METHOD FOR TREATING HYPERCHOLESTEROLEMIA WITH UNSUBSTITUTED POLYDIALLYLAMINE

BACKGROUND OF THE INVENTION

Reabsorption of bile acids from the intestine conserves lipoprotein cholesterol in the bloodstream. Conversely, blood cholesterol levels can be diminished by reducing reabsorption of bile acids.

One method of reducing the amount of bile acids that are reabsorbed and, thus, reducing serum cholesterol is the oral administration of compounds that sequester the bile acids and cannot themselves be absorbed. The sequestered bile acids are excreted.

Compounds which have been suggested for bile acid sequestration include various ion exchange polymers. One such polymer is cholestyramine, a copolymer of divinylbenzene and trimethylammoniummethyl styrene. It has been long recognized that this polymer is unpalatable, gritty, and constipating. More recently, various polymers have been suggested which are characterized by hydrophobic substituents and quaternary ammonium radicals substituted upon an amine polymer backbone (Ahlers, *et al.* U.S. Patent 5,428,112 and 5,430,110 and McTaggart, *et al.*, U.S. Patent 5,462,730, which are incorporated herein by reference). In some cases, these polymers have had disappointing efficacy and require complex processes for their manufacture.

Thus, there is still a need to discover superior bile acid sequestrants.

SUMMARY OF THE INVENTION

The invention relates to the unexpected discovery that a new class of ion exchange resins have improved bile salt sequestration properties. The polymers, or resins, employed in the invention comprise non-absorbable, and optionally cross-linked polydiallylamines. The polydiallylamines of the invention are characterized by one or more monomeric units of the formulae:

remove a significant quantity of bile acids from the patient and, thus, to lower the serum cholesterol level of the patient. The patient can be an animal, for example, a mammal, or a human.

As described above, the polymers employed in the invention comprise non-absorbable, optionally cross-linked polydiallylamines characterized by the formula above. Importantly, the polymers can be characterized by the substantial absence of substituted or unsubstituted alkyl substituents on the amino group of the monomer, such as obtained in the alkylation of an amine polymer. That is, the polymer can be characterized in that the polymer is substantially free of alkylated amine monomers.

The polymer can be a homopolymer or a copolymer. Where copolymers are manufactured with a diallylamine monomer, the comonomers are preferably inert, non-toxic and/or possess bile acid sequestration properties. Suitable examples of additional comonomers include substituted and unsubstituted acrylate, substituted and unsubstituted acrylamide, substituted and unsubstituted methacrylate, substituted and unsubstituted methacrylamide, allylamine, triallylamine, allyl alcohol, substituted and unsubstituted vinyl amine and substituted and unsubstituted vinyl alcohol. In one embodiment, the additional monomer is sulfur dioxide. Preferably, the monomers are aliphatic. Most preferably, the polymer is a homopolymer, i.e. a homopolydiallylamine.

Preferably, the polymer is rendered water-insoluble by branching and/or crosslinking. The cross-linking agent can be characterized by functional groups which react with the amino group of the monomer. Alternatively, the crosslinking group can be characterized by two or more vinyl groups which undergo free radical polymerization with the amine monomer. Suitable multifunctional co-monomers include triallylamine, tetraallyleammonium salts, bis(diallylamine)s (such as alkylene bis(diallylamine)s), diacrylates, triacrylates and tetraacrylates, dimethacrylates, diacrylamides, diallylacrylamide and di(methacrylamides). Specific examples include ethylene bis(diallylamine), hexamethylene bis(diallylamine), ethylene glycol diacrylate, propylene glycol diacrylate, butylene glycol diacrylate, ethylene glycol dimethacrylate, butylene glycol dimethacrylate, methylene bis(methacrylamide), ethylene bis(acrylamide), ethylene bis(methacrylamide), ethylidene bis(acrylamide), ethylidene bis(methacrylamide),

polymers have molecular weights which range from about 2,000 Daltons to about 500,000 Daltons, preferably from about 5,000 Daltons to about 150,000 Daltons. Crosslinked polymers, however, are not generally characterized by molecular weight. The crosslinked polymers discussed herein should be sufficiently crosslinked to resist adsorption from the gastrointestinal tract.

As described above the polymer can be administered in the form of a salt, or as a partial salt. By "salt" it is meant that the nitrogen groups in all or some of the repeat units are protonated to create a positively charged nitrogen atom associated with a negatively charged counterion.

The anionic counterions can be selected to minimize adverse effects on the patient, as is more particularly described below. Examples of suitable counterions include Cl^-, Br^-, $CH_3OSO_3^-$, HSO_4^-, SO_4^{2-}, nitrate, HCO_3^-,CO_3^{2-}-acetate, lactate, phosphate, hydrophosphate, methanesulfonate, fumarate, malate, pyruvate, malonate, benzoate, glucuronate, oxalate, acetylglycinate, succinate, propionate, butyrate, ascorbate, citrate, tartrate, maleate, folate, an amino acid derivative, a nucleotide, a lipid, or a phospholipid. The counterions can be the same as, or different from, each other. For example, the reaction product can contain two different types of counterions.

