A Novel Oral Vehicle for Poorly Soluble HSV-Helicase Inhibitors: PK/PD Validations

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Purpose. The current study describes the design and validation of a novel oral vehicle for delivering poorly water-soluble herpes simplex virus (HSV)-helicase inhibitors in preclinical pharmacokinetic (PK) and pharmacodynamic (PD) evaluations.

Methods. Poorly water-soluble compounds were used in solubility and drinking compliance tests in mice. A preferred vehicle containing 0.1% bovine serum albumin (BSA), 3% dextrose, 5% polyethylene glycol (PEG) 400, and 2% peanut oil, pH 2.8 with HCL (BDPP) was selected. This vehicle was further validated with oral PK and *in vivo* antiviral PD studies using BILS 45 BS.

Results. Solubility screen and drinking compliance tests revealed that the BDPP vehicle could solubilize BILS compounds at 0.5–3 mg/ml concentration range and could be administered to mice without reducing water consumption. Comparative oral PK of BILS 45 BS in HCL or BDPP by gavage at 40 mg/kg showed overlapping PK profiles. *In vivo* antiviral efficacy and potency of BILS 45 BS in BDPP by oral gavages or in drinking water were confirmed to be comparable as that achieved by gavage in HCL solution.

Conclusions. These results provide a protein-enriched novel oral vehicle for delivering poorly water-soluble antiviral compounds in a continuous administration mode. Similar approaches may be applicable to other poorly soluble compounds by gavage or in drinking solution.

KEY WORDS: antiviral; pharmacokinetic; pharmacodynamic; oral vehicle; solubility.

INTRODUCTION

Oral drug dosage represents the most convenient and common route for drug administration in human pharmaceutics as well as in preclinical drug evaluations (1–4). Yet, oral drug absorption is a very complex process dependent on several factors including compound solubility and permeability, formulation, and physiologic variables such as regional permeability differences, pH, luminal and mucosal enzymology, intestinal motility, among others (5–8). Recent advances in combinatorial chemistry and high throughput screening have led to the rapid increase of poorly soluble compounds in early drug discovery. Oral delivery of these compounds to animals for pharmacokinetic (PK) and pharmacodynamic (PD) evaluations has been an important part of drug discovery and/or development, but proven to be very challenging (3,9). Depending on the physicochemical properties of tested compounds, different formulations have been designed for these compounds, including solutions at nonphysiologically high or low pH, utilization of co-solvents, surfactants, cyclodextrins, mixed micellar solutions, fat/oil emulsions, or (nano-, micro-) suspensions (9–12).

Our HSV-helicase research has led to the discovery of a series of orally active inhibitors against HSV infections (13). Due to their poor aqueous solubility, these compounds (including BILS 45 BS) were administered by oral gavage in an acidic HCL solution (14). Like most poorly soluble compounds, BILS 45 BS and its analogs also demonstrate high plasma protein binding. The current study explored the application of their high protein binding property in a novel oral vehicle design: using a protein-enriched solution to deliver the compounds in the drinking solution for continuous drug administration. Because the animals get their oral dosage by spontaneous drinking, this mode of drug administration can be used conveniently to a fairly large number of mice over lengthy periods of time, with less distress to animals and investigators (15–18). In addition, it has been suggested that drug administration in the drinking mode, in comparison to the discrete oral gavages, to be advantageous in terms of providing a constant and continuous oral drug administration, with feasibility to achieve higher doses, with reduced toxicity (15–18). The current study further evaluated the applicability of the designed oral vehicle for administration of poorly soluble HSV-helicase inhibitors in the drinking mode for preclinical PK and PD evaluations.

MATERIALS AND METHODS

HSV-Helicase Inhibitors (BILS Compounds) and Other Chemicals

Acyclovir (ACV) and BILS compounds were synthesized in-house. The molecular structure of BILS 45 BS was shown in an early publication (14). Bovine serum albumin, fraction V (BSA), and peanut oil were purchased from Sigma (St Louis, MO, USA), Polyethylene glycol (PEG) 400 was purchased from Aldrich Chemical Company Inc. (Milwaukee WI, USA) and dextrose and HCL from Anachemia (Montreal, Canada).

Animals

Female hairless and nude mice (5–6 weeks of age, Charles River, Quebec, Canada) were used for all experiments, following protocols authorized by the institutional animal care committee, adhered to the guidelines of the Canadian Council on Animal Care (Ottawa, Canada). Nude mice were housed in microisolator cages inside semi-rigid isolators with sterile food, water, and bedding. All experiments using nude mice were carried out within Class II-type safety cabinets (Nuaire, Plymouth, Minnesota, USA).

Water Consumption Screen

Several solutions with sufficient solubilization power for poorly soluble compounds were screened in female hairless and nude mice (Charles River, Quebec, Canada) for water consumption compliance. Animals were divided into 5 to 6 per group. Water was used as the blank control and compared with all tested solutions for daily consumption. The mice were individually ear-marked and their body weights were re-

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corded every other day. Water consumptions, calculated by the weight difference, were recorded daily and presented as % of blank control.

Pharmacokinetic Study

Female hairless mice (n = 4 for each group per time point) were dosed with BILS 45 BS by oral gavage at the dose of 40 mg/kg (1.6 mg/ml, 25 ml/kg) in either 0.03 N HCL (pH 1.6) or BDPP (0.1% BSA, 5% dextrose, 5% PEG400, 2% peanut oil, pH 2.8). Blood samples were collected at designated time points as shown in results via cardiac puncture. Plasma was obtained by centrifugation and stored at –20°C until analyzed. Aliquots of plasma (250 μl) were extracted with 3 ml of organic mixture containing 80% ethyl ether and 20% hexane. After vortexing for 30 s, the solvents were separated by centrifugation at 2500 rpm for 10 min at 4°C. Each solvent extract was then transferred to a 3.5-ml polypropylene tube and evaporated to dryness under a nitrogen gas stream. The dried extracts were reconstituted with 100 μl of 50% acetonitrile in milli-Q water. Compounds used for the standard curve were prepared in blank mice plasma. Plasma extracts were analyzed using an HPLC system (Waters Limited, Mississauga, ON, Canada) consisting of a controller model 600-MS, a 625 LC pump, a 700 Satellite WISP automatic injector set at 10°C to minimize sample evaporation, and a diode array detector model 996 with the system management Millennium 2010 version 2.10. Fifty microliters of the reconstituted sample extracts was injected into a 2.1 mm × 150 mm Symmetry C-8 (Waters Limited, Mississauga, ON, Canada) column at 40°C. The mobile phase contained acetonitrile (A) and Milli-Q water (B). A gradient from 25 to 100% A in 17 min was used. The flow rate was set at 0.25 ml min^{-1}. BILS 45 BS was detected at a wavelength of 294 nm.

The apparent solubility of BILS compounds in BDPP vehicle was assessed by the clarity of the solution prior to the addition of peanut oil and confirmed by filtration through a 0.45-μm filter and then analyzed by HPLC. Samples of BILS 45 BS (0.5 mg/ml) in BDPP vehicle were also taken on days 0, 1, 2, 3, 8, and 10, to determine the stability of the compound in this vehicle.

Pharmacodynamic Study (*in Vivo* Antiviral Activity)

Athymic nude mice (female, nu/nu, CD1 from Charles River, Quebec, Canada) were inoculated with ACV-resistant HSV-1 thymidine kinase deficient (*dl*sptk) or polymerase (PAA^r5) mutants under halothane anesthesia by needle scarification and rubbing for 10 s with 10 μl of virus stock (10^7 pfu), on an area of about 1 cm^2 on each side of the dorsal skin. ACV was dissolved in the drinking water at 0 to 5 mg/ml and provided to infected mice immediately following inoculation for 10 days. BILS 45 BS at the concentration of 0 to 0.5 mg/ml in BDPP vehicle was also given during the same treatment duration. Cutaneous lesions were scored based on a standard criterion: 0, no lesions; 1, discrete vesicles; 2, two or more open lesions; 3, separate ulcerations; 4, zoster-band formations. Under current experimental conditions, topical lesions became visible within 2-3 days, and peaked within about 10–13 days. Although some degree of spontaneous regression occurred in animals infected with PAA^r5 mutants, topical lesions persisted over the whole duration of experiment. Only a few animals (less than 10%) of the PAA^r5-infected mice developed systemic disease (disseminated infections, neurologic and physical abnormality and mortality) following severe topical lesions. Therefore, systemic disease was not used for drug evaluation.

