Tioconazole (TCZ) is an imidazole antifungal agent with broad spectrum activity. Percutaneous absorption and intracutaneous distribution of TCZ solution have been compared with TCZ cream, miconazole nitrate (MCZ) solution and bifonazole (BFZ) solution following a single topical application to abdominal skin of guinea pigs. Following application of TCZ solution, TCZ concentrations in the stratum corneum, epidermis-cutis and subcutaneous tissue were higher than those after TCZ cream application suggesting superior percutaneous penetration after TCZ solution application. The percutaneous penetration after applications of MCZ solution and BFZ solution was comparable to that of TCZ cream, but inferior to that of TCZ solution. TCZ concentrations in the stratum corneum were much higher than those in epidermis-cutis and subcutaneous tissue after applications of both TCZ formulations. The majority of applied TCZ remained in the stratum corneum at high levels for a long duration. TCZ concentrations in the stratum corneum within 24 h after applications of both TCZ formulations were more than several hundred times higher than the minimum inhibitory concentrations against most of the dermatophytes and yeasts. The effectiveness of both TCZ formulations against dermatophytes may be due to this favorable pharmacokinetic property in the skin tissues, together with its potent antifungal activity. Percutaneous absorption of TCZ after applications of both formulations was negligible suggesting that these treatments are unlikely to produce systemic side effects.

Key words  tioconazole; percutaneous absorption; intracutaneous distribution; miconazole; bifonazole; guinea pig

MATERIALS AND METHODS

Materials  TCZ 1% (w/v) solution (lot No. L52-10) and cream (lot No. 391203) were supplied by Pfizer Japan Inc. (Tokyo, Japan). MCZ 1% (w/v) solution (lot No. 016E1) and BFZ 1% (w/v) solution (lot No. 6203) were obtained commercially from Taisho Pharmaceutical Co., Ltd. (Tokyo, Japan) and Bayer Yakuhin, Ltd. (Osaka, Japan). MCZ, an internal standard (IS) for TCZ, was supplied from Sigma Chemical Co. (St. Louis, U.S.A.). TCZ (lot No. R-11), IS for BFZ, was supplied from Sigma Chemical Co. (St. Louis, U.S.A.). TCZ (lot No. R-11), IS for MCZ, was supplied by Pfizer Japan Inc. (Tokyo, Japan). Itraconazole, IS for BFZ, was extracted from commercial preparations purchased from Janssen Kyowa Co., Ltd. (Tokyo, Japan). All other chemicals used were of reagent grade.

Animals  Guinea pigs of the Hartley strain (Japan SLC, Shizuoka, Japan) weighing approximately 400 g were used in the study. The animals were kept in an animal room main-
tained at a temperature of 23 ± 2 °C and a relative humidity of 55 ± 5%, and pellets (CG-7; Oriental Yeast, Tokyo, Japan) and water were provided ad libitum. They were used in the experiments after acclimation under the same conditions. The fur over the abdominal skin was sheared using electric hair clippers and a shaver before drug application.

**Administration** Two hundreds microliters of TCZ solution, TCZ cream, MCZ solution or BFZ solution was topically applied using a micro syringe to the sheared abdominal skin (3 × 3 cm²) of guinea pigs. At 24 h after application (or immediately before the sampling of specimens at 0.5, 2 or 6 h time points), the drug applied abdominal skin site was wiped with damp absorbent gauze to remove the drugs which were not absorbed or penetrated into the skin tissues.

**Sampling of Specimens** Four guinea pigs per each time point were sacrificed by drawing blood directly from the heart under ether anesthesia at 0.5, 2, 6, 24, 48, 120 h after application of TCZ solution, at 2, 6, 24, 48, 120 h after application of TCZ cream, and 0.5, 2, 48 h after application of MCZ solution or BFZ solution. The stratum corneum samples were obtained on 10 sheets of vinyl tape (3 × 3 cm²) by applying the adhesive tape to the test site of abdominal skin and removing the tape. This procedure was repeated until specimens were collected on all 10 sheets. The weight of abdominal stratum corneum samples obtained was considered as 21.2 mg on the basis of the mean difference of the weight of vinyl tapes between the pre- and post-application in the preliminary study. After extirpation of the abdominal skin tissue from the same test site, each tissue sample was divided by means of a razor into epidermis-cutis and subcutaneous tissue. Plasma was prepared by centrifugation of the obtained blood. All specimens were kept at −20 °C until the analysis was performed.

