R34000, A Dioxolane Imidazole in the Therapy for Experimental Coccidioidomycosis*

Comparison with Miconazole and Econazole

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Comparisons were made on the therapeutic influence of three imidazole drugs in experimental lethal coccidioidomycosis of mice. When administered by the intramuscular route, miconazole and a closely related structural analogue, econazole, were effective in preventing death, restricting fungal replication in the lungs, and minimizing the extent of extrapulmonary dissemination. Neither drug was as effective when administered by the oral route as by the intramuscular route. This contrasted sharply with results obtained using R34000, a dioxolane imidazole. It was very highly effective when administered by the oral route and less so by intramuscular injection. All orally treated mice survived a challenge lethal to more than 80 percent of control animals. Plasma or serum concentrations of orally administered R34000 in mice and in man exceeded the minimum inhibitory concentration for a virulent strain of *Coccidioides immitis*.

Recent studies have shown that the synthetic imidazole, miconazole, prevented death in experimental murine coccidioidomycosis. Other studies in man have pointed to the usefulness of the drug in managing human coccidioidomycosis, including infections with dissemination to the meninges.

A second drug, econazole, which is very closely related to miconazole in structure, has now been examined in experimental coccidioidomycosis of mice. As will be described, econazole was as therapeutically effective as miconazole by intramuscular administration. Both miconazole and econazole were of lesser value when administered orally; however, a third imidazole drug, R34000, was very effective in oral treatment. It produced blood concentrations in mice and in a human volunteer that were in excess of the minimum inhibitory concentration for *Coccidioides immitis*. This report describes therapeutic trials in mice with the three imidazole drugs.

**Materials and Methods**

**Drugs**

R34000, miconazole, and econazole were synthesized by a commercial manufacturer (Janssen Pharmaceutica). Miconazole and econazole were supplied at a concentration of 10 mg/ml in an aqueous vehicle containing the following per milliliter: 0.115 ml of polyethoxylated castor oil, 1.62 mg of methylparaben, and 0.18 mg of propyl paraben. The vehicle was nontherapeutic; it was also noninhibitory to *C immitis* at the concentrations employed for studies on inhibition in vitro. R34000 was supplied as a crystalline powder. It is relatively insoluble in water and was therefore utilized in one of three solvents or solvent mixtures for different purposes. In studies on the effect of R34000 administered by the oral route, the drug was given in different doses by lavage (0.2 ml) in No. 200 polyethylene glycol. Undiluted No. 200 polyethylene glycol was employed as placebo at the same volumes as it occurred in the drug solution and by the same regimens. When R34000 was administered intramuscularly, it was suspended in a solution containing 5 percent No. 200 polyethylene glycol and 1 percent polysorbate 80 (Tween 80; polyethylene sorbitan monooleate mixture) in 0.9 percent sodium chloride. Control animals were treated with this vehicle. For studies in vitro, R34000 was initially dissolved in No. 200 polyethylene glycol at a concentration of 10 μg/ml. This solution and subsequent dilutions were made in water and were used to determine inhibition of endospores of *C immitis* on agar. The No. 200 polyethylene glycol vehicle and dilutions of it were noninhibitory.

**Assay of Drugs in Vitro**

The relative inhibitory activities of the three drugs were determined on Sabouraud agar, and the dose-response relationships served also as the basis for biological assays of drug concentrations in mouse plasma. Reference standards were prepared in normal mouse plasma for the purpose. One study was concerned with determining the concentration of R34000 in human serum, and in this case the standards were made in the same menstruum obtained from a human being who had not ingested the drug. The method of assay was a modification of one described by H. Van Landuyt (unpublished report to Janssen Pharmaceutica, Sept 9, 1974). A base of 65 ml of Sabouraud medium containing 1.5 percent agar was allowed to solidify in Petri plates (150 mm in diameter and 25 mm in depth). Over this base was poured 9 ml of a seed layer with 10^4 endospores per milliliter in the
same medium modified to contain 0.75 percent agar. The endospores were freshly grown, as described elsewhere. Solutions of the drug to be assayed (0.3 ml) in plasma, water, or No. 200 polyethylene glycol solution were introduced into cylinders 8.0 mm in diameter that had been placed on the agar. After diffusion for 24 hours at 3°C, the assemblies were incubated for 72 hours at 34°C before zones of growth inhibition were measured.