Topical lesion data were presented in terms of mean and standard error of the mean (SEM). Daily lesion scores and the areas under the curve (AUC) of lesion scores were compared for statistical significance by the analysis of variance followed with Student-Newman-Keuls multiple comparisons using SAS software (SAS Institute, Cary, NC, USA). A value of $p < 0.05$ was considered statistically significant.

RESULTS

Most potent BILS compounds including BILS 45 BS are poorly soluble in aqueous media (<10 μg/ml). Although acidic solutions can be used to increase their solubility, it was observed that these solutions reduced water consumption in mice, particularly when compounds were incorporated. After testing several different combinations and approaches, it was discovered that a new vehicle with 0.1% BSA had a good solubility power for BILS 45 BS. Although there was a reduced drinking compliance when 0.1% BSA was included in the drinking vehicle, the addition of dextrose (3%) brought the water consumption to the similar level as the water control (102–113%, Fig. 1). However, when BILS 45 BS was incorporated at the concentration of 1 mg/ml, the water consumption was 24–26% less than the water control. The solution containing BILS 45 BS had a smell that might have reduced the drinking compliance. To solve this problem, several flavors were tested. Addition of 0.1% peppermint oil caused a further decrease in water consumption to 56%. Replacing it with 0.05% vanilla, or further increase of dextrose to 8% failed to bring the water consumption back to normal.

At the same time, it was discovered that some compounds such as BILS 111, 163, and 22 BS even had lower solubility, and it was necessary to include 5% polyethylene glycol 400 (PEG400) to the BSA (0.1%) and dextrose (5%) based solution. Although this vehicle alone did not have a problem with drinking compliance, inclusion of 0.5 mg/ml of

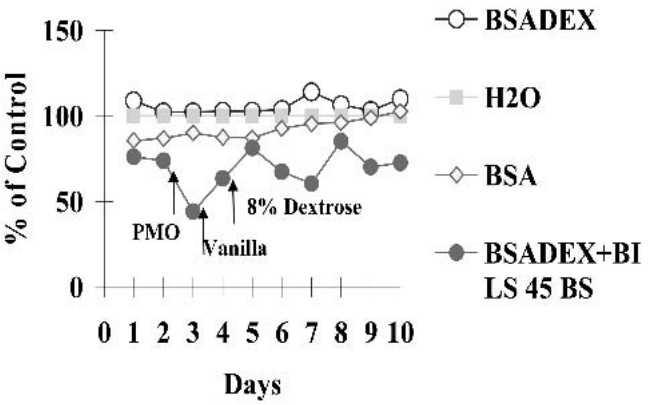

Fig. 1. Water consumption in hairless mice expressed as percentage of control (H_2O). BSA, 0.1% in H_2O; BSADEX, 0.1% BSA, 3% dextrose; BSADEX+BILS 45 BS, 1 mg/ml BILS 45 in 0.1% BSA, 3% dextrose. On day 2, 0.1% peppermint oil (PMO) was added to the last group only. On day 3, the peppermint oil was replaced with 0.05% vanilla. On day 4, the vanilla was replaced with 8.11% dextrose.

BILS 111 BS, BILS 163 BS, or BILS 22 BS reduced water consumption to 62%, 56%, or 69% of the water control, respectively (Fig. 2). Addition of 2% peanut oil increased water consumption to a similar level as the water control (Fig. 2).

The vehicle containing 0.1% BSA, 5% dextrose, 5% PEG400, 2% peanut oil (pH2.8, BDPP) in nude mice actually increased water consumption than that in the control water group. Inclusion of BILS 45 BS at 0.5 mg/ml brought the water consumption back to the similar level as the water control (Fig. 3).