**Drug Assay** The TCZ concentrations in plasma, stratum corneum, epidermis-cutis and subcutaneous tissue (except plasma and subcutaneous tissue at 0.5 h after application of TCZ solution), and MCZ and BFZ concentrations in stratum corneum and epidermis-cutis were measured by an HPLC-UV method as follows. A pre-determined volume of IS solution was added in each test tube. Chromatographic separation was carried out at ambient temperature on the reverse-phase analytical column, Capcell pak C18 (5 μm particle size, 250 × 4.6 mm i.d.) at a flow rate of 1 ml/min. The UV wavelength was monitored at a 220 nm for TCZ and MCZ, and 255 nm for BFZ. The mobile phase was a 50 mM phosphate buffer containing 5 mM octanesulfonate (pH 2.5): acetonitrile (1:1, v/v) for TCZ and MCZ assay, and a 0.05% (w/v) ammonium acetate (pH 6.8): acetonitrile (2:3, v/v) for BFZ assay.

**TCZ and MCZ** Lower limits of quantification of TCZ were 1 ng/ml for plasma, 47 μg/g (1 μg/21.2 mg) for stratum corneum and approximately 0.2 μg/g for skin tissues. For MCZ, those were 236 μg/g (5 μg/21.2 mg) for stratum corneum and approximately 2 μg/g for epidermis-cutis.

Plasma: To each tube, 1 ml of plasma, 0.5 ml of 0.1 M NaOH and 5 ml of hexane were added, and the tubes were shaken vigorously for 5 min and then centrifuged at 3000 rpm for 5 min. After transferring the hexane layer to another test tube, 1 ml of 0.1 M HCl was added, and the tube was shaken and centrifuged, and then the organic layer was removed and discarded. To the remaining aqueous layer, 0.5 ml of 0.3 M NaOH and 6 ml of hexane were added, and the tube was shaken and centrifuged. The organic layer was transferred to another test tube and evaporated to dryness under a stream of nitrogen. The residue was dissolved in HPLC mobile phase to prepare the HPLC sample.

**Stratum Corneum:** The vinyl tapes with stratum corneum samples were each placed in a test tube containing 8 ml of methanol, and these samples were placed under ultrasonic treatment for 1.5 h. After the ultrasonic treatment, the vinyl tapes were taken out and the solvent was evaporated to dryness. The residue was dissolved in 6 ml of hexane, and 1 ml of 0.1 M HCl was added. The tubes were shaken and centrifuged, and then the hexane layer was removed. The remaining aqueous layer was washed again by hexane and then 0.3 ml of 1 M NaOH and 6 ml of hexane were added. The HPLC samples were prepared by the same procedures as those used in the case of plasma.

**Epidermis-Cutis and Subcutaneous Tissue:** Skin tissue samples were weighed and each was placed in a test tube. To each tube, 2 ml of 1 M NaOH was added and the tube was incubated over night to dissolve the specimen. After incubation, the solvent was extracted with 6 ml of ethyl acetate. The ethyl acetate layer was transferred to another tube and evaporated. To each tube, 6 ml of hexane and 2 ml of 0.1 M NaOH were added for reverse extraction. To the remaining aqueous layer, 0.3 ml of 1 M NaOH and 6 ml of hexane were added. The HPLC samples were prepared by the same procedures as those used in the case of plasma.

**BFZ** Lower limits of quantification were 94 μg/g (2 μg/21.2 mg) for stratum corneum and approximately 5 μg/g for epidermis-cutis.

