Oral Formulation of a Novel Antiviral Agent, PG301029, in a Mixture of Gelucire 44/14 and DMA (2:1, wt/wt)

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ABSTRACT

To develop an oral formulation for PG301029, a novel potent agent for the treatment of Hepatitis C virus infection, that not only has very low aqueous solubility but also degrades rapidly in water. The solubility of PG301029 was determined in water, various aqueous media, and several neat organic solvents. The stability of PG301029 was monitored at room temperature in buffers for 4 days, and in several neat organic solvents for up to 8 mo. Drug concentrations were measured by high-performance liquid chromatography (HPLC). Based on solubility and stability data, Gelucire 44/14 and DMA (N,N-dimethylacetamide) at a weight ratio of 2 to 1 were chosen as the formulation vehicle. After the vehicle was prepared, it was maintained in liquid form at ~40ºC until the PG301029 was dissolved. The final formulation product was a semisolid at room temperature. The bioavailability of the formulation was tested on 4 female BALB/c mice. PG301029 is insoluble in all tested aqueous media, while its solubility is promising in DMA. This compound is unstable in aqueous media and some organic solvents; however, it is stable in DMA. This proposed formulation is able to hold up to 10 mg/mL of drug and is stable at 4ºC. The shelf life for this formulation stored at 4ºC is extrapolated to be greater than 4 years. This formulation dramatically increases the bioavailability of PG301029. This nonaqueous formulation solves the stability, solubility, and bioavailability problems for PG301029. This semisolid formulation can easily be incorporated into soft elastic capsules.

KEYWORDS: PG301029, Gelucire 44/14, DMA (N,N-dimethylacetamide), oral formulation, HCV

INTRODUCTION

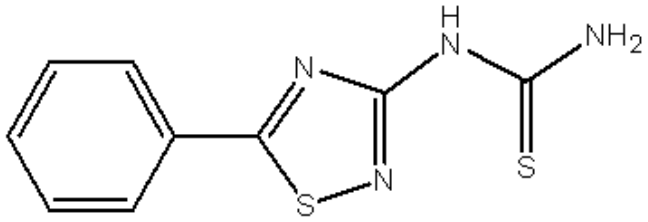
It is important to develop new regimens for treating Hepatitis C virus (HCV) infections because nearly 4 million people in the United States are estimated to be infected and ~10 000 lives are lost as a result of treatment failure each year. The current treatment for HCV infection is a combination of ribavirin and interferon. This regimen has several disadvantages. It is expensive and requires long-term treatment and has limited efficacy, working in only ~25% of patients. Significant side effects are associated with this treatment, including anxiety, arthralgias, autoimmune phenomena and associated disorders, chills, cough, depression, fatigue, fever, headache, hemolytic anemia, irritability, mild alopecia, myalgias, personality changes, psychiatric effects and associated disorders, reduced white blood cell counts, reduced platelet count, risk of birth defects, sleep disturbances, and thyroid dysfunction.[1-2] A novel antiviral compound, PG301029 (Figure 1), was found to have high efficacy and low toxicity on the inhibition of HCV. The therapeutic index of PG301029 was found to be 100-fold greater than ribavirin, and the oral LD_{50} in mice is greater than 6000 mg/kg.[1,3] However, making this compound into a feasible formulation is a challenging task owing to its extremely low water solubility (~0.7 µg/mL) as well as its instability in aqueous solutions. In order to overcome these problems, effort can be placed on either reducing particle size of the drug or developing a novel vehicle for the drug.

A nanoparticle formulation of PG301029 suspended in an aqueous medium was developed having as high as 91% bioavailability.[4] However, that formulation, unfortunately, used an aqueous medium. It would not be able to provide a long enough shelf life to be of practical value. In order to overcome the low solubility and poor stability problems of PG301029 in aqueous media, nonaqueous vehicles were investigated in this study.

MATERIALS AND METHODS

Materials

PG301029 ((5-Phenyl-2,5-dihydro-[1,2,4]thiadiazol-3-yl)-thiourea, $C_9H_8N_4S_2$) was donated by Procter and Gamble Co (Cincinnati, OH). Gelucire 44/14 was a gift from Gattefosse



PG 301029 (CAS No. 97149-60-5)

Figure 1. PG 301029 structure.