The stability of BILS 45 BS in BDPP solution was analyzed by HPLC on days 0, 1, 2, 8, and 10. It was found that the compound recovery from day 0 to 10 fluctuated from 106.19% to 85.22% over the 10 days. At a concentration of 3 mg/ml, BILS 45 BS had a recovery of 96.71% to 104.03% from the vehicle over the 7 days tested.

Oral pharmacokinetic profiles following gavage of 40 mg/kg BILS 45 BS in either acidic solution or BDPP were compared (Fig. 4). The compound concentration in both vehicles was 1.6 mg/ml. The acidic solution contained 0.03 N HCL (pH 1.6, as that previously published, Ref. 14). It was clear that both vehicles showed an overlapping plasma concentration profile, suggesting that BDPP delivered BILS 45 BS as efficiently as the simple acidic solution.

In vivo antiviral activity of BILS 45 BS in nude mice was examined against ACV-resistant HSV-1 PAA^{r}5 mutant. In this model, HSV-1 PAA^{r}5-induced cutaneous lesions reached maximum within ~10 days and partial regression started at ~2 weeks postinoculation. Although oral gavage of lower dose of ACV failed to show antiviral activity (19), continuous drug administration of ACV allowed higher daily doses of ACV (up to 5 mg/ml or 1391 mg/kg/day) being administered to achieve the maximum effects (% reduction in AUC of topical lesions). In comparison, BILS 45 BS in BDPP (0.5 mg/ml) resulted in a much more efficacious antiviral activity (96% reduction of AUC of the lesion score, Fig. 5).

The dose-dependent effects of BILS 45 BS in BDPP drinking solution were further examined at the concentration range of 0.1–0.5 mg/ml (Figs. 6 and 7). Under current experimental conditions, it was observed that the water consumption was dramatically higher in the vehicle group, reproducing the observation in previous experiments. Inclusion of BILS 45 BS gradually reduced the consumption to the water control level. Nevertheless, average daily water consumption within each group was highly reproducible over the 10-day administration period, allowing a nicely graded dose-response evaluation (Fig. 6). Figure 7 illustrates the clear dose-dependent effects of BILS 45 BS on reducing both the mean lesion score and AUC, with estimated 50% effective dose (ED_{50}) of 91.9 mg/kg/day against HSV-1 PAA^{r}5-induced cutaneous lesions. Similar effects were also observed for BILS 45 BS against ACV-resistant *dl*sptk mutants, with an estimated ED50 of 95.6 mg kg^{-1} day^{-1} (Fig. 8). These ED_{50} values were slightly higher than those achieved with 10 days oral gavages of BILS 45 BS in the HCL solution (61 and 56.7 mg kg^{-1} day^{-1} against PAA^{r}5 and *dl*sptk mutants, respectively), as published previously (14).

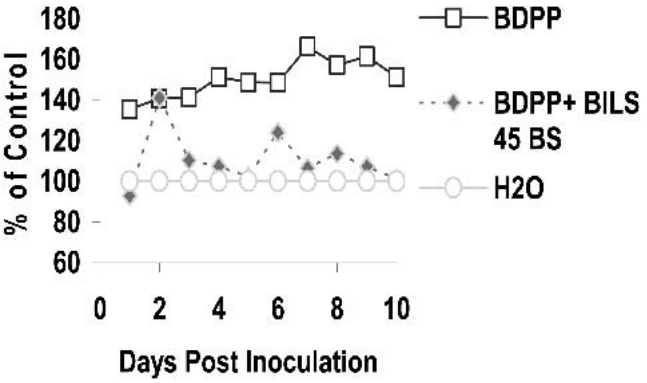

Fig. 3. Water consumption in nude mice expressed as percentage of control (H_2O). BDPP, 0.1% BSA, 5% dextrose, 5% PEG400, 2% peanut oil; BDPP+ BILS 45 BS, BILS 45 BS (0.5 mg/ml) in BDPP solution.

