Design of Thermosensitive Hydrogel for Extended-Release of Praziquantel

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DESIGN OF THERMOSENSITIVE HYDROGEL FOR EXTENDED-RELEASE OF

PRAZIQUANTEL

A Thesis
Presented for the
Master of Science Degree
The
Department of Pharmaceutics and Drug Delivery
The University of Mississippi

by

SHENG FENG

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ABSTRACT

Parasitic diseases are a severe threat to people and animals. Praziquantel is the most common anti-parasitic drug with high efficiency, and it has been used to treat parasitic diseases for many years. However, it has very strong gastrointestinal and liver metabolism that leads to a strong first-pass effect and a short half-life. Additionally, the frequent oral administration was inconvenient for the large livestock. To overcome these drawbacks, this study aimed to develop an extended-release thermosensitive hydrogel formulation that was applicable to the injectable administration. The praziquantel-loaded hydrogel formulation based on poloxamer 407 (20%, w/v) was prepared, and two strategies for modifications of poloxamer 407 hydrogel were studied. One of them was the formulation consisting of PEG-DSPE/TPGS mixed micelles-poloxamer 407 hydrogel hybrid system; another was the poloxamer 407 hydrogel adding with HPMC as an adhesive. These hydrogel formulations had a reversible sol-gel transition property at approximate 26 °C and obtained high loading efficiencies and storage stability. In-vitro release studies, as well as in-vivo pharmacokinetic studies, were conducted to evaluate the extended-release effect, and the bioavailability of several optimized formulations of praziquantel was calculated. The in-vitro release of praziquantel was prolonged when loading into poloxamer 407 hydrogel. Both the modifications of poloxamer 407 hydrogel, by adding HPMC as well as
utilizing PEG- DSPE/TPGS mixed micelle as a secondary delivery vehicle obtained the relatively better extended-release profiles than the original poloxamer 407 hydrogel. The pharmacokinetic studies indicated that the poloxamer 407 hydrogel formulation has a relatively high bioavailability and prolonged release profile, but both two modifications failed to obtain a significant improvement of an extended-release characteristic compared with the original hydrogel solution.
ACKNOWLEDGMENTS

I would like to thank my advisor Dr. Chalet Tan for supporting my projects. During my two-years master study, she always patiently encouraged me and guided me to be a better researcher. She let me know how to be more accurate and efficient doing experiments, as well as be humble and never stop learning. And thank Dr. Seongbong Jo and Dr. Walt Chambliss for being part of my committee members, their kind advice is so helpful and important to me.

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Finally, I thank my family and my parents for supporting and encouraging me through my study.
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I. INTRODUCTION

Parasitic diseases are a severe threat to people and animals, especially in tropical countries, sub-tropical countries and most agricultural countries. Schistosomiasis, caused by the infection from parasitic worms such as Schistosoma mansoni, Schistosoma haematobium, and Schistosoma japonicum, is a typically parasitic disease resulting in several chronic symptoms, and it has been considered the most severe helminthic disease in terms of high morbidity and mortality. Approximately 200 million people worldwide are affected by schistosomiasis, and almost 800 million people are at risk for schistosomiasis.

Praziquantel (Figure 1), which was discovered in 1975, is a broad-spectrum antiparasitic drug. Owing to its high efficacy, praziquantel has been the most essential anti-parasitic drug for human and animals. It has been widely used for schistosomiasis treatment and other trematode infections or even cestode infections for several decades\textsuperscript{1,2}. So far, the most widely available formulation of praziquantel on the market is the oral formulation, associated with the therapeutic regimen of 20 mg/kg, three times a day at intervals of 4–6 hours or as a single dose of 40 mg/kg. However, the bioavailability of the oral delivery of praziquantel
is extremely low because of the strong first-pass effect, which means about 99% of the drug is metabolized before entering to circulation\textsuperscript{3}. The pharmacokinetic study demonstrates that the elimination half-life ($t_{1/2}$) of praziquantel is

![Chemical structure of praziquantel](image)

**Figure 1.** Chemical structure of praziquantel

between 1 and 1.5 h, and more than 90% drug will be eliminated within 24 h. The fast elimination of praziquantel and short mean residence time (MRT) lead to frequent dosing\textsuperscript{4,5}. Additionally, frequent oral delivery of praziquantel is not convenient especially for the administration to the large livestock. Therefore, it is necessary to develop an injectable, extended-release formulation to improve the bioavailability of the drug and prolong the dosing intervals.

