Effective Therapy of Enteric Hyperoxaluria: *In Vitro* Binding of Oxalate by Anion-Exchange Resins and Aluminum Hydroxide

M. F. LAKER * and A. F. HOFMANN ˣ

Received August 11, 1980, from the *Division of Gastroenterology, Department of Medicine, University of California at San Diego Medical Center, San Diego, CA 92103.* Accepted for publication March 2, 1981. *Present address: Department of Clinical Biochemistry, Royal Victoria Infirmary, Newcastle-upon-Tyne NE1 4LP, England.

Abstract □ Hyperabsorption of dietary oxalate is a major factor in the pathogenesis of enteric hyperoxaluria and a frequent complication of inflammatory bowel disease and ileojejunal bypass surgery. Successful treatment requires reduction in oxalate intake or inhibition of absorption of dietary oxalate by oral ingestion of oxalate binding agents. To identify such agents, oxalate binding by anion-exchange resins, gums, and aluminum hydroxide was measured under conditions that simulated those present in the intestinal lumen. Of the agents tested, those that bound oxalate best were colestipol and aluminum hydroxide. Strongly basic anion-exchange resins readily bound oxalate only in the absence of chloride. These results suggest that colestipol and aluminum hydroxide administration might reduce dietary oxalate absorption in patients with enteric hyperoxaluria.

Keyphrases □ Oxalate—*in vitro* binding by various anion-exchange resins and aluminum hydroxide □ Binding agents—*in vitro* binding of oxalate by anion-exchange resins and aluminum hydroxide □ Enteric hyperoxaluria—dietary oxalate, *in vitro* binding by anion-exchange resins and aluminum hydroxide

Nephrolithiasis is a frequent complication of inflammatory bowel disease (1, 2), ileal resection (3–5), and ileojejunal bypass (6, 7). The stones are usually composed of calcium oxalate; hyperoxaluria, which often occurs in patients with ileal dysfunction, is a major predisposing factor.

Enteric hyperoxaluria is due, at least in part, to enhanced absorption of dietary oxalate (8, 9). The pathogenesis of this condition is complex and probably involves alterations in the physical state of dietary oxalate in the intestinal lumen as well as an increase in colonic permeability to the oxalate anion. In hyperoxaluric patients, malabsorbed fatty acids are thought to prevent normal precipitation of oxalate in the gut by preferentially binding intraluminal calcium to form insoluble calcium soaps (10, 11). Free oxalate then is absorbed in increased amounts, in part because the permeability of the colon to the oxalate anion is increased by abnormally elevated intraluminal concentrations of bile acids and fatty acids (12–14).

This explanation suggests that enteric hyperoxaluria can be treated successfully by decreasing dietary oxalate, the free oxalate concentration in the intestinal lumen, or the colonic permeability. To decrease intraluminal oxalate, agents capable of binding or precipitating oxalate could be administered. To decrease the concentration of fatty acids or bile acids in the colonic lumen, fat intake could be reduced or bile acid or fatty acid binding resins could be administered.

BACKGROUND

In this study the *in vitro* binding of oxalate by a number of nonabsorbable substances was examined. Cholestyramine, a nonabsorbable ion-exchange resin already reported to bind oxalate *in vitro* (9, 15), was used successfully by some investigators (3, 9), but not others (7, 16), to reduce oxalate excretion in patients with enteric hyperoxaluria. Aluminum antacids are essentially nonabsorbable and also were reported to decrease oxalate excretion in patients with enteric hyperoxaluria (11). However, since both cholestyramine and aluminum hydroxide are potent binders of bile acids (17–20) and fatty acids (21) *in vitro*, their ability to diminish urinary oxalate excretion in patients with enteric hyperoxaluria might result from bile acid or fatty acid binding in the colon, which would *diminish the enhanced mucosal permeability, thus reducing the increased* oxalate absorption. This effect on mucosal permeability might contribute more to diminishing oxalate absorption than that attributable to intraluminal oxalate binding. Thus, an unequivocal interpretation of clinical investigations of agents capable of binding not only oxalate but also bile acids and fatty acids is impossible.

Information is needed on the relative affinity *in vitro* of these binding agents for oxalate. Binding studies with these and several other potential therapeutic agents for oxalate are reported here. Binding of oxalate by calcium was not studied since the solubility product of calcium oxalate is well known and since calcium administration to patients with enteric hyperoxaluria has been reported to increase urinary calcium excretion, which may be undesirable in patients at risk for nephrolithiasis (22, 23).