DISCUSSION

A novel oral vehicle (BDPP) was described for poorly soluble HSV-helicase inhibitors, with good solubilization power (≥0.5 mg/ml for all tested compounds) and compliance in mice. Oral gavages of BILS 45 BS in BDPP showed a comparable PK profile to that in HCL solution, validating the PK application of BDPP. BILS 45 BS in BDPP drinking so-

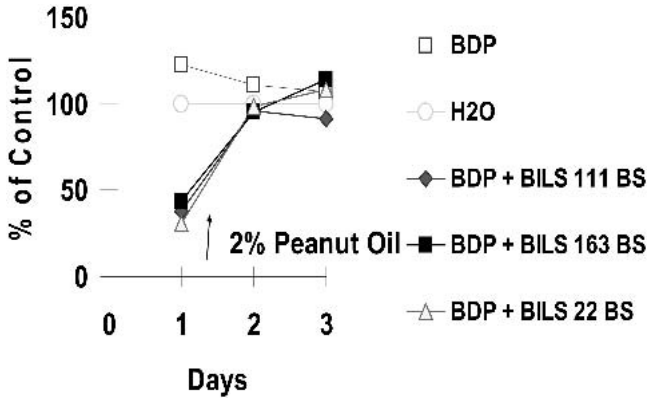

Fig. 2. Water consumption in hairless mice expressed as percentage of control (H2O). BDP, 0.1% BSA, 3% dextrose, 5% PEG400; BDP + BILS compounds were all tested at 0.5 mg/ml with 0.1% BSA, 5% dextrose, and 5% PEG400. After observation on day 1, peanut oil (2%) was added to all solutions containing BILS compounds.

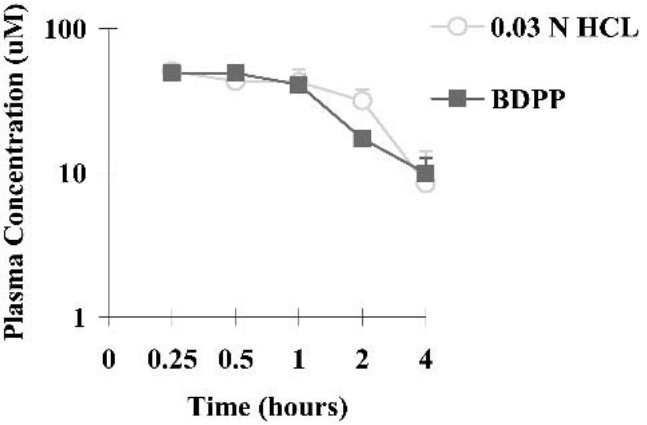

Fig. 4. Comparable oral PK of BILS 45 BS following oral gavage at 40 mg/kg in HCL (0.03 N HCL, pH 1.6) or BDPP solution (n = 4). Female hairless mice were dosed and sampled as described in the text.

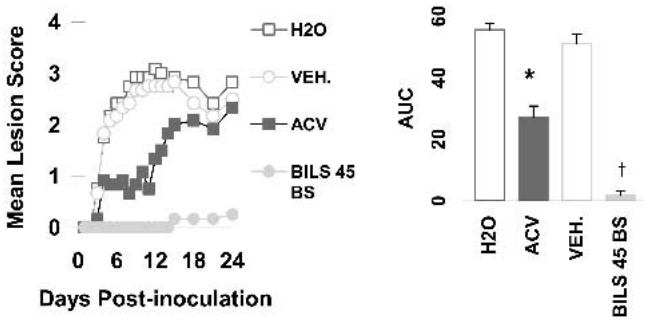

Fig. 5. Comparative effects of ACV (5 mg/ml) and BILS 45 BS (0.5 mg/ml) against HSV-1 PAA^{r}5 infection. Nude mice were infected with HSV-PAA^{r}5 (10^7pfu) and treated as detailed in the text. The mean lesion scores and areas under the curve (AUC) were calculated with n = 12 in each treatment. ACV was dosed at 5 mg/ml in H_2O, BILS 45 BS at 0.5 mg/ml in BDPP solution (VEH.), for 10 days, respectively. *p < 0.05 from vehicle control; †p < 0.05 from lower doses; n = 12 for each treatment.

