Improving Cyclodextrin Complexation of a New Antihepatitis Drug With Glacial Acetic Acid

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ABSTRACT

The purpose of this study was to develop and evaluate a solid nonaqueous oral dosage form for a new hepatitis C drug, PG301029, which is insoluble and unstable in water. Hydroxypropyl-β-cyclodextrin (HPβCD) and PG301029 were dissolved in glacial acetic acid. The acetic acid was removed by rotoevaporation such that the drug exists primarily in the complexed form. The stability of formulated PG301029 was determined upon dry storage and after reconstitution in simulated intestinal fluid (SIF), simulated gastric fluid (SGF), and water. Formulated PG301029 was found to be stable upon storage and can be reconstituted with water to a concentration 200 times that of the intrinsic solubility. Once reconstituted, the powder dissolves rapidly and PG301029 remains stable for 21 hours in SGF, SIF, and water. The unique use of acetic acid and HPβCD results in a solid dosage form of PG301029 that is both soluble and stable in water.

KEYWORDS: Hydroxypropyl-β-cyclodextrin, acetic acid, solid dose, oral formulation, complexation, solubility, stability, PG301029.

INTRODUCTION

Approximately 4 million people in the United States and up to 500 million worldwide are infected with the hepatitis C virus. In the United States, approximately 10 000 people die annually from hepatitis C–related illnesses.[1]

Interferon and ribavirin are currently used to treat hepatitis C. A treatment program combining both of these drugs is considered to be most effective in establishing a sustained virological response. According to Rossi and Wright,[2] the advent of pegylated interferon has brought increased effectiveness compared with that of standard interferon, for monotherapeutic administration as well as for combination therapy with oral ribavirin. Nevertheless, these approaches are reported to be unsuccessful in more than 50% of treated patients.[3]

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In addition to having a low success rate, interferon is known to cause a multitude of side effects. Some of these side effects, including depression, suicidal ideation, compromised kidney function, and thyroid disease,[4,5] are serious enough to dramatically decrease patient compliance. Another challenge to patient adherence is that interferon must be administered parenterally, as it is not well absorbed orally.[5]

In cell-based assays, PG301029 has been found to show 3 times the potency,[6] 100 times the therapeutic index, and far fewer side effects in comparison to interferon and ribavirin.[7] In addition, PG301029 reduces toxicity by 100 to 200 times when given in conjunction with interferon and ribavirin.[6] Furthermore, this nonpolar molecule is not likely to present gastrointestinal permeability problems. Provided the existing solubility and stability challenges can be overcome, the drug can be given orally.

PG301029 (5-phenyl-3-thioureido-1,2,4-thiadiazole), shown in Figure 1, has a molecular weight of 236.32, and it melts and decomposes at ~245°C. The molecule has a calculated logP of 2.7, and 4 calculated pK_as. The 2 acidic pK_as are greater than 10, and the 2 basic pK_as are below 2.[8-10]

Unfortunately, PG301029 has a very low aqueous solubility of 0.7 μg/mL and degrades in water. In addition, the drug's lack of a usable pK_a makes it difficult to formulate. If PG301029 can be formulated for oral administration to solve the aqueous stability and solubility issues, that formulation may become valuable in treating hepatitis C.

Many publications have provided evidence that inclusion complexation can increase drug solubility and stability.[11-13] The benzene moiety of PG301029 makes it an ideal candidate for inclusion complexation with hydroxypropyl-β-cyclodextrin (HPβCD).[14,15] Loftsson et al[16] have shown that the formation of complexes can be inceased between HPβCD and various acidic and basic drugs by ionizing the drug in aqueous media. In fact, Kruss et al,[17] Loftsson,[18] and Loftsson and Masson[19] have filed patent applications proposing variations on this ionization approach for enhancing complexation efficiency. Because PG301029 is not ionizable and not stable in aqueous media, an alternative approach is presented in which the drug is dissolved and complexation forced without exposing PG301029 to water. The novelty of the formulation proposed is in the use of neat acetic acid as a solvent and not an ionizing agent. Neat acetic acid is employed as the ideal solvent to dissolve

