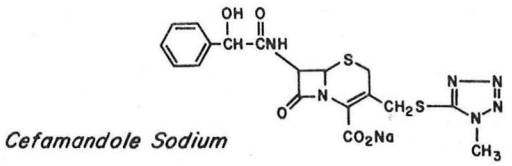
in humans. The therapeutic ratio for these drugs is correspondingly high. 5). The first generation cephalosporins (excepting cefaclor for *H. influenzae*) are ineffective against significant pathogens such as Enterobacter sp., *Pseudomonas aeruginosa*, most strains of *H. influenzae*, *Serratia marcescens*, indole + Proteus sp., *Bacterocides fragilis* and the Enterococcus.

The second generation cephalosporins (cefamandole, cefoxitin and cefuroxime) expanded the antimicrobial spectrum of these compounds. Cefuroxime is not available for use in the U.S. (Figure 13). To determine antimicrobial sensitivities, separate tests for cefamandole and cefoxitin had to be performed. Cefamandole was effective against a broader spectrum of Gram negative bacilli, including β-lactamase + and - strains of *H. influenzae*.

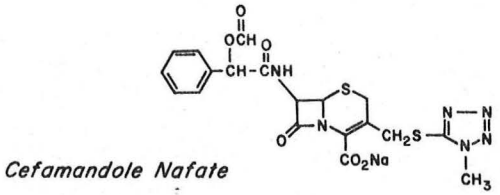
Figure 13



Cephalothin



Cefamandole Sodium



Cefamandole Nafate

The development of resistance in strains of Enterobacter sp. has been noted for cefamandole. A comparison of sensitivities of different bacteria to cefamandole and cefoxitin has been compiled at PMH by Dr. Paul Southern.

In total, between 1979 and 1981, development of resistance to the antibiotics has been slight. *Serratia marcescens, citrobacter diversus* and *citrobacter freundii* have become increasingly resistant to cefoxitin. About 85% of *B. fragilis* isolates have been found to be sensitive to cefoxitin. Both antibiotics have emerged as useful additions to the therapeutic armamentarium. Although effective against *S. aureus*, these drugs have moved away from the efficacy noted with the prototype drug, cephalothin. Tillotson has reported a series of seven consecutive patients with *S. aureus* endocarditis which represented therapeutic failures with cefamandole. Neither drug penetrates the meninges in therapeutic qualities. Organisms such as *Pseudomonas aeruginosa, Serratia marcescens, acinetobacter cocalaceticus* and the Enterococcus continue as therapeutic problems unsolved by these second generation drugs.

The third generation cephalosporins represent further advances to broaden the antimicrobial spectrum of these drugs (Table 16).

Table 16

THIRD GENERATION CEPHALOSPORINS

Cefotaxime	Cefsulodin
Moxalactam	Thienamycin
Cefoperazone	Cefotiam
Ceftizoxime	Ceftriaxone
Ceftazidime	Ceforanide

Placement of any of these drugs on the hospital formulary would necessitate the microbiology laboratory performing sensitivity studies for each drug.

TABLE 17

SALIENT FEATURES OF THE THIRD GENERATION CEPHALOSPORINS

	Metabolism	Metabolite < active than parent drug	Excretion	t 1/2 (hours)	CSF penetration (%)	Dosage affected by dialysis	Comments
Cefotaxime	Y	Y	$R_{GF,Tp}$	1.1	5-8	N	No dosage adjustment except with severe renal failure
Moxalactam	N		R_{GF}	2.1	20-25	Y	
Cefoperazone	N		H,R_{GF}	1.9	5-8	N	No dosage adjustment in renal failure
Ceftazidime	N		R_{GF}	1.8			
Ceftizoxime	N		R	1.3			
Cefsulodin	Y	Y	R	1.7			
Thienamycin							Nephrotoxic in humans
Cefotiam							
Ceftriaxone	N		R	7.3	Y		CSF penetration in rats and rabbits $\geq$ Moxalactam
Ceforanide			$R_{GF,T}$	3.1		N	

Y yes, N no, H hepatic, R renal,
GF glomerular filtration, T tubular secretion not affected by prebenecid, Tp tubular secretion affected by probenecid.

