Development of an Unconventional *In Vitro* Drug Release Test Method for a Bile Acid Sequestrant, DMP 504, Tablet

Lei-Shu Wu,[1,2] Timothy J. McCormick,[1] Rong-Kun Chang,[1] Josephine Pang,[1] Tom McCummings,[1] Miriam Ramos,[1] and Munir A. Hussain[1]

Received November 3, 1998; accepted March 23, 1999

KEY WORDS: reverse-binding; dissolution; *in vitro* release; bile acid sequestrant; DMP 504.

INTRODUCTION

DMP 504 is a water insoluble, cross-linked polymeric bile acid sequestrant. It is synthesized by cross linking of 1,10-dibromodecane and 1,6-diaminohexane. DMP 504 is intended to be used as a non-systemic cholesterol lowering agent. The primary mechanism of action in vivo is its capability of binding bile salts with a slow dissociation rate until fecal excretion occurs. This process reduces the reabsorption of the bile salts, which triggers the consumption of plasma cholesterol for bile salts syntheses in the liver. The efficacy of a bile acid sequestrant is related to its binding capacity, binding affinity, and rates of association and dissociation for the major bile salts (1–2). Luner and Amidon used a multiple mixing tank model, which simulated the transit of bile salt and sequestrant in gallbladder, stomach, jejunum, ileum and colon, to predict the effect of bile acid sequestrant on the bile salt excretion (3). A good correlation was found in the fecal bile salt output between simulation and patient data provided that bile salt binding, reversible binding, and reabsorption were taken into consideration.

In vitro equilibrium binding and kinetic studies revealed that DMP 504 exhibited a higher maxmium binding capacity, a greater affinity and a slower dissociation rate than cholestyramine to the major bile salts (4). In addition, the binding of the bile salts to DMP 504 exhibited more cooperativity than to cholestyramine (4). The results of an *in vivo* hamster study indicated that DMP 504 was superior to cholestyramine in the excretion of fecal bile salts/sterols, the elevation of cholesterol 7-α-hydroxylase activity and the reduction of total serum cholesterol and HDL cholesterol (5).

A film coated DMP 504 tablet formulation was developed. Since DMP 504 is a water insoluble polymer (6), a conventional dissolution method is not feasible for evaluating the drug release from the tablets. In this communication, development of an unconventional in-vitro test method using a reverse-binding technique is reported; the linearity of the standards and binding calibration curves are demonstrated; the recovery of the bile salt in the presence of the placebo blend is investigated; drug release from tablets is evaluated; and drug release profiles of DMP 504 and two other bile acid sequestrant commercial products, Questran and Colestid, are compared.

MATERIALS AND METHODS

Materials

Various lots of DMP 504 drug substance manufactured by Lonza, AG (Walliserwerke, CH-3930, Visp, Switzerland) and sodium cholate obtained from Sigma (St. Louis, MO, USA) were used for the filter check, linearity, and recovery tests. DMP 504 tablets (750 mg strength) manufactured at DuPont Pharmaceuticals Company (Wilmington, DE, USA), Questran tablets (1 g strength anhydrous cholestyramine) from Bristol-Myers Squibb Co. (Princeton, NJ, USA), and Colestid tablets (1 g strength colestipol hydrochloride) from the Upjohn Company (Kalamazoo, MI, USA) were used for the release studies. A placebo powder blend was prepared by DuPont Pharmaceuticals Company and used for the recovery test. Whatman 0.45-μm PTFE Autovial Syringeless Filters (Whatman Science, Ann Arbor, Mi, USA) and Gelman Nylon Acrodisc filter (Gelman Inc., USA) were used for sample filtration. Reagent grade potassium phosphate, monobasic (EM Science, Gibbstown, NJ, USA) was used to prepare the drug release medium and standard solutions. Milli-Q water (Millipore Corp., Milford, MA, USA) was used for both the drug release medium and the HPLC mobile phase. HPLC grade methanol (EM Science, Gibbstown, NJ, USA) and reagent grade glacial acetic acid (J. T. Baker Inc., Phillipsburg, NJ, USA) were used in the preparation of the mobile phase for HPLC analysis.

Apparatus

A Distek 2100 (Distek Inc., Monmouth Junction, NJ, USA) dissolution apparatus using USP Apparatus 2 (teflon coated paddle) at 50 rpm was developed and validated for release testing of DMP 504 tablets. Since Colestid exhibited a slower drug release rate, paddles stirred at 100 rpm was used in this study. A stainless steel wire sinker was used to hold the tablet at the bottom of the plastic dissolution flask. The medium for the drug release test was heated and deaerated through an Erweka Dissofill, model DSF3 (Erweka Instruments Inc., Milford, CT, USA).

