Enhancement of 3-Hydroxy-3-methylglutaryl–Coenzyme A (HMG–CoA) Reductase Inhibitor Efficacy Through Administration of a Controlled-Porosity Osmotic Pump Dosage Form

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An extended-release osmotic dosage form was designed for gastrointestinal delivery of the water-soluble tromethamine salt of the β-hydroxyacid form of simvastatin, a potent HMG–CoA reductase inhibitor and cholesterol lowering agent. The cholesterol lowering efficacy and systemic plasma drug levels resulting from peroral administration of this dosage form, relative to a powder-filled capsule oral bolus, were evaluated in dogs. A twofold improvement in cholesterol lowering efficacy was realized with the controlled-release dosage form that was accompanied by a drug AUC and C_{max} that were 67 and 16%, respectively, of those achieved with the bolus dosage form. These results suggest that extended-release dosage forms have the potential for a dose-sparing advantage in the administration of HMG–CoA reductase inhibitors for the treatment of hypercholesterolemia.

KEY WORDS: 3-hydroxy-3-methylglutaryl–CoA reductase (HMG–CoA reductase) inhibitor; simvastatin; extended release; osmotic pump; cholesterol lowering efficacy.

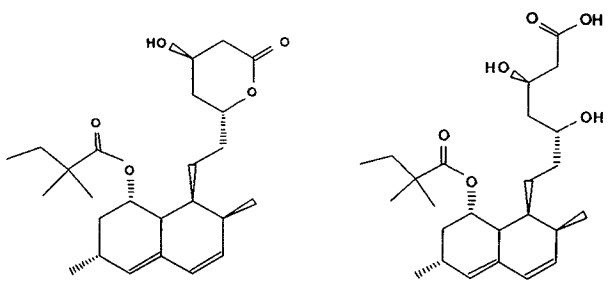
INTRODUCTION

Simvastatin (I) is a lactone that hydrolyzes to the corresponding β-hydroxyacid (II), a potent inhibitor of 3-hydroxy-3-methylglutaryl–Coenzyme A reductase (HMG–CoA reductase) and thus of de novo cholesterol synthesis (1,2) (Scheme I). As the primary site of cholesterol synthesis and regulation, the liver is the target organ for HMG–CoA reductase inhibitors. Vickers *et al.* (3) have shown that the liver extracts the lactone form of simvastatin to a much greater extent than the β-hydroxyacid form. An ideal dosing scheme would provide therapeutic levels of inhibitor to the liver at a rate that results in a hepatic extraction ratio approaching unity, thereby minimizing the systemic HMG–CoA reductase inhibitor levels. In principle, this may be accomplished by a portal drug infusion. Osmotically actuated dosage forms have been extensively investigated (4–6) as peroral drug delivery systems with similar *in vitro/in vivo* performance. Recent advances in osmotic pump technology have extended the utility of these dosage forms (7–9). In the studies reported here osmotic pump devices were designed and fabricated with microporous coats and the *in vitro* release of the water-soluble tromethamine salt of the β-hydroxyacid (i.e., tromethammonium · II) was characterized. A peroral dosing trial in dogs designed to evaluate cholesterol lowering efficacy and systemic plasma drug levels following osmotic pump dosing relative to bolus dosing of the water-soluble ammonium salt of the β-hydroxyacid (i.e., ammonium · II) was completed.