This would entail additional expense in addition to the considerable cost of the drug to the patient. It is estimated that the cost of cefotaxime to the patient per gram would approximate $25. One gram of moxalactam would cost the patient between $25 to $30. Compiling drug after drug of similar mechanism and type on the hospital formulary leads to confusion on the part of the physician so that dosage errors occur. An attempt must be made considering the different needs and usages of the major services of the hospital to arrive at a consensus of what constitutes a rational policy toward the third generation cephalosporins. Since the first drugs to be licensed in the U.S. are cefotaxime, moxalactam and cefoperazone, analysis will be centered on these agents. Salient features of all the third generation cephalosporins are given in Table 17. Cefotaxime is metabolized to a microbiologically less active compound, desacetylcefotaxime. It is not significantly removed by hemodialysis and because of its metabolism there is no dosage adjustment except with severe impairment of renal function. It has been suggested that there is no adjustment in dosage until the creatinine clearance <5; below this level the dosage should be reduced 50%. Moxalactam has the best penetration into the CSF. It has a longer half-life than cefotaxime, is removed significantly by dialysis and the dose must be adjusted in fenal failure. (Table 18 - next page). Cefoperazone is excreted primarily into bile. High concentrations are reached in the biliary tract. There is no dosage adjustment in renal failure. Cefoperazone does penetrate the CSF and a patient with pneumococcal meningitis has been treated successfully with that drug. The treatment of CNS infections has been limited but evidence indicates that moxalactam may be effective in sensitive Gram negative bacillary infections. A case of pneumococcal meningitis occurring during moxalactam therapy has

TABLE 18

Maintenance Dosage Guide for Patients with Renal Impairment

Creatinine Clearance ml/min/1.73 m^2	Renal Function	Life-Threatening Infections-- Maximum Dosage	Less Severe Infections
>80	Normal	4 gm q. 8 h.	0.5-2 gm q. 8-12 h.
50-80	Mild Impairment	3 gm q. 8 h.	0.5-1 gm q. 8 h.
25-50	Moderate Impairment	2 gm q. 8 h. OR 3 gm q. 12 h.	0.25-1 gm q. 12 h.
2-25	Severe Impairment	1 gm q. 8 h. OR 1.25 gm q. 12 h.	0.25-0.5 gm q. 8 h.
<2	0	1 gm q. 24 h.	0.25-0.5 gm q. 12 h.

been reported. Ceftazidime and cefsulodin have enhanced activity against *Pseudomonas aeruginosa*. Thienamycin has remarkable *in vitro* antibacterial activity and there have only been a few isolates tested that have been resistant (corynebacterium sp.). Human testing has not been reported because of nephrotoxicity in humans. Ceftriaxone has a prolonged half-life as has ceforanide. Ceftriaxone has an antibacterial spectrum similar to cefotaxime and in experimental animals penetrates the CSF as well or better than moxalactam.

In vitro susceptibility testing reveals that β-lactamase + and - strains of *H. influenzae* are susceptible to cefotaxime, moxalactam and cefaperazone.

In general, moxalactam was the single best antimicrobial against aerobic Gram negative bacilli excluding *P. aeruginosa* (Table 19). Cefoperazone was

Table 19 Number of strains examined and percentage of cultures inhibited by antibiotic concentrations of 32 μg/ml or more. Ref. 9

Organism	No. of Strains	Percentage of Strains Inhibited by ≥ 32 μg/ml of Antibiotic						
		Cefo-perozone	Cefo-taxime	Moxilactam LY127935	Cefur-oxime	Cefa-mandole	Cefox-itin	Cephalo-thin
Escherichia coli	49	2	0	0	0	2	2	10.2
Klebsiella sp.	37	5.4	0	0	5.4	13.5	5.4	13.5
Enterobacter sp.	35	0	11.4	0	22.9	34.3	91.4	91.4
Citrobacter freundii	6	0	0	0	0	0	83.3	83.3
Serratia sp	26	46.2	0	0	88.5	76.9	30.8	100
Proteus mirabilis	43	1.6	0	0	14.0	4.7	0	34.9
Proteus sp (indole-positive)	18	0	0	0	77.8	50.0	11.1	100
Salmonella/Shigella sp	29	0	0	0	10.3	3.6	6.9	17.2
Pseudomonas/Aeromonas sp	58	8.6	27.6	41.4	100	100	100	100
Other oxidative-fermentative bacteria	21	71.4	28.6	52.4	61.9	61.9	71.4	81.0
Total (percentage of all organisms)	322	11.2%	8.1%	3.4%	39.4%	37.9%	38.8%	57.8%