**Therapeutic Trials**

Female albino mice (eight weeks old; mean weight, 29 g) were anesthetized by exposure to vapor from a mixture of diethyl ether, chloroform, and anhydrous alcohol (ethanol) (3:2:1, volume/volume). The animals were infected intranasally with 0.05 ml of a suspension of arthrospores of *C. immitis* (strain Silveira) in a 0.9 percent solution of sodium chloride. The infecting dose was determined by triplicate pour plates of serial decimal dilutions of the suspension on 2 percent glucose, 1 percent yeast extract agar (2XGYE). Drugs (or placebo) were then administered by the routes and schedules described further, beginning on the third day after infection or later. Fungal numbers in different organs were determined by plating homogenates of the organs on 2 percent glucose, 1 percent yeast extract agar containing antibiotics directly and after dilution in a 0.9 percent solution of sodium chloride.

**RESULTS**

The structural configurations and empirical formulae of the imidazole drugs examined in this study are shown in Figure 1. Miconazole and econazole are closely related; in both, there is a methyl ethyl ether chain substituted at the β-position with an imidazole nitrate group. The α-positions of the ether have chlorinated phenyl substituents in the 2 and 4 positions, whereas one of econazole's phenyl rings contains only a single chlorine in the 4 position. Thus, econazole differs from miconazole by the absence of one chlorine atom.

R34000 differs markedly from miconazole and econazole. A 1,3-dioxolane ring is attached to the methylene group that is bonded to the imidazole nitrogen. The compound contains a single 2,4-dichlorophenyl group attached to the dioxanyl structure at the 2 position, and a biphenyl group is bridged to the 4 position by an oxymethyl group. Both the latter structure and the dioxolane ring do not occur in miconazole and econazole.

The three drugs were inhibitory in vitro, at high dilution, to the endospore phase of *C. immitis* (Fig 2). Econazole was somewhat more active in vitro than the other compounds on Sabouraud agar, and R34000 was least active; however, the relative activities of the drugs in vitro should be qualified at this juncture, since Van den Bossche and associates found that the inhibitory activities of the drugs were dependent on the medium. In nutritionally lean media, R34000 showed considerably greater inhibitory activity in vitro than miconazole (H. Van den Bossche, G. Willemsens, and F. Cornelissen, unpublished data; obtained from Janssen Research Products Information Service Report, February 1976).

The in vitro activity of R34000 appeared to be little influenced by the presence of plasma (Fig 2), but this feature currently may be variable; a second lot of drug, synthesized several months later, showed twofold to fourfold diminution in inhibitory activity when plasma was present. Nevertheless, both lots were less affected in vitro by binding to plasma proteins than either miconazole or econazole (Fig 2).

The capacity of the three imidazole drugs to preserve life in mice challenged intranasally with otherwise lethal doses of arthrospores of *C. immitis* is re-
viewed in Table 1. Deaths are shown at 30 days after infection; the final mortality, as discussed earlier, was essentially established before this time. Intramuscular treatment with miconazole (72 mg/kg twice daily) was highly effective when administered over days 3 to 21 after infection (experiment 1) or days 5 to 12 after infection (experiment 6). Similarly, econazole in intramuscular doses of 36, 54, or 72 mg/kg twice daily over days 5 to 20 after infection (experiment 7) was comparably effective.

R34000 had a profound lifesaving influence when given by the oral route over days 3 to 21 (after infection) in doses of 43 mg/kg twice daily (experiment 1), 29 mg/kg twice daily (experiment 2), or 43 mg/kg twice daily by a regimen interrupted intermittently after the 15th day after infection (experiment 3). The drug was less effective when treatment was discontinued on the 16th day (experiment 5). Oral treatment with econazole, on the other hand, was less effective than intramuscularly injected econazole (experiment 7). Orally administered R34000 was superior to the same drug given intramuscularly (experiment 2). Finally, orally administered R34000 was more efficacious than miconazole given by the oral route (experiments 4 and 5).