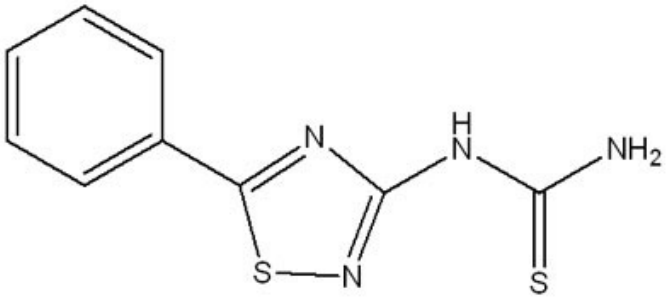

Figure 1. The structure of PG301029.

HPβCD and PG301029 together, and solvent evaporation increases the degree of complexation as the solution becomes increasingly concentrated. The final product is a dry powder consisting of HPβCD, PG301029, and acetic acid where the drug predominantly exists in the complexed form. The powder can be reconstituted with water to an increased drug concentration, as compared with the intrinsic solubility, and the PG301029 remains stable.

Materials and Methods

Materials

The PG301029 used in this study was good laboratory practice (GLP) grade and donated by Procter & Gamble (Cincinnati, OH). All other materials used were at least reagent-grade, and all of the studies were done at room temperature: 25 ± 2°C.

Melting Point Determination

The melting point and subsequent decomposition of PG301029 were measured by differential scanning calorimetry (TA Instruments 910, model 910001–901, TA Instruments, New Castle, DE) at a heating rate of 5°C/minute.

Determination of PG301029 Intrinsic Solubility

The apparent intrinsic aqueous solubility of the drug was determined, in triplicate, by oversaturating Millipore (reverse osmosis) water with the raw drug, rotating for 2 hours, and filtering prior to analyzing the clear solution by high-pressure liquid chromatography (HPLC). To minimize possible degradation, all samples were rotated with enough excess drug to allow constant dissolution as potential degradation occurred. In addition, the samples were analyzed within 15 minutes after filtration.

Determination of Solubility Dependence on pH

The solubility of PG301029 was measured in 0.1M HCl, unbuffered water, 0.1M sodium phosphate, and 0.1 glycine/NaOH buffer solutions at pH values of 1, 6, 7.5, and 10, respectively. Each sample was prepared by oversaturating the solution with the drug, followed by sonication and agitation by vortex. The triplicate samples of each solution were filtered prior to being analyzed by HPLC according to the assay described below. Again, aqueous degradation was minimized by analyzing all samples within 15 minutes of filtration.

Preparation of PG301029 in HPβCD/Acetic Acid Formulation

HPβCD (Cavasol W7 HP, Wacker Biochem Corp, Adrian, MI) and PG301029 were initially dissolved together in glacial acetic acid (ACS Reagent, Spectrum Quality Products, Inc, Gardena, CA) at a 1/0.0022/10 weight ratio or a 100/1/1 000 000 molar ratio, respectively. Dissolution was facilitated by alternating vortexing with sonicating until the solution was clear, as evidenced by the absence of a Tyndall effect when the solution was illuminated by a laser pointer. The acetic acid was then removed by rotoevaporation for ~30 minutes. This allowed the solution to become gradually more concentrated, thereby encouraging complexation. Once all of the liquid is removed, a solid white powder consisting of HPβCD, PG301029, and residual acetic acid remains.

This rather high (100/1) molar ratio of cyclodextrin to drug was found to best facilitate the drug's complexation as well as to best allow aqueous reconstitution to a clear solution. The final component proportions were determined as follows. First, HPβCD and PG301029 were dissolved to their maximum solubilities (10% wt/wt and 0.2% wt/vol, respectively) in glacial acetic acid. Second, after the acetic acid was removed and the powder was thoroughly dried, weighed aliquots of the powder were reconstituted with increasing volumes of water and filtered to remove precipitate. Third, the resulting solutions were analyzed by HPLC for soluble PG301029 concentration. The volume of the solution with the highest soluble drug concentration was chosen as that most desirable for reconstitution. Last, the amount of drug necessary in the initial acetic acid dissolution step was calculated so filtration would be unnecessary after reconstitution.

