

# Potato Glycoalkaloids Adversely Affect Intestinal Permeability and Aggravate Inflammatory Bowel Disease

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**Summary:** *Background:* Disruption of epithelial barrier integrity is important in the initiation and cause of inflammatory bowel disease (IBD). Glycoalkaloids, solanine (S), and chaconine (C) are naturally present in potatoes, can permeabilize cholesterol-containing membranes, and lead to disruption of epithelial barrier integrity. Frying potatoes concentrates glycoalkaloids. Interestingly, the prevalence of IBD is highest in countries where fried potatoes consumption is highest. *Objective:* To further understand the role of potato glycoalkaloids on intestinal barrier integrity, we examined the effect of varying concentrations of solanine and chaconine on intestinal permeability and function. *Methods:* Solanine (0–50  $\mu\text{M}$ ), chaconine (0–20  $\mu\text{M}$ ), or a 1:1 mixture (0–20  $\mu\text{M}$ ) were exposed to T84 cultured epithelial monolayers for varying periods of time to determine concentration response effect on epithelial permeability. Next, a 1:1 mixture (5  $\mu\text{M}$ ) of solanine-to-chaconine (C:S) was exposed to sheets of normal murine small intestine, mounted in Ussing chambers, from control and interleukin-10 gene-deficient mice to determine whether glycoalkaloids af-

ected intestine from mice with a genetic predisposition for IBD greater than controls. Finally, the effects of glycoalkaloids on colonic histologic injury were examined in mice orally fed amounts of glycoalkaloids that would normally be consumed in a human diet. *Results:* Glycoalkaloids embedded and permeabilized the T84 monolayer epithelial membrane bilayer in a concentration-dependent fashion, with C:S > C > S. In vitro Ussing chamber experiments also illustrated a concentration-dependent disruption of intestinal barrier integrity in animals with a genetic predisposition to develop IBD, but not in control animals. Similarly, in vivo oral feeding experiments demonstrated that C:S ingestion, at physiologic concentrations, aggravated histologic colonic injury in mice genetically predisposed to developing IBD. *Conclusion:* Concentrations of glycoalkaloids normally available while eating potatoes can adversely affect the mammalian intestine and can aggravate IBD. **Key Words:** Crohn's disease—Inflammatory bowel disease—Glycoalkaloids—Solanine—Chaconine—Barrier integrity—Permeability.

## INTRODUCTION

Inflammatory bowel disease (IBD) is the result of an unchecked intestinal inflammatory response. While the exact etiology and pathogenesis of IBD remain unclear, initiation of the inflammatory response is likely caused by luminal environmental factors, very likely bacteria or bacterial products, serving as activating antigens. By sieving through a permeable epithelial barrier, this initiating factor leads to an increased exposure of the mucosal immune system to luminal antigens and toxins. In the

genetically susceptible individual, this process results in an inappropriate and unrestrained mucosal immune response and ensuing tissue injury.

In animal models, primary disruption of the epithelial barrier is, by itself, sufficient to initiate a sustained IBD. Indeed, we have previously shown that the interleukin-10 (IL-10) gene-deficient mouse model of IBD has an intestinal permeability defect that is present prior to the development of any intestinal injury (1). Nevertheless, the role of a primary epithelial barrier disruption in human IBD remains a matter of debate (2,3).

The fact that human IBD appears to be a disease of developed countries, and is seen within one generation of arrival from developing countries, strongly suggests an environmental association. The nature of this environmental factor remains obscure, but both microbiologic

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agents and orally ingested components of foods have been implicated (4).

Glycoalkaloid toxins present in potatoes and other members of the *Solanaceae* plant family have been identified as the compounds by which these plants defend themselves against fungi, bacteria, and other parasites. The glycoalkaloids present in potatoes, and subsequently consumed in the human diet, are alpha-solanine and alpha-chaconine. Glycoalkaloids, like digitonin, form a covalently bound complex with cholesterol in the cell wall. Cholesterol is oriented at right angles to the plane of the membrane bilayer. The lipