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New Microporous Cholestyramine Analog for Treatment of Hypercholesterolemia

RENATO DE SIMONE *, FRANCO CONTI ‡, M. ROSA LOVATI ‡, MARINA SIRTORI ‡, ENZO COCUZZA *, and CESARE R. SIRTORI ‡x

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Abstract □ A new, microporous, uniformly reticulated preparation of cholestyramine is described. The preparation, cholpor, has a higher exchange capacity for chloride than does cholestyramine and swells very little in water. It is 15–20% more potent than cholestyramine in the *in vitro* binding of sodium cholate; moreover, the binding velocity is considerably higher than that of cholestyramine. Colestipol hydrochloride, also used as a reference anion-exchange resin, is about half as potent as the other two resins; its binding velocity is similar to that of cholpor. Cholpor may be prepared in a suspension form of good palatability. Preliminary clinical findings in short-term trials showed a cholesterol-lowering effect similar to that of cholestyramine with lower doses and fewer side effects.

Keyphrases □ Cholestyramine analog—cholpor synthesized, *in vitro* binding of sodium cholate and cholesterol lowering effect in humans evaluated □ Cholpor—cholestyramine analog synthesized, *in vitro* binding of sodium cholate and cholesterol lowering effect in humans evaluated □ Antihypercholesterolemic activity—cholestyramine analog synthesized, *in vitro* binding of sodium cholate and cholesterol lowering effect in humans evaluated

Lowering of plasma cholesterol appears to be a major goal in the prevention of atherosclerosis (1). Anion-exchange resins are the most effective pharmaceutical agents in the clinical management of hypercholesterolemia (2); cholestyramine[1], a styrene-divinylbenzene copolymer with a free quaternary ammonium group, is currently the most widely used agent. Although the clinical efficacy of cholestyramine is not disputed, its unpleasant taste as well as the difficulty encountered by patients in mixing it with water upon each administration makes continued treatments rather difficult (3). The newer anion-exchange resin, colestipol hydrochloride[2] (4), is better tolerated but also requires several daily extemporary preparations.

This report describes a new microporous analog of cholestyramine, cholpor, which may be prepared in suspension form, is of good palatability, and is easily accessible to the patient.

EXPERIMENTAL

Analytical Methods—Swelling of the resins in water was determined by adding 1 g of cholestyramine[1], colestipol hydrochloride[2], or cholpor to enough water (10–30 ml) to provide a suspension. After 24 hr, the suspension was shaken and the volume of resin under water was determined.

Exchange Capacity—Chloride-ion absorption from a 5% NaCl solution, after regeneration of the resin with a 4% NaOH[3] solution, was measured by titrating with 0.1 *N* HCl[3] the sodium hydroxide resulting from the resin and sodium chloride reaction.

Pharmacology—Binding of sodium cholate[4] *in vitro* was estimated essentially according to Whiteside *et al.* (5). Preparations of cholestyramine (100–200 mesh), colestipol hydrochloride (60–150 mesh), and cholpor (60–80 mesh) were added to scintillation vials[5] containing 10 ml of 0.02 *M* phosphate buffer with 2 mg of sodium cholate/ml.

The significance of the differences in the various experiments was determined by the Student *t* test.

RESULTS AND DISCUSSION

Chemistry—Cholpor (6) is obtained by starting with a porous matrix. Porosity is first achieved during preparation of the copolymer. During chloromethylation, porosity is protected by maintaining reaction conditions that do not alter the physical structure of the matrix (low reaction temperature, low acidity of the catalysts, and marked swelling of the polymer chains with appropriate solvents).

Before polymerization, a mixture of compatible organic substances, chemically inert, is added to the monomers. During polymerization, this "porogenic" mixture regulates the molecular weights of the growing monomeric chains by causing precipitation when the chain length exceeds solubility. The copolymer precipitates in the form of microspheres, which, in the presence of the last nonreacted monomers, stably bind to each other. This copolymer is thus composed of numerous microspheres, and the empty space between one microsphere and another constitutes porosity.