the most active of the three drugs against *P. aeruginosa* but had limited activity against Serratia sp. and Acinetobacter sp. (not shown in the table). Moxalactam has lesser activity than cefotaxime and cefoperazone against Gram positive cocci (Table 20). The Enterococcus and *Listeria monocytogenes* are not reliably inhibited by either cefotaxime, moxalactam or cefoperazone. Anaerobic bacteria are inhibited by all three antibiotics with perhaps moxalactam showing the greatest activity (Table 21).

TABLE 20

Table 20. Comparative *in vitro* activity of seven cephalosporin/cephalosporin-like antibiotics against gram-positive cocci. Ref. 9

Organism (No. of Isolates)	Geometeric Mean of MIC and Range (μg/ml)						
	Cefo-perozone	Cefo-taxime	LY127935	Cefur-oxime	Cefa-mandole	Cefox-itin	Cephalo-thin
Staphylococcus aureus (P'nase positive) (31)	1.71 1–2	1.37 1–2	4.41 4–8	0.94 0.5–1	0.4 0.25–0.5	1.78 1–2	0.24 0.125–0.25
Staphylococcus aureus (P'nase negative) (16)	1.19 1–2	1.13 1–2	4.86 4–8	0.92 0.5–1	0.30 0.125–1	1.6 0.5–4	0.16 0.06–0.25
Streptococcus pneumoniae (16)	0.036 0.015–0.25	0.003 < 0.003–0.03	1.19 0.5–4	0.024 < 0.003–0.125	0.092 0.03–0.25	0.48 < 0.003–2	0.013 0.003–0.06
Beta-hemolytic streptococci group A (13)	0.12 0.06–0.25	0.002 < 0.003–0.007	0.62 1–4	0.01 0.007–0.015	0.07 0.03–0.125	0.81 0.5–1	0.035 0.015–0.06
Beta-hemolytic streptococci non group A (12)	0.25 0.125–0.5	0.03 < 0.003–0.125	21.4 1–32	0.059 0.007–0.5	0.13 0.03–0.5	2.99 0.5–8	0.17 0.015–0.5
Enterococci (30)	14.9 8–32	14.9 1–> 250	512 > 250	46.2 8–> 250	26 8–64	388 32–> 250	26 8–> 250

All three drugs appear safe but a summary of the published figures (Table 22) causes some skepticism that complications are being under-reported. No cases of pseudomembranous enterocolitis were reported. The increased prothrombin time was corrected by vitamin K administration and was due to antibiotic induced alterations in bowel flora in persons thought to have diminished body stores of that vitamin. Ethanol intolerance with a

TABLE 21

Table 21 Comparative activity of beta-lactam antibiotics against anaerobic bacteria. Ref. 9