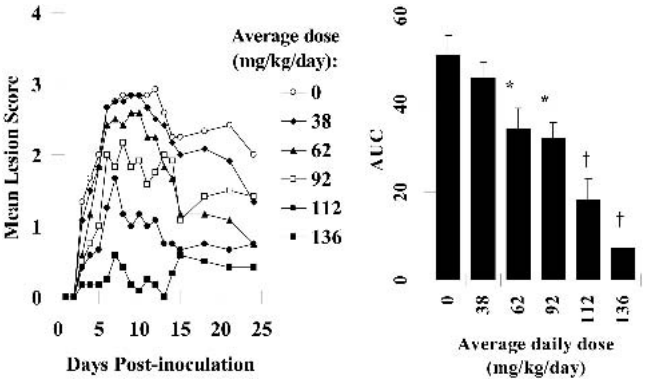

Fig. 7. Dose response of BILS 45 BS in BDPP solution against HSV-1 PAA^{r}5 infection in nude mice. Nude mice were infected with HSV-PAA^{r}5 (10^7pfu) and treated as detailed in the text. (*p < 0.05 from vehicle control; †p < 0.05 from lower doses; n = 12 for each treatment).

lution demonstrated excellent dose-response against ACV-resistant HSV-1 infections, validating the PD application of BDPP. The designed BDPP solution provides the feasibility for convenient and continuous drug administration for poorly soluble drugs at higher doses and lower risks.

Continuous oral compound administration in the drinking mode has been suggested as advantageous in preclinical drug evaluations using HSV-infected mouse models (15). These advantages include convenience of treating a fairly large number of infected mice over lengthy periods with the drug (both ACV and bromovinyldeoxyuridine) given *ad libitum* in the drinking water, and the regularly maintained drug levels with this mode of drug administration (16–18). Although there has been a concern on the uncertainty and regularity of oral drug intake by administration via the drinking solution, it has been demonstrated that the daily consumption in mice was surprisingly constant (15,19). In a preclinical model of ACV-resistant HSV-1 infection in nude mice, our laboratory demonstrated the feasibility to evaluate drug efficacy of higher doses of ACV in the drinking water (19). Unlike potent antiviral effects observed against wild-type HSV-1 infections, ACV at up to 289 mg kg^{-1} day^{-1} (1 mg/ml in drinking water) did not have any effect on HSV-1 PAA^{r}5 induced diseases. Increasing the dose to 871 mg kg^{-1} day^{-1} (3 mg/ml) reached a maximum protection of ACV in this disease model of 48%, similar to that achieved with 5 mg/ml, 1391 mg kg^{-1} day^{-1} in drinking water. These studies demonstrated the feasibility of delivering higher doses of ACV to achieve maximum efficacy without introducing any side effects, as well as the approach to study drug combinations via different routes of drug administration to minimize chemical or drug-drug interactions in the dosing solution or at the administration sites (19).

The current study explored the application of plasma protein binding properties in oral drug delivery. Serum albumin is one of the major proteins in human and animal plasma. It is the natural carrier of fatty acids in the blood. Because of its capacity to bind a wide variety of exogenous and endogenous substrates, albumin has been used to remove toxins, for drug delivery and for coating *in vivo* devices (20–21). In terms of drug delivery, albumin was commonly used as a macromolecular carrier to prepare microspheres (22). The current study, for the first time, explored the direct application of protein binding as an approach in oral drug delivery. The results showed that simple inclusion of BSA in the drinking solution, combined with a low percentage of PEG and dextrose was sufficient and effective to solublize and continually

