Ketoconazole, an oral antifungal: laboratory and clinical assessment of imidazole drugs

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Summary

Miconazole, a parenterally administered imidazole antifungal agent has been shown to produce responses in systemic fungal infections in man. Ketoconazole, an analogue, can be given by mouth. It is inhibitory *in vitro* at low concentrations to most fungi. Blood levels after oral administration to animals and man greatly exceed these inhibitory concentrations for several hours. The efficacy of this drug has been demonstrated in animal models. Initial clinical evaluation has produced responses to therapy with 200-400 mg/day in 13 of 16 evaluable patients with systemic and superficial fungal infections, involving 10 fungal pathogens. No toxicity has been noted to date in these human studies. Ketoconazole is a promising agent needing further extensive evaluation.

Introduction

Miconazole is an imidazole drug, inhibitory *in vitro* against almost all pathogenic fungi (Van Cutsem and Theinpont, 1972). It is effective against experimental fungus disease in animals (Levine *et al.*, 1975). It is effective used topically for cutaneous, oral and vaginal infections in man (Anonymous, 1974; Sawyer *et al.*, 1975; Brincker, 1977). The drug has been under study or in general clinical use in several countries as parenteral therapy against systemic fungal infections in man (Stevens, 1977). This year (1978) it will be released for general clinical use in the U.S.A. During its evaluation stage, the authors studied several human fungal infections. More than 100 courses of treatment for coccidioidomycosis were

involved, most of them failures of or relapses after amphotericin B therapy. In such refractory cases, 35-67% have objective clinical responses, depending on the form of the disease (Stevens, 1977). Chronic pulmonary disease is the most responsive and meningitis the least. Every patient with paracoccidioidomycosis studied responded to therapy (Stevens *et al.*, 1978). Miconazole therapy seems to be the treatment of choice for petriellidiosis, since *Petriellidium boydii* is resistant to other available drugs (Lutwick *et al.*, 1979). Other workers have demonstrated the effectiveness of this compound against oesophageal candidiasis (Verhaegen, 1977) and chronic mucocutaneous candidiasis (Fischer *et al.*, 1977), as well as the diseases already mentioned. Side effects are principally hyponatraemia, anaemia, nausea and pruritus, and are reversible. A potential problem with this agent is relapse after successful therapy (Stevens, 1977). It was suggested that this related to the relative brevity of the courses of treatment for which there are several reasons. It was decided that admission to hospital was necessary because of the rapid disappearance from the blood of the drug and the resultant desire to give multiple doses per day. Recent analysis of experience with one manifestation of coccidioidal disease has now clearly indicated that in responders, relapse is associated with short courses of treatment (S. C. Deresinski and D. A. Stevens, *in press*).

These results were encouraging and further molecular modifications of the side chains on the imidazole ring were made by the chemists at Janssen Pharmaceutica, Beerse, Belgium. Several compounds were screened and tested (Levine, 1976) with the object of finding one that combined the most *in vitro*

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fungal inhibition, least animal toxicity, best absorption after oral administration, and most effectiveness in animal models. The compound thus brought to clinical trials was R41,400, now known as ketoconazole, and its differences from miconazole include the presence of both dioxolan and piperazine rings. Ketoconazole is soluble in acid, and can be given by mouth (van Cutsem *et al.*, unpublished).

Ketoconazole: *in vitro* susceptibility

Table 1 shows the results of the studies with several fungal and actinomycotic pathogens carried out by the authors using previously published methods (Levine *et al.*, 1975; Galgiani and Stevens, 1976). Many were highly susceptible ($MIC \leq 1$ mg/l). This degree of susceptibility will be related subsequently to achievable body fluid levels. *Candida* spp. are quite susceptible, while the most remarkable susceptibility was noted with *Paracoccidioides* sp. This latter degree of susceptibility parallels that seen with miconazole (Stevens *et al.*, 1978). This is significant in relation to the clinical results with *Paracoccidioides* sp. It should be noted that those methods of *in vitro* susceptibility testing whose results vary with inoculum size will show variability of inoculum size with ketoconazole (Brass and Stevens, 1978), as with miconazole (Galgiani and Stevens, 1976).

TABLE 1. Ketoconazole susceptibility *in vitro*

33 Strains	MIC (mg/l)
<i>Paracoccidioides brasiliensis</i>	0.002(2)*, 0.004(2)
<i>Blastomyces dermatitidis</i>	0.005
<i>Candida albicans</i>	0.02, 0.125, 0.25(2), 0.39(3), 0.78(4)
<i>C. parapsilosis</i>	0.25(2)
<i>Coccidioides immitis</i> (mycelia phase)	0.2(2), 0.4(2), 0.5, 0.58, 0.6†, 0.64
<i>Nocardia brasiliensis</i>	3.2
<i>Aspergillus</i> sp.	5.5, 6.4, 100
<i>Sporothrix schenckii</i>	6.4
<i>Torulopsis glabrata</i>	10
<i>Actinomyces madura</i>	25

* Numbers in parentheses = number of strains with identical MIC.

† Also tested in endospore phase, MIC = 0.4 mg/l.

