Relationship Among Physicochemical Properties, Skin Permeability, and Topical Activity of the Racemic Compound and Pure Enantiomers of a New Antifungal

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Received August 7, 1991; accepted July 18, 1992

The topical antifungal Sch-39304 is a racemic compound comprised of two enantiomers, Sch-42427 and Sch-42426, only one of which (Sch-42427) is pharmacologically active. The pure enantiomers have a lower melting point and, therefore, a higher solubility than the racemic compound. Because of these differences in physicochemical properties, the concentration of the pure enantiomers in vehicles and in the skin was predicted to be an order of magnitude higher than the racemic compound. It was hoped that the pharmacological activity would also be higher. By measuring the flux of the chiral forms through human cadaver skin, the expected differences in skin solubility were confirmed. However, only a minimal difference between racemate and active enantiomer was observed in the lesion scores using a guinea pig dermatophyte model. By fitting the data to the E_{max} pharmacodynamic model, it is demonstrated that the maximum effect occurs at a concentration lower than the saturated concentration of the less soluble racemic compound. The data illustrate that the efficacy of topically active compounds may not be linearly related to drug concentration in either the vehicle or the skin.

KEY WORDS: topical activity; racemic compounds; chirality; enantiomers; antifungals.

INTRODUCTION

The varying activities and pharmacokinetics among chiral forms of pharmaceutically active compounds have been well documented (1–6). The chirality of a compound is critical to receptor-binding interactions; therefore, differences in biological activity are common. Chirality can also effect the way in which molecules fit together in a crystal. Two enantiomers may crystallize together in a racemic compound or true racemate in which half of the positions in the unit cell are replaced by the opposite enantiomer, which may result in a different crystal structure from the pure enantiomers (7,8). If the difference in crystal structure results in a change in the melting point, then the solubilities of the pure enantiomers may be different from that of the racemic compound (8,9). This difference in solubility between enantiomers and the racemic compound may also affect physiological phenomena, such as membrane permeability, pharmacokinetics, and ultimately pharmacological effects.

In this investigation, certain physicochemical properties (melting point, solubility) and skin permeation of the racemic compound (Sch-39304) and pure enantiomers (Sch-42426 and Sch-42427) of a novel topical antifungal (Fig. 1) were characterized. Mathematical models were then applied to relate the physicochemical properties of the chiral forms to maximum pharmacological effect.

THEORY

For a given vehicle, the concentration of drug in the skin, C_m, as well as the flux through the skin, J, will be at a maximum (represented by a superscript asterisk) when in contact with a saturated solution of drug, C_d^*,

$$C_m^* = k_p C_d^* = J^* h / D_m \quad (1)$$

where k_p, D_m, and h are the partition coefficient, diffusivity, and skin thickness, respectively (10). For a racemic compound, r, and pure enantiomer, e, the activity coefficient, γ, entropy of fusion, ΔS, and diffusivity, D, may be assumed to be the same because of the similarity in their molecular structures after melting (8,9,11). Since the saturated concentration of a compound can be related to its melding point, T_m (9), the concentration or flux ratios of two compounds, differing only with respect to melting points, may be given by

$$J_r^*/J_e^* = C_r^*/C_e^* = e^{-A(T_{mr} - T_{me})/T} \quad (2)$$

where the constants A and B are equal to $\Delta S/R$ and γ, respectively.

Since the partition coefficient is a ratio of concentrations in one solvent vs the other, the melting-point term cancels. Therefore, no differences should be observed in the partitioning behavior of the racemic compound vs the pure enantiomer.

The E_{max} model can be used to relate concentration in the skin and pharmacological effect (12). When inhibition of some biological phenomenon is the measure effect, E, the expression is

$$E = E_{ND} - [E_{max} C_m / (C_{m50} + C_m)] \quad (3)$$

where E_{ND}, E_{max}, and C_{m50} are the effect with no drug, maximum effect, and concentration at 50% of E_{max}, respectively. In the case of an inhibition relationship, the E_{max} is, by convention, the difference between the effect when no drug is administered and the maximum inhibition of effect obtained with drug. Generally the maximum inhibition is reached when enzymes or active transport processes are saturated.