The picture that emerged from these studies, ie that R34000 was the drug of choice for oral therapy in murine coccidioidomycosis while miconazole and econazole were preferable for intramuscular therapy, was reinforced by findings on blood concentrations of the drugs. A single oral dose of 1 mg (35 mg/kg) of R34000 produced plasma concentrations of between 2 μg/ml and 3 μg/ml within 30 minutes (Fig 3). This level exceeded the minimum inhibitory concentration by 20-fold to 30-fold, as may be calculated from the data in Figure 2. The plasma concentration remained above 1.5 μg/ml for at least five hours. Intramuscularly administered R34000 was detected in low concentrations only after a three-hour delay. The same oral dose of econazole yielded a high plasma concentration within one hour, but it declined rapidly and sooner than was the case when it was administered intramuscularly. Miconazole given orally did not produce plasma concentrations of drugs as high as it did with the intramuscular route, and decay was more rapid when the drug was administered by the oral than by the intramuscular route.

The occurrence of high concentrations of R34000 in the blood after ingestion of the drug has also been...
### Table 1—Influence of Imidazole Drugs Intramuscularly (IM) or per os (PO) on Mortality in Mice Infected Intranasally with Arthrospores of C. immitis (Strain Silveira)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Drug, Days of Treatment Route</th>
<th>Infected Dose (No. arthrospores)</th>
<th>Control/Placebo*</th>
<th>Percent Mortality at 30 Days after Infection (No. Dead/Total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R34000, POS 3-21 MI 3-21</td>
<td>PO 64 70 (7/10)</td>
<td>18 mg/kg b.i.d. 29 mg/kg b.i.d. 36 mg/kg b.i.d. 43 mg/kg b.i.d. 54 mg/kg b.i.d. 72 mg/kg b.i.d.</td>
<td>0 (0/10)</td>
</tr>
<tr>
<td>2</td>
<td>R34000, POS 3-21 MI 3-21</td>
<td>PO 59 87 (13/15)</td>
<td>18 mg/kg b.i.d. 29 mg/kg b.i.d. 36 mg/kg b.i.d. 43 mg/kg b.i.d. 54 mg/kg b.i.d. 72 mg/kg b.i.d.</td>
<td>0 (0/14)</td>
</tr>
<tr>
<td>3</td>
<td>R34000; POS 4-15, 18-22, 25-29</td>
<td>PO 54 83 (15/18)</td>
<td>18 mg/kg b.i.d. 29 mg/kg b.i.d. 36 mg/kg b.i.d. 43 mg/kg b.i.d. 54 mg/kg b.i.d. 72 mg/kg b.i.d.</td>
<td>0 (0/17)</td>
</tr>
<tr>
<td>4</td>
<td>Miconazole, POS 5-16</td>
<td>PO 26 100 (5/5)</td>
<td>18 mg/kg b.i.d. 29 mg/kg b.i.d. 36 mg/kg b.i.d. 43 mg/kg b.i.d. 54 mg/kg b.i.d. 72 mg/kg b.i.d.</td>
<td>87 (13/15)</td>
</tr>
<tr>
<td>5</td>
<td>R34000, POS 5-16</td>
<td>PO 33 40 (2/5)</td>
<td>18 mg/kg b.i.d. 29 mg/kg b.i.d. 36 mg/kg b.i.d. 43 mg/kg b.i.d. 54 mg/kg b.i.d. 72 mg/kg b.i.d.</td>
<td>27 (4/15)</td>
</tr>
<tr>
<td>6</td>
<td>Miconazole, POS 5-12</td>
<td>IM 74 50 (10/20)</td>
<td>18 mg/kg b.i.d. 29 mg/kg b.i.d. 36 mg/kg b.i.d. 43 mg/kg b.i.d. 54 mg/kg b.i.d. 72 mg/kg b.i.d.</td>
<td>5 (1/20)</td>
</tr>
<tr>
<td>7</td>
<td>Econazole, POS 5-20</td>
<td>PO 29 50 (5/10)</td>
<td>18 mg/kg b.i.d. 29 mg/kg b.i.d. 36 mg/kg b.i.d. 43 mg/kg b.i.d. 54 mg/kg b.i.d. 72 mg/kg b.i.d.</td>
<td>27 (4/15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IM 61 80 (8/10)</td>
<td>18 mg/kg b.i.d. 29 mg/kg b.i.d. 36 mg/kg b.i.d. 43 mg/kg b.i.d. 54 mg/kg b.i.d. 72 mg/kg b.i.d.</td>
<td>53 (8/15)</td>
</tr>
</tbody>
</table>