Another function of the porogenic mixture is to avoid cross-linking of the macromolecules, allowing a uniform reticulation. Cross-linking inside the porous cavities would alter them, so that the specific properties of the resin, *i.e.*, velocity of diffusion, particularly important in the macroanion exchange, would be lost.

To achieve porosity in cholpor, several methods were tried (6). One method used squalane[3]–1-octanol[3]–paraffin[3] (mp 42–45°) (70:20:10), with the ratio of monomers to porogens being 1.2:1.1. The porogenic mixture, following copolymerization, was removed by prolonged extraction in a soxhlet extractor with a swelling solvent mixture (ether[3]–methylene chloride[3]). Proof of the completed extraction was provided by the final measurement of the extracted porogenic mixture. The general charac-

[1] Cuemid, courtesy of Merck Sharp & Dohme, Rahway, N.J.
[2] Colestid, courtesy of The Upjohn Co., Kalamazoo, Mich.
[3] Carlo Erba, Milan, Italy.
[4] Merck, Darmstadt, West Germany.
[5] Packard Instruments, Downers Grove, Ill.

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Table I—Characteristics of One Preparation of Cholpor Compared with Cholestyramine and Colestipol Hydrochloride

	Cholpor	Cholestyramine	Colestipol Hydrochloride
Copolymer type	Styrene	Styrene	Epoxide
Monomer	Styrene-divinylbenzene	Styrene-divinylbenzene	Diethylenetriamine-epichlorohydrin
Reticulation[a], % wt.	11.3	2	Not significant
Exchange capacity for chloride, mEq/g	4.5	2.9–3.1	4.5
Mean diameter of pores[b], Å	~190	0	0
Specific surface[c], m^2/g	82	<1	<1
Real density[d], g/cm^3	1.11	1.09	1.07
Apparent density[e], g/cm^3	0.50	0.44	0.60
Porosity[f], cm^3/g	0.6	0	0
Granulometry, μ	250–177	150–80	250–100

[a] Percentage in weight of the divinylbenzene monomer in the total mixture of monomers. [b] Reference 7. [c] Reference 8. [d] Determined with a helium–air comparison pycnometer, model 930, Beckman Instruments, Palo Alto, Calif. [e] Determined with an SM3 dilatometer (3-mm capillary). [f] Difference between the reciprocals of apparent density and real density.

teristics of this preparation, as well as those of cholestyramine and colestipol hydrochloride, are reported in Table I.

The exchange capacity for chloride of different preparations of cholpor ranged between 3.9 and 5.7 mEq/g; this capacity exceeded that found with several preparations of cholestyramine, *i.e.*, 2.9–3.1 mEq/g. The exchange capacity of colestipol hydrochloride was similar to that of cholpor, *i.e.*, 4.5 mEq/g. Swelling of cholpor in water was about one-fifth that found with cholestyramine and about one-half that of colestipol hydrochloride (Fig. 1).

Pharmacology—In the first experiment (Fig. 2), a fixed amount of sodium cholate (2 mg/ml) and a fixed amount of each resin (30 mg) were incubated; the pH of the buffer solution was varied from 5 to 8. In the second experiment (Fig. 3), increasing amounts of each resin were added to the sodium cholate solution (pH 6). In the third experiment (Fig. 4), the percentage of binding was measured at successive time intervals after addition of 30 mg of each resin to the sodium cholate solution (pH 6).

Incubations always were carried out at 25° in a Dubnoff-type bath[6]. Unbound cholate was determined by filtering the content of each vial on paper[7] and measuring cholate spectrophotometrically[8] according to Kier (9).

Binding of sodium cholate *in vitro* was significantly higher for cholpor, as compared to cholestyramine and colestipol hydrochloride, in all experiments (Figs. 2–4). Colestipol had a binding capacity of about one-half that of the other two resins, thus confirming previous data (10), whereas cholpor was about 15–20% more potent than cholestyramine. The binding capacity of all three resins was scarcely modified by the pH of the buffer solution (Fig. 2).

