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LACK OF ANTIVIRAL ACTIVITY OF KETOCONAZOLE ALONE OR IN COMBINATION WITH THE ACYCLIC NUCLEOSIDE GANCICLOVIR AGAINST A HERPES VIRUS TYPE 2 INFECTION IN MICE

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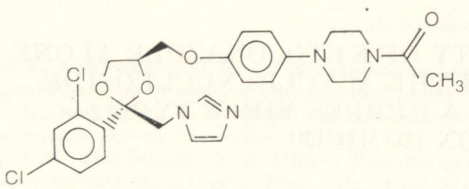
Summary. — Ketoconazole and ganciclovir were tested for antiviral activity, each alone and in combination, against a herpes simplex virus type 2 (HSV-2) systemic infection in Swiss-Webster mice. When given once daily for 5 days starting 24 hr after infection, the ED_{50} for ketoconazole either alone or in combination was >60 mg/kg; for ganciclovir, the ED_{50} was 7.1 mg/kg alone and 10.8 mg/kg in combination. Thus, ketoconazole did not potentiate or antagonize the antiviral activity of an acyclic nucleoside. Consequently, AIDS patients could perhaps receive ketoconazole and ganciclovir simultaneously for fungal and viral opportunistic infections without interference with their respective efficacies.

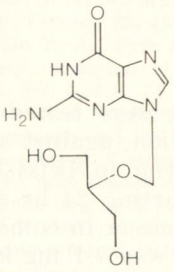
Key words: *ketoconazole*; *ganciclovir*; *HSV-2*; *antiviral activity*

Human immunodeficiency virus (HIV), the aetiological agent of Acquired Immunodeficiency Syndrome (AIDS), selectively infects and debilitates helper T-lymphocytes. This severe defect in the immune system renders patients with AIDS highly susceptible to opportunistic infections. *Cryptococcus*, *Candida*, herpes virus, and cytomegalovirus are several common causes of opportunistic infections in immunocompromised AIDS patients. In the clinic, ganciclovir and acyclovir are the treatments of choice for CMV and herpes infections, respectively. Cryptococcal meningitis in AIDS patients is presently treated with Amphotericin B and often followed by maintenance therapy with ketoconazole. In addition, ketoconazole is also given for treatment of *Candida* esophagitis and oral thrush.

In recent *in vitro* studies, Pottage *et al.* (1986) demonstrated that ketoconazole possessed antiviral activity against HSV-1 and HSV-2 and synergistic antiviral activity when it was combined with acyclovir. The present study was designed to compare the efficacy of ketoconazole and ganciclovir, either alone or in combination, against a HSV-2 systemic infection in mice, in order to determine whether or not a synergistic or antagonistic interaction might occur when the two were given concurrently. Ketoconazole (cis-(±)-1-acetyl-4[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3 dioxo-



Ketoconazole



Ganciclovir

Fig. 1.
Chemical structures for ketoconazole and ganciclovir
Top: Ketoconazole
Bottom: Ganciclovir

Table 1. Effect of treating with Ketoconazole or Ganciclovir either alone or in combination against a systemic HSV-2 infection in mice*

Treatment regimen		Response to treatment				ED_{50}	
Ganciclovir (mg/kg)	Ketoconazole (mg/kg)	No. of Survivors/ Total	Percent alive	Time to death (days) Median	Range	Ganciclovir	Ketoconazole
Saline	—	0/20	0	7	6—11		
12	—	14/20	70	14	11—20	7.1	
4	—	11/20	55	12.5	11—15		
1.3	—	1/20	5	10	8—14		
12	30	11/20	55	12	11—17	10.8	30
4	30	5/20	25	12.5	8—15		
1.3	30	5/20	25	11	8—15		
—	60	0/20	0	8	5—11		
—	30	0/20	0	8	5—10		> 60
—	10	0/20	0	8	6—12		
4	60	4/20	20	11	4—19		
4	30	7/20	35	12.5	8—19	4	> 60
4	10	4/20	20	11	6—16		

* Ketoconazole was given orally, and ganciclovir was given subcutaneously. All treatments were given once daily for 5 days, starting 24 hr after infection.

lan-4-yl]methoxy]phenyl]-piperazine) was initially solubilized in 0.1 mol/l hydrochloric acid and then diluted >20 fold in sterile water for oral administration. Ketoconazole was tested at 60, 30, and 10 mg per kg per day. Ganciclovir [9-(1,3-dihydroxy-2-propoxymethyl)guanine, DHPG] was solubilized in sterile physiological saline and administered subcutaneously at 12, 4, and 1.3 mg per kg per day. Both ketoconazole and ganciclovir were synthesized by Syntex Research (Fig. 1).