Organism	No. of Isolates	Antibiotic	MIC Range (μg/ml)	50% MIC (μg/ml)	90% MIC (μg/ml)
Bacteroides fragilis	86	Cefoperazone	0.5–128	16	32
		Cefoxitin	0.5–128	4	8
		LY127935	≤ .125–256	1	32
		Cefotaxime	0.5–256	4	64
		Cefamandole	2–> 256	64	256
		Cephalothin	2–> 256	64	256
Bacteroides melaninogenicus	10	Cefoperazone	≤ 0.125–8	0.5	2
		Cefoxitin	0.5–2	1	2
		LY127935	≤ 0.125–8	0.5	2
		Cefotaxime	≤ 0.125–2	0.5	1
		Cefamandole	≤ 0.125–64	4	16
		Cephalothin	≤ 0.125–64	4	32
Bacteroides bivius disiens	20	Cefoperazone	0.5–8	1	4
		Cefoxitin	0.5–8	2	4
		LY127935	0.5–16	2	8
		Cefotaxime	≤ 0.125–8	0.5	8
		Cefamandole	0.5–16	2	16
		Cephalothin	0.5–32	4	16
Other *Bacteroides* sp	9	Cefoperazone	≤ 0.125–8	1	8
		Cefoxitin	≤ 0.125–16	2	8
		LY127935	≤ 0.125–1	0.5	1
		Cefotaxime	≤ 0.125–8	1	8
		Cefamandole	≤ 0.125–32	4	32
		Cephalothin	≤ 0.125-64	8	64
Fusobacterium sp	8	Cefoperazone	≤ 0.125–0.5	≤ 0.125	0.5
		Cefoxitin	≤ 0.125–1	≤ 0.125	1
		LY127935	≤ 0.125–1	0.5	1
		Cefotaxime	≤ 0.125–1	≤ 0.125	1
		Cefamandole	≤ 0.125–2	0.5	2
		Cephalothin	≤ 0.125–2	1	2
Clostridium sp	14	Cefoperazone	≤ 0.125–0.5	≤ 0.125	0.5
		Cefoxitin	≤ 0.125–1	≤ 0.125	1
		LY127935	≤ 0.125–1	0.5	1
		Cefotaxime	≤ 0.125–1	≤ 0.125	1
		Cefamandole	≤ 0.125–2	0.5	2
		Cephalothin	≤ 0.125–2	1	2
Peptostreptococcus sp *Peptococcus* sp	8	Cefoperazone	≤ 0.125–8	1	8
		Cefoxitin	≤ 0.125–16	2	8
		LY127935	≤ 0.125–1	0.5	1
		Cefotaxime	≤ 0.125–8	1	8
		Cefamandole	≤ 0.125–32	4	32
		Cephalothin	≤ 0.125–64	8	64

TABLE 22

THIRD GENERATION CEPHALOSPORINS

SIDE - EFFECTS

	Cefotaxime	Moxalactam	Cefoperazone
# patients	2500	1680	521
Hypersensitivity reactions (%)		3.2	
Rash	2.2	1.3	2.9
+ Coombs test	6.0		
Thrombophlebitis	0.5	2.4	
↑ SGOT, SGPT		2.5	2.9
Diarrhea	0.4	2.1	0.1
↑ prothrombin time		0.4	
Eosinophilia	0.1	1.5	2.3
Leukopenia	0.1	0.2	
ETOH intolerance		0.1	
Overgrowth of resistant bacteria &/or fungi			

disulfuram mechanism was found in one person receiving moxalactam. More important is the overgrowth of resistant organisms and suprainfection. Suprainfections with Enterococcus, *Pseudomonas aeruginosa* and fungi should be expected and may be a limiting factor in therapy. The development of resistance during therapy has been poorly studied for each of the three drugs.

CONCLUSION.

Cefotaxime, moxalactam & cefoperazone represent advances in the therapy of bacterial infections. To choose between them is difficult but requires setting up goals about what can and should be accomplished by placing one of these antibiotics on the formulary at a particular institution. Two attainable goals at PMH are: 1) to diminish aminoglycoside usage and the attendant risks of nephrotoxicity and ototoxicity and 2) to treat Gram negative bacillary infections of the central nervous system more effectively. Although moxalactam is not without its drawbacks, it is the most effective antibiotic against aerobic Gram negative bacilli, except for *P. aeruginosa*. This would include bacteria presently that are resistant to aminoglycoside drugs. Moxalactam also penetrates the CSF most effectively and studies increasingly suggest its utility in neonatal meningitis and in Gram negative bacillary meningitis in the neurosurgical patient.

P. aeruginosa is a pathogen that overall would not be treated well with moxalactam but the future addition of a drug like ceftazidime to the formulary or one of the ureido-penicillins (piperacillin, azlocillin or mezlocillin) plus continued usage of the aminoglycoside antibiotics should be considered as further steps to help contain that organism.