Cholestyramine and cholpor differed in the velocity of binding *in vitro*. Whereas cholpor reached 80% of its maximal binding capacity after just 2 min and 100% after 10 min, the maximal binding for cholestyramine was found after 20 min of incubation (Fig. 4). Colestipol hydrochloride had a binding velocity similar to that of cholpor.

Pilot Clinical Study—After the chronic toxicity tests in animals, a 3-week clinical trial in patients was designed to evaluate both the hypocholesterolemic activity and the general acceptance of cholpor. Twenty patients with type IIA hyperlipoproteinemia (11) were selected (five females and 15 males, ages 37–72 years). All had been following a therapeutic diet, low in cholesterol and saturated fats (12), for at least 3 months and had stable weights. Eight out of the 20 patients had been treated previously with cholestyramine but had discontinued because of side effects.

Cholpor was made available in plastic ampuls containing 3 g of resin suspended in a solution of sorbitol[3] (3.4 g), yellow orange[9] (E 102 and E 124) (2.0 mg), and *p*-hydroxybenzoic acid esters[10] (14 mg) in demineralized water to a final volume of 17 ml. The drug was given immediately before meals. All patients were started with 1 ampul/day. As suggested by Farah *et al.* (13), plasma cholesterol (14) and triglycerides (15) were estimated twice weekly and eventual dose increases were decided at the end of each week; the dose was not increased if plasma cholesterol levels had dropped more than 20%. One patient, with severe familial hypercholesterolemia, was treated in a metabolic ward; the dose was initially 9 g/day and gradually was increased to 15 g/day.

This short-term clinical trial indicated an excellent palatability of the resin. Only two patients dropped out of the study, one (5%) because of

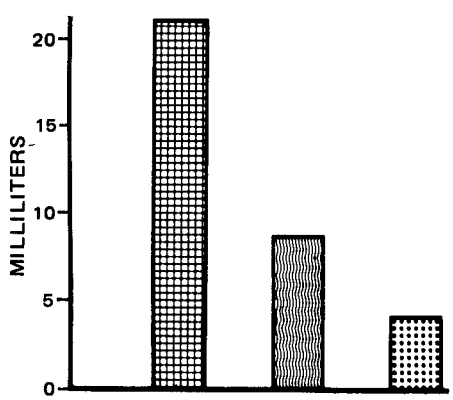

Figure 1—*Swelling of cholestyramine (left), colestipol hydrochloride (middle), and cholpor (right) after 24 hr in water. One gram of each resin was placed in an appropriate amount of water, and the volume was determined after 24 hr. Each column gives the mean volume (milliliters of water) of three different lots of each resin.*

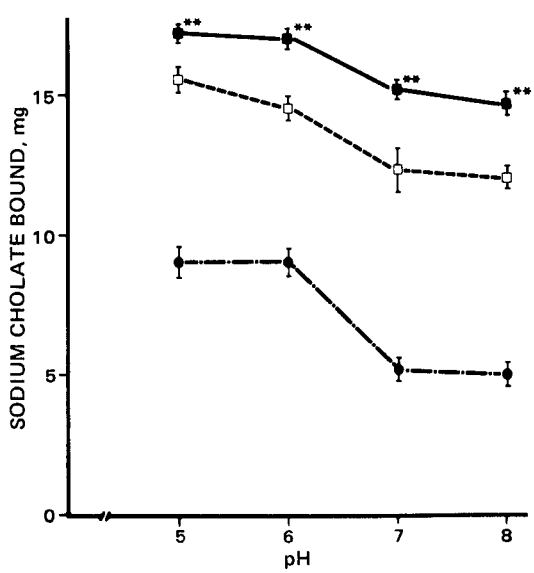

Figure 2—*Milligrams of sodium cholate bound by each resin after 30 min (2 mg of cholate/ml in 10 ml of 0.02 M phosphate buffer with 30 mg of each resin). The pH of the solution was appropriately changed before incubation. Each point gives the mean ± SD from six determinations. Key: ■, cholpor; □, cholestyramine; ●, colestipol hydrochloride; ★, $p < 0.05$; and ★★, $p < 0.01$ as compared to cholestyramine.*

[6] Bagno Valentini, Milan, Italy.
[7] Whatman type 42 ashless filter papers.
[8] Gilford model 2400 spectrophotometer, Gilford Instrument Laboratories, Oberlin, Ohio.