Poloxamer is a series of triblock copolymers consisting of a specific ratio of polyoxyethylene (PEO) and polyoxypropylene (PPO). Poloxamer 407, a widely used copolymer, has a molecular weight of 12 kDa containing 70\% PEO units and 30\% PPO unit\textsuperscript{6}. As the temperature increases, the poloxamer 407 molecule begins to self-assemble into micelles. This micellization is driven by the dehydration of the hydrophobic polyoxypropylene block that becomes progressively less soluble as the polymer concentration or temperature increases. The
aggregation of several monomers occurs to minimize the interactions of the PPO blocks with the solvent. Thus, the core of the aggregates is made from the insoluble blocks (polyoxypropylene) while the soluble portion (polyoxyethylene) forms the shell of the micelles. The hydrophobic drug is able to be encapsulated into hydrophobic PPO core while the existence of hydrophilic PEO moiety makes poloxamer well-dissolved in water. When the temperature sequentially increases over critical gelling temperature (CGT), the gelation consequently happens due to the ordered packing of micelles\(^7\) (Figure 2). Poloxamer 407 hydrogel aqueous solution possesses the reversible thermo-gelation property at the concentration of 20\% or higher. Within the concentration of 20\%-40\%, the sol-gel transition temperature is lower than body temperature, it means after the injectable administration of cold, liquid-form hydrogel solution to the body (37 °C), it will then become a semi-solid gel in a short time.

Poloxamer 407 hydrogel is a very suitable vehicle for drug delivery system owing to its low toxicity, high biocompatibility and high solubilizing capacity for the hydrophobic drugs\(^8\). Despite many advantages of poloxamer 407 hydrogel, it still has some limitations. The hydrogel structure is not able to maintain for a long time in physiological conditions. The network easily breaks down since the weak mechanical strength makes it susceptible to the dilution of poloxamer 407 by the influx of \textit{in-vivo} water, which results in a burst release of the drug\(^9\). To modify the poloxamer 407 hydrogel for obtaining a better extended-release system, several strategies have been studied including 1) increasing the drug-hydrogel interactions by physically or covalently crosslinking method\(^10\). 2) increasing the viscosity and density of the gel network by physical mixing with some additives\(^6\) or by chemical grafting with polymeric materials\(^11,12\). 3) co-formulating particulate systems (such as micelles) as a secondary delivery vehicle into the
hydrogel matrix forming a composite hydrogel network\textsuperscript{13}. Hydroxypropylmethylcellulose (HPMC) is a well-known mucoadhesive, and it has been demonstrated that the addition of HPMC to hydrogel solution is able to improve the extended-release profiles of the drug. Possibly HPMC reinforces the strength between each poloxamer 407 micelles, meanwhile, the net structure of poloxamer 407 hydrogel becomes denser, leading to an increased viscosity which prolongs the dissolution of the hydrogel as well as the drug release\textsuperscript{14} (Figure 3).

As stated above, loading the hydrophobic drug into the micelle-hydrogel hybrid system is also a promising strategy to modify the hydrogel-based formulation. If we first load the drug into a secondary delivery vehicle, such as micelles, then the drug is going to release from the secondary delivery vehicle and then release from the hydrogel network, consequently the release of drug from the micelle-hydrogel hybrid system will be prolonged (Figure 4). In our previous study, an efficient and versatile delivery system for the hydrophobic drug with the polymeric micelles consisting of PEG-DSPE and TPGS was developed. The conjugates of polyethylene glycol (PEG) and diacyl lipids, such as PEG-distearoylphosphatidylethanolamine (PEG-DSPE), formed stable micelle in which a number of sparingly water-soluble drugs could be incorporated. TPGS is a derivative of Vitamin E, which is obtained by conjugating PEG with Vitamin E succinate via the esterification reaction. TPGS is an amphiphilic polymer that has both the advantages of Vitamin E and PEG in applications of drug delivery. The previous studies showed that at a 1:2 molar ratio, PEG-DSPE/TPGS mixed micelles could encapsulate paclitaxel and tanespimycin (17-AAG) with high stability and efficiency, and the release of the drug was significantly prolonged\textsuperscript{16}. 