Preparation of PG301029 Physical Mixtures

Two physical mixtures with component ratios equal to those of the final formulation were prepared. One mixture consisted of HPβCD/PG301029 (454.5/1) (wt/wt) or (100/1) molar ratio, and the other consisted of HPβCD/acetic acid/PG301029 (454.5/50/1) (wt/wt/wt) or (100/200/1) molar ratio. Both were prepared by trituration for 5 minutes using a mortar and pestle.

HPLC Assays

PG301029 concentrations were assayed by HPLC using an Agilent 1100 series photo diode array detector (Agilent Technologies, Palo Alto, CA). A Restek pinnacle octyl amine, 5 μm, 150 × 4.6 mm column (Restek Chromatography Products, Bellefonte, PA) was used to separate eluents while running a mobile phase of acetonitrile and water in a 32:68 concentration ratio. The mobile phase flow rate was set to 1 mL/min, and the column temperature was controlled at 37°C. Each injection volume was 20 μL. The wavelength of detection was 254 nm, and the run and PG301029 retention times were 10 and 6 minutes, respectively. Acetic acid concentrations were assayed using the same conditions, except the detection wavelength was 210 nm and the retention time was 2.5 minutes.

Both HPLC assays were validated for linearity and precision. Linearity was confirmed for PG301029 covering concentrations in the range of 0.13 μg/mL to 220.00 μg/mL with an R^2 value greater than 0.999. Linearity was also achieved for acetic acid for concentrations ranging from 0.01% to 0.1% by volume. The R^2 value for acetic acid was greater than 0.980. The intraday and interday relative standard deviations for both assays were less than 1%. The PG301029 assay is able to separate degradation products from the drug and acetic acid peaks.

Characterization of HPβCD/Acetic Acid/PG301029 Formulation

Formulated PG301029 Concentration

To determine the drug concentration present in the proposed solid formulation, ~10 mg aliquots of the solid powder were weighed and then dissolved, in quadruplicate, in 2 mL of the HPLC mobile phase. All samples were analyzed according to the HPLC assay detailed above.

Determination of Residual Acetic Acid

To determine the acetic acid present in the proposed solid formulation, a standard curve was established by diluting acetic acid with mobile phase into 3 different known concentrations. Then, ~10 mg aliquots of the solid powder were weighed and then dissolved, in quadruplicate, in 2 mL of the HPLC mobile phase. All samples were analyzed according to the HPLC assay detailed above. Final concentrations of acetic acid were calculated according to the original weight of the powder sample dissolved.

Characterization of Residual Acetic Acid

Approximately 20 mg aliquots of the formulation were weighed and heated in an attempt to remove the residual acetic acid. Two samples were heated at 60°C for 4 hours, and 2 others were heated at 120°C for 12 hours. All samples were subsequently reconstituted and analyzed by HPLC for acetic acid and drug concentration.

Differential Scanning Calorimetry Formulation Characterization

The solid HPβCD/acetic acid/PG301029 formulation, pure HPβCD, and the HPβCD/PG301029 physical mixture were each separately analyzed by differential scanning calorimetry (TA Instruments 910, model 910001–901). Each sample was heated from room temperature to 300°C at a rate of 5°C/minute.

Physical Stability Upon Reconstitution

The physical stability of the HPβCD/acetic acid/PG301029 formulation upon reconstitution was determined in Millipore water at pH 6.1. The necessity of the acetic acid step was also investigated. The 2 physical mixtures and the HPβCD/acetic acid/PG301029 formulation were reconstituted as follows: ~10 mg aliquots of each powder were weighed and then dissolved in 2 mL of Millipore (reverse osmosis) water. After 30 seconds of vortexing, the HPβCD/PG301029 physical mixture and the HPβCD/acetic acid/PG301029 formulation solutions were filtered and the soluble drug concentrations were obtained in triplicate, for each powder, using the HPLC assay described above. The HPβCD/acetic acid/PG301029 physical mixture did not require HPLC analysis because the mixture did not dissolve when reconstituted.