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(Westwood, NJ). Water for injection was purchased from VWR (West Chester, PA). All other solvents and chemicals were of reagent or high-performance liquid chromatography (HPLC) grade and used without further purification as purchased from Aldrich (Milwaukee, WI) or VWR.

Methods

Solubility of PG301029

The solubility of PG301029 was determined in water, various aqueous media, and several neat organic solvents by phase solubility analysis.[5] Excess drug was added into duplicate 4-mL screw-capped vials that contained ~1 mL of the tested solvent. The vials were rotated end-over-end at a constant speed of 8 rpm on a Labquake rotator (Barnstead International, model no 415110, Dubuque, IA) at room temperature for 3 days. The samples were filtered with 0.45-μm Acrodisc syringe filters (Pall Gelman Laboratory, Ann Arbor, MI) and then diluted to the proper concentrations before they were analyzed by HPLC.

The organic solvents used were ethanol (EtOH), triacetin, propylene glycol (PG), acetic acid, diacetin, dimethyl isosorbide (DMI), polyethylene glycol 400 (PEG 400), tetramethylurea, dioxane, N,N-dimethylacetamide (DMA), DMA:PEG 400 (1:1), N-methyl-2-pyrrolidinone (NMP), dimethyl sulfoxide (DMSO), and methylsulfonic acid (MeS).

Stability of PG301029 in Neat Organic Solvent and Water

The stabilities of PG301029 in DMA, EtOH, DMI, PEG 400, NMP, and PG were monitored up to 8 mo at room temperature (RT), while its stability in water (pH 7.67) was monitored up to 4 days. Solutions of PG 301029 in each solvent were prepared in duplicate at a concentration less than its previously measured solubility. After the PG301029 was added into each solvent, the suspension was vortexed for ~1 minute. They were then filtered with 0.45-μm Acrodisc syringe filters. The initial concentration of each solution was determined by HPLC. At each time point, drug concentration was measured by HPLC, and the percentage of drug remaining was calculated from the measured drug concentration divided by the initial drug concentration.

Vehicle Selection

Based on the drug solubility and stability, along with the consideration of excipient safety, DMA was chosen as the solvent to dissolve PG301029. However, the DMA solution of PG301029 precipitates drastically when mixed with water. The following excipients were selected to test their ability to retard or prevent drug precipitation: Gelucire 44/14, Labrasol, Tween 80, soy oil, PVP (kollidon 17PF), and sodium lauryl sulfate (SLS). Each excipient was mixed with DMA at certain ratios to prepare the vehicle. PG301029 was then dissolved in the vehicle at a concentration of 10 mg/mL. An aliquot part of 10 μL and 100 μL of formulation was each added into 1 mL of water to make the mixtures with a water/formulation ratio of 100 and 10, respectively. The diluted mixture was vortexed for 3 seconds, and its physical appearance was recorded. Half of the mixture was filtered by a 0.45-μm filter and diluted to analyze its concentration by HPLC, while the other half was used to characterize the drug precipitation by a polarized microscope.

Formulation Preparation and Its Stability

A formulation of Gelucire 44/14 and DMA at a weight ratio of 2 to 1 was chosen as the vehicle for further study. The formulation is prepared by mixing Gelucire 44/14 and DMA at a weight ratio of 2 to 1 and keeping it at ~40ºC to maintain its liquid form until all PG301029 is dissolved. The final product is a semisolid at room temperature. The stability of this formulation with PG301029 was monitored at 4ºC, RT (25ºC ± 2ºC), and 45ºC for 9 mo.