Ketoconazole pharmacology in animals

The peak blood levels, reached at one hour, after administration to mice of oral doses of various sizes suggested a disproportionate increase of active drug in the serum with increase in dose of drug administered (Table 2). If true, this would suggest saturation of binding sites for the drug or of some body compartment in rapid equilibrium with the blood, or of some early metabolic or excretory pathway (saturable first-pass effect). As can be seen, these levels

TABLE 2. Peak blood concentration of ketoconazole in the mouse one hr after oral administration

Dose in mg/kg	(Peak blood concentration in mg/l/hr)
10	(3)
40	(13)
80	(57)
160	(135)

exceed considerably the MICs for the pathogens tested. The intermediate dose tested gave detectable levels for 5 hr, but as shown in Table 3 higher doses gave detectable blood levels for at least 8 hr.

TABLE 3. Concentration in mg/l of ketoconazole in blood of mouse at various times after oral administration

Time (hr)	Oral dose	
	80 mg/kg	160 mg/kg
1	57	135
2	57	65
3	28	68
4	17	21
5	13	16
6	5	15
7	4	11
8	2	6

Ketoconazole in experimental models

These pharmacological observations are consistent with some early experiments in animal models. The results of studies with ketoconazole therapy of experimental coccidioidomycosis are summarized in Table 4. The method of infection is intranasal inoculation with arthrospores, and treatment is initiated after the infection is established. As can be seen, therapy once daily for 17 days is completely protective against an infecting dose that kills > 60% of untreated mice. Viable organisms persist, however, in the healthy survivors after treatment, as noted with miconazole (Levine *et al.*, 1975). No development of resistance by the pathogen after *in vivo* therapy could be shown. However, with higher doses and more prolonged therapy, eradication of

TABLE 4. Ketoconazole-experimental murine coccidioidomycosis

Treatment	Dead/total	Survivors – Visceral* culture neg.
None	25/39	0/14
40 mg/kg daily for 17 days	0/10	0/10
80 mg/kg twice daily for 46–96 days	0/18	9/18

* Lung, liver, spleen and kidney.

all pathogens from the host can be achieved. This could not be achieved with tolerable doses of miconazole.

Preliminary experiments, by the present authors, in pulmonary blastomycosis produced by intranasal inoculation of 60 colony-forming units of *Blastomyces dermatitidis* in a murine model as described by Harvey *et al.* (1978) show that therapy begun on day 4 was completely protective against an LD₅₀ challenge (60 days' observation). (The mortality amongst 30 mice was 7 where no treatment was given; 0/30 where 160 mg/kg of ketoconazole was given (5 times/week).)

Ketoconazole pharmacology in man

These pre-clinical studies produce very interesting correlations with the early clinical data. Van Cutsem *et al.* (unpublished) have studied the pharmacokinetics following oral administration of 200 mg to man. Peak blood levels of 6 mg/l were reached at 2 hr, and levels exceeded 1 mg/l for ≥ 4 hr. Their later studies indicated that plasma levels after higher doses were relatively higher than after lower doses (e.g. peak levels after 100 mg were 25% of those after 200 mg), consistent with the authors' data for mice as presented above. It also appears that less drug is needed for man on a per-weight basis, to produce blood levels comparable to those in mice.

The authors performed protein-binding studies with equilibrium dialysis methods (Stevens, Levine and Deresinski, 1976) in human sera. At serum levels of 50 and 25 mg/l the binding was 93% and 91% respectively.

Initial clinical evaluation of ketoconazole (Table 5)

A total of 18 patients have been studied to date by the present authors although evaluation is preliminary and not complete. Thus far, treatment up to 180 days (36 g) has been recorded. The highest dose given has been 200 mg twice daily (2 patients); the rest of the patients have received 200 mg daily.

Six men with paracoccidioidomycosis were treated; 5 of them objectively responded to therapy, although

4 remain on prolonged maintenance therapy, as previously suggested (Stevens *et al.*, 1978).

Case 1 (aged 32 years) had a 5-month history of painful mouth ulcers. Ulcers of the oral mucous membranes measured up to 2.5 cm in diameter. Scrapings of the ulcers and sputum cultures grew *Paracoccidioides brasiliensis*. The mucosal lesions and associated pain were decreased after 2 weeks and healed after one month of therapy. At this point the patient was unable to raise sputum.

Case 2 (37 years) relapsed, 2 months after a remission induced by a short course of miconazole, with haemoptysis, positive sputum smears and pulmonary infiltrates. On therapy, his X-ray cleared, and haemoptysis and sputum production disappeared. His CF titre and immunodiffusion tests with *P. brasiliensis* (Restrepo-M and Moncada, 1970) have fallen from 1/512 to 1/256 and 3 bands to 1 band respectively.

Case 3 (56 years) had at least 7 months of epigastric pain. A gastric lesion biopsied at surgery revealed *P. brasiliensis*, sputum smears were also positive, as was a tongue lesion measuring 5 × 8 mm. Therapy with sulphonamides was attempted but discontinued because of associated vomiting. His epigastric pain decreased after 1 week of therapy with ketoconazole, and the tongue lesion cleared after 15 days. At 2.5 months of therapy his serology was unchanged as were pulmonary infiltrates on X-ray.