Determination of Aqueous Chemical Stability of PG301029

The aqueous stability of both the unformulated raw drug and the HPβCD/acetic acid formulated PG301029 was determined for 21 hours in 3 systems: unbuffered Millipore water at pH 6.1, simulated gastric fluid (SGF) at pH 1.2, and simulated intestinal fluid (SIF) at pH 7.5. The SGF and SIF were prepared according to USP XVII instructions but without the pepsin and pancreatin. All of the drug concentration determinations were obtained in triplicate using the HPLC assay described above.

Unformulated PG301029

The initial unformulated drug concentrations were prepared in triplicate by adding an excess of PG301029 to a 4 mL volume of the aqueous solution. Each vial was rotated for 2 hours, filtered by a 0.45 μm Acrodisc syringe filter (Pall Corp, East Hills, NY), and assayed both immediately and approximately every 2 hours thereafter, for a 21-hour period.

Formulated PG301029

The HPβCD formulated PG301029 solutions were prepared by reconstituting ~200 mg aliquots of the solid formulation in 2 mL of aqueous solution. These solutions were also assayed for drug concentration immediately upon reconstitution as well as every 2 hours for the 21 hours that followed.

Determination of Chemical Stability of PG301029 in Dry Powder Formulation

The drug's stability in the proposed solid HPβCD formulation, upon storage, was determined by HPLC assay of the drug concentration immediately after reconstitution in water. This was done in quadruplicate immediately after initial formulation preparation and at 4, 5, 6, 8, 10, and 13 months of storage at room temperature. All stability studies were performed at room temperature.

Statistical Methods

All of the values herein are reported as means and standard deviations of the replicate determinations. Simple linear regression was used to establish the precision of the HPLC assays. Specifically, coefficients of determination (R^2 values) and slopes were compared to determine interday and intraday replicability. All of these calculations were made using Microsoft Excel.

Results and Discussion

Unformulated PG301029 Characterization

The melting point of the compound is 245°C, above which it decomposes. The apparent intrinsic aqueous solubility of PG301029 is 0.7 μg/mL. The solubility of PG301029 is also 0.7 μg/mL at pH values of 1, 6.1, 7.5, and 10.

Previously, Wong et al[20] reported the PG301029 solubility to be 50 μg/mL. This discrepancy with our measured 0.7 μg/mL is believed to be due to degradation and the difference in the 2 methods of analysis. To determine the drug concentration, Wong et al used spectrophotometry, which is unable to separate the drug from its degradation products. HPLC enables the differentiation of the drug from its degradation products.

HPLC Assays

Figure 2 shows sample chromatograms for PG301029. The bottom chromatogram shows peaks at 4 minutes and 5.5 minutes, which represent degradation products of PG301029. This degradation was observed as the peaks at 4 and 5.5 minutes increased in size as the PG301029 peak decreased in size over time while in water.

Formulated PG301029 Solubility and Stability

The presence of the benzene ring in PG301029 was the guiding factor in formulating with cyclodextrin. A benzene ring is known to be the ideal structure to snugly form an inclusion complex with HPβCD.[14,15] A relatively large amount of cyclodextrin is used in the current formulation.

