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Letter to the Editor

Protonation of ketoconazole in relation to fungistatic activity

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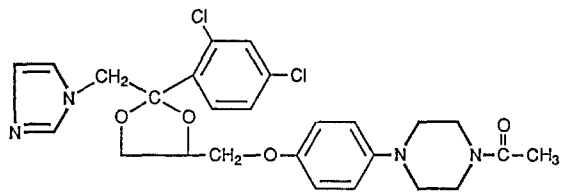
Antifungal imidazole-containing drugs are primarily fungistatic, although some can kill under certain conditions [1]. Fungistatic action is due largely to inhibition of a cytochrome P-450-dependent enzyme system that converts lanosterol to ergosterol, a fungal cell membrane component [2, 3]. The imidazole group behaves as a weak base and pKa values range from 5.7 for bifonazole [4] to 6.3, 6.5, and 6.7 for clotrimazole, ketoconazole, and miconazole, respectively [2]. In view of evidence that *Candida* sp. maintain a cytoplasmic pH of 5.7–6.7 [3], a substantial fraction of any intracellular imidazole would be nonprotonated; i.e. the form that binds to target enzyme [2, 3]. Since nonprotonated imidazole also appears to be required for cellular uptake [5], it is not surprising that an acidic environment usually decreases drug activity. For most of these agents, however, the pH effect is quite modest. Generally, MICs are increased <10-fold as pH is decreased from 8 to 5 [6–10] and even down to pH 3–4 [7, 9, 11].

Several years ago Minagawa et al. [12] reported that in contrast to other imidazoles, the activity of ketoconazole against *C. albicans* is enormously influenced by pH. MICs decreased in excess of 1000-fold as growth medium pH was raised from 3 to 8. Tests by others based on inhibition of ergosterol synthesis [3] and on reduction in MIC [8, 13] have supported this finding. Ketoconazole, in addition to an imidazole, also possesses a piperazine group (pKa ~ 3) [3]. Minagawa et al. [12] attributed shifts in MIC to degree of piperazine protonation. Intended or not, this questioned whether protonation of the imidazole group plays any significant role in ketoconazole activity.

Susceptibility of ketoconazole activity to environmental pH is probably a reflection of drug uptake that is governed by both piperazine and imidazole group protonation. Since neutral substrates are generally more permeable, drug molecules bearing a nonprotonated piperazine as well as a nonprotonated imidazole should be preferred for cell uptake. In this regard, there is evidence that the piperazine group plays a key role in the interaction of ketoconazole with cell membranes [14] and that maximal uptake of ketoconazole by *C. albicans* occurs between pH 6.5 and 7.0 [3, 15]. Although Minagawa et al. [12] reported over a 1000-fold decrease in MIC as pH was raised from 3 to 8, they observed only an 8- to 16-fold decrease in ketoconazole MIC as pH was raised from 5 to 8. This latter and rather modest change agrees quite well with data obtained for other imidazole-containing drugs [6–10], and probably reflects increases in the fraction of neutral imidazole group as pH is increased above 5.0 (see Table 1). At pH 5.0 and above essentially all of the piperazine is nonprotonated and, therefore, probably not involved in any shift of MIC in this pH range. From pH 5.0 to 3.0, however, the situation is quite different. Over this acidic range, essentially all of the imidazole remains in the protonated form, but piperazine undergoes a very

Table 1. Protonation of the imidazole and piperazine moieties of ketoconazole at different pHs.



pK a = 6.5 pK a = 3.0

%neutral: %protonated[a]	pH	%neutral: %protonated[a]
<<1 : >>99	3.0	50 : 50
<1 : >99	4.0	91 : 9
3 : 97	5.0	99 : 1
24 : 76	6.0	>99 : <1
50 : 50	6.5	>>99 : <<1
76 : 24	7.0	>>99 : <<1

a = *as calculated from the Henderson-Hasselbalch equation:* $pH = pKa + \log \frac{[nonprotonated]}{[protonated]}$

marked change (i.e. a 50% decrease in the neutral form). It is suggested that as pH is increased from 3.0 to 5.0, the 100- to 200-fold reductions in ketoconazole MIC observed by Minagawa et al. [12] are a result of decreases in piperazine protonation, but above pH 5.0, further reductions in MIC are due to decreases in imidazole group protonation.

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