MATERIALS AND METHODS

Simvastatin (I), ammonium · II (Merck, Sharp & Dohme Research Laboratories, West Point, PA), tromethamine free base, mannitol (Aldrich Chemical Company, Milwaukee, WI), polyvinylpyrrolidone (Povidone 29-32K, GAF Corporation, Wayne, NJ), butylated hydroxyanisole (BHA; Eastman Chemical Products Incorporated, Kingsport, TN), and magnesium stearate (Fisher Scientific, Fair Lawn, NJ) were used as received. Cation-exchange resin (Dowex 50 × 8-100, 8% cross-linked styrene–divinylbenzene copolymer, Aldrich Chemical Company, Milwaukee, WI) was sequentially rinsed in a sintered glass funnel with deionized water (3 × 80 ml, 60°C), methanol (3 × 80 ml), deionized water (1000 ml), sodium hydroxide (3 *N*, 3 × 80 ml), deionized water (1000 ml), hydrochloric acid (3 *N*, 5 × 80 ml), and deionized water until the pH of the rinse water eluting out of the funnel equaled the pH of the rinse water added into the funnel. The resin was dried before use. Cellulose acetate 398-30 (CA-398-30) and cellulose acetate 320S (CA-320S) (Eastman Chemical Products, Kingsport, TN), sorbitol (Aldrich Chemical Company, Milwaukee, WI), and polyethylene glycol 400 (PEG 400, Sigma Chemical Company, St. Louis, MO) were used as received to form the coats of controlled porosity. All other reagents were reagent grade and used without further purification.

Tromethammonium · II was obtained by hydrolysis of I. Compound I (25 g, 0.06 mol) was dissolved in methanol



Compound I Compound II

Scheme I

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(150 ml). A solution of sodium hydroxide (3 g, 0.075 mol) in 20 ml water was then added to hydrolyze the lactone ring. After 5 min the solution (light orange color) was neutralized by the slow addition of phosphoric acid to a final pH of 5. The resultant II was extracted into ethyl acetate (2 × 300 ml) and dried over sodium sulfate, and the bulk solvent was evaporated. The residual oil was dried *in vacuo* (16 hr) to remove the remaining solvent. The resulting solid was accurately weighed, then dissolved in methanol (100 ml), and 1 equivalent of tromethamine free base added. Upon complete dissolution of the tromethamine, methanol was removed under vacuum (rotary evaporator, 60°C), and the oil obtained dissolved in acetone (100 ml). Crystallization was observed upon storage (25°C, 16 hr). Three crops of crystals were successively isolated (suction filtration) and dried (25°C, *in vacuo,* 18 hr) to give a 76% yield of tromethammonium · II. The X-ray powder diffraction pattern verified the isolate was crystalline. Elemental analysis was consistent with the tromethammonium · II monohydrate. FTIR and NMR spectra were consistent with the structure.

Core tablets were prepared from an aqueous granulation of tromethammonium · II, tromethamine free base, mannitol, Dowex 50 × 8-100, Povidone 29-32K, and BHA. The granulation was dried (40°C, 24 hr) and sized (No. 18 U.S. Standard Sieve). The granulation was lubed with magnesium stearate and compressed into core tablets with a ⅜-in. round standard concave die. A controlled porosity wall was applied to these core tablets by fluidized-bed (Uni-Glatt, Glatt Air Techniques, Ramsey, NJ) spray-coating techniques. The coating solution was CA-398-30, CA-320S, sorbitol, and PEG 400 dissolved in a water:methanol:methylene chloride (1:10:15, by parts) solvent blend. The core and coat compositions are summarized in Table I. The *in vitro* release (USP paddle dissolution Method 2, 50 rpm, 37°C, VanKel Industries, Edison, NJ) of tromethammonium · II from these devices was followed by two procedures. In the first procedure (Procedure A) devices were added to isoosmotic HCl buffer (900 ml, pH 1.2) for 4 hr, then transferred into isoosmotic phosphate buffer (900 ml, pH 8.0, 0.07 *M* phosphate) for the remainder of the release experiment. In the second procedure (Procedure B) devices were added to the isoosmotic phosphate buffer for the entire release experiment. At each sampling time the devices were transferred into fresh buffer (900 ml) and sodium dodecyl sulfate (SDS; 0.6%, w/w) was dissolved in the previous medium to assure solubilization of the released drug. Samples taken with and without the added SDS were compared. In all cases mass balance recovery of drug was obtained with SDS. An ~90% recovery was observed in samples that did not contain SDS.

Table I. Osmotic Pump/Tromethammonium · II Formulation Composition

Granulation component	mg/core tablet	Coat component[a]	Parts
Tromethammonium · II	25.4[b]	CA-398-30	1.00
		CA-320S	0.33
Mannitol	100	Sorbitol	0.96
Tromethamine free base	105	PEG 400	0.27
Dowex 50 × 8	45		
Povidone 29-32K	25		
Mg stearate	1.5		
BHA	0.06		

[a] 350-μm coat applied.
[b] Equivalent to 20 mg ammonium · II.