[9] Curt Georgi, Milan, Italy.
[10] Paracombin, Formenti Boots, Milan, Italy.

Table II—Results of a 3-Week Study with Cholpor in Hypercholesterolemic Patients

Final Daily Dose, g	Number of Patients	Age Range, years	Pretreatment Lipid Levels[a], mg/dl		Posttreatment[a], mg/dl	
			Plasma Cholesterol[b]	Triglycerides[b]	Cholesterol	Triglycerides
6	4	37–68	265.7 (7.1)	129.2 (27.2)	196.5 (16.3)[c]	135.0 (20.3)
9	13	37–72	317.4 (17.3)	145.7 (14.0)	240.6 (17.7)[d]	148.0 (14.0)
15	1	49	480	130	242	190

[a] Mean (*SEM*). [b] The mean of three pretreatment determinations was used for each patient. [c] $p < 0.01$ *versus* pretreatment. [d] $p < 0.001$ *versus* pretreatment.

constipation (she had the same symptom with cholestyramine previously) and the other for family reasons. A transient, very mild constipation was noted in three more patients. The final results (Table II) show that 6 g of resin/day was sufficient in four cases to achieve a cholesterol reduction of more than 20%. A dose of 9 g/day (given in two or three daily administrations, according to the patient's convenience) was adequate in 13 cases. The case treated in the hospital had a normalization of serum cholesterol levels with 15 g/day. Many of the patients have continued treatment for longer periods, generally maintaining the results achieved in this short trial.

Cholpor was prepared with the objective of eliminating some major problems of the available cholestyramine preparations, *i.e.*, bad taste and smell, relatively low activity, and constipating effect. Bad taste, mostly due to the release of trimethylamine groups, is reduced upon hydration, when the concentration of active groups is lower than in the dry state, and may be eliminated by the addition of a binding agent (16). The activity of cholestyramine, on the other hand, may be improved if all functional groups are readily accessible. A microporous, very uniformly reticulated resin, by making functional groups wholly available, may increase the velocity of the reaction.

Constipation is also probably a consequence of the relatively low activity of cholestyramine; the high doses employed in therapy require, in fact, large volumes of liquid (4 g corresponds to a final volume of approximately 85 ml). Probably the bulky masses of hydrated resin in the intestine, following the normal draining of liquid, leave a solid residue that the intestinal contractions do not easily eliminate.

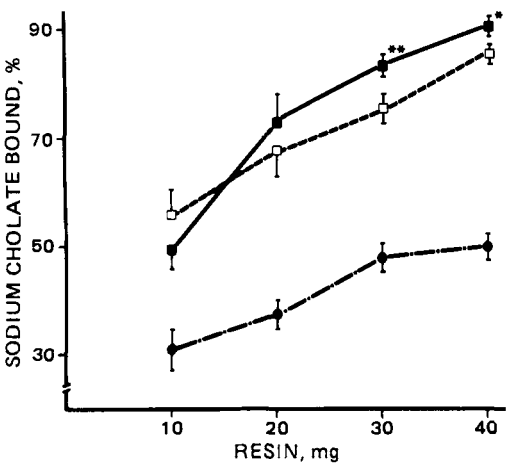

Figure 3—*Percentage of binding of sodium cholate (2 mg/ml in 10 ml of 0.02 M phosphate buffer, pH 6) after 30 min of incubation in the presence of increasing amounts of each resin (means ± SD of six determinations). Key: same as Fig. 2.*