4
Figure 2. Schematic diagram to show the formation of poloxamer 407 hydrogel

The present study aimed to design an injectable formulation of praziquantel based on thermosensitive poloxamer 407 hydrogel, which was also able to obtain the extended-release characteristic. Two strategies for modifying the poloxamer 407 hydrogel were investigated. The in vitro release studies and in vivo pharmacokinetics studies were conducted, and the results demonstrated that the poloxamer 407 hydrogel formulation increased the water solubility of praziquantel as well as prolonged the release profile, and the bioavailability of the drug was significantly improved compared with oral delivery. Two modifications of hydrogel obtained better extended-profile during in vitro release study, but did not show significant improvement in the pharmacokinetic study. The possible reason was finally discussed.
**Figure 3.** Schematic diagram to show the network structure of HPMC-added poloxamer 407 hydrogel

**Figure 4.** Drug releases from micelle-hydrogel dual system
II. MATERIAL AND METHOD

Chemicals and instrumentation

Praziquantel was purchased from Sigma-Aldrich Chemical Company (USA). Poloxamer 407 was a gift from BASF Chemical Company (US). 1,2-Distearoyl-sn-Glycerol-3-Phosphoethanolamine-N-[Methoxy (Polyethylene glycol)-2000] (PEG-DSPE) was purchased from Corden Pharma (Cambridge, MA). D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) was a gift from Eastman Chemical Company (Kingsport, TN). Hydroxypropylmethylcellulose (HPMC, E4M) was a gift from Dr. Michael A. Repka’s lab. Analyses were performed on an Agilent 1260 Infinity Analytical SFC System combined with an Agilent 1260 Infinity ELSD Evaporative Light Scattering Detector. Acetonitrile (ACN, HPLC grade) was purchased from Fisher scientific Chemical Company (US).

Preparation of praziquantel incorporating PEG-DSPE/TPGS mixed micelles

The praziquantel-loaded mixed micelles were prepared using thin-film evaporation method\textsuperscript{16}. Briefly, 10 mg TPGS, 10.2 mg PEG-DSPE and 1 mg praziquantel were dissolved in 1.2 ml chloroform in a round-bottom flask, then the chloroform was removed under vacuum and
dried overnight. The film was then hydrated with 100 µl 10 mM HEPES-buffered saline (PH 7.4) with vigorous vortexing for 5 min. The mixed solution was sonicated at room temperature for 10 min, then it was centrifuged at 12,000 g for 5 min to form a clear micellar dispersion (PZQ/M). The micellar solution was kept at 4 °C.

**Preparation of praziquantel-incorporated PEG-DSPE/TPGS micelles-poloxamer 407**

hydrogel hybrid system

The poloxamer 407 hydrogel was prepared using ‘cold method’\(^{17}\). Briefly, poloxamer 407 (20%, w/v) was added to cold water, then the mixed solution was kept at 4 °C with continuous stirring overnight to ensure the complete dissolution of poloxamer 407. Praziquantel-incorporated micelle was slowly added to 1 ml hydrogel solution at 4 °C with gentle stirring. Finally, the sodium chloride (0.9 %, w/v) solution was added to obtain PZQ/M/P407 hydrogel solution (1mg/ml).

**Preparation of praziquantel loaded poloxamer 407 hydrogel and poloxamer 407/HPMC**

hydrogel

To make a comparison with the effect of the praziquantel-loaded micelle, 1mg praziquantel dissolved by ethanol was added to 1ml hydrogel solution using the same method described above to obtain PZQ/P407 hydrogel solution. For the preparation of praziquantel loaded poloxamer 407/HPMC hydrogel, HPMC 1% (w/v) was first added to hot water, after the HPMC was completely swelled, it was shifted to 4 °C with continuous stirring until the solution became totally clear and transparent. Then 1 mg praziquantel dissolved by ethanol was slowly added to 1
ml poloxamer 407/HPMC hydrogel solution to obtain PZQ/P407/HPMC hydrogel solution (1 mg/ml). All the hydrogel solutions were kept at 4 °C for further use.