* Placebo for all drugs given per os was No. 200 polyethylene glycol per os by the same regimen. Placebo for intramuscular administration of R34000 was the vehicle, 5 percent No. 200 polyethylene glycol, 1 percent polysorbate 80 (Tween 80) in 0.9 percent solution of sodium chloride administered intramuscularly by same regimen. Control for intramuscular administration of miconazole and econazole was no treatment. Animals were infected on day 1.

ascertained in a human being. A 65 kg (143 lb) man ingested 1 gm of R34000 (15 mg/kg). A specimen of his serum obtained before ingestion of the drug showed no drug. Specimens of blood drawn at 2, 4, 8, and 24 hours after ingestion of the drug assayed, respectively, 1.7 ng/ml of serum, 4.0 ng/ml of serum, 3.3 ng/ml of serum, and 0.5 ng/ml of serum. These levels were not grossly divergent from corresponding plasma levels in mice after oral treatment with 1 mg of R34000 (35 mg/kg), as described in Figure 3.

It was clear in earlier studies with mice that very high intramuscular doses of miconazole (75 mg/kg twice daily from days 5 to 20 after infection) did not eradicate C. immitis from the lungs, liver, or spleen. In the present study, this was also the case with both econazole and R34000 and with combinations of miconazole and R34000. Even the administration of R34000 over a 54-day period (Table 2) did not result in biological cure. Despite the drug's remarkable capacity to preserve life, minimize extrapulmonary involvement, and restrict fungal replication in the lungs (Table 2), all surviving animals that were tested yielded C. immitis on culture.

### Discussion

The usefulness of miconazole in managing human coccidioidomycosis2,3 and its efficacy in a model of murine coccidioidomycosis led us to investigate two other imidazoles in the murine model. Both econazole and R34000 were as effective as miconazole, but the unique capacity of orally administered R34000 to control the sequelae of coccidioidal infection attracts attention. Earlier, unpublished data by J. Van Cutsem and D. Thienpont (Janssen Research Product Information Service Report, March 1976) showed the efficacy of orally administered R34000 in the treatment of systemic candidiasis of chickens.

Further studies in other hosts should indicate if R34000 qualifies as a candidate for a trial in man. In particular, toxicologic investigations should be made. Toxicity was not a factor of our investigation; however, it is apparent from the data in Table 2 that a cumulative dose of 0.1 gm over a period of 54 days was nonlethal to mice. Furthermore, these animals treated with 43 mg/kg twice daily did not manifest superficial symptoms of toxicity (despite their coccidioidal disease); they gained weight, and
Their fur coats remained unruffled. Other mice that had been examined at necropsy after three weeks of treatment showed no macroscopic evidence of extrathoracic disease are in accord with the possibility that advantages may be seen. Two other attributes of the drug mentioned earlier—should be emphasized in this context. During treatment with R34000, the animals showed no gross signs of illness, even during the critical period at 15 to 20 days after infection, when more than 80 percent of the control animals died. Secondly, serum concentrations achievable in man after ingestion of a single 1 gm dose of R34000 exceeded for 24 hours the minimum inhibitory concentration for the strain of C immitis used in this study. These initial observations emphasize the desirability of continuing studies with the drug.

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REFERENCES
4 Hoeprich PD, Goldstein E: Miconazole therapy for coccidioidomycosis. JAMA 230:1153, 1974