HPLC Assay

The following HPLC assay was developed: an Agilent 1100 HPLC system was used to determine drug concentration (1100 autosampler, 1100 quaternary pump with degasser, 1100 thermostated column compartment, and 1100 diode array detector) (Agilent, Palo Alto, CA). A Pinnacle Octyl Amine column maintained at 37ºC was used as the stationary phase (150 × 4.6 mm, 5 μm, Resteck, Bellefonte, PA), and a combination of acetonitrile with water at the ratio of 32 to 68 was used as the mobile phase. The flow rate was controlled at 1.0 mL/min. The effluent was monitored at 254 nm for 10 minutes with the drug retention time 6.0 minutes. The injection volume was 20 μL. This HPLC assay was validated with linearity and precision. Area under the curve (AUC) was linear in the concentration range of 0.13 μg/mL to 220.00 μg/mL with an R^2 value greater than 0.999. The relative SD was 0.93%, intraday and interday. Neither the degradation products nor the solubilization agents interfered with the drug peak. All samples were analyzed in duplicate. Because of the low solubility and instability of PG301029 in aqueous media, each sample was first diluted with DMA to the proper concentration and diluted with mobile phase immediately before injection.

Bioavailability of the Formulation

A formulation of 1 mg/mL of PG301029 in a mixture of Gelucire and DMA (2:1) was prepared for bioavailability study. Female BALB/c mice were used. The formulation was dosed by oral gavage on 4 mice with a PG301029 concentration of 5

Table 1. Solubility of PG301029 in Aqueous and Organic Media*

Vehicle	Solubility (mg/mL)
Aqueous	
Deionized water (instant)	0.00065
Deionized water (3 days)	0.00073
Phosphoric acid (H_3PO_4, 0.1M)	0.00065
Sulfuric acid (H_2SO_4, 0.1M)	0.00064
MeS (0.1M)	0.00065
Caffeine (2%)	0.0082
Nicotinamide (20%)	0.038
PG:EtOH:0.01N HCL (4:1:5)	0.041
Organic	
EtOH	0.29
Triacetin	0.58
PG	0.89
Acetic acid	1.23
Diacetin	1.33
DMI	5.55
PEG 400	6.06
Tetramethylurea	8.10
Dioxane	12.90
DMA	19.79
DMA:PEG 400 (1:1)	23.63
NMP	69.78
DMSO	84.51
MeS	82.91

*MeS indicates methylsulfonic acid; PG, propylene glycol; EtOH, ethanol; DMI, dimethyl isosorbide; PEG 400, polyethylene glycol 400; DMA, N,N-dimethylacetamide; NMP, N-methyl-2-pyrrolidinone; and DMSO, dimethyl sulfoxide.

mg/kg. Plasma levels of the drug were analyzed at 0.5, 1, 2, 4, and 8 hours after the mice were dosed. Details of plasma handling were described by Johnson et al. (unpublished data).

RESULTS

Solubility of PG301029

PG301029 is found to have low solubility in the tested aqueous media with an intrinsic solubility of 0.7 µg/mL in deionized water (Table 1). However, it has a promising solubility in several tested organic solvents, such as dioxane, DMA, NMP, DMSO, and MeS (Table 1).

Stability of PG301029 in Neat Organic Solvents and Water

As displayed in Figure 2, PG301029 is stable in DMA, EtOH, PEG 400, NMP, and PG for up to 8 mo. Figure 2 also shows that PG301029 is extremely unstable in pH 7.67 buffer and DMI. In fact, PG301029 is found to be unstable in all tested buffers from pH 1.2 to pH 7.7 (Johnson et al, unpublished data).

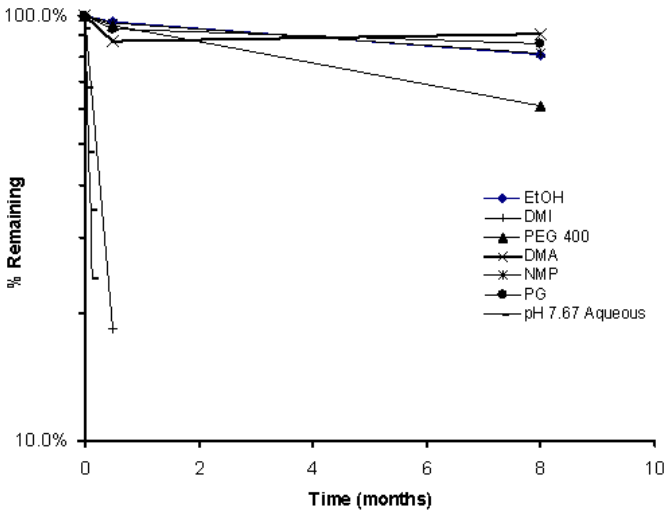

Figure 2. Stability of PG301029 in neat organic solvents and pH 7.67 buffer.