**Measurement of Lower Critical Solution Temperature (LCST) and Gelation Time**

The temperature and time of the sol-gel transition were determined using tube-inverting method\(^\text{18}\). For the testing of LCST, 500 µl hydrogel solution was added in a 1.7 ml Eppendorf tube under digital dry bath, heating from 20 °C to 37 °C at a rate of 0.5 °C /min. Each minute, the tube was inverted 180 ° to observe the hydrogel gelling behavior. The temperature when the hydrogel solution did not flow for 15 s was determined as LCST. For the determination of gelation time, 500 µl hydrogel solution was added in a 1.7 ml Eppendorf tube at room temperature, then it was instantaneously transferred to a water bath at 37 °C. The gelling behavior was observed every 30 s by the tube-inverting method. The time until the hydrogel solution did not flow for 10 s was determined as gelation time.

**Quantification of praziquantel by HPLC analysis**

The method was developed on the instrument consisting of Agilent HP1260 HPLC system with UV detection. The separation was carried out on a reversed phase column Luna C18 (150 mm × 3 mm, 2.5 µm particle size: Phenomenex\(^\text{TM}\), USA). α-naphthoflavone was used as the internal standard at a concentration of 2 µM, and the detection wavelength for praziquantel and α-naphthoflavone were 198 nm and 281 nm, respectively. The Column temperature was 30 °C. A gradient elution method of A: sodium phosphate buffer (20 mM, pH 3.0) and B: acetonitrile (0-9 min 60% A, 9.1-16.5 min 40% A) was used for the analyses of the samples, and 50 µl sample
was injected into the column each time with the mobile phase at a flow rate of 0.5 ml/min.

**Calibration curves**

The *in vitro* calibration curve was prepared by diluting praziquantel stock solution (10 mM) with mobile phase (acetonitrile/sodium phosphate buffer, 40:60) to varying concentration of praziquantel (0, 0.5, 1, 5, 10 and 20 µM), each sample was added with α-naphthoflavone at a fixed concentration of 2 µM. The plasma calibration curve was prepared using six plasma samples collecting from mice (50 µl each) spiked with the same concentration interval as above, α-naphthoflavone was added to each sample at the concentration of 2 µM.

The plasma samples were analyzed by protein precipitation method using organic solvent\(^1\). Acetonitrile (250 µl) was dropwise added at five times the volume of each plasma sample and fully vortexed for 5 min, then centrifuged at 1200 g for 5 min. The organic phase was removed by speed vacuum evaporation, then the residue was reconstituted with 60 µl mobile phase. The reconstituted solution was centrifuged at 12,000 g for 5 min, then the supernatant was collected and injected to HPLC for analysis.

**In-vitro release studies**

*In-vitro* release studies were performed using a membrane-less dissolution method (Figure 5). The advantage of this model was that the hydrogel formulations were in direct contact with the release medium, and thus the formulation factors which affecting the drug release were able to be readily discerned\(^2\). For the studies of praziquantel release from different formulations, 0.5 g PZQ/M/P 407 hydrogel solution, PZQ/P 407/HPMC hydrogel solution and PZQ/P407
hydrogel solution were added to the glass vials under water bath at 37 °C for 5min. After the completion of the sol-gel transition, 5 ml release medium (phosphate buffered saline, pH 7.4) containing SDS solution (0.5 %, w/v) was carefully added to a glass vial in the water bath, layering over the hydrogel. The entire release medium was removed at the predetermined time point and fresh release medium was changed to the glass vial. For the release study of the praziquantel-incorporated micelle, the 0.5 g micellar solution was added to a cylindrical dialysis tube (cellulose membrane), and the other steps were as same as the membrane-less dissolution method above. The removed release medium was subjected to HPLC analysis to determine the cumulative release amount of praziquantel.