EXPERIMENTAL

Adsorbents—The adsorbents investigated were ion-exchange resins, an uncharged hydrophobic resin, guar gum, and various types of aluminum hydroxide.

Three types of strongly basic anion-exchange resins were used. The first (SB_r) is a widely used bead resin with quaternary ammonium groups attached to a styrene–divinyl benzene copolymer lattice[1]. The second, cholestyramine[2], is chemically identifical to SB_r but was milled to reduce its particle size; it is marketed as a hypocholesterolemic agent. The third (SB_d) is a resin used for ion-exchange gel permeation chromatography; it consists of dextran beads containing quaternary ammonium groups[3].

Three weakly basic anion-exchange resins also were used. The first, colestipol[4], is a synthetic ion-exchange resin prepared by cross-linking tetraethylenepentamine with epichlorohydrin; the polymer, which has primary and secondary amine groups, is marketed as a hypocholesterolemic agent. The second (WB_r), a widely used bead resin, contains mostly tertiary amine groups on an epoxy lattice[5]. The third (WB_c), diethylaminoethylcellulose containing diethylamine groups on a cellulose lattice, is used for ion-exchange chromatography of proteins[6].

The uncharged hydrophobic resin (UH_r) consisted of beads of divinyl–benzene styrene copolymer with a macroreticular surface[7]. Guar gum[8] is a natural gum containing primary and secondary amino groups.

Aluminum hydroxide was used both in the form of proprietary antacid preparations ($AHPF_1$[9] and $AHPF_2$[10]) and as dried powder[11]. Three types

[1] Dowex-1, Dow Chemical Co. (sold as AG1-X4 by Bio-Rad Laboratories, Richmond, Calif.).
[2] Provided by Mead Johnson Co., Evansville, Ind.
[3] QAE-Sephadex, Pharmacia, Inc., Piscataway, N.J.
[4] Provided by The Upjohn Co., Kalamazoo, Mich.
[5] Dowex-3, Dow Chemical Co. (sold as AG3-X4A by Bio-Rad Laboratories, Richmond, Calif.).
[6] DEAE Cellulose, Schleicher and Schuell, Keene, N.H.
[7] XAD-2, Rohm & Haas, Philadelphia, Pa. (purchased from Mallinckrodt, St. Louis, Mo.).
[8] Bradford Co., Los Angeles, Calif.
[9] Amphogel (AHCFI), Wyeth Laboratories.
[10] Alternagel (AHCFII), Stuart Pharmaceuticals.
[11] Three aluminum hydroxide preparations (Reheis, Barcroft, and Chattem) were generously supplied by the Rorer Group through the courtesy of John Eckman.

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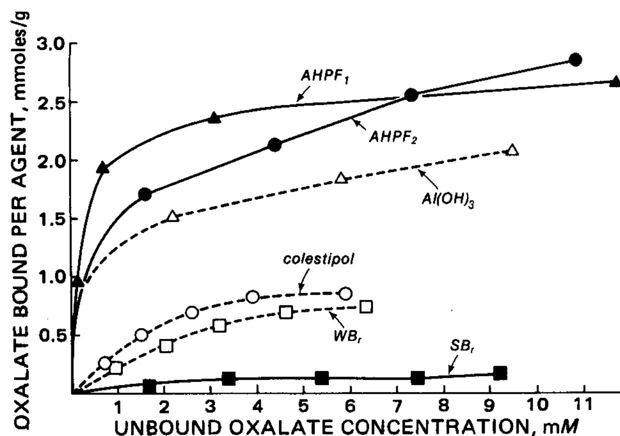

Figure 1—*Equilibrium isotherms for oxalate binding to anion-exchange resins and aluminum hydroxide in histidine buffer (pH 6.5, 37°; 0.15* M *chloride ion).*

of aluminum hydroxide powder were studied.

Ion-exchange resins were conditioned before use by stirring with two volumes of 1 *N* NaOH, decanting the supernate, and washing the resin with distilled water. The resin was converted to the chloride form by stirring with two volumes of 1 *N* HCl. After repeated washings with glass-distilled water until the supernate was no longer acidic (pH > 6), resins were dried at room temperature.

Next, UH_r was conditioned by washing with methanol followed by distilled water and then was stored in water. The dextran- and cellulose-based ion exchangers, as well as the guar gum, were preswollen in buffered saline solution (150 m*M* NaCl, 20 m*M* L-histidine, pH 6.5).

Aluminum hydroxide preparations were used without initial conditioning.