**Stratum Corneum:** The vinyl tapes with stratum corneum samples were treated as same as TCZ and MCZ cases. After ultrasonic treatment and evaporation, 6 ml of hexane and 1 ml of 0.5 M H₂SO₄ were added to each tube for reverse extraction. The tubes were shaken and centrifuged, and the hexane layer was removed. The remaining aqueous layer was washed again by hexane and then 1 ml of 1 M NaOH and 6 ml of ethyl acetate were added. The tube was shaken and centrifuged, and the organic layer was transferred to another test tube and evaporated to dryness. The residue was dissolved in HPLC mobile phase to prepare the HPLC sample.

**Epidermis-Cutis:** Epidermis-cutis samples were treated as same as TCZ and MCZ cases. After incubation, the solvent was extracted with 6 ml of ethyl acetate. The ethyl acetate layer was transferred to another tube and evaporated. To each tube, 6 ml of hexane and 1 ml of 0.5 M H₂SO₄ were added for reverse extraction. The HPLC samples were prepared by the same procedures as those used in the case of stratum corneum.

**Standard Curve** To create a standard curve for each specimen, the pre-determined volume of standard solution for each test drug (TCZ, MCZ or BFZ) was added to a corresponding blank specimen (plasma or each skin tissue) before processing by the method described above. From the results, the peak ratio (each test drug/IS) was calculated and plotted against the added drug concentration to make a calibration curve by linear regression. Linear regression yielded a correlation coefficient of 0.99 or above.

**Pharmacokinetic Analysis** The pharmacokinetic analysis of mean concentration profiles of TCZ was performed by
non-compartmental methods using an analysis program WinNonlin Professional (version 3.1, Pharsight Corporation). The maximum concentration ($C_{\text{max}}$) and Time to $C_{\text{max}}$ ($T_{\text{max}}$) were taken directly from the recorded data. Elimination half-life ($t_{1/2}$) was calculated as $\ln 2/k_{el}$, where $k_{el}$ was the slope of the terminal phase of the log concentration–time points by the linear regression. The area under the concentration–time curve from time zero to the time ($t$) of the last measurable concentration ($AUC_{t}$) was calculated by the linear trapezoidal method. The area under the concentration–time curve from time zero to $\infty$ ($AUC_{\infty}$) was calculated as $AUC_{t} + (C_{l}/k_{el})$, where $C_{l}$ was the concentration at the last measurable time ($t$). Because the terminal phase was not identified, the $t_{1/2}$ and $AUC$ for the plasma and stratum corneum could not be calculated and $AUC_{120}$ was presented for stratum corneum instead of $AUC$.

**RESULTS**

Following a single application of TCZ solution and TCZ cream, the TCZ concentration profiles are shown in Fig. 1, and the pharmacokinetic parameters are summarized in Table 1. The TCZ concentrations in the stratum corneum were much higher than those in other skin tissues following applications of both TCZ formulations. $C_{\text{max}}$ in the stratum corneum of 12242 and 1597 $\mu$g/g were obtained at 0.5 and 6 h after applications of solution and cream, respectively. These concentrations were 3536 and 402 $\mu$g/g at 120 h after applications and maintained high levels for a long duration. $AUC_{120}$ were 455964 and 57524 $\mu$g·h/g, respectively. The concentrations at 120 h after applications were higher than those at 48 h after applications for both TCZ formulations.

At 120 h after applications the fur over the sheared abdominal skin grew again, and this fur was come out and clung to vinyl tape on the occasion of the stratum corneum sampling. This means that TCZ existed near the fur (e.g. sebaceous gland or hair follicle) was counted among TCZ concentrations in the stratum corneum. These may cause higher concentrations at 120 h after applications.

TCZ concentrations in epidermis-cutis and subcutaneous tissue reached a peak at 6 h after application of solution and the $C_{\text{max}}$ were 418.64 and 20.33 $\mu$g/g, respectively. These decreased thereafter with the $t_{1/2}$ of 15.5 and 28.4 h, and $AUC$ were 18118 and 843 $\mu$g·h/g for epidermis-cutis and subcutaneous tissue, respectively. Following cream application, TCZ concentrations in epidermis-cutis and subcutaneous tissue showed $C_{\text{max}}$ of 67.17 and 3.31 $\mu$g/g at 24 h after application. $AUC$ were 4112 and 237 $\mu$g·h/g, and $t_{1/2}$ were 14.8 and 30.6 h for epidermis-cutis and subcutaneous tissue, respectively. The highest TCZ concentrations in plasma (0.017 and 0.0007 $\mu$g/ml) were obtained at 6 and 24 h after applications of solution and cream, respectively.