![Diagram](image.png)

**Figure 5.** The schematic diagram of the membrane-less dissolution method
Pharmacokinetic study in mice

The mice were randomly divided into six groups (n=3), four groups of mice were administered with subcutaneous injection of the praziquantel-incorporated micelle, PZQ/M/P407 hydrogel solution, PZQ/P407/HPMC hydrogel solution and PZQ/P407 hydrogel solution with the same dosage of 10 mg/kg, respectively. For the other two groups of mice, one group was administered free praziquantel which dissolved in DMSO (10 mg/kg) via the tail vein injection, and another group of mice was orally injected with praziquantel which was dissolved in ethanol, Tween 80 and phosphate buffer (1:2:7, v/v/v) mixed solution at a dosage of 60 mg/kg. After the subcutaneous injection, whole blood was collected via the retro-orbital bleeding at 30, 90 and 150 min. The time points of collecting the blood after intravenous injection were 2, 15, 30, 60 and 90 min, while for oral injection were 30, 60, 90 and 150 min. The blood sample was centrifuged at 4 °C (12,000 g, 5 min), then the plasma was obtained by collecting the supernatant. The plasma samples were stored at -20 °C for further analysis. The internal standard was added to the plasma samples at a concentration of 2 µM, then the samples were analyzed using the same method described before.
III. RESULTS AND DISCUSSION

Encapsulation efficiency and storage stability

For praziquantel-loaded PEG-DSPE/TPGS micelles, the final transparent micellar solution contains 32 mM praziquantel, 200 mM PEG-DSPE and 400 mM TPGS. At a 1:2 molar ratio of PEG-DSPE and TPGS, the mixed micelle incorporated more than 90% of drug which indicated a high encapsulation rate. Both the PZQ/P407, PZQ/M/P 407 and PZQ/P 407/HPMC hydrogel solution provided more than 90% drug loading without any precipitation. Praziquantel concentration in the micelle dispersion remained practically unchanged (>95%) at 4 °C for one week, indicating that the drug-incorporated micelles were stable at 4 °C and can be stored for at least one week. Besides, the stability duration should be extended for a more viable formulation. Further study in order to increase storage stability should be conducted.

Lower Critical Solution Temperature (LCST) and Gelation Time

To form a thermosensitive hydrogel, the percentage of poloxamer 407 should be 20% (w/v) or above, the gelling temperature was directly determined by the content of poloxamer 407 in the hydrogel solution. For the poloxamer 407 solution in which the concentration of poloxamer 407 was lower than 20%, the polymeric solution flowed freely at body temperature (37 °C) and did
not form gel at all. Generally, for poloxamer 407 at the concentration between 20%-30%, the viscosity increased after being taken out of the refrigerator, but still allowed the free flow of the polymer solution at room temperature, making the injection possible. But for the concentration of 30% poloxamer 407 or above, the gelation temperature was as low as about 14 °C, making injection very difficult. So only 20% (w/v) poloxamer 407 hydrogel solution was chosen in this study. The sol-gel transition of PZQ/M/P407 and PZQ/ P407 hydrogel solution happened when the temperature was higher than 26 °C and the corresponding gelling time is approximately 80 s. For PZQ/P407/HPMC hydrogel solution, the gelling temperature was also 26 °C but it took only 40 s to form a gel (Figure 6). Potentially HPMC facilitated the packaging of poloxamer 407 micelles, making the micellization much easier to occur, therefore it formed a gel more quickly.

**Figure 6.** The sol-gel transition of the hydrogel

**HPLC assay and calibration curves**

The retention times of praziquantel and α-naphthoflavone were 8.9 min and 14.9 min, respectively (Figure 7). The linear ranges, for both the calibration curve of praziquantel *in vitro* and in plasma, were 0.5-20 μM (Figure 8). The mean recoveries of praziquantel in plasma at the
concentration of 0.5-20 µM including the internal standard in all cases were higher than 85%.
The results reflected essentially 85% recovery from the spiked plasma and indicated the sample
preparation procedure was not interfering.

**Figure 7.** Chromatogram of the standard solution of praziquantel (10 µM) and the internal
standard (α-naphthoflavone: 2 µM), with retention times of 8.9 min and 14.9 min, respectively.

