CLINICAL INVESTIGATION

Chiral Stability of an Extemporaneously Prepared Clopidogrel Bisulfate Oral Suspension

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OBJECTIVES: The purpose of this study was to evaluate the chiral stability of clopidogrel bisulfate in an extemporaneously compounded oral suspension for a period of 60 days.

METHODS: A 5 mg/mL oral suspension of clopidogrel bisulfate was prepared from commercially available Plavix tablets. The clopidogrel suspension was then evenly divided between two light-resistant prescription bottles and stored either under refrigeration (4°C) or at room temperature (25°C). Samples were drawn from the stored suspensions immediately after preparation and on days 7, 14, 28, and 60. Samples were subsequently analyzed at each time point by high-performance liquid chromatography using a reversed-phase column, with chemical stability defined as the retention of at least 90% of the initial intact clopidogrel concentration measured. To determine the chiral stability of the suspension, samples were also analyzed by high-performance liquid chromatography using a chiral column to investigate possible enantiomeric inversion. Chiral stability was defined as the retention of at least 90% of the initial concentration of the suspension as the S-enantiomer, the active moiety of Plavix.

RESULTS: Regardless of storage conditions, the oral suspension of clopidogrel retained at least 98% of the active S-enantiomer for 60 days after preparation. Compared with the clopidogrel suspension stored in the refrigerator, more chiral inversion was noted in the clopidogrel suspension stored at room temperature.

CONCLUSIONS: Our investigation of chiral stability indicates that a 5 mg/mL clopidogrel oral suspension stored under refrigeration and at room temperature maintains chiral stability as the active S-enantiomer.

INDEX TERMS: chiral, clopidogrel bisulfate, compounded oral suspension, enantiomers, high-performance liquid chromatography

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INTRODUCTION

Clopidogrel bisulfate (Plavix; Bristol-Myers Squibb, Bridgewater, NJ), an antithrombotic drug, is marketed as a bisulfate salt and is indicated for the reduction of cardiovascular events, such as myocardial infarction and stroke. Chemically, clopidogrel is a thienopyridine derivative prodrug that requires hepatic activation to exert its antithrombotic activity (Figure 1).¹ The active metabolite irreversibly inhibits the platelet adenosine diphosphate 2Y₁₂ receptor, resulting in selective inhibition of adenosine diphosphate-induced platelet aggregation.² Furthermore, clopidogrel is an enantiomeric drug, with its S-configuration being active following metabolic

conversion (Figure 2).³ The R-enantiomer of the activated form of clopidogrel is devoid of anti-thrombotic activity and has the potential to evoke convulsions when used in large doses in animals.⁴ Consequently, Plavix is marketed exclusively as the S-enantiomer of clopidogrel.

The use of clopidogrel is increasing in pediatric patients with complex congenital heart disease, stroke, and Kawasaki disease.⁵ The 2008 Platelet Inhibition in Children on Clopidogrel (PICOLO) study established that infants and young children achieve clinically relevant platelet inhibition following a small dose of clopidogrel (0.2 mg/kg).² Unfortunately, clopidogrel bisulfate is presently available commercially only in tablet form (75 and 300 mg). This makes administration of the

Figure 1. Metabolic conversion of prodrug clopidogrel to active metabolite that inhibits adenosine diphosphate–induced platelet aggregation.

small doses often required for pediatric patients challenging. Because liquid formulations are needed to provide accurate weight-based dosing in infants and children, pharmacists may extemporaneously compound a liquid product.⁶ Preparation of an extemporaneous oral suspension usually involves trituration of the tablet in a mortar, with the addition of an appropriate suspending vehicle in a small portion to form a paste. Subsequently, the selected liquid vehicle is mixed in portions to the final volume. In the absence of measured stability data, the United States Pharmacopeia suggests that the compounded product be stored under refrigeration and the application of a beyond-use date be limited to 14 days.⁷

A previous study demonstrated that extemporaneously compounded clopidogrel suspension (5 mg/mL) was stable for 60 days when stored under refrigeration and/or at room temperature.⁸ Unfortunately, the possibility of chiral inversion was not investigated. Because the R-enantiomer is devoid of antithrombotic activity and large doses in animals have been reported to cause seizures, it is imperative to understand whether clopidogrel can undergo chiral inversion when prepared in oral suspension.^{4,9} The objective of this study was to prepare an extemporaneous clopidogrel oral suspension and investigate the possibility of chiral inversion during the 60-day storage period.

