Transdermal Delivery of Praziquantel: Effects of Solvents on Permeation Across Rabbit Skin

Xin-Sheng ZHENG, Cun-Zheng Duan, Zhi-Dong Xiao, and Bao-An Yao

Department of Chemistry, College of Science, Huazhong Agricultural University; and College of Animal Husbandry and Veterinary Medicine, Huazhong Agricultural University; Wuhan 430070, China.

To explore a new method for the transdermal delivery of praziquantel (PZQ), the effects of solvents on permeation across rabbit skin were investigated. The solubility of PZQ in five different solvents, ethylene glycol monophenyl ether (EGPE), 1,4-dioxane, tetrahydrofuran, dimethyl sulfoxide, and oleic acid, were measured with a UV–Vis spectrophotometer. The determination of the n-octanol/water partition coefficient of PZQ in the five different solutions and assay of serum concentration following PZQ transdermal administration in rabbits were performed using HPLC. The results indicated that the transdermal absorption of the drug was related to the partition coefficient and lipophilic characteristics of the solvent. The optimal solvent for PZQ transdermal delivery was EGPE in our protocol. The solubility of PZQ in EGPE is >400 mg/ml, and the apparent partition coefficient of PZQ in the solution is 0.895 (log P value). After transdermal administration of PZQ in EGPE solution, the bioavailability is 2.85-fold that after oral administration. The serum drug concentration was maintained at 4.0 µg/ml over 4 h, which is sufficient for the treatment of schistosomiasis. At the same time, no apparent side effects were found on the skin. EGPE may thus be a promising vehicle for the transdermal delivery of PZQ in the future.

Key words praziquantel; transdermal delivery; partition coefficient; solubility; solvent

Praziquantel (PZQ) is widely used in the treatment of schistosomiasis and it is also effective in other trematode and cestode infections. Because it is insoluble in water and other common solvents, PZQ can be only administrated orally. However, the main limitation on the therapeutic effectiveness of PZQ is poor bioavailability, gastrointestinal side effects associated with high peaks, superinfection, etc. Transdermal delivery would avoid numerous problems with the oral route and has been well documented. The administration of PZQ via the dermal route has received increased attention in recent years. PZQ transdermal paper towels were developed for the prevention and treatment of schistosomiasis. PZQ penetrating agents for animal skin are available for the treatment of cestodes and flukes on mice with worm reduction rates of more than 80%. Despite the recent progress, one problem frequently encountered in PZQ transdermal delivery is that the concentration of PZQ in penetrating agents is too low for therapy in heavy animals. For maintenance therapy, large volumes of penetrating agents are often needed when PZQ is used for transdermal administration in large animals such as cattle. That is infeasible, however, because a large volume of penetrating agents is difficult to absorb and lost easily.

It is still a challenge to find an ideal solvent in which the solubility of PZQ is sufficiently high to meet the needs of large animals without toxicity. We investigated the effects of solvents on permeation across rabbit skin and found an excellent solvent in our protocol, ethylene glycol monophenyl ether (EGPE), for transdermal delivery of PZQ by measuring the solubility of PZQ in five different solvents, the n-octanol and water apparent partition coefficients of PZQ in five different solutions, and the blood drug concentration following PZQ transdermal administration in rabbits.

MATERIALS AND METHODS

PZQ powder and tablets were purchased from Shinpoong Pharmaceutical Co., Ltd. (Korea). PZQ standard samples were obtained from the Sixth Pharmaceutical Factory of Shanghai (Shanghai, China). EGPE, 1,4-dioxane, tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), and oleic acid (OA) were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and were of analytical grade. Methanol and ethyl acetate purchased from Sinopharm Chemical Reagent Co., Ltd., were of chromatographic grade. Water was of HPLC grade deionized with a Milli-Q50 purification system (Millipore, Milford, MA, U.S.A.). Other reagents were of analytical grade.

New Zealand rabbits (1.90—2.20 kg) were provided by the Laboratory Animal Center of Huazhong Agricultural University (HZAU) and use of the animals was reviewed and approved by the HZAU Animal Care and Use Committee. The Principles of Laboratory Animal Care (NIH publication No. 86—23, revised 1985) were followed.