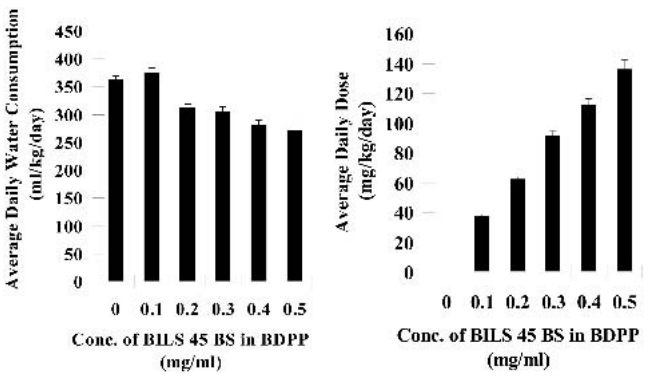

Fig. 6. Average daily water consumption and dose of BILS 45 BS in BDPP solution in HSV-1 PAA^{r}5 infected nude mice (over 10 days). Nude mice were infected with HSV-PAA^{r}5 (10^7pfu) and treated as detailed in the text.

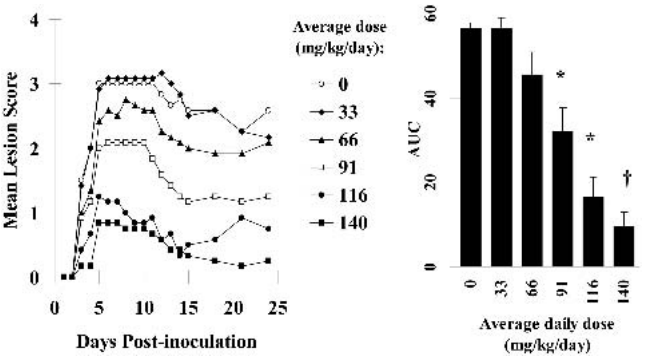

Fig. 8. Dose response of BILS 45 BS in BDPP solution against HSV-1 *dl*sptk infection in nu/nu mice. Nude mice were infected with HSV-*dl*sptk (10^7pfu) and treated as detailed in the text. (*p < 0.05 from vehicle control; †p < 0.05 from lower doses; n = 12 for each treatment).

deliver poorly water-soluble antiviral compounds in infected animals.

One of the concerns of including protein in the drinking solution was its potential effect on the rate and extent of oral absorption. It has been reported that the effect of protein on the dissolution rate and solubility was dependent on the pH, the nature of the protein and protein concentration (23). The effects of protein on the dissolution rate of their tested compound, phenytoin, was much higher than that can be accounted for by normal binding. The authors suggested that protein binding within the diffusion layer was of a different order to that in the bulk solution (23). This increased dissolution rate and solubility may have complicated effects on the transfer rate (24). The apparent transfer rate through their everted gut preparations could be negatively affected due to the decreased free drug concentration, yet the increased solubility and concentration gradient in the presence of protein could also increase the apparent transfer rate (24). Strong anti-inflammatory effects of freeze-dried indomethacin–olive oil–albumin complex were associated with its increased drug exposure (25–26). Similar approaches have been extended to several other poorly water-soluble drugs including meclinzine, prednisolone, norfloxacin and doxorubicin (27–28). In the current study, the BDPP vehicle demonstrated a good solubility power to deliver poorly water-soluble HSV helicase inhibitors. When BILS 45 BS was orally administered by gavages, a comparative PK profile was obtained with either simple acidic solution or BDPP vehicle, indicating that the designed BDPP vehicle did not render either dissolution or absorption barriers for the tested compounds. The slightly higher ED_{50} values achieved with this vehicle in drinking mode were not consistant with the literature suggestion that this mode of drug administration was advantageous than that by oral gavages. This may be attributable to the fact that the time to reach the therapeutic level in the beginning is critical. With oral gavages, the first dose was administrated as a bolus at 3 h post-inoculation. In the drinking mode, it may take longer time to reach the therapeutic level at the beginning. Alternatively, inter-experimental variability may also contribute to these small differences of ED_{50} values.

In today's drug discovery, more and more poorly soluble compounds have been introduced (4,29). These hydrophobic molecules usually have high plasma protein binding properties as well (20). Preclinical PK/PD evaluations of these compounds have been a continuous challenge for R&D scientists, particularly for studies using infected animals where prolonged, frequent dosing is usually required but not very convenient to carry out. The novel vehicle presented in the current study, or similar approaches, may be used to deliver these poorly soluble compounds in preclinical PK/PD evaluations.

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