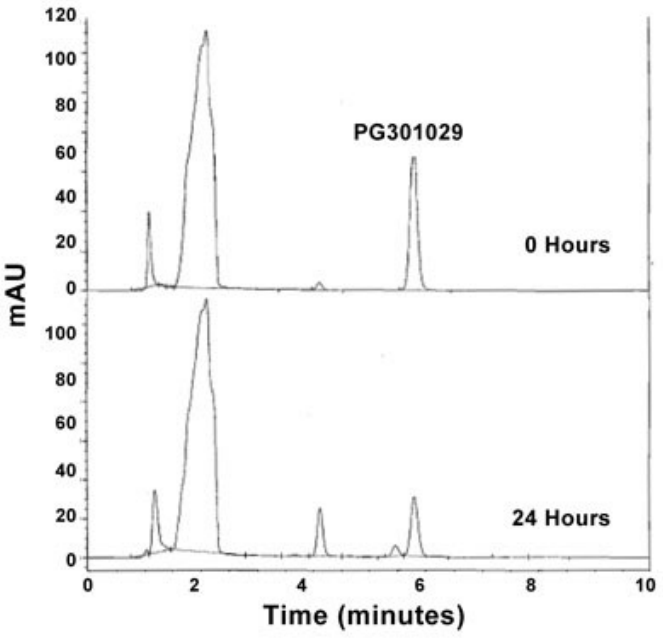

Figure 2. High-pressure liquid chromatography (HPLC) chromatograms for the same sample of PG301029 in water over a 24-hour period. The reduction in size of the drug peak and the increase in size of the other peaks over time suggest degradation of PG301029 in water.

This ensures that the PG301029 is primarily in the complexed form.[16] Unique to the proposed formulation is the use of acetic acid, which is ideal because it is generally regarded as safe (GRAS) and because both the drug and the cyclodextrin are soluble enough in acetic acid to obtain a solution at the desired concentration. In addition, PG301029 is stable in acetic acid. PG301029 is stable for 13 months upon dry storage in the solid formulation.

Once the solid formulation is introduced into water, the reconstituted pH is ~3 and the proposed vehicle increases the soluble drug concentration to 0.2 mg/mL. As indicated in Figure 3, raw PG301029 is most stable in SIF at pH 7.5 and relatively unstable in water; the degradation is most pronounced in SGF at pH 1.2. In contrast, the drug is stable under all 3 aqueous conditions in the HPβCD/acetic acid formulation. Error bars represent the percent relative standard deviation of the mean determined percent concentrations.

The mechanism of increasing the stability of PG301029 is unknown. It is possible that the presence of the cyclodextrin simply provides steric hindrance or that the cyclodextrin hydroxyl groups interact with the sulfur group. In any case, degradation is prevented for 21 hours.

Final Formulation Component Concentrations

The final solid powder consists of HPβCD, acetic acid, and PG301029 in a molar ratio of 100/200/1. The residual acetic

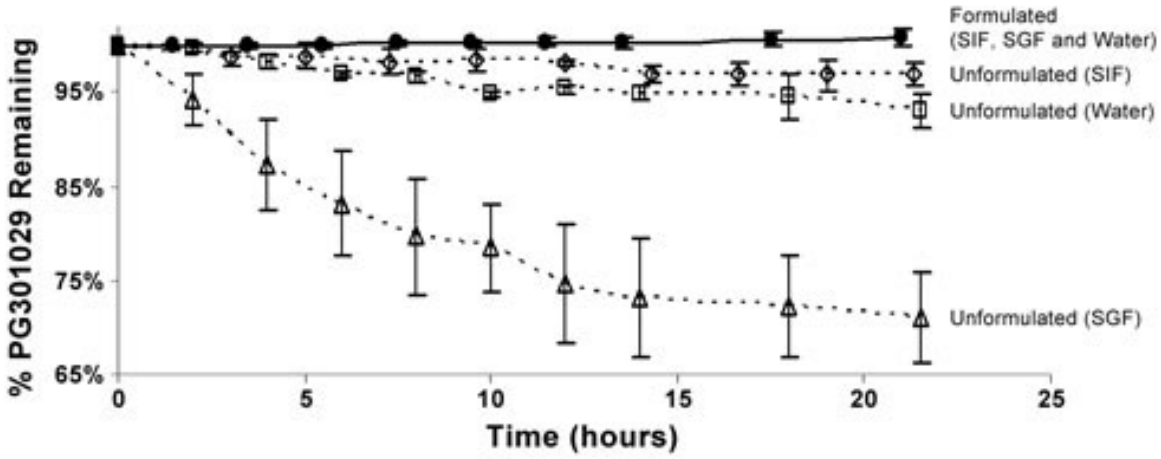

Figure 3. A comparison of the stability of unformulated PG301029 and PG301029 formulated in hydroxypropyl-β−cyclodextrin/acetic acid in simulated intestinal fluid (SIF), simulated gastric fluid (SGF), and water.

acid content in the solid formulation is 10% by weight. Once the powder is reconstituted in water, the final concentration is 1%.