The polymers according to the invention can be administered orally to a patient in a dosage of about 1 mg/kg/day to about 10 g/kg/day, preferably between about 1 mg/kg/day to about 200 mg/kg/day; the particular dosage will depend on the individual patient (e.g., the patient's weight and the extent of bile salt removal required). The polymer can be administered either in hydrated or dehydrated form, and can be flavored or added to a food or drink, if desired, to enhance patient acceptability. Additional ingredients such as other bile acid sequestrants, drugs for treating hypercholesterolemia, atherosclerosis or other related indications, or inert ingredients, such as artificial coloring agents can be added as well.

Examples of suitable forms for administration include pills, tablets, capsules, and powders (e.g., for sprinkling on food). The pill, tablet, capsule, or powder can be coated with a substance capable of protecting the composition from disintegration in the esophagus but will allow disintegration of the composition in the stomach and mixing with food to pass into the patient's small intestine. The polymer can be

mesyl, acyl or glycidyl group. Examples of such compounds include epichlorohydrin, succinyl dichloride, butanedioldiglycidyl ether, ethanedioldiglycidyl ether, pyromellitic dianhydride and dihaloalkanes. The crosslinking agent can also be an α,ω-alkylene diisocyanate, for example $OCN(CH_2)_pNCO$, wherein p is an integer from about 2 to about 20.

The polymer can also be crosslinked using a crosslinking agent which incorporates one functional group which incorporates into the polymerizing chain and a second functional group which can react with amine groups in a second polymer chain. Examples include glycidyl methacrylate, glycidyl acrylate, acryloyl chloride, methacryloyl chloride, 3-bromopropylacrylate, 3-bromopropylmethyldiallylammonium chloride, and 3-chloropropyldiallylamine.

The invention will now be described more specifically by the examples.

Example 1 - Preparation of Poly(diallylammonium chloride)

Concentrated hydrochloric acid (507.0 g; 37%) was charged to a 5L, 3-neck round bottomed flask and agitated with a mechanical stirrer. The flask was cooled to <5°C with an ice bath. Diallylamine (635.0 ml) was added dropwise to the stirring hydrochloric acid over a three hour period using an addition funnel capped with a pierced rubber septum. The stirring solution temperature was maintained at <10°C. After the addition was completed, the ice bath was removed and the mixture was allowed to warm to room temperature. Concentrated hydrochloric acid (7.3 g) was added to the solution. Water (368.7 g) was added to the solution and it was allowed to sit overnight.

The stirring solution was purged with nitrogen gas for 30 minutes at room temperature. 2,2'-Azobis[2-amidinopropane]dihydrochloride (6.87 g) was added as 34.4 g of a 20% aqueous solution. The solution was heated to 60°-80°C for six and one-half hours. 2,2'-Azobis[2-amidinopropane] dihydrochloride (6.87g) was added as a 20% aqueous solution. The solution was stirred and heated overnight (16 hours).

2,2'-Azobis[2-amidinopropane]dihydrochloride (6.87 g) was added as a 20% aqueous solution. The solution was stirred and heated for another 16 hours, then cooled to room temperature.

was then transferred to glass drying trays and dried in a 60°C forced air oven for several days. The final dry weight was 176.2 g.

Example 4 - Crosslinked Polydiallylamine

Using the same procedure as in Example 3, the neutralized polymer solution was crosslinked at 20 mole %. Epichlorohydrin (21.07 mL) was added to 900.0 g of the neutralized polymer solution. The final dry weight was 163.3 g.

Example 5 - Crosslinked Polydiallylamine

Using the same procedure as in Example 3, the neutralized polymer solution was crosslinked at 10 mole %. Epichlorohydrin (10.54 mL) was added to 900.0 g of the neutralized polymer solution. The final dry weight was 164.2 g.

Example 6 - Crosslinked Polydiallylamine

Using the same procedure as in Example 3, the neutralized polymer solution was crosslinked at 4.5 mole %. Epichlorohydrin (4.74 mL) was added to 900.0 g of the neutralized polymer solution. The final dry weight was 176.2 g.

Example 7 - Copolymer of Diallylamine and Methylenebisacrylamide

A solution of diallylammoniuim chloride (73.53 g of 68% aqueous solution), methylenebisacrylamide (2.93 g, 0.019 mol), 2,2'-azobis(2-amidinopropane) dihydrochloride (V50) (0.5 g) and water (27 mL) was heated at 70°C under a nitrogen atmosphere. Water (100 mL) was added after 15 minutes of reaction. An additional 0.5 g of V50 was added after 3 hours and again after 4 more hours. After keeping the reaction at 70 C for a total of 72 hr, it was cooled to room temperature. The resulting material was filtered and washed with 2 M NaCl (400 mL), and filtered and washed with water (2.5 L) and filtered again. The washed polymer was dried at 60°C in a forced-air oven gave 18.8 g of a solid (0.36 g/g yield, IPS=18.4)

Example 8 - Copolymer of Diallylamine and Acrylamide

A solution of diallylammonium chloride (73.53 g of 68% aqueous solution), methylenebisacrylamide (2.93 g, 0.019 mol), 2,2'-azobis(2-amidinopropane)