If moxalactam is accepted on the formulary, its use should be restricted because of its expense and until further experience can be gained with the compound. One potential way to restrict its use initially would be to perform susceptibility studies only on Gram negative bacteria that are isolated from the CSF and others that are found resistant to the first and second generation cephalosporin compounds (cephalothin, cefamandole and cefoxitin). Since antibiotic audits have been mandated by the JCAH, the introduction of moxalactam into the hospital would be an ideal setting for a prospective audit so

that physicians can learn in an ongoing manner how best to use the drug and to correct mistakes in its use. Further experience with the drug should enable physicians to select specific problems in which the drug might be used empirically.

References

1. Abstracts. 12th International Congress of Chemotherapy. Florence, Italy 19-24 July 1981. International Society of Chemotherapy, 1981, pp 1-301.

2. Cefoperazone: A Clinical Review. Program and Abstracts. Cefoperazone Symposium & ICAAC Abstracts, New Orleans, La. Sept. 21, 1980. *Excerpta Medica 1980*, pp 1-26.

3. Cefotaxime: A New Cephalosporin Antibiotic. Eds. A.M. Geddes, J.F. Acar & H. Knothe. *The J. of Antimicrobial Chemotherapy*. 6(Suppl. A) The British Society for Antimicrobial Therapy. Academic Press (London) Sept. 6, 1980, pp 1-303.

4. Current Chemotherapy and Infectious Disease. Proceedings of the 11th International Congress of Chemotherapy and the 19th Interscience Conference on Antimicrobial Agents and Chemotherapy. Vol. 1. 1-5 Oct. 1979, Boston, Mass. Eds. John D. Nelson and Carlo Grassi. The American Society for Microbiology, Washington, DC, 1980, pp 1-1764.

5. FK 749 (SK&F 88373-Z) Investigational Use Circular. Confidential. Research & Development Division, Smith Kline & French Laboratories. Nov. 1979, pp 1-35.

6. Future Prospects and Past Problems in Antimicrobial Therapy: The Role of Cefoxitin. A Symposium Held at the Harvard Club of Boston, Boston, Mass. May 24-25, 1978. Eds. E.H. Kass, and D.A. Evans. *Reviews of Infectious Diseases*. 1(1): Jan-Feb, 1979, pp 1-244.

7. Moxalactam. Investigator's Monograph. Lilly Research Laboratories-Indianapolis, Indiana, pp 1-116.

8. Program and Abstracts. 20th Interscience Conference on Antimicrobial Agents and Chemotherapy 22-24 Sept. 1980 New Orleans, La. American Society for Micropiology, pp 1-768.

9. Special Issue on Cefoperazone Sodium: Proceedings of the First International Symposium Boston, Oct. 1979. Co-Chairmen: Dr. Harold Neu & Dr. Yasushi Ueda. *Clinical Therapeutics* 3: 1980, pp 1-208.

10. Symposium on Cefamandole. Boca Raton, Florida March 17-19, 1977. Ed. Robert C. Moellering, Jr. *J. Infect. Dis.* 17(Suppl): May 1978, pp S1-S194.

11. Brogden, R.N., Heel, R.C. et al. Cefoxitin: A review of its antibacterial activity, pharmacological properties and therapeutic use. *Drugs* 17, 1979, pp 1-37.

12. Delgado, D.G., Brau, C.J. et al. Clinical and laboratory evaluation of cefamandole in the therapy of *Haemophilus* spp. bronchopulmonary infections. *Antimicrob. Agents & Chemother.* 15(6): June 1979, pp 807-812.

References - cont'd.

13. Farrar, W.E., Jr. and Gramling, P.K. Antistaphylococcal activity and β-Lactamase resistance of newer cephalosporins. *The J. of Infect. Dis.* 133(6): June 1976, pp 691-695.

14. Greenberg, R.N., Scalcini, M.C. et al. Cefamandole therapy in anaerobic infections. *Antimicrob. Agents & Chemother.* 15(3): Mar. 1979, pp 337-341.

15. Greenwood, D., Pearson, N.J. & O'Grady, F. Cefuroxime: a new cephalosporin antibiotic with enhanced stability to enterobacterial β-lactamases. *J. Antimicrob. Chemother.* 2, 1976, pp 337-343.