Reagents and Standards Preparation

A phosphate buffer (0.02M at pH 7.0) was used as a dilution medium. A sodium cholate stock solution, in the phosphate buffer, at a concentration of 6.21 mM was used as the medium for the in-vitro drug release of the 750 mg strength tablets, the filter check, and the recovery test. The standard solutions were prepared by a series of dilutions of the stock solution (at 10, 25, 50, 75, and 100%) using the phosphate buffer dilution medium.

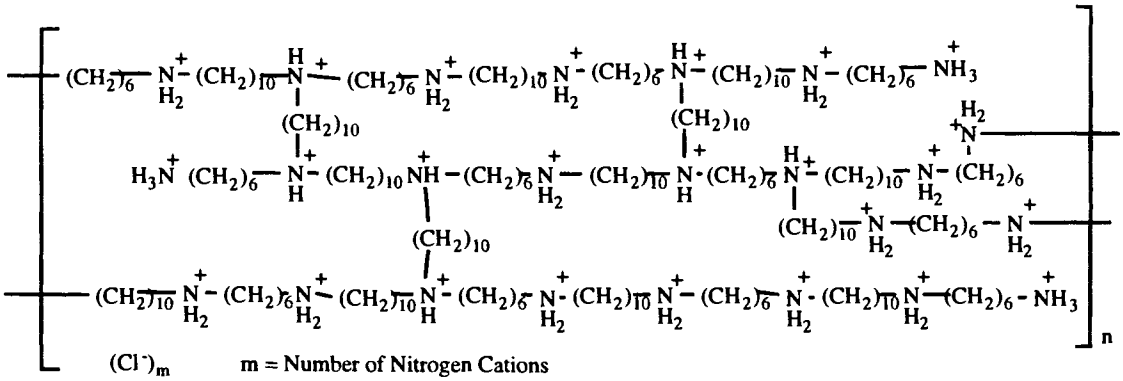
Filter Check

Two milliliters of 10, 50, and 100% standard solutions were filtered through either a 0.45-μm Whatman PTFE Autovial

[1] DuPont Pharmaceuticals Company, Experimental Station, P.O. Box 80400, Wilmington, Delaware 19880-0400.

[2] To whom correspondence should be addressed. (e-mail: lei-shu.wu@dupontpharma.com)

0724-8741/99/0700-1136$16.00/0



Scheme 1.

Filter or a 0.45-μm Gelman Nylon Acrodisc filter. The filtered and un-filtered standard solutions were analyzed by HPLC.

Recovery Test

A cholate recovery study, in the presence of placebo, was conducted by placing the placebo blend into 900 mL of sodium cholate stock solution (n = 3) at 37°C. The mixture was stirred with the paddle at 150 rpm for 2 minutes to disperse the powder and then stirred at 50 rpm for 30 minutes. A 2-mL aliquot was taken from each flask, filtered through PTFE filter, and analyzed by HPLC to determine the concentration of unbound cholate.

In Vitro Drug Release and HPLC Analysis

The medium used for in-vitro drug release testing was 900 mL of 2.67 mg/mL sodium cholate stock solution in pH 7.0, 0.02 M phosphate buffer at 37.0 ± 0.5°C. Agitation was accomplished via the USP Apparatus 2 (paddle) at 100 rpm. DMP 504 film coated tablet was placed in the center of a stainless steel coil and dropped to the bottom of the flask. Two-milliliter aliquots were taken at various timepoints, filtered through a 0.45-μm Whatman PTFE Autovial Syringeless Filter, and analyzed.

HPLC was performed on a system consisting of a pump (Model 510), an autoinjector (Model 717 Wisp), a refractive index detector (Model 410), and a column oven programmed by a temperature control module and operated at 37°C, all from Waters Associates, Milford, MA, USA. All seperations were performed on a Zorbax® RX-C_8 column (4.6 mm × 150 mm) using a mixture of methanol (720 mL), glacial acetic acid (2 mL), and water (280 mL) as a mobile phase at a flow rate of 1.5 mL/min. The concentration of unbound cholate was determined. The chromatographic data were acquired and analyzed on a computer equipped with a data acquisition and analysis software program (Multichrom 2 software, Thermo LabSystem).

Binding Calibration Curve

The extent of cholate bound by DMP 504 varied when the ratio of drug substance to sodium cholate was altered. As shown in Fig. 1, a linear relationship was found in the range of DMP 504 to cholate ratio (w/w) up to 0.4 by plotting percentage bound cholate as a function of the ratio. Therefore, different concentrations of cholate must be used to evaluate dissolution profile of different strengths of DMP 504 tablets. A binding calibration curve was established by using 10, 20, 40, 60, 85, and 115% of the label claim of DMP 504 and a fixed excess amount of sodium cholate. A volume of 900 mL of sodium cholate stock solution (2.67 mg/mL) was placed in each of six dissolution flasks, with paddles stirred at 200 rpm. The solution was heated to 37°C. Various amounts of DMP 504 drug substance (10–115% of 750-mg) were added into each flask. A 2-mL aliquot was taken at 120 minutes, filtered and analyzed by HPLC to determine the concentration of unbound cholate. The bound cholate concentration was obtained by subtracting the unbound cholate concentration from the initial cholate concentration.