Characterization of Residual Acetic Acid

At 60°C for 4 hours and 120°C for 12 hours, ~10% of the remaining acetic acid was removed, resulting in a final average residual concentration of 9%. Analyses of the PG301029 concentration showed that in both temperatures the drug degraded by 10% or more.

Aree et al[21] have reported that acetic acid forms an inclusion complex with cyclodextrin when HPβCD is dissolved in 10% acetic acid and allowed to recrystallize through slow solvent evaporation. It is unknown whether the residual acetic acid present in the proposed dry powder formulation is part of the complex. However, the fact that the remaining acetic acid was not easily removed combined with the final stoichiometric ratio of 2 acetic acid molecules per cyclodextrin molecule, suggests that the acetic acid is, to some degree, involved in the complex. Through x-ray diffraction, Aree et al[21] have shown that a complex between HPβCD and acetic acid can be accomplished through hydrogen bonding. They show that the acetic acid carboxyl hydrogen bonds with the hydroxyl groups of the cyclodextrin while the 2 carbons of the acid are positioned inside the nonpolar cavity.

Based on the data of Aree et al,[21] it is possible that the aromatic ring of the PG301029 is snugly enmeshed between the 2 carbon chains of 2 acetic acid molecules and that a network of hydrogen bonds readily occurs between the 2 carboxyls, the cyclodextrin hydroxyls, and the PG301029 amines. Furthermore, it is possible that the presence of the acetic acid molecules in the complex shields the PG301029 molecule from aqueous degradation.

Differential Scanning Calorimetry Characterization

Because the PG301029 concentration in the formulation is so low and the DSC pan size can accommodate only 5 mg in a sample, the solid HPβCD/acetic acid/PG301029 formulation and the HPβCD/PG301029 physical mixture were expected to produce very small drug endotherms. Therefore, the scans were expanded to facilitate examination of the temperature range surrounding the known melting point of PG301029. The HPβCD component of both scans is comparable to that of pure HPβCD. This indicates, in both cases, the presence of uncomplexed cyclodextrin. The physical mixture also shows an endotherm corresponding to the melting point of the pure drug, at 245ºC, while the proposed formulation does not. This evidence suggests that the PG301029 present in the proposed formulation is complexed while it is not complexed in the physical mixture. Additional evidence to support the existence of the complex between PG301029 and HPβCD lies in the prolonged stability of the drug upon reconstitution of the formulation; this stability is most dramatically observed under acidic conditions.

Physical Stability of PG301029 Upon Reconstitution

The use of acetic acid is shown to facilitate the complex, because the soluble PG301029 concentration upon reconstitution in water is only 0.046 mg/mL for the HPβCD/PG301029 physical mixture. This is compared with a soluble concentration of ~0.2 mg/mL when acetic acid is used to form the complex. In addition, the method in which the acetic acid is used and then evaporated is important, as evidenced by the fact that the ternary physical mixture of HPβCD, acetic acid, and PG301029, in equivalent ratios, does not dissolve upon aqueous reconstitution.

CONCLUSION

Through use of only HPβCD and glacial acetic acid, an oral dosage form is presented in which the solubility and the chemical stability of PG301029 are substantially increased in aqueous media. The HPβCD provides a shield for the drug from the hydrophobic effect and from aqueous degradation. Specifically, once dissolved in water, the vehicle increases the soluble concentration by a factor of 285 (from 0.7 μg/mL to 0.2 mg/mL) and degradation is prevented for 21 hours after reconstitution in water and simulated gastrointestinal fluids. The formulation can be given orally in solid form or reconstituted with water at a 1/10 (wt/wt) ratio immediately before administration.

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