Adsorption Studies—Oxalate binding was studied by incubating 50 mg of adsorbent (wet weight for UH_r, volume containing 50 mg of aluminum hydroxide for $AHPF_1$ and $AHPF_2$, and dry weight for the other adsorbents) with 10 ml of oxalate-containing solution. The solutions contained varying amounts of unlabeled oxalic acid (2–25 m*M*), a constant amount of [^{14}C]oxalic acid[12] (20 μCi/liter), sodium chloride (150 m*M*), and L-histidine (20 m*M*). The pH was adjusted to 6.5 with hydrochloric acid.

Premixed solutions and adsorbents were added to 20 ml-glass serum storage vials, which were sealed with butyl rubber septa and crimped aluminum closures. The sealed bottles were kept in a 37° oven and continuously rotated. Samples were centrifuged, and the supernate was aspirated; its radioactivity was determined by liquid scintillation counting (0.2 ml was mixed with 10 ml of scintillant[13]). Each study was performed in duplicate.

The binding affinity of each adsorbent for oxalate was characterized by using adsorption isotherms to summarize the data (24). The amount of oxalate bound at equilibrium was determined for several starting

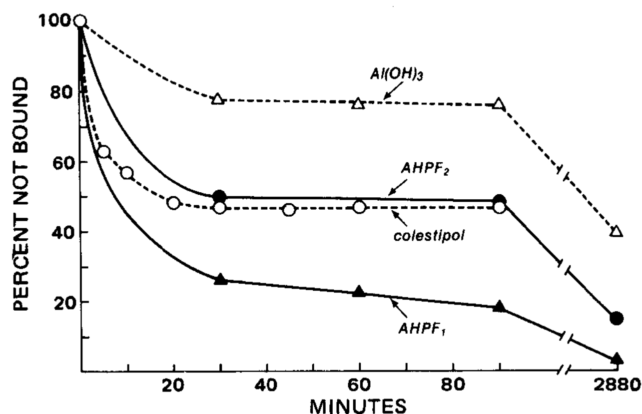

Figure 2—*Kinetics of oxalate binding to aluminum hydroxide or colestipol (pH 6.5, 37°; 0.15* M *chloride ion).*

[12] New England Nuclear, Boston, Mass.
[13] Ready-Solv HP, Beckman Instruments, Fullerton, Calif.

Table I—Oxalate Binding at Equilibrium by Selected Adsorbents in the Presence of Isotonic Saline or Simulated Intestinal Content (Containing Fatty Acid and Bile Acid Anions)

Adsorbent	Incubation Solution Oxalate Concentration, m*M*	Oxalate Bound, mmoles/g of adsorbent: With 0.15 *M* Saline	With Simulated Intestinal Content	Reduction in Binding, %
SB_r	10	0.16	0.10	37
WB_r	10	0.74	0.52	30
Colestipol	10	0.85	0.44	48
$AHPF_1$	25	2.38	0.74	69
Aluminum hydroxide powder	25	1.84	0.10	94

concentrations (2–10 m*M* for resins and 5–25 m*M* for aluminum hydroxide preparations). Specimens in which adsorbent and isotope were omitted were included as controls. The kinetics of oxalate binding were studied by repeated sampling of containers with a single oxalate concentration (7.5 m*M*).

To test whether oxalate binding to SB_r was influenced by the presence of chloride ion in the incubation medium, oxalate binding to SB_r was defined in the absence of chloride ion. Thus, SB_r was incubated with 10 ml of a solution containing 7.5 m*M* oxalic acid, [^{14}C]oxalic acid (20 μCi/liter), and 20 m*M* L-histidine, and the pH was adjusted to 6.5. Oxalate binding at equilibrium was determined as already described.

To determine whether bile acid or fatty acid anions that are present in intestinal content in humans might competitively inhibit oxalate binding, oxalate binding was measured in simulated intestinal content (25). The incubation solution contained 130 m*M* sodium chloride, 20 m*M* L-histidine, 7.5 m*M* glycocholic acid, 7.5 m*M* chenylglycine, 2.5 m*M* taurocholic acid, 2.5 m*M* chenyltaurine, 6.7 m*M* monoolein, and 13.3 m*M* oleic acid; the pH of the solution was adjusted to 6.5 (19). Unlabeled oxalate (10 m*M* for resins and 25 m*M* for aluminum hydroxide preparations) and [^{14}C]oxalic acid (20 μCi/liter) were included. Vials were sampled until equilibrium of binding was achieved.