Compounds I and II were assayed by HPLC (Shimadzu Corporation, Kyoto, Japan). An acidified (0.75 ml of 70% phosphoric acid/liter of mobile phase) methanol:water (3:1, by volume) mobile phase was pumped at a rate of 1.5 ml/min through a C_8 column (25 cm, RP-8 Spheri-5, Brownlee Labs Inc., Santa Clara, CA). Peaks were detected and quantitated by UV absorbance at 238 nm. A linear detector response ($r^2 > 0.99$) with zero intercept was observed over the concentration range of interest (1–40 mg/L).

In vivo studies were performed in a crossover study in beagle dogs to determine the extent of serum cholesterol reduction associated with oral administration of tromethammonium · II released from an osmotic pump compared to an oral bolus (dry-filled capsules) of ammonium · II. Seven beagle dogs (three females and four males) were maintained on a fixed and recorded diet. In all cases the dogs were fed prior to blood sampling and dosage form administration. The diet was initiated 1 week prior to baseline blood sampling. Blood was sampled on the second and fifth days of each week throughout the trial. Blood samples were collected for 3 weeks prior to the initiation of dosing to establish untreated baseline cholesterol levels. Oral bolus dosing (100 mg/day of ammonium · II for 28 days) was initiated first to confirm that the specific animals selected were responsive to the drug before administering the drug in the controlled-release osmotic pump dosage form. Upon termination of dosing blood sampling was continued to confirm return to baseline cholesterol levels as plasma levels of the HMG–CoA reductase inhibitor returned to zero. Once return to baseline levels was demonstrated, oral dosing of tromethammonium · II in the osmotic pump configuration was initiated. Each dog received five devices per day as a single dose for 28 days, to realize a total dose of tromethammonium · II equivalent to 100 mg ammonium · II per day. All serum cholesterol assays were performed by a contract laboratory (Lawrence Memorial Hospital, Lawrence, KS) utilizing a cholesterol esterase procedure standardized against the Abell–Kendal reference method (10). On day 16 (steady state) of both dosing regimes blood samples were taken at 0.5, 1, 2, 3, 4, 6, and 24 hr and analyzed by an enzyme inhibition assay (1,11) for the total amount of HMG–CoA reductase inhibitors (including metabolites) present in plasma. From this data C_{max} and AUC pharmacokinetic parameters were calculated.

RESULTS AND DISCUSSION

Compound I has a water solubility of 0.03 mg/ml in water at room temperature. This low solubility would preclude effective delivery from an osmotic pump dosage form. However, tromethammonium · II has a water solubility of >40 mg/ml in water at room temperature and becomes a feasible candidate for osmotic pump delivery. The *in vitro* release profiles of tromethammonium · II from the osmotic pump formulation (Table I) are shown in Fig. 1. Release profiles

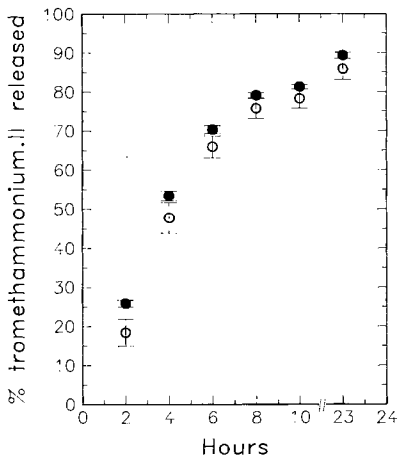

Fig. 1. *In vitro* release profiles from tromethammonium · II/osmotic pump devices as determined by Procedure A (○; n = 6) and Procedure B (●; n = 6). Standard deviations are included.