Measurement of Solubility Solubility was measured by adding excess amounts of PZQ into screw-capped vials containing EGPE, 1,4-dioxane, THF, DMSO, and OA, respectively. The suspensions were stirred vigorously at a constant temperature for at least 48 h with a SHA-B constant temperature shaker (Guohua Electric Co., Changzhou, China). After equilibration, an aliquot of solution was filtered quickly through a prewarmed 0.45-µm Millipore filter (Millipore, Bedford, MA, U.S.A.). Then the solution was appropriately diluted with methanol, and the concentration was determined using a Nicolet Evolution 300 UV–Vis spectrophotometer (Thermo Electron Co., U.S.A.). The solubility of PZQ was calculated based on the standard curves and diluted multiples. The solubility of PZQ at different temperatures was determined according to the same method described above.

Determination of Apparent Partition Coefficients The

© 2008 Pharmaceutical Society of Japan
apparent partition coefficients of PZQ were determined using n-octanol/phosphate buffer solution (PBS, pH 7.4) at 37 °C. Equal volumes (20 ml) of n-octanol and PBS were placed in a screw-capped test tube and stirred vigorously for 24 h at 37 °C. After equilibration, the aqueous phase saturated with n-octanol and oil phase saturated with PBS were separated by centrifugation at 4000×g.

A series of PZQ solutions 100 mg/ml were prepared by dissolving PZQ in EGPE, 1,4-dioxane, THF, DMSO, and OA, respectively. Two milliliters of these solutions of PZQ were dissolved in 15 ml of presaturated n-octanol in assay tubes, stoppered, and agitated for 10 min. Subsequently, 15 ml of presaturated PBS was added, and then the tubes were stoppered and agitated for 1 h. Thereafter, the n-octanol and aqueous phases were separated by centrifugation at 4000×g for 10 min, diluted with methanol, and analyzed with HPLC according to the method described below. The apparent partition coefficients (P) were the ratio of concentrations of PZQ in the n-octanol phase and the concentrations in the aqueous phase:

\[ P = \frac{C_{\text{oct}}}{C_{\text{aq}}} \]

Where \( C_{\text{oct}} \) is the concentration of PZQ in the n-octanol phase, and \( C_{\text{aq}} \) is the concentration of PZQ in the aqueous phase.

**In Vivo Absorption** Twenty-four hours prior to drug administration, an Oster clipper (Oster Model 5-01, U.S.A.) was used to shave the abdominal fur of rabbits in an area of approximately 6 cm². All of the rabbits were fasted but provided with water. The solutions of PZQ were transdermally administered via the prepared abdominal areas at a dose of 200 mg/kg (w/w) body weight. The concentration of PZQ in the solution of the EGPE, 1,4-dioxane, THF, DMSO, and OA was 200, 200, 100, 100, and 100 mg/ml (w/v), respectively.

To compare the bioavailability after oral administration, 6 other rabbits were administrated oral PZQ tablets at a dose of 200 mg/kg (w/w) by body weight. Prior to transdermal and oral administration, the 10-ml blank blood samples were collected from the femoral artery to establish calibration curves and determine of recovery rates.

Blood samples were then taken 5, 10, 15, 30, 45, 60, 120, 180, and 240 min from the femoral artery after PZQ administration and centrifuged at 3500×g for 10 min. Serum was separated and kept at −20 °C until analysis.

Serum samples 800 μl were extracted with ethyl acetate 1 ml by shaking vigorously for 10 min. The upper organic layer was collected after centrifugation at 2500×g for 5 min. The extraction was repeated three times, and the extraction was placed into a clean tube and evaporated to dryness under a stream of nitrogen at 60 °C. The residue was dissolved in methanol 400 μl. An aliquot (20 μl) was injected into the HPLC apparatus. The concentration of PZQ was calculated from the regression equation given below.

HPLC analysis was performed with an Agilent 1100 system (Agilent, U.S.A.) consisting of a VWD absorbance detector, chromatographic column (Eclipse×XDB-C18, 4.6×150 mm i.d., 5 μm, Agilent, U.S.A.), and vacuum degasser (G1379A, Agilent, U.S.A.). The column temperature was maintained at 40 °C. The mobile phase was a mixture of methanol and double-distilled water (70:30, v/v). After degassing, the eluents were pumped at a flow rate of 0.6 ml/min. The detector wavelength was set at 210 nm.