Following a single application of MCZ solution and BFZ solution, the concentrations of these drugs are shown in Fig. 2 with above mentioned TCZ solution data. The MCZ and BFZ concentrations in the stratum corneum showed the highest levels of 1869 and 722 $\mu$g/g at 2 h after application. These concentrations were 705 and 178 $\mu$g/g at 48 h after application. The MCZ and BFZ concentrations in epidermis-cutis also showed the highest levels at 2 h after application and those were 13.4 and 27.2 $\mu$g/g, respectively. These decreased to 7.6 and 3.4 $\mu$g/g at 48 h after application, respectively.

**DISCUSSION**

Following applications of TCZ solution and cream, the TCZ remained at high levels for a long duration in the stra-

![Fig. 1. Tioconazole (TCZ) Concentration Profiles in the Stratum Corneum, Epidermis-Cutis, Subcutaneous Tissue, and Plasma Following a Single Topical Application of TCZ Solution (A) and TCZ Cream (B) to Abdominal Skin of Guinea Pigs](image)

Each point with the bar represents the mean±S.D. of four guinea pigs. There is no data of subcutaneous tissue and plasma at 0.5 h after application of TCZ solution and all specimens at 0.5 h after application of TCZ cream.

![Fig. 2. Concentration Profiles in the Stratum Corneum (A) and Epidermis-Cutis (B) Following a Single Topical Application of Tioconazole (TCZ) Solution, Miconazole Nitrate (MCZ) Solution and Bifonazole (BFZ) Solution to Abdominal Skin of Guinea Pigs](image)

Each value with the bar represents the mean±S.D. of four guinea pigs.

**Table 1. Mean Pharmacokinetic Parameters of Tioconazole (TCZ) Following a Single Topical Application of TCZ Solution and TCZ Cream to Abdominal Skin of Guinea Pigs**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Stratum corneum</th>
<th>Epidermis-cutis</th>
<th>Subcutaneous tissue</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_{\text{max}}$ ($\mu$g/g)</td>
<td>$T_{\text{max}}$ (h)</td>
<td>$AUC_{120}$ ($\mu$g·h/g)</td>
<td>$C_{\text{max}}$ ($\mu$g/g)</td>
</tr>
<tr>
<td>Solution</td>
<td>12242</td>
<td>0.5</td>
<td>455964</td>
<td>418.64</td>
</tr>
<tr>
<td>Cream</td>
<td>1597</td>
<td>6</td>
<td>57524</td>
<td>67.17</td>
</tr>
</tbody>
</table>
tumor corneum, the main site where the fungus inhabits, and the concentrations at 24 h after applications were 4363 and 768 μg/g, respectively. TCZ inhibits the growth of a broad range of dermatophytes and yeasts, usually at concentrations of 6.25 μg/ml or less. The TCZ concentrations within 24 h after applications of both formulations were more than several hundred times higher than these minimum inhibitory concentrations against most of the dermatophytes and yeasts. TCZ may be excreted from the stratum corneum mainly by the normal turnover of keratinocytes. Although the basic structure of the skin of a guinea pig is similar to the human, the stratum corneum is thin, and percutaneous absorption and permeability of substances is generally high compared with the human. The turnover period of keratinocytes in guinea pigs might differ from human. Therefore there is a possibility that this result is not directly applied to human. However Kashin et al. have reported that the results of their clinical studies show no clinically or statistically significant differences in efficacy between once a daily and twice daily treatment regimens with TCZ cream for the treatment of superficial dermatophyte infections. The results obtained in our present study support the usefulness of once a daily applications reported by Kashin et al.