Example 11 - Copolymer of Diallylamine, a Functionalized Acrylic Ester and an Acrylic Ester Cross-linking Monomer

A solution of diallylammonium chloride (22.06 g of a 68% aqueous solution), tetrahydrofurfuryl methacrylate (18.72 g), ethyleneglycol dimethacrylate (4.36 g) and 2,2'-azobis(2-amidinopropane) dihydrochloride (2.03 g of an 18.8% aqueous solution) was heated at 65°C under a nitrogen atmosphere for 24 hours. The resulting material was suspended in methanol (300 mL), stirred 15 minutes and filtered. This methanol wash and filtration was repeated twice more. The polymer was similarly washed three times with water (500mL). The material was suspended in water (500 mL) and this mixture was acidified with concentrated HCl to pH 2.0. Filtration, and drying at 60°C in a forced-air oven gave 19.9 g of a solid.

Example 12 - Copolymer of Diallylamine and Glycidylmethacrylate

A solution of diallylammonium chloride (29.42 g of a 68% aqueous solution), glycidylmethacrylate (2.13 g), MeOH (25 mL), and 2,2'-azobis(2-amidinopropane) dihydrochloride (1.18 g of an 18.8% aqueous solution) was heated at 65°C under a nitrogen atmosphere for 12 hours. After cooling to room temperature, methanol (25 mL) was added and the pH of the solution was adjusted to 10 with the addition of 50% aqueous NaOH, and allowed to stir at room temperature. The reaction solution turned to a solid mass after about 2 hours, and was allowed to stand for 22 hours. The resulting gel, was suspended in MeOH (300 mL), stirred and filtered. This methanol wash and filtration were repeated twice more. The polymer was then suspended in water (1 L). Concentrated HCl was added to this suspension until pH 2.0 and stirred 0.5 hours. Filtration and drying in a forced-air oven at 60°C gave 6.0 g of a solid.

Example 13 - Copolymer of Allylamine, Diallylamine, Triallylamine and a Bis(diallylamino)alkylene salt

A solution of allylammonium chloride (25.0 g of a 60% aqueous solution), diallylammonium chloride (66.81 g of a 67% aqueous solution), triallylammonium chloride (40.87 g of a 68% aqueous solution), 1,6-bis(diallylmethylammoniium) hexane dibromide (5.0 g), and 2,2'-azobis(2-amidinopropane) dihydrochloride

Example 16 - *In Vivo* Testing

Male Golden Syrian Hamsters were group housed in shoe box cages and acclimated for approximately 1 week in our animal facility. Animals were fed rodent chow (brown color) and water ad libitum. The hamsters were then transferred to metabolism cages and housed individually. Following a 24 hour fast (water ad libitum), animals were presented a casein-based purified diet (white color) with 10% fat added plus the drug to be evaluated. Fecal material was collected from 9 hours after the casein-based diet was presented for 39 additional hours. The white fecal pellets (drug-containing casein-based diet) were lyophilized and ground to a homogeneous powder. One gram of the ground fecal pellet was extracted in a solution consisting of methanol and 500 mM aqueous NaOH (4:1; v/v) at 100°C and 1500 psi for 15 minutes. A 500 µL aliquot of the extract was evaporated and reconstituted in 1500 µL bovine calf serum:0.9% saline (1:1) and analyzed enzymatically, utilizing a test kit for bile acids (Sigma Chemical Co., St. Louis, MO) for bile acid concentration.

Table

Polymer	Dose (% in feed)	Fecal Bile Acids (µmol/g)
None	None	1.34
Example 6	.10	2.19
Example 6	.15	3.44
Example 6	.20	3.72
Example 6	.25	3.48
Cholestyramine	0.30	3.00
Colestipol	0.30	2.81

This example shows that crosslinked polydiallylamine is a highly potent bile acid sequestrant, with *in vivo* activity greater than current commercial products, Colestipol and Cholestyramine.

CLAIMS

This Sheet Has Been Replaced



What is claimed is:

1. A method for removing bile salts from a patient comprising administering to said patient a therapeutically effective amount of a polydiallylamine polymer, said polymer characterized in that the polymer is substantially free of alkylated amine monomers.

2. The method of Claim 1 wherein said polymer is crosslinked by means of a multifunctional crosslinking agent, said agent being present in an amount from about 0.5-50% by weight, based upon the combined weight of monomer and crosslinking agent.

3. The method of Claim 2 wherein said crosslinking agent is present in an amount from about 2.5-20% by weight, based upon the combined weight of monomer and crosslinking agent.

4. The method of Claim 2 wherein said crosslinking agent comprises epichlorohydrin.

5. The method of Claim 2 wherein said crosslinking agent is a bis(diallylammonium)dialkylene ion.

6. The method of Claim 1 wherein the polymer is a homopolymer.

7. A method of Claim 1 wherein the polymer is a copolymer.

8. A method of Claim 7 wherein the copolymer comprises the monomers diallylamine, allylamine, and triallylamine.