MATERIALS AND METHODS

Materials

Sch-39304, the racemic compound of active isomer Sch-42427 and inactive isomer Sch-42426 were obtained from Schering Plough or subsidiaries. The chiral purities of the compounds are given in Table I. ^{14}C-Sch-39304, ^{14}C-42426, and ^{14}C-Sch-42427 (chiral purity of 99.9, 96.0, and 99.6, re-

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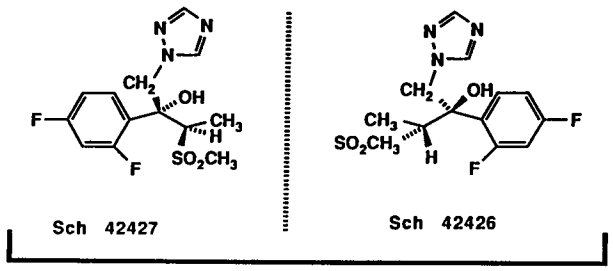

Fig. 1. Structure of the RR enantiomer, Sch-42427, and the SS enantiomer, Sch-42426; the two crystallize together to form the racemic compound, Sch-39304.

spectively, and radiochemical purity of 99.2, 94.3, and 98.2%, respectively) were synthesized by Schering-Plough Radiochemistry Department. All excipients used in formulations or buffer systems were NF, USP, or Food Grade.

Human cadaver skin from the abdomen of adult, white males was obtained untreated, microtomed to 400 μm, and kept frozen at −20°C until use. The vehicle used in the *in vitro* and *in vivo* studies was composed of polyethylene glycol 400, glycerol, and ethanol, at a volume ratio of 45:45:10, and contained unlabeled compound at the designated concentration. For the skin permeation studies 25 μCi of the respective ^{14}C-labeled compound was added.

Quantitation of the Chiral Form

The amount of each chiral form of the drug was determined by HPLC using a YMC C-4 wide-pore column (300 Å, 6.5-μm particle size, 25 cm × 4.1-mm i.d.), with a mobile phase of water and acetonitrile (96:4) containing 10 ml/L of triethylamine and 0.022 *M* β-cyclodextrin. The apparent pH of the mobile phase was adjusted to 5.0 with glacial acetic acid. Detection was by UV at 254 nm.

Solubility and Partitioning Studies

Equilibrium solubility studies and octanol:water partitioning studies were conducted at 25°C. The concentration of drug in each solvent was determined after filtration using the HPLC method described above, which was specific for each chiral form.

Skin Permeation Studies

Approximately 1 cm^2 of skin was clamped between the donor and the receptor chambers of a Franz diffusion apparatus. Approximately 500 μl of the vehicle containing a saturated solution of one of the drugs was applied to the stratum corneum and covered with parafilm for the duration of the study. The receptor solution, pH 7.4 buffer containing 6% Oleth-20 (Amerchol, Edison, NJ), was removed once a day and replaced with fresh buffer. The amount of radioactivity in each sample was determined with a liquid scintillation counter (LKB, Gaithersburg, MD).

In Vivo Studies

Trichophyton mentagrophytes was grown on Sabouraud dextrose agar slants for 10 days at 28°C. The growth was washed off with Sabouraud broth and blended in a Waring blender for 15 sec at 5-sec intervals. Groups of 9 or 10 male guinea pigs (Charles River) weighing 250–300 g were used. Animals were shaved on the right side with electric clippers and remaining hairs were then removed with a safety razor. The skin was rubbed gently with medium sandpaper in a circular motion and the inoculum was then swabbed onto the abraded area. Animals were then rested for 72 hr. After resting, and just prior to the first treatment, animals were cultured to confirm infection. The three compounds were tested topically by spreading an aliquot (0.3 ml) of drug in vehicle with a glass rod over the infected area of each animal twice a day for 10 consecutive days. Lesions were graded daily by

Table I. Properties of Sch-42427, Sch-42426, and Sch-39304

Property	Sch-42427	Sch-42426	Sch-39304
Isomer content (%)	99.9 RR	99.7 SS	50:50 SS, RR
Melting point (°C)	152	152	212
Specific rotation (deg)[a]	−39	+39	0
Water solubility [mg/ml (SD)]	2.5 (0.02)	2.5 (0.01)	0.2 (0.02)
Vehicle solubility [mg/ml (SD)][b]	39.2	37.7	3.6
Octanol:water partition coefficient[c]	3.8		4.4
Skin permeation rate from saturated solutions [μg/cm^2-hr (SD)]	60.9 (22.7)	43.8 (12.2)	6.70 (2.19)
Crystal structure (a, b, c; Å)[d]	Orthorhombic a = 11.5 b = 13.4 c = 9.66		Monoclinic a = 9.12 b = 10.9 c = 15.6 β = 102.8°

[a] From a 0.5% methanol solution at room temperature.
[b] Vehicle was composed of polyethylene glycol 400:glycerol:ethanol (45:45:10).
[c] Equal to $C_{octanol}/C_{water}$, where C is the concentration; the aqueous phase was kept at a constant pH of 7.0 with 0.05 *M* phosphate buffer.
[d] Determined by mathematical analysis of powder diffraction pattern.