Stock solutions of PZQ (50 μg/ml) were prepared by dissolving a given weight of PZQ in methanol in a 50-ml volumetric flask and further diluted at appropriate times to obtain a series of stock solutions of 25, 12.5, 6.25, and 3.125 μg/ml. To an aliquot of 800 μl of the solutions, 800 μl of blank serum was added. Then the mixtures were shaken vigorously for 10 min, and the suspended solutions were extracted three times with 2 ml of ethyl acetate. The organic layer was placed in a clean tube and evaporated to dryness under a stream of nitrogen at 60 °C. The residues were dissolved in 800 μl of methanol. An aliquot (20 μl) was injected into the HPLC apparatus. The calibration curve was drawn by plotting the peak area against the serum concentration of PZQ.

Statistical Analysis Statistical analysis of the blood concentration data was performed using Agilent Offline Station N2000 (Agilent). The limit of detection is 0.035 μg/ml at a signal-to-noise ratio of 3:1. The average extraction recovery rate of the method was 90.80±3.79%, and the method recovery rate is 99.37±3.38% from different concentrations. The average relative standard deviations (RSD) for intra- and interday variation are less than 5% and 6%, respectively. The AUC was estimated using the trapezoidal rule. Student’s t-test was used for statistical analysis, and a value of p<0.05 was considered significant.

**RESULTS AND DISCUSSION**

**Solubility of PZQ** It is important to find an appropriate solvent to dissolve PZQ because it is not soluble in water and only slightly soluble in common solvents. To screen appropriate solvents for the preparation of transdermal agents, the solubility of PZQ in the selected solvents were measured and the results are shown in Fig. 1.

The solubility of PZQ in EGPE was the highest (>400 mg/ml) among the solvents examined in the temperature range from 25 to 45 °C. The solubility varied slightly with the temperature change. Maximum solubility was 490 mg/ml at 37 °C.

The solubility of PZQ in 1,4-dioxane changed markedly
with the temperature change. It remained 220—284 mg/ml in the temperature range from 25 to 35 °C, increased markedly from 35 to 37 °C, and remained 440 mg/ml at temperatures higher than 37 °C. The solubility of PZQ in DMSO, OA, and THF fluctuated slightly with temperature and remained at a relatively low level.

According to the solubility data shown in Fig. 1, the solubility of PZQ in EGPE and 1,4-dioxane from 25 to 40 °C are >280 mg/ml, much higher than the concentration reported in the patents (50, 125 mg/ml), respectively. Based on the solubility of PZQ, EGPE and 1,4-dioxane will be appropriate solvents for the transdermal administration of PZQ for large animal in the future.

**Apparent Partition Coefficient of PZQ**

The apparent partition coefficients of PZQ dissolved in different solvents between n-octanol and aqueous phases (pH 7.4) are shown in Table 1. The log \( P \) value of PZQ dissolved in EGPE was the highest, followed by that of PZQ dissolved in OA and 1,4-dioxane. That of PZQ dissolved in DMSO was the lowest among the five solvents selected. The different \( P \) values are result from of the different distribution of PZQ solutions between the n-octanol and aqueous phases. The lipophilic effects of PZQ in solvents are affected by the solvation. Higher \( P \) values of PZQ dissolved in EGPE and OA could be attributed to the lipophilic effects of both solvents, which are insoluble in water and soluble in organic solvents. The lower \( P \) value of PZQ dissolved in DMSO could be related to the hydrophilicity of the solvent.

**Partition Coefficient of PZQ Dissolved in Different Solvents at 37 °C (\( n=4 \), S.D.)**

<table>
<thead>
<tr>
<th>Solvents</th>
<th>DMSO</th>
<th>1,4-Dioxane</th>
<th>EGPE</th>
<th>THF</th>
<th>OA</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P )</td>
<td>0.680±0.005</td>
<td>1.251±0.008</td>
<td>7.847±0.009</td>
<td>1.091±0.004</td>
<td>4.493±0.005</td>
</tr>
<tr>
<td>( \log P )</td>
<td>−0.167</td>
<td>0.182</td>
<td>0.895</td>
<td>0.0378</td>
<td>0.653</td>
</tr>
</tbody>
</table>

**Transdermal Absorption of PZQ**

After transdermal administration, side effects such as red, swelling, and pruritus on local skin were not seen, and the rabbits moved normally. EGPE and DMSO are often used as permeation enhancers and EGPE is widely used in cosmetics, showing that the transdermal agents are safe. But detailed research is needed when the agents are used in the clinical treatment of schistosomiasis.