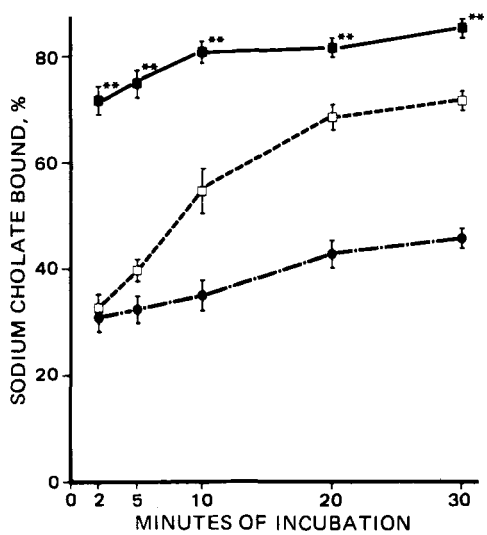

Figure 4—*Percentage of binding of sodium cholate (conditions similar to Fig. 3) in the presence of 30 mg of each resin after increasing intervals of incubation (means ± SD of six determinations). Key: same as Fig. 2.*

CONCLUSIONS

Results obtained with cholpor confirm that a porous, uniformly reticulated preparation of cholestyramine may be better suited for clinical use in hypercholesterolemia than the parent preparation. Cholpor has a higher exchange capacity for chloride than cholestyramine and a remarkably higher *in vitro* capacity for bile acids; it also binds bile acids *in vitro* significantly more rapidly than does cholestyramine. Moreover, possibly because of its uniform reticulation, cholpor swells very little in water, allowing the preparation of suspensions for clinical use.

The reported short-term clinical trial showed that relatively low doses of cholpor may be sufficient for the treatment of the most frequent forms of hypercholesterolemia. With these dosages, constipation is seldom encountered. Doses in the general range of cholestyramine may be necessary for the more severe forms. Longer studies will be needed to confirm both the efficacy and safety of cholpor.

Cholpor may provide a solution to some of the biggest obstacles in a wide-scale administration of anion-exchange resins for hypercholesterolemia. Its satisfactory palatability, ready availability in suspension form, and low constipating effect, in addition to the rapid *in vitro* activity which may allow administration immediately before meals, are possibly relevant for its use even for relatively mild forms of hypercholesterolemia where physicians may find cholestyramine powder too demanding for the patient.

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Crystal and Molecular Structure of Quinidine

RUTH DOHERTY *, WALTER R. BENSON ‡x, MILLARD MAIENTHAL ‡, and JAMES McD. STEWART *

Received January 25, 1978, from the **Chemistry Department and Computer Science Center, University of Maryland, College Park, MD 20742,* and the ‡*Division of Drug Chemistry, Food and Drug Administration, Washington, DC 20204.* Accepted for publication April 27, 1978.

Abstract □ The structure of the free base quinidine was determined by single crystal X-ray diffraction. Quinidine crystallizes from absolute ethanol as the ethanolate, with the molecular formula $C_{20}H_{24}N_2O_2 \cdot C_2H_6O$ and molar mass 370.491 units. It crystallizes in the orthorhombic space group $P2_12_12_1$ with unit cell dimensions a = 1321.1(3), b = 989.3(2), and c = 1651.5(3) pm. The measured density was 1.15 g/cm^3; the density calculated for $Z = 4$ was 1.164 g/cm^3. The diffraction data were collected by using MoKα radiation. A final R value of 0.055 was obtained. Evidence for intermolecular hydrogen bonding was found. The crystal analysis is in agreement with the structure proposed by other methods. The absolute configuration is based on the published structure of 10-bromo-10,11-dihydroepiquinidine.