16. Griffith, R.S., Black, H.R. et al. Cefamandole: In Vitro and clinical pharmacokinetics. *Antimicrob. Agents & Chemother.* 10(5): Nov. 1976, pp 814-823.

17. Hall, W.H., Opfer, B.J. & Gerding, D.N. Comparative activities of the Oxa-β-Lactam LY 127935, cefotaxime, cefoperazone, cefamandole, and ticarcillin against multipe-resistant gram-negative bacilli. *Antimicrob. Agents & Chemother.* 17(2): Feb. 1980, pp 273-279.

18. Hinkle, A.M., LeBlanc, B.M. & Bodey, G.P. In Vitro evaluation of cefoperazone. *Antimicrob. Agents & Chemother.* 17(3): Mar. 1980, pp 423-427.

19. Hoesprich, K.D. & Huston, A.C. Induction of resistance in *staphylococcus aureus* and *Klebsiella pneumoniae* by exposure to cephalothin and cefotoxin. *The J. Infect. Dis.* 133(6): June 1976, pp 681-685.

20. Lang, S.D.R., Edwards, D.J. & Durack, D.T. Comparison of cefoperazone, cefotaxime, and moxalactam (LY127935) against aerobic gram-negative bacilli. *Antimicrob. Agents & Chemother.* 17(3), Mar 1980, pp 488-493.

21. Markowitz, S.M. and Sibella, D.J. Comparative susceptibilities of clinical isolates of *Serratia marcescens* to newer cephalosporins, alone and in combination with various aminoglycosides. *Antimicrob. Agents & Chemother.* 18(5): Nov. 1980, pp 651-655.

22. Miki, F. and Shiota, K. Cefotaxime in lower respiratory tract infections compared to cefazolin. *J. Antimicrob. Chemother.* 6(Suppl. A), pp 169-175.

23. Minor, M.R., Sande, M.A., et al. Cefamandole treatment of pulmonary infection caused by gram-negative rods. *J. Antimicrob. Chemother.* 2: 1976, pp 49-53.

24. Mohr, J.A., Griffiths, W., et al. Clostridial myonecrosis ('Gas Gangrene') during cephalosporin prophylaxis. *JAMA* 239(9), Feb. 27 1978, pp 847-849.

References - cont'd

25. Neu, H.C., Aswapokee, N. et al. Antibacterial activity of a new 1-Oxa cephalosporin compared with that of other β-Lactam compounds. *Antimicrob. Agents & Chemother.* 16(2): Aug. 1979, pp 141-149.

26. Norrby, R. Newer cephalosporins and cephamycins--A review. *Scand. J. Infect. Dis.* Suppl. 13, 1978, pp 83-87.

27. Schurman, D.J., Hirsham, H.P. & Borton, D.S. Cephalothin and cefamandole penetration into bone, synovial fluid, and wound drainage fluid. *The J. Bone & Joint Surgery, Inc.* 62-A(6): Sept. 1980, pp 981-985.

28. Shelton, S., Nelson, J.D. & McCracken, G.H., Jr. In Vitro susceptibility of gram-negative bacilli from pediatric patients to moxalactam, cefotaxime, Ro 13-9904, and other cephalosporins. *Antimicrob. Agents & Chemother.* 18, Sept. 1980, pp 476-479.

29. van Winzum, C. Clinical safety and tolerance of cefoxitin sodium: an overview. *J. Antimicrob. Chemother.* 4(Suppl. B), 1978, pp 91-104.

30. Watanakunakorn, C. and Glotzbecker, C. Comparative *In Vitro* activity of LY 127935 (6059-S), seven cephalosporins, three aminoglycosides, carbenicillin, and ticarcillin. *The J. of Antibiotics* XXXII:10, Oct. 1979, pp 1019-1024.

31. Young, J.P.W., Husson, J.M. et al. The evaluation of efficacy and safety of cefotaxime: a review of 2500 cases. *J. Antimicrob. Chemother.* 6:Suppl. A., 1980, pp 293-300.