METHODS

A 5 mg/mL oral suspension of clopidogrel was prepared according to a previously published method, without modification.⁸ Briefly, for each 2 oz of suspension, four 75-mg tablets of clopidogrel bisulfate (Plavix; lot 1A71140) were triturated in a porcelain mortar to a fine powder. Next, 30 mL of Ora-Plus (Paddock Laboratories Inc, Minneapolis, MN; lot 1437415) and 30 mL of Ora-Sweet (Paddock Laboratories; lot 1235891) were combined in a separate container. Approximately 30 mL of the Ora-Plus:Ora-Sweet mixture was geometrically added to the mortar, and the mixture was levigated to a uniform suspension. The mixture was transferred to a 2-oz light-resistant prescription bottle. Finally, the mortar was rinsed with the Ora-Plus:Ora-Sweet mixture with a quantity sufficient to bring the final volume of the suspension to a final volume of 60 mL. This suspension was divided into 2 light-resistant, amber prescription bottles (Apothecary Products Inc, Burnsville, MN), with 1 bottle stored protected from light at a monitored refrigerated temperature (2°C–8°C), and 1 bottle stored protected from light at a monitored room temperature (20°C–25°C). Three aliquots (50 µL) were sampled from each oral suspension at various intervals (0, 7, 14, 28, and 60 days) thereafter and stored at –80°C pending assay via high-performance liquid chromatography (HPLC).

Figure 2. The S- and R-enantiomers of clopidogrel.

Quantitative analysis of clopidogrel was conducted using an HPLC system consisting of a Waters 1525 Binary HPLC Pump (Milford, MA), a Waters 717 Plus autoinjector/autosampler, a Waters dual-absorbance ultraviolet detector, and a reversed-phase C8 analytical column (150 × 4.6 mm inner diameter; 5 μ m; Spherisorb, Supelco, St Louis, MO). The mobile phase consisted of potassium phosphate (Sigma-Aldrich, St Louis, MO) at a concentration of 0.1 M plus acetonitrile (Sigma-Aldrich) at a ratio of 60:40, respectively, buffered at a pH of 2.5. The mobile phase solution was infused at a flow rate of 1 mL/min. The standards were injected at a volume of 10 μ L, and sharp peaks were obtained at a retention time of 8.7 minutes at an absorbance of 225 nm. Standard preparation solutions of clopidogrel were prepared by serial dilution and measured in triplicate. The squared correlation coefficients for the calibrated range (0.0001–0.1 mg/mL) were linear ($r^2 = 0.99991$). Analysis of matrix blanks showed no coeluting peaks at the retention time of the drug peaks of interest. The SEM for replicate injections did not exceed 2%.

Within-day and between-day coefficients of variation of the standard solution were 3% and 6%, respectively. Recovery was not assessed because samples were analyzed in test matrix (i.e., no extractions were necessary). For analysis, frozen samples collected from the clopidogrel oral suspension were allowed to thaw and were centrifuged at 11,200 × g for 5 minutes (Eppendorf Centrifuge 5415R; Hauppauge, NY). The supernatant was subsequently collected and diluted with 950 μ L of mobile phase for injection into the HPLC. As with the clopidogrel standards, samples were injected at a volume of 10 μ L and measured in triplicate. The concentration of clopidogrel in each sample was determined based on the extrapolation of integration values obtained to those previously measured in the standard curve (GraphPad Prism Software, La Jolla, CA).

The stability-indicating capability of the assay

for clopidogrel was determined by preparing 1 mg/mL aliquots and mixing one with either sulfuric acid (0.05 M; Mallinckrodt, Mansfield, MA) or sodium hydroxide (0.1 M; Sigma-Aldrich) at a dilution of 1:5. The clopidogrel solutions were heated to 80°C under reflux conditions for 1 hour and assayed under the HPLC conditions described above. No interfering peaks were observed under these conditions.

Two aliquots (50 μ L) were collected from the oral suspensions of clopidogrel at 0, 7, 14, 28, and 60 days and stored at –80°C until assay. Analysis of chiral stability of clopidogrel was performed using the same HPLC conditions described above; however, the reversed-phase C8 analytical column was replaced with a chiral column capable of resolving the R- and S-enantiomers of clopidogrel (4.0 × 150 mm; 5 μ m; Chiral-AGP, Sigma-Aldrich). The mobile phase used with the chiral column consisted of 16% acetonitrile, 1 mM *N,N*-dimethyloctylamine (Sigma-Aldrich), and 10 mM ammonium acetate (Sigma-Aldrich) buffered at a pH of 5.5. Under these conditions, standard solutions (0.1 mg/mL) of the S-enantiomer of clopidogrel (Toronto Research Chemicals, Toronto, ON, Canada) and the R-enantiomer of clopidogrel (Toronto Research Chemicals) were resolved on the chiral column at retention times of 14.9 and 20.4 minutes, respectively. Subsequently, samples collected from the oral suspensions of clopidogrel were centrifuged for 5 minutes at 11,200 × g after thawing. The supernatant (50 μ L) was then collected and diluted with 950 μ L of mobile phase and injected in triplicate for analysis by HPLC. In order to determine the amount of chiral inversion of clopidogrel over time, the integrations for each enantiomer of clopidogrel determined in collected samples at each time point were summed. Subsequently, the same individual integrations of each of the enantiomers were divided by the summed integrations and multiplied by 100 to obtain the percent amounts of each enantiomer at that respective time point.