Keyphrases □ Quinidine—crystal and molecular structure, X-ray diffraction determination □ X-ray diffraction—determination, crystal and molecular structure of quinidine □ Cardiac depressants—quinidine, crystal and molecular structure determined by X-ray diffraction

The drug substance quinidine (I) contains several naturally occurring impurities (1). Commercial samples of I contain 3–22.1% of 10,11-dihydroquinidine (II). Compounds I and II are both potent antiarrhythmic agents, II being significantly more effective than I (2). Although structures for I and II have been determined by other techniques (3–7), no absolute configuration studies by single crystal X-ray diffraction have been reported. Because of the therapeutic significance of I and II, X-ray diffraction studies were undertaken.

One crystal study (8) was reported for 10-bromo-10,11-dihydroepiquinidine (III), a derivative of 10,11-dihydroepiquinidine (IV). Compounds II and IV differ only in the configuration about the carbon bearing the hydroxyl group. In the study of III, evidence was found for intramolecular hydrogen bonding between the hydroxyl group and the nitrogen in the quinuclidine ring. From models of II and IV, it was concluded that intramolecular hydrogen bonding in II and I is less favorable than in III and IV because of steric interactions of the quinoline and

Table I—Crystal Structure Analysis of Quinidine Ethanolate ($C_{20}H_{24}N_2O_2 \cdot C_2H_6O$)

Property	Analysis
Unit cell dimensions	a = 1321.1(3), b = 989.3(2), c = 1651.5(3) pm
Space group	$P2_12_12_1$
Density, g/cm^3	1.15 (measured), 1.164 ($Z = 4$) (calculated)
Radiation	MoKα
Number of observations	Total number of reflections = 1404, number of reflections less than 3σ above background = 586
Scattering factors	C,N,O (Ref. 11); H (Ref. 12)
Method of solution	Direct methods for partial structure, translation function, Fourier transform to complete
Method of refinement	Full-matrix least-squares
Weights	Unit
Programs used	XRAY76
Final R value ($\Sigma \Delta F/\Sigma\|F_0\|$)	0.055
Final difference synthesis	No significant peaks
Atomic coordinates	See Table II
Bond lengths	See Fig. 1
Bond angles	See Fig. 2
Hydrogen bonds between	O-12–H-12- - -N-1 O-9–H-91- - -N-1′

Distance from	Distance, pm
O-12–N-1	281(1)
H-12–N-1	160(8)
O-9–N-1′	279(1)
H-91–N-1′	243(8)

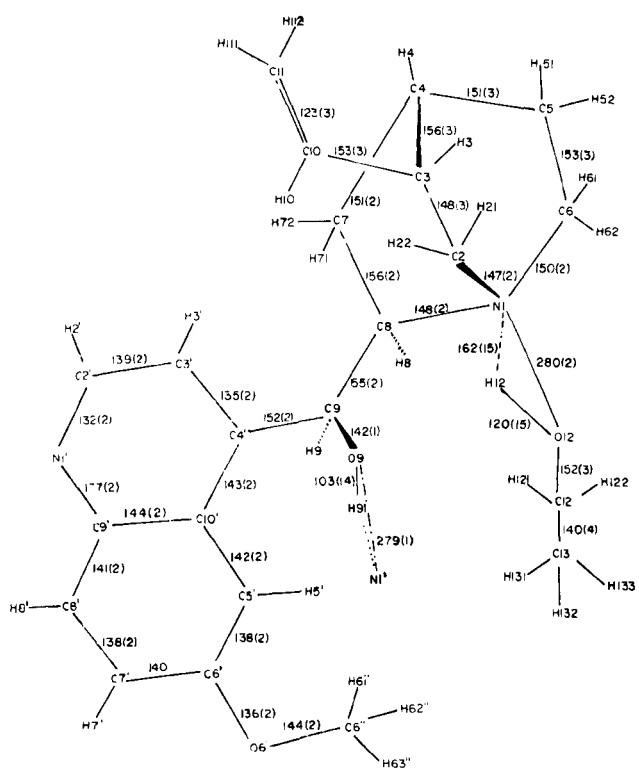

Figure 1—*Bond lengths (pm) for quinidine ethanolate.*

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