Vehicle Selection

Among all the combinations of the tested excipients with DMA as vehicles, only the formulation with Gelucire 44/14 and DMA in a ratio of 2:1 gives clear solutions after it is diluted with water at water/formulation ratios of 10 and 100. In addition, under polarized microscope, very few small drug crystals were observed for the mixtures. All of the other diluted mixtures were milky, and a larger amount of bigger crystals was observed. The measured drug concentration in the filtrates of the diluted mixtures further indicated that the Gelucire/DMA (2:1) formulation held the most drug in solution with 71% and 98% drug remaining, respectively, for the mixtures at water/formulation ratios of 10 and 100. The rest of the diluted mixtures held much less drug in solution. In fact, there was less than 5% of drug remaining in all of the filtrates for formulations that did not contain Gelucire 44/14.

Clearly, the formulation in Gelucire 44/14 and DMA at a weight ratio of 2 to 1 is the best choice to retard or prevent drug crystal formation when it contacts water. This formulation was chosen for the future study.

Stability of PG301029 in Formulation

Figure 3 presents the stability of PG301029 in the formulation of Gelucire 44/14 and DMA at a weight ratio of 2 to 1. It is stable at 4ºC, less stable at RT (25ºC ± 2ºC), and even less stable at 45ºC. The shelf life for this formulation stored at 4ºC is extrapolated to be greater than 4 years.

Bioavailability of the Formulation

Figure 4 depicts the mouse plasma PG301029 concentration vs time profile over 8 hours. The AUC of this formulation was calculated to be 355 ng/mL/h.

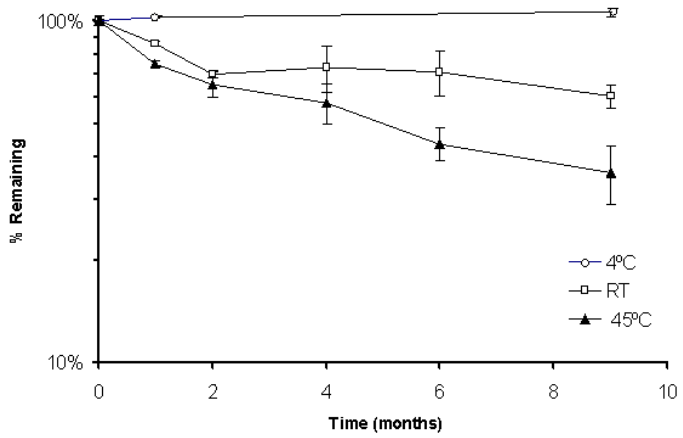

Figure 3. Stability of PG301029 in Gelucire 44/14 and DMA (2:1) at 4ºC, RT, and 45ºC.

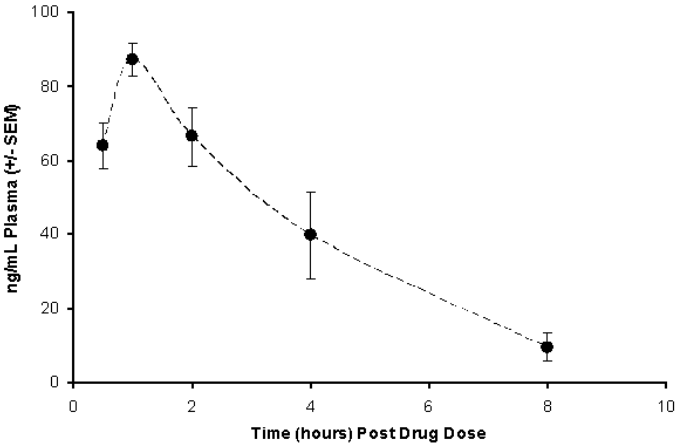

Figure 4. Plasma concentration-time profile of PG301029 after oral administration of 1 mg/mL PG301029 in Gelucire 44/14 and DMA (2:1) to female BALB/c mice (5 mg/kg).