**In vitro release study**

The praziquantel release profiles from different hydrogel solutions were studied (Table 1).
As shown in Figure 9, the release of praziquantel from poloxamer 407 hydrogel was much
slower compared to the release of praziquantel from TPGS/PEG-DSPE mixed micelle, indicating
the network structure consisting of small poloxamer 407 micelles of hydrogel formulation
blocked
Figure 8. The calibration curves of praziquantel: *in vitro* (A); in plasma (B).

the release of the drug more effectively. For the PZQ/P407/HPMC hydrogel, the praziquantel released much more slowly than poloxamer 407 hydrogel only, probably because the HPMC chains bridged the poloxamer micelles, leading to a more compact network structure consisting of interconnected micelles. The increasing of densification and viscosity of the hydrogel resulted in the extension of the drug release profiles\textsuperscript{21}. The PZQ/M/P407 hydrogel obtained the most obvious extended-release characteristic compared to the other formulations, demonstrating it would take a much longer time for the drug to release from the micelle-hydrogel dual system. But there was a defect of the release study. The release medium was not under continuous stirring during the process of drug release. In this case, it may not be a homogeneous solution but forming a concentration gradient of the drug which released from the tested formulation, therefore the drug release profile here would be a little different from the ideal condition.
**Figure 9.** The release kinetics of praziquantel from PEG-DSPE/TPGS micelle and several hydrogel formulations

**Table 1.** The release half-lives (t₁/₂, release) of praziquantel from PEG-DSPE/TPGS micelle and several hydrogel formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>t₁/₂, release (h)</th>
<th>Goodness of fit (R²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG-DSPE/TPGS micelle</td>
<td>1.45</td>
<td>0.9596</td>
</tr>
<tr>
<td>PZQ/P407 hydrogel</td>
<td>5.5</td>
<td>0.9596</td>
</tr>
<tr>
<td>PZQ/P407/HPMC hydrogel</td>
<td>9.47</td>
<td>0.9792</td>
</tr>
<tr>
<td>PZQ/M/P407 hydrogel</td>
<td>10.45</td>
<td>0.9792</td>
</tr>
</tbody>
</table>