RESULTS

When compounded by the previously described recipe,⁸ the resulting 5 mg/mL clopidogrel bisulfate dosage form was a pharmaceutically elegant suspension. Samples of the suspension, stored either in the refrigerator (4°C)

Table 1. Concentration of Clopidogrel Found in Oral Suspension During a Period of 2 Months as Determined by High-Performance Liquid Chromatography Analysis

Temperature	Initial Concentration	Duration of Storage			
		Day 7	Day 14	Day 28	Day 60
4°C	5.0 ± 0.2	5.2 ± 0.2	5.1 ± 0.3	5.1 ± 0.5	5.1 ± 0.1
25°C	5.1 ± 0.2	5.5 ± 0.2	5.1 ± 0.1	5.3 ± 0.2	5.2 ± 0.1

or at room temperature (25°C), retained physical stability throughout the course of the experiment and were easily resuspended upon shaking. No changes in either odor or appearance of the suspensions were noted at either temperature during the duration of the study. Aliquots of the sample suspensions were collected at various time points during the course of the experiment and analyzed for intact clopidogrel by HPLC plus a reversed-phase column. No degradation in intact clopidogrel was noted during the duration of the study when the suspension was stored at either 4°C or 25°C. (Table 1)

Because Plavix as marketed consists of only of the S-enantiomer, an effort was made to determine the amount of chiral inversion that may occur in an extemporaneously compounded suspension. The reversed-phase column on the HPLC was replaced with a chiral column, and standards of each enantiomer were obtained. In order to determine whether these enantiomers could be resolved by the chiral column, stock solutions of each were prepared and injected into the HPLC. The S-enantiomer and the R-enantiomer were easily resolved, with approximately 5 minutes between their retention times on the chiral column (Figure 3).

Subsequently, samples of the suspensions collected at various intervals were injected into the HPLC equipped with the chiral column to determine if inversion of the S-enantiomer to the R-enantiomer was occurring. When the oral suspension of clopidogrel was refrigerated, little to no inversion was noted over the course of the experiment. (Table 2) At room temperature, slightly more inversion to the R-enantiomer was noted during the duration of the study.

DISCUSSION

Clopidogrel bisulfate (Plavix) is increasingly used for cardiovascular indications in pediatric patients. The lack of a commercially available liquid formulation of clopidogrel requires that a suspension be compounded. Stability of an

extemporaneously compounded suspension was previously studied using HPLC with a reversed-phase column.⁸ The authors concluded that the suspension was physically and chemically stable for 2 months when stored at either room temperature or in the refrigerator. Whether stored in the refrigerator or at room temperature, clopidogrel remained intact throughout the course of the study period. Furthermore, the suspensions displayed no loss of physical stability (maintained pleasant odor, suspendability, pharmaceutical elegance) during the study period. These findings are consistent with previous reports.⁸

Plavix is marketed only as the active S-enantiomer because the R-enantiomer is devoid of activity. Non-enzymatic *in vitro* chiral inversion of the S- to the R-enantiomer has been demonstrated previously.³ A limitation of the reversed-phase column that was used in the stability study above is that inversion of the S-enantiomer to the R-enantiomer cannot be assessed; therefore, we investigated the possibility of chiral inversion in an oral suspension compounded from 75-mg Plavix tablets that were suspended in Ora-Plus and Ora-Sweet vehicle.

Figure 3. Resolution of the S-enantiomer (A) and R-enantiomer (B) of clopidogrel.

Ten microliters of stock solution (0.1 mg/mL) of each enantiomer was injected into a chiral column, and the retention of each was determined to be 14.9 and 20.4 minutes for the S- and R-enantiomers, respectively.

Table 2. Percent of R-enantiomer of Clopidogrel Found in Oral Suspension During a Period of 2 Months as Determined by High-Performance Liquid Chromatography Analysis With a Chiral Column*

Temperature	% of R-enantiomer Enantiomer				
	Initial Concentration	Day 7	Day 14	Day 28	Day 60
4°C	0.22 ± 0.28	0.26 ± 0.11	0.15 ± 0.04	0.19 ± 0.06	0.31 ± 0.02
25°C	0.11 ± 0.03	0.21 ± 0.16	0.43 ± 0.06	0.76 ± 0.17	1.13 ± 0.22

*Samples were injected in duplicate. Data are presented as mean percent of R-enantiomer measured ± SD.

Chiral stability due to inversion was also assessed in the clopidogrel suspension using HPLC fitted with a chiral column. A proper chiral analysis requires adequate resolution of the respective enantiomers. In the case of clopidogrel, the enantiomers were clearly resolved via the method used. Analysis of the collected clopidogrel samples indicated that more inversion occurred in the clopidogrel suspension stored at room temperature compared with that stored in the refrigerator. Even at room temperature, less than 2% of clopidogrel in the extemporaneously compounded suspension inverted from the S-enantiomer to the inactive R-enantiomer over the duration of the study.

We conclude that an extemporaneously prepared clopidogrel suspension is chemically and physically stable at either room temperature or under refrigeration for up to 60 days. Regardless of storage conditions, clopidogrel bisulfate remained in its active S-enantiomer (>98%) throughout the 60-day study. This extemporaneously compounded suspension remains a useful alternative option for the dosing of pediatric patient populations.

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Abbreviations HPLC, high-performance liquid chromatography; PICOLO, Platelet Inhibition in Children on Clopidogrel

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