Experiments also were carried out to determine whether substances present in the liquid component of one of the two proprietary formulations of aluminum hydroxide ($AHPF_1$) influenced oxalate binding. An aliquot was centrifuged, and the supernatant fraction was removed. The aluminum hydroxide was resuspended in water and recentrifuged, with the supernate being discarded, and this procedure was repeated three times. The initial supernate was then mixed with aluminum hydroxide powder. The equilibrium binding was studied with the dried $AHPF_1$ residue as well as the suspension of aluminum hydroxide powder, the oxalate concentration in the incubation mixture being 15 mmoles/liter.

RESULTS

Equilibrium Isotherms—Colestipol and WB_r had a greater affinity for oxalate than SB_r (Fig. 1). Binding to SB_d or WB_c (not shown) was considerably less than that to SB_r. The weak binding of oxalate to SB_r was due to the chloride ion in the medium; when chloride ion was omitted, binding increased threefold.

In addition, aluminum hydroxide, either as a proprietary antacid formulation or as a dried powder, readily bound oxalate. Binding of oxalate to $AHPF_1$ was identical whether the suspension or the washed solid phase obtained by centrifugation was used. Thus, all binding was attributable to particulate aluminum hydroxide. The binding isotherms for all aluminum hydroxide powders were similar, so only data for one of them are shown. No binding by guar gum or UH_r was detected, and WB_c only bound oxalate weakly (7%).

In Table I, oxalate binding by selected adsorbents in the presence or absence of simulated intestinal content is compared. Less oxalate was bound by all the adsorbents tested when constituents of intestinal content, *i.e.*, bile acid and fatty acid anions, were included. The decrease was most marked for aluminum hydroxide preparations, with $AHPF_1$ binding only 31% and aluminum powder binding only 5% of the amount of oxalate adsorbed in the absence of intestinal content. Oxalate binding to colestipol and WB_r was also less in simulated intestinal content, being 52 and 70%, respectively, of that observed when chloride was the only anion present.

Binding Kinetics—Oxalate was adsorbed more rapidly by colestipol

and WB_r (not shown) than by aluminum hydroxide (Fig. 2). The binding to aluminum hydroxide had two rates. Initially, there was rapid uptake, binding being more rapid for proprietary antacid formulations of aluminum hydroxide than for the powders. For both adsorbents, several days were required for equilibrium to be obtained.

DISCUSSION

These results indicate that oxalate, in the presence of simulated intestinal content, binds better to aluminum hydroxide and weak anion-exchange resins (colestipol and WB_r) than to a strongly basic ion-exchange resin. They also show that the binding is rapid to ion-exchange resins but consists of a slow and fast component for aluminum hydroxide.

Although cholestyramine has been reported to be an effective oxalate adsorbent *in vitro* (11, 16), the data suggest that effective binding occurs only if chloride is omitted from the incubation. This could explain the findings of Caspary *et al.* (16) who demonstrated that cholestyramine did not bind oxalate *in vivo*. The data suggest that the reported efficacy of this resin in the treatment of enteric hyperoxaluria (3, 9) is due more to its binding of bile acid and fatty acids than to its binding of oxalate. Such binding should diminish colonic permeability to oxalate, which would be interpreted as reducing its absorption.

Colestipol and WB_r bound less oxalate when bile acids and fatty acids were present in the incubation mixture; however, both resins were effective binding agents under experimental conditions simulating those present in the small intestinal lumen.

Aluminum hydroxide preparations had a greater capacity than colestipol or WB_r for oxalate binding but required much longer for equilibrium to be reached, a factor that could diminish clinical efficacy. Although the commercial antacid preparations bound oxalate more rapidly than the dry powder preparations, similar amounts were bound by the two forms of oxalate within 30 min. Whether these differing rates of binding *in vitro* have clinical significance is unknown.

These *in vitro* binding data indicate that both aluminum antacids and weakly basic anion exchange resins might prove effective in adsorbing oxalate within the intestinal lumen and thus act to diminish the hyperoxaluria occurring in patients with intestinal disease. Preliminary clinical studies support these *in vitro* binding studies (26). Nonetheless, several recent reports (27, 28) claimed that oral calcium reduces oxalate absorption and that urinary calcium levels do not show a reciprocal increase.

Although hyperoxaluria contributes to nephrolithiasis in such patients, other factors, such as diminished urinary volume (29) and hypocitraturia (30, 31), are involved. Thus, reduction in oxalate excretion is only one of several alternative approaches for reducing the lithogenicity of urine.

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