Concentrations of PZQ in serum samples were determined using the HPLC method. The serum concentration–time profiles of PZQ dissolved in different solvents are shown in Fig. 2.

PZQ is rapidly absorbed, reaching the peak concentration \( C_{max} \) 30 min after transdermal administration in solution. Among the five solutions, the \( C_{max} \) value of EGPE solution was highest with a peak height of 18.36 μg/ml, followed by that of DMSO with a peak height of 11.74 μg/ml. The \( C_{max} \) values of OA solution, OA solution, and THF solution were 1.58, 3.02, and 3.47 μg/ml respectively. The blood concentration of PZQ declines rapidly in rabbits after 30 min. Although the variation in serum concentration is marked from 45 to 240 min, a therapeutic concentration is maintained (from 5 to 240 min was 6.85 μg/ml·h\(^{-1}\)). The relative bioavailability of EGPE, DMSO, OA, THF, and 1,4-dioxane solution via the transdermal route relative to oral PZQ tablets was 2.85, 1.89, 1.68, 0.67, and 0.54 respectively. Thus higher bioavailability was obtained compared with oral dosing when PZQ in EGPE, DMSO, and OA was transdermally administered.

It is clear that the use of different solvents for PZQ results in different permeation profiles and trends. The transport of PZQ through the skin includes the following processes: the partition into the stratum corneum (SC) from the vehicle, diffusion in the SC and partitioning from the SC into viable tissue. The partitioning into the SC from the vehicle is affected by the lipophilic properties of PZQ in the vehicle. Partitioning from the SC into viable tissue is influenced by the hydrophilicity of PZQ in solution. Therefore transdermal agents should have appropriate hydrophilicity and lipophilicity.

The diffusion in the SC is influenced by the diffusion coefficient and the concentration gradient within the skin in accordance with Fick’s law. The diffusion coefficient can be increased by partitioning of solvents into the SC rapidly to aid drug diffusion and disrupting the ordered intercellular lipids with solvents. EGPE has these features and therefore satisfies the diffusion coefficient. DMSO, a penetration enhancer, allows increased diffusion of drug in the skin. Previous detailed mechanistic studies indicated that there was an OA pool in the SC which increases bilayer fluidity. The concentration gradient of drug within the skin is also influenced by the ability of the solution to partition into the SC and its ability to partition from the SC into the viable tis-
A high \( \log P \) value makes the partitioning from the SC into viable tissue difficult. However, a low \( \log P \) value makes partitioning from vehicle into the SC inefficient. The generally accepted range of \( \log P \) value for maximum permeation is between 1 and 3.\(^{1,2}\)

Our results show that the value of \( \log P \) of PZQ dissolved in EGPE is near 1; the values of the other solutions are far outside the accepted range. The optimal \( \log P \) value explains why PZQ EGPE solution has the advantage of partitioning into the SC from the vehicle and the partitioning into viable tissues from the SC compared with other solutions tested. The PZQ EGPE solution with the highest \( \log P \) value in the tested solutions for transdermal delivery exhibits the largest partition trends (Fig. 2).

CONCLUSIONS

The transdermal absorption of drugs is related to the partition coefficient and lipophilic characteristics of the solvent used. High solubility of PZQ in the solvent is able to decrease the volume of transdermal agent applied at certain dosages.

From our results, the solubility of PZQ in EGPE (415 mg/ml) is the highest among the five solvents at room temperature. When the PZQ EGPE solution is used for transdermal administration, the serum drug level maintained at 4.0 \( \mu \)g/ml over 4 h is sufficient for the treatment of schistosomiasis without apparent side effects. The bioavailability is 2.85-fold that after oral administration. It is clear that the bioavailability of transdermal PZQ delivery is much higher compared with oral administration. Hence the higher solubility and bioavailability make the solution of PZQ in EGPE a promising vehicle for the transdermal delivery of PZQ.

Acknowledgments The authors thank the College of Animal Husbandry and Veterinary Medicine, Huazhong Agricultural University, for supplying some experiment equipment. The authors also thank Prof. Daju Wang for her help in the experiments.

REFERENCES