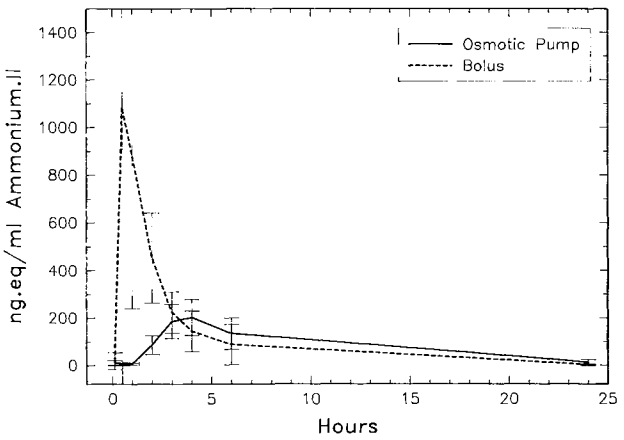

Fig. 2. Mean plasma concentration time profiles (±SD; n = 7) following administration of bolus or osmotic pump dosage forms.

generated by Procedure A, designed to simulate the extremes of the variable pH conditions within the gastrointestinal tract, and Procedure B (constant pH conditions) were very similar. Any conversion of the water-soluble tromethammonium · II into the water-insoluble, and hence poorly releasable, lactone at low pH was insufficient to alter substantially the observed release profiles. The devices released ~60% of the drug load *in vitro* with zero-order kinetics over a 6-hr period. Within 10 hr, 75–80% of the drug was released, with the remaining drug released at a substantially slower rate. After 24 hr, ~90% of the drug load had been released.

The *in vivo* cholesterol lowering efficacy results of the osmotic pump compared to the bolus dosage form have been summarized in Table II. The osmotic pump dosing regime was twice as effective (statistically significant; one-way ANOVA with post hoc Tukey W comparison; $P < 0.05$) at lowering cholesterol levels as the bolus dosing regime at an equivalent total daily dose. The mean total plasma HMG–CoA reductase inhibitor concentration time profiles (blood samples collected on day 16 of the respective dosing regimes) are shown in Fig. 2 and the relevant pharmacokinetic parameters are given in Table III. Area under the HMG–CoA reductase inhibitor · time curve (AUC) values were calculated using the trapezoidal rule to the last measurable inhibitor concentration. The observed mean AUC after the osmotic pump dosing was 67% that of the bolus (one-way ANOVA with post hoc Tukey W comparison; $P < 0.10$). The mean C_{max} after the osmotic pump dosing was 16% that of the bolus (one-way ANOVA with post hoc Tukey W comparison; $P < 0.05$). In the present studies, C_{max} and AUC represent measures of systemic drug burden that have been substantially reduced through administration of the controlled release osmotic pump dosage form. Combining the twofold improvement in efficacy with the lowered systemic drug burden leads to a 3- to 12-fold therapeutic advantage from the controlled-release dosage form. The increased therapeutic efficacy from the extended release osmotic pump relative to the bolus at the same daily dose suggests that a dose-sparing advantage may be possible through the controlled release of HMG–CoA reductase inhibitors.

Table II. Maximum Reduction of Serum Cholesterol in Dogs Following 28 Days' Administration of Ammonium · II[a]/Boluses or Tromethammonium · II[b]/Osmotic Pumps

Dog No.	Baseline cholesterol (mg/100 ml)	Maximum percentage decrease in serum cholesterol	
		Bolus	Osmotic pumps
02	186	9	36
03	200	21	31
37	218	24	44
66	203	10	25
81	160	23	43
84	185	18	23
85	192	18	37
Mean ± SD	192 ± 18	17 ± 6	34 ± 8

[a] Dose, 100 mg/day.
[b] Dose, equivalent of 100 mg/day of ammonium · II.

Table III. Pharmacokinetic Summary[a]

Dog No.	C_{max} (ng · eq/ml)		AUC (ng · eq · hr/ml)	
	Bolus	Osmotic pump	Bolus	Osmotic pump
01	571	203	3520	2450
03	1950	213	4060	2430
37	249	274	3340	3180
66	566	127	1910	990
81	652	113	1690	1140
84	990	187	2100	1130
85	3940	319	4450	2930
Mean[b]	1270	205	3010	2035
SD	1300	74	1105	926

[a] Sixteenth dosing day.
[b] Geometric mean.

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