DISCUSSION

PG301029 has been found to be efficacious in treating Hepatitis C and presents fewer side effects than treatments currently on the market. However, as revealed in Table 1 and Figure 2, this drug not only has very low aqueous solubility but also degrades rapidly in water. Jia et al[4] reported a virtual bioavailability for this drug by using a nanoparticle formulation. This formulation is not likely to provide a long enough shelf life as it is prepared in an aqueous medium.

A nonaqueous vehicle seems the best choice to formulate this drug. As indicated in Table 1, PG301029 can be dissolved in several neat organic solvents to form true solutions. However, it is unlikely that these solutions would provide good bioavailability because drastic precipitation is produced when they contact water. The addition of excipients to retard or prevent the precipitation when the solutions are mixed with water would greatly improve the formulation.

In this study, DMA was chosen as the organic solvent because each milliliter of DMA can dissolve PG301029 up to 20 mg (Table 1). DMA is generally regarded as a safe excipient for oral use since it was approved by the Food and Drug Administration as an inactive ingredient in various injection products.[6-8] In fact, DMA itself was studied as an antitumor agent in clinical trials with a dose of 100 to 610 mg/kg/d for 2 to 5 consecutive days.[9] Furthermore, the chemotherapy agent, Amsacrine (Amsidyl) is formulated with DMA.[10] Each cycle of Amsidyl is usually given over 3 to 5 days with between 57 and 159 g of DMA being given for each patient.[9]

Gelucire 44/14 was chosen as the additional excipient as it helps the solution to form a stable and fine dispersion to increase drug solubility when it contacts water.[11] Gelucires have amphiphilic properties and are generally recognized as safe (GRAS). They are a family of excipients derived from mixtures of mono-, di-, and triglycerides with PEG esters of fatty acids.[12] Gelucire 44/14 appears as a waxy solid at RT with a melting point of 44°C and a hydrophilic-lipophilic balance (HLB) value of 14. It enhances bioavailability of a poorly water soluble drug because it forms a very fine emulsion, improves the wettability of the drug, and increases the solubility of the drug when it contacts with gastrointestinal (GI) fluid at 37°C.[12-16] The mechanism for Gelucire 44/14 to improve the bioavailability of a poorly water soluble drug is related to its ability to act as a dispersing or emulsifying agent for the liberated drug.[17]

This study proposes a PG301029 oral formulation produced by dissolving the drug into a vehicle of Gelucire 44/14 and DMA in a ratio of 2:1. This formulation solves stability, solubility, and bioavailability problems for PG301029. The solubility of the drug is increased by using DMA as the solvent, and its stability is increased as it prevents PG301029 aqueous degradation until administration.

PG301029 was orally administered at 5 g/kg to BDF-1 mice,[3] and the nanoparticle formulation of PG301029 was orally administered at 500 mg/kg to Nude mice (P and G unpublished data). Assuming linear pharmacokinetics, the AUC from 0 to 8 hours by orally dosing PG301029 and nanoparticle formulation would be 6 ng/mL/h and 384 ng/mL/h, respectively, for a 5-mg/kg dose. The 8 hours AUC of this formulation was calculated to be 355 ng/mL/h (Figure 4). Clearly, this formulation provides a comparable bioavailability to the nanoparticle formulation in mice.[3] In addition, it provides higher bioavailability than any of the previously proposed formulations (P and G unpublished data). The increased bioavailability of PG301029 from this formulation may rely on the fact that Gelucire 44/14 acts as a dispersing and emulsifying agent when the formulation comes in contact with GI fluid.[17] No toxic side effects have been observed by dosing this formulation to mice.

Conclusion

The nonaqueous formulation of Gelucire 44/14 and DMA at a weight ratio of 2 to 1 solves the stability, solubility, and bioavailability problems for PG301029. Basically, the solubility of the drug is increased by using DMA as the solvent, its bioavailability is increased by using Gelucire 44/14 as a dispersant, and its stability is increased by preventing contact with water until administration. Using this formulation, the solubility of PG301029 can be enhanced to as high as 10 mg in 1 g of the vehicle. The liquid formulation can be filled into soft elastic capsules before it solidifies. This would offer the convenience of unit dose delivery.

Acknowledgments

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