The total initial amount of cholate used for the study could be obtained by multiplying the concentration of the stock solution by 900. The specific amount of DMP 504 could be obtained by dividing the amount of DMP 504 by the total initial

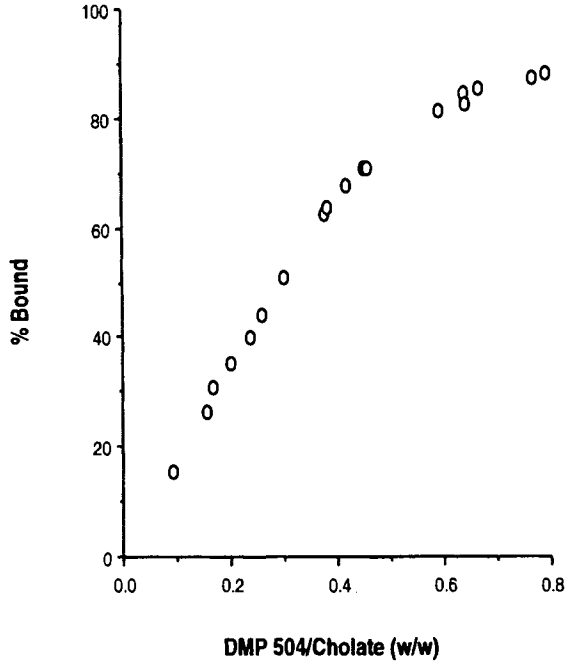


Fig. 1. Percentage bound cholate as a function of DMP 504 to cholate weight ratio.

amount of cholate. Therefore, binding calibration curve could be established by plotting the bound cholate (A_b) as a function of the specific amount of DMP 504 (SW).

$$A_b = (C_{ini} - C_{unb}) \times 900$$

$$SW = \frac{W_{504}}{C_{ini} \times 900}$$

where,

A_b = amount of bound cholate
C_{ini} = the initial concentration of cholate solution
C_{unb} = the concentration of unbound cholate
SW = specific weight of DMP 504, the weight ratio between DMP 504 and cholate
W_{504} = weight of DMP 504

Since the drug substance used to prepare Questran and Colestid tablets were not available to establish their binding calibration curve, the percent drug release for these two commercial products was estimated through normalizing the results of percent bound.

$$\%Re = \frac{B_t}{B_{eq}}$$

where,

%Re = percentage of drug release
B_t = the amount of bound cholate at time t
B_{eq} = the amount of bound cholate at equilibrium

RESULTS AND DISCUSSION

Linear regression of the peak area as a function of the cholate concentration of five standards was performed, and the correlation coefficient ranged from 0.99999 to 1.00000. This demonstrated linearity of the HPLC assay method.

Two types of filters (nylon and teflon) were evaluated to determine potential loss of cholate to the filter. No significant loss of cholate on either type of filters was found (Table 1). The Whatman PTFE Autovial Syringeless Filter (teflon) was selected for further studies.

Complete recovery of the cholate, in the presence of placebo, was obtained in the recovery test (Table 2). This indicates that no binding occurred between the excipients of the placebo and sodium cholate; therefore, the loss of cholate observed during in-vitro drug release testing can be attributed to binding to DMP 504.

Table 1. Percent Recovery of Sodium Cholate After Filtration Using Telfon and Nylon Filters

Level	% Recovery of Sodium Cholate (Mean ± RSD, n = 3)	
% of Cholate Stock Solution	Whatman Teflon Filter	Gelman Nylon Filter
10%	96.3 ± 0.9%	98.5 ± 0.2%
50%	98.5 ± 0.3%	98.1 ± 0.3%
100%	99.1 ± 0.1%	98.3 ± 0.4%

Table 2. Recovery of Sodium Cholate in the Presence of Placebo

Level	% Recovery of Sodium Cholate
% of Cholate Stock Solution	(Mean ± RSD, n = 3)
50%	100.3 ± 0.1%
100%	100.4 ± 0.1%

Drug release from a tablet is a dynamic process. The ratio between the drug available for cholate binding and the concentration of unbound cholate is changing constantly during the release process. In addition, the extent of cholate binding to DMP 504 varies when the ratio of drug substance to cholate is altered. A linear relationship was observed between the ratio of drug to cholate in a range of 0–0.4 and the bound cholate in a range of 0–66% (Fig. 1). Therefore, it was necessary to choose a dissolution medium with an appropriate cholate concentration which would maintain this linear relationship during the entire dissolution process for a specific strength of tablet. It was found that a 900 mL of sodium cholate solution at 2.67 mg/mL met the requirement for a 750 mg tablet. A calibration curve, with a correlation coefficient range of 0.996–0.998 was obtained by plotting the amount of cholate bound as a function of the specific amount of DMP 504. This binding calibration curve and the amount of bound cholate at different sampling points were used to compute the amount of DMP 504 released from the tablet at various time intervals.