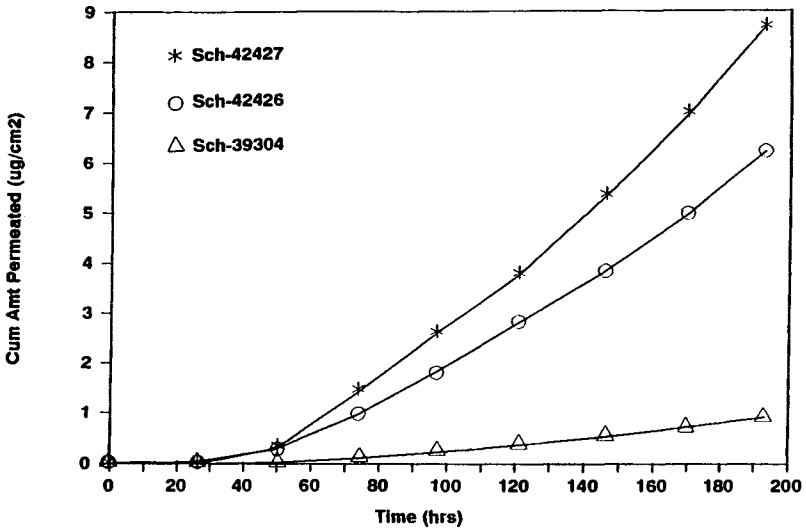

Fig. 2. *In vitro* permeation profile of Sch-42427, Sch-42426, and Sch-39304 through human cadaver skin.

the same person at the same time each day under the same lighting conditions. The lesions were scored from 0 (least severe) to 5 (most severe). The final group lesion scores were determined by taking the sum of the lesion scores for each animal in the group at the end of the experiment and dividing it by the number of animals per group.

RESULTS AND DISCUSSION

The physicochemical properties of each drug are given in Table I. Conversion of one chiral form to another was not observed. As expected the lower melting pure enantiomers exhibited a higher solubility than the racemic compound, while the partition coefficient values were similar. Inserting the melting point values (in °K) and solubility values for Sch-42427 and Sch-39304 into Eq. (2) resulted in values for A of 12.6 and 11.9 from the aqueous and vehicle solubility values, respectively. Yalkowsky (9) reported that for aromatic compounds, every 100° increase in melting point results in an approximately 10-fold decrease in solubility; and for alkanes the effect is even greater. Therefore, the calculated values for A are reasonable and support the assumptions used in arriving at Eq. (2).

Table I also lists the values for maximum flux for each compound obtained from human cadaver skin permeation profiles given in Fig. 2. As expected from the similarity in their melting points, the two enantiomers exhibit similar flux values. However, the maximum flux of Sch-39304 is an order of magnitude lower than that of either of the enantiomers. The ratio of flux values for Sch-42427 compared to Sch-39304 calculated from the skin permeation experiments is 9.1. A theoretical flux ratio of 11.2 was calculated by inserting into Eq. (2) the melting point values and the value for A (of 11.9) calculated above. Based on Eq. (1) the concentration of Sch-42427 in the skin may be 9–11 times greater than that of Sch-39304.

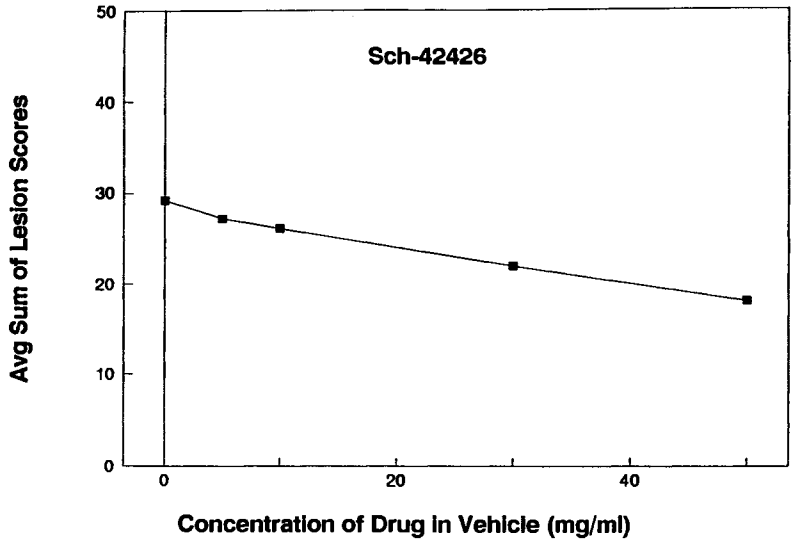

Fig. 3. Plot of *in vivo* data (average sums of lesion score) for Sch-42426.