The concentrations in the stratum corneum reached \( C_{\text{max}} \) at 0.5 h after application of solution, and this concentration was 7.7 times higher than \( C_{\text{max}} \) at 6 h after application of cream. \( AUC_{\infty} \) of solution was 7.9 times higher than that of cream. The TCZ concentrations in epidermis-cutis and subcutaneous tissue reached a peak at 6 h after application of solution and 24 h after application of cream. These concentrations in epidermis-cutis and subcutaneous tissue decreased thereafter with the \( t_{1/2} \) of approximately 15 and 30 h, respectively, after both treatments. As same as the concentrations in the stratum corneum, the concentrations in epidermis-cutis and subcutaneous tissue after solution application were higher than those after cream application. These suggest that percutaneous penetration of TCZ after application of TCZ solution was superior to that after application of TCZ cream. This finding has been also observed in other drugs (e.g., BFZ, sertaconazole, flutrimazole, and salicylic acid). Ingredients of TCZ solution formulation are easy to volatilize, and the majority of TCZ in solution formulation is distributed over the surface of the stratum corneum soon after the applications. On the other hand, TCZ in cream formulation must be released from the vehicle and partitioned into the stratum corneum. Therefore not all TCZ in cream formulation are distributed over the skin surface. These may cause more rapid and superior percutaneous penetration of TCZ from the solution formulation.

Low plasma concentrations of TCZ after applications of both formulations suggest that the percutaneous absorption of TCZ was negligible. These treatments are therefore unlikely to produce systemic side effects. In fact no systemic side effects or clinically relevant changes in laboratory parameters, indicative of drug toxicity, have been noted in therapeutic trials.

Following applications of MCZ solution and BFZ solution, the highest MCZ and BFZ concentrations in the stratum corneum at 2 h after application were comparable to and lower than \( C_{\text{max}} \) after application of TCZ cream, and these were 1/6.6 and 1/17 of \( C_{\text{max}} \) after TCZ solution application. The concentrations in the stratum corneum after TCZ solution application were 3 to 8 times and 12 to 23 times higher than those after MCZ and BFZ applications, respectively, at any sampling points. The MCZ and BFZ concentrations in epidermis-cutis were almost same or lower than those after TCZ cream application and much lower than TCZ solution application. Antifungal activity of TCZ against dermatophytes and yeasts is equal or stronger in comparison with those of MCZ and BFZ. These findings also support that once a daily applications of both TCZ formulations show the excellent efficacy for treating dermatophytes as same as MCZ solution and BFZ solution.

Skin permeation rates are generally affected by the molecular weight, charge and distribution coefficient (i.e., n-octanol–water partition coefficient) of penetrants. MCZ and BFZ are imidazole derivatives as same as TCZ, but there is a little difference in the physicochemical properties (molecular weight, solubility, lipophilicity and so on) among these three drugs. This difference is considered to be the cause of the different percutaneous penetration of these drugs. Rougier et al. and Surber et al. have reported that the penetration flux value of a substance depends on its partition coefficient between stratum corneum and vehicle. The difference of percutaneous penetration among these four formulations may originate in the difference of their ingredients.

Patzschke et al. have reported that the equivalent concentrations of 200 to 1000 μg/cm² are detected in the stratum corneum after a single topical application of \(^{14}\)C]BFZ as a 1% solution to human subjects. The concentrations up to 20 and 3 μg/cm² are found in the lower layers of the epidermis and the cutis, respectively. These BFZ concentrations in the skin were similar to those obtained in our present study. MCZ concentrations in the stratum corneum at each sampling point and in the epidermis-cutis at 48 h after application were higher than the corresponding BFZ concentrations. This is consistent with the data reported by Veronese et al. suggesting the longer skin retention time of MCZ in effective therapeutic concentrations than BFZ.

In conclusion, percutaneous penetration after application of TCZ solution was superior to that after applications of MCZ solution and BFZ solution, and those of TCZ cream was comparable to MCZ solution and BFZ solution. The effectiveness of both TCZ formulations against dermatophytes may be due to this favorable pharmacokinetic property in the skin tissues, together with its potent antifungal activity. These findings support that once a daily applications of both TCZ formulations may show the excellent efficacy for treating superficial dermatophyte or yeast infections of the skin as same as MCZ solution and BFZ solution. The percutaneous absorption of TCZ after applications of both formulations was negligible suggesting that these treatments are unlikely to produce systemic side effects.

REFERENCES