Case 4 (43 years) had a 4-month history of neck nodules, with spontaneous purulent drainage, and weight loss. Chest X-ray showed infiltrates and fibrosis. Cultures of sputum and drainage yielded *P. brasiliensis*. The neck lesions started to heal by the 10th day of therapy and the draining sinus had cleared by the 18th day. By the second month of therapy only a scar remained in this area, the patient regained some weight. He has now returned to work, sputum production has ceased, and the X-ray has begun to clear.

Case 5 (17 years) had relapsed 14 months after combined amphotericin B + sulphonamide + miconazole therapy, with disseminated cutaneous disease. The skin lesions showed improvement after one week of treatment. Therapy was stopped after 1.5 months of treatment, and the lesions had cleared. Relapse, however, occurred 15 days later.

The sixth patient is non-evaluable; this emaciated 57-year-old with a 13-month history of weight loss, fever, dyspnoea and sputum production, had bilateral alveolar infiltrates on X-ray. He died, after only 6 days' therapy, of undiagnosed causes, with hypotension and congestive heart failure.

Two patients with cutaneous sporotrichosis were treated. A 24-year-old man had 7 skin lesions, and concurrent, diagnosed, cutaneous tuberculosis. The

TABLE 5. Clinical results - Ketoconazole

	Response	No response	Non-evaluable
Paracoccidioidomycosis	5	0	1
Sporotrichosis	1	0	1
Candidiasis	2	0	0
Coccidioidomycosis	1	0	0
Histoplasmosis	1	0	0
Pityriasis versicolor	1	0	0
Tinea and p. versicolor	2	0	0
Mycetoma	0	2	0
Chromoblastomycosis	0	1	0
Total no. of patients:	13	3	2

lesions cleared completely on 3 months' therapy concurrent with antituberculous therapy, and *Sporothrix schenckii* could not then be grown from the skin. He is in remission 3 months after discontinuation of therapy. A 22-year-old woman was treated for one week, and showed no change.

Two patients with candidiasis were treated. A 29-year-old man with chronic mucocutaneous candidiasis of 8 years' duration had previously shown temporary responses to nystatin, tolnaftate, and miconazole. His mouth, perineum and nails were involved, and *Candida albicans* was cultured. The lesions cleared after the third day of therapy, which was discontinued after 8 days. Cultures were then negative. He remains in remission 6 months later. A 13-year-old girl with chronic oral candidiasis cleared her disease with daily therapy, which has been gradually reduced to a maintenance dose of 200 mg once a week, and she remains in remission 6 months after therapy was started.

A 21-year-old man with a 3-month history of coccidioidomycosis of the tibia, with overlying sinus tracts and cutaneous involvement was treated for 3 months. In this time the lytic lesion in the tibia cleared, as did the skin, and cultures became negative for *Coccidioides immitis*.

A 32-year-old man with severe pharyngeal and miliary pulmonary histoplasmosis, with positive marrow cultures responded promptly and was discharged from hospital after 3 weeks' therapy, after his pharyngeal cultures had reverted to negative. After 2 months' therapy all clinical evidence of disease had cleared, and the chest X-ray was clearing. He remains in remission 6 months later.

Two men, aged 16 and 18 years, both had concurrent pityriasis versicolor (*Malassezia furfur*) and tinea (*Epidermophyton floccosum*) of the legs and feet (and, in one case, also the trunk). Three weeks' treatment cleared their lesions, and skin scrapings were negative on culture. They remain in remission 3 and 4 months after termination of therapy.

An 18-year-old man with pityriasis versicolor was cured on 2 weeks' therapy, and remains in remission 3 months later.

A 42-year-old diabetic man with chromoblastomycosis of 6 years' duration was treated for 3 weeks, without change.

Two patients with mycetomas failed to respond. One 65-year-old man had a mixed *Monosporium apiospermum*/*Aspergillus* sp. infection of the ankle and foot of 18 years' duration. A 64-year-old man had a *Nocardia brasiliensis* infection of the femur and lower leg of 10 years' duration. Therapy of 43 and 56 days respectively produced no change.

Toxicology of ketoconazole in man

No subjective symptoms were noted by any of the

patients on therapy. The haematocrit, haemoglobin, leukocyte count and differential were performed before, during, and after therapy in 12 patients; serum glucose and creatinine determinations and urine analysis in 11; platelet count, serum calcium, cholesterol, protein, SGOT, bilirubin, sodium, potassium, chloride in 10; serum phosphate and prothrombin time in 9; partial thromboplastin time and slit lamp eye examinations in 6; and serum bicarbonate in 4 patients. None of these determinations showed any abnormalities induced by the drug, with the possible exception of one patient who had a transient rise in SGOT which returned to normal, even though he remained on therapy at the same dose.

Conclusion

Ketoconazole appears to be a promising antifungal agent with desirable pharmacological properties and limited toxicity. Further extensive clinical studies are warranted.

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