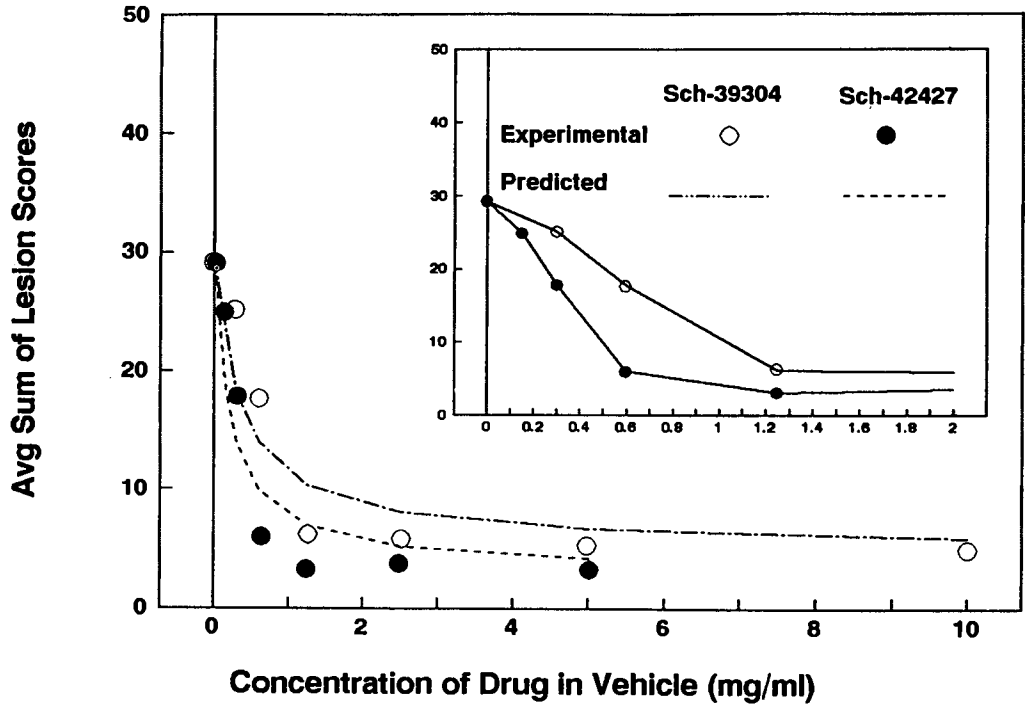

Fig. 4. Plot of *in vivo* data (average sums of lesion score) for Sch-42427 and Sch-39304 in guinea pig dermatophyte model. Inset is the region $<E_{max}$, where it can be observed that the concentration of Sch-39304 is 2× that of Sch-42427 for a given effect.

In Vivo Results

The results of the *in vivo* study are summarized in Figs. 3 and 4. The lesion scores resulting from no treatment and vehicle alone were 34.4 and 29.2, respectively. Sch-42426 exhibited negligible activity in the dermatophyte model. At concentrations lower than those needed to achieve the maximum decrease in lesion scores (Fig. 4, inset), the concentration of Sch-42427 needed to achieve a given lesion score is one-half that of Sch-39304. This is consistent with Sch-39304 being a 50:50 racemic compound of Sch-42426, which has negligible activity, and Sch-42427. The maximum effect (i.e., lowest lesion score) was observed at a Sch-42427 concentration of 1.25 mg/ml and a Sch-39304 concentration of 10 mg/ml (although there is little difference from the effect obtained at a Sch-39304 concentration of 2.5 mg/ml). The E_{max} was calculated from the difference between the highest (at zero drug concentration) and the lowest values of the lesion scores. The value for C_{m50} was calculated by fitting an equation to the experimental data using nonlinear regression and then solving the resulting equation for the concentration at $E_{max}/2$. These values were inserted into Eq. (3), to give the predicted relationships shown in Fig. 4. Since the lesion scores for Sch-42426 did not reach a minimum plateau (maximum effect), even at concentrations of 50 mg/ml, the predicted values for Sch-42426 could not be calculated. The values for E_{max} and C_{m50} were 24.2 and 0.35, respectively, for Sch-39304 and 26.1 and 0.21, respectively, for Sch-42427. Considering the inherent variability in biological data, the fit of the E_{max} model with the experimental results is fairly good.

CONCLUSIONS

It is interesting to note that even though the solubility of Sch-42427 is almost 10 times that of Sch-39304, there is very little difference between the maximum effect achieved with the two drugs. The reason may be due to the relationship between the solubility and the C_{E50}. From Fig. 4, the maximum effect is achieved at a Sch-39304 concentration of 1.25 mg/ml, well below the solubility limit of the drug in the vehicle (3.6 mg/ml). Even though the exact concentration of drug in the skin is not known, it is known, from Eq. (1), that the maximum concentration of drug in the skin is achieved at this point (3.6 mg/ml). Thus the concentration needed to achieve the maximum effect is lower than the maximum concentration of drug in either the vehicle or the skin. Therefore increasing the solubility of drug in the vehicle/skin, as observed with Sch-42427, may have no effect on the E_{max}. A general conclusion which can be drawn from this study is that the efficacy of topically active drugs may not correlate linearly with the thermodynamic activity of the drug in the vehicle or the skin.

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