The virus used in this study was herpes simplex virus type 2 (HSV-2), G strain. It was propagated in African green monkey (Vero) cells and titered in human laryngeal carcinoma (Hep-2) cells. The HSV-2, Hep-2, and Vero cells were obtained from the American Type Culture Collection, Rockville, Md.

Female Swiss-Webster mice were employed for this investigation. The animals, weighing 18–20 grams, were obtained from Simonsen Labs in Gilroy, CA. Groups of 20 mice were infected intraperitoneally with approximately 1.5×10^4 PFU per mouse. This HSV-2 challenge was approximately seventy-five LD_{50} doses and produced 100 per cent mortality in saline-treated controls.

Starting 24 hours after infection, groups of 20 mice received oral treatments of ketoconazole either alone or in combination with subcutaneous treatments of ganciclovir at the above listed doses. Treatments were given once a day for 5 days. Mice were observed for mortality for 21 days after challenge.

As seen in Table 1, a dose response was demonstrated when ganciclovir was administered alone. At doses of 12 or 4 mg per kg, the compound significantly increased the number of survivors when compared with the saline control ($P < 0.001$, two-tailed Fisher probability tested [Maxwell, 1961]). The effective dose at which 50 per cent of the mice survived (ED_{50}) was 7.1 mg per kg as calculated using probit analysis (Finney, 1964). A dose response was also seen when ganciclovir was administered in combination with an ineffective dose of ketoconazole (30 mg/kg). However, in this combination the ED_{50} of ganciclovir was not less than ganciclovir alone, indicating that the dose response seen was the effect of ganciclovir itself. Efficacy was not seen when ketoconazole was given by itself. In addition, when ketoconazole was combined with a slightly effective dose of ganciclovir (4 mg/kg), the activity, as demonstrated by the increase in the number of survivors, was similar to that of ganciclovir alone at 4 mg/kg.

In vitro studies examining treatment of HSV-1 and -2 with ketoconazole in human lung (HL) and Vero cells have shown a dose-dependent reduction in viral titre and, when combined with acyclovir, obvious synergy was demonstrated. Pottage *et al.* proposed that this antiviral activity of ketoconazole could be attributed to interference with lipid metabolism and the consequent production of a detective viral envelope (Pottage *et al.*, 1986). However, in the present *in vivo* study ketoconazole did not demonstrate anti-HSV-2 activity in mice from 10–60 mg/kg (or at 100 mg/kg in a separate test, data not shown). Efficacy of ketoconazole has been reported against numerous other pathogens using the same dose levels as in this investigation. At 60 and 30 mg/kg, ketoconazole significantly increased the survival of mice subjected to lethal challenges of *Trypanosoma cruzi* (McCabe *et al.*, 1983, 1984). In humans, ketoconazole has been shown to be effective at comparable levels against paracoccidiodomycosis, histoplasmosis, coccidiodo-

mycosis, blastomycosis, as well as a variety of yeast and fungal infections (Smith *et al.*, 1984; van Tyle and Hawkins, 1984).

Thus, although synergy was observed *in vitro* when ketoconazole was combined with acyclovir against HSV-2 (Pottage *et al.*, 1986), under the conditions of the present *in vivo* study ketoconazole did not potentiate the antiviral activity of the acyclic nucleoside, ganciclovir. Perhaps of greater interest to the clinician is that no antagonistic interaction between ketoconazole and ganciclovir was evident. Consequently, patients with AIDS might possibly be treated simultaneously for fungal and viral opportunistic infections without risking interference with their respective efficacies.

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