The in-vitro drug release profile for film coated DMP 504 tablets is shown in Fig. 2. DMP 504 was completely released from the tablets within 10 minutes.

A comparison of the release profiles of DMP 504, Questran, and Colestid tablets is also given in Fig. 2. The

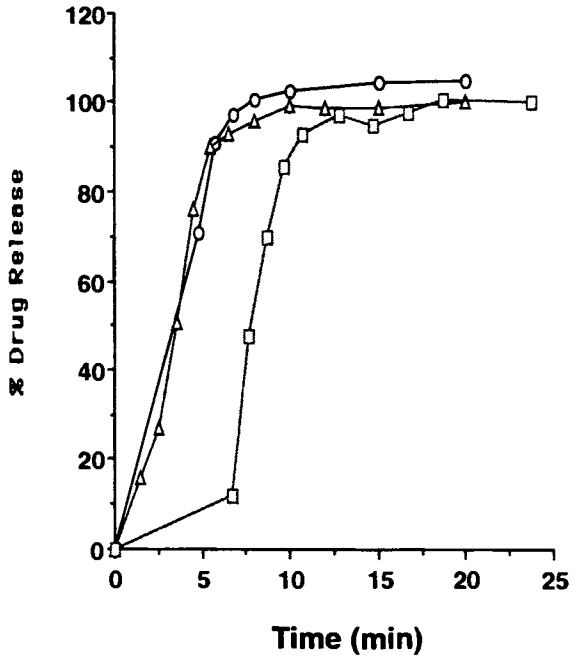


Fig. 2. *In vitro* drug release profiles for DMP 504 (○), questran (Δ), and colestid (□) tablets. The study was conducted at a stirring rate of 100 rpm. Data represent the mean of three experiments.

profiles for the Questran and Colestid tablets were normalized to correct for differences in binding capacity. DMP 504 and Questran tablets exhibited a comparable drug release profile; however, Colestid tablets showed a slower drug release rate.

CONCLUSIONS

Since DMP 504 is a water insoluble polymer, the evaluation of *in vitro* drug release from tablets can not be accomplished using a conventional dissolution method. An unconventional reverse binding technique was developed to examine the drug release from tablets using the USP apparatus 2 at 100 rpm with 900 mL of cholate phosphate buffer solution as a dissolution medium. DMP 504 was completely released from the film coated tablet within 10 minutes using this test method. The drug release rate of Colestid tablet was slower than those of DMP 504 and Questran.

ACKNOWLEDGMENTS

The contributions of Dr. Paul Hovsepian and Dr. Mark Schreiber are greatly appreciated.

REFERENCES

1. G. M. Benson, C. Haynes, S. Blanchard, and D. Ellis. In vitro studies to investigate the reasons for the low potency of cholestyramine and colestipol. *J. Pharm. Sci.* **82**:80–86 (1993).
2. P. E. Luner and G. L. Amidon. Equilibrium and kinetic factors influencing bile sequestrant efficacy. *Pharm. Res.* **9**:670–676 (1992).
3. P. E. Luner and G. L. Amidon. Description and simulation of a multiple mixing tank model to predict the effect of bile sequestrans on bile salt excretion. *J. Pharm. Sci.* **82**:311–318 (1993).
4. P. Gillies, L. Grimminger, G. Figuly, J. Jensen, S. Royce, and E. Shimshick. DMP 504: A hydrogel bile acid sequestrant; part 1 - Equilibrium binding and kinetics. Abstract and presentation in XII International Symposium on *Drugs Affecting Lipid Metabolism*, 139 (1995).
5. J. Billheimer, R. Fischer, S. Germain, G. Figuly, J. Jensen, S. Royce, M. Gorko, J. Hainer, and P. Gillies. DMP 504: A hydrogel bile acid sequestrant; part 2 - Mechanism of action studies in the hamster. Abstract and presentation in XII International Symposium on *Drugs Affecting Lipid Metabolism*, 139 (1995).
6. K. S. Raghavan, R-K. Chang, J. Pang, G. Figuly, and M. A. Hussain. Physical and chemical properties of DMP 504, a polyalkylammonium-based bile acid sequestrant. *Pharm. Dev. Tech.* **2**:233–241 (1997).