**Pharmacokinetics of praziquantel in mice**

To explore the bioavailability of subcutaneous administration of praziquantel-loaded formulations, the mice treated by intravenous injection of free praziquantel were set as the
control group. As shown in Figure 10 A, the plasma concentration-time profile demonstrated that the subcutaneous injection of PZQ/P407 hydrogel formulation obtained a relatively high bioavailability (Table 2), and the drug elimination was much slower. The comparison of oral administration (60 mg/kg) and subcutaneous injection of PZQ/P407 hydrogel (10 mg/kg) was shown in Figure 10 B. Although the oral dosage of praziquantel was six times higher than the subcutaneous dosage of hydrogel formulation, the area under the curve (AUC) of oral delivery was remarkably lower than the PZQ/P 407 hydrogel, indicating either a strong first-pass effect of the drug or the drug was not completely soluble in the gastrointestinal tract after oral administration. As illustrated in Table 2, the absolute bioavailability of subcutaneous injection of the hydrogel formulation of praziquantel was approximately 20 folds higher than oral administration. To modify the PZQ/P407 hydrogel for a better-extended release property, the PZQ/M/P 407 hydrogel and PZQ/P407/HPMC hydrogel were tested. However, both two modified hydrogel formulation failed to obtain a significant improvement of extended-release characteristic. The possible reasons were discussed here: 1) for PZQ/M/P 407 hydrogel, the pharmacokinetics of the subcutaneous injection of praziquantel-incorporated PEG-DSPE/TPGS micelle was compared with the intravenous injection of free drug. The results showed that the drug elimination following subcutaneous injection of the micellar solution was almost as fast as the intravenous injection of free drug, indicating the drug release from the micelle was much faster than release from the poloxamer 407 hydrogel. It meant the latter would be the rate limiting step, the total release rate should be more dependent on the drug release from the hydrogel. Therefore, the introduction of micelle as the secondary delivery vehicle did not radically contribute to prolonging the drug release characteristic. 2) for PZQ/P407/HPMC hydrogel: The hydrophobic and hydrophilic drugs distributed in different domains of the
hydrogel. The hydrophobic drug tends to partition into the hydrophobic PPO domain, while the hydrophilic drug partition into the hydrophilic PEO domain. Drug molecules distributed in the hydrophilic domain released by diffusing through the hydrophilic channels, drug distributed in hydrophobic domain released by the hydrogel erosion. Possibly, despite HPMC reinforce the poloxamer 407 micelles, form a more compact and denser network, it may mainly affect outside hydrophilic domain, in that case, the release of the hydrophobic drugs from the internal hydrophobic pores of the hydrogel would not be significantly prolonged (Figure 11).
Figure 10. The pharmacokinetic profiles of the formulations. The comparison between intravenous injection of free drug and subcutaneous injection of PZQ/ P407 hydrogel (A); the comparison between oral delivery of praziquantel and subcutaneous injection of PZQ/ P407 hydrogel (B); the comparison between PZQ-loaded micelle solution and several hydrogel formulations (C); the comparison of intravenous injection of free drug and subcutaneous injection of praziquantel-loaded micelle (D).
Figure 11. The schematic diagram of the drug distribution in the hydrogel and the drug release
Table 2. The half-lives ($t_{1/2}$), the area under the curves (AUC) and the bioavailability (F) of different routes or formulations for the administration of praziquantel.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>$t_{1/2}$ (h)</th>
<th>AUC (µM*h)</th>
<th>F (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.v. free praziquantel</td>
<td>0.321</td>
<td>355.615</td>
<td>100</td>
</tr>
<tr>
<td>p.o. free praziquantel</td>
<td>0.333</td>
<td>35.85</td>
<td>2.1</td>
</tr>
<tr>
<td>Praziquantel-loaded micelle</td>
<td>0.323</td>
<td>290.48</td>
<td>81.7</td>
</tr>
<tr>
<td>PZQ/P 407 hydrogel</td>
<td>0.963</td>
<td>311.71</td>
<td>87.7</td>
</tr>
<tr>
<td>PZQ/P 407/HPMC hydrogel</td>
<td>1.269</td>
<td>331.96</td>
<td>93.3</td>
</tr>
<tr>
<td>PZQ/M/P 407 hydrogel</td>
<td>1.409</td>
<td>350.52</td>
<td>98.6</td>
</tr>
</tbody>
</table>
IV. CONCLUSION

The current study succeeded in preparing a thermosensitive hydrogel based on poloxamer 407 for the extended-release of praziquantel with a high loading efficiency. The liquid hydrogel solution will easily form a semi-gel after administered into the body. The HPLC methodology and the plasma analysis procedures were well-developed with the linear calibration curves and a high recovery ratio, respectively. The model of *in vitro* release study can still be modified to maintain a ‘perfect sink condition’.

By the hydrogel formulation, the solubility of praziquantel was significantly increased, and both the results of *in vitro* release study and *in vivo* pharmacokinetic study suggested that the hydrogel formulation obtained a better extended-delivery of praziquantel. All the bioavailability of subcutaneous injection of the poloxamer 407-based hydrogel formulations were dramatically higher than oral administration. Owing to the convenience of the injectable administration to large livestock, the hydrogel-system formulations would be an alternative delivery strategy for praziquantel. However, although poloxamer 407 copolymers rapidly formed a semi-solid gel *in vivo*, the gel-like property was not able to maintain for a long time in physiological conditions due to the weak strength of the interconnecting poloxamer 407 micelles. In this study, the PEG-DSPE/TPGS mixed micelle-poloxamer 407 hydrogel hybrid system and poloxamer 407/HPMC hydrogel formulation was prepared to overcome the limitation of the original poloxamer 407 hydrogel. The results of *in vitro* release study indicated that both two modified hydrogel
formulations obtained a better extended-release characteristic of praziquantel. However, two modifications of hydrogel were not able to obtain a significantly extended-release profile, and the possible reasons were discussed. On the basis of these discoveries, other micelles or even other materials, which can be investigated as novel secondary delivery vehicles to modify the hydrogel. The further modification of poloxamer 407 hydrogel formulation which can obtain a better extended-release characteristic will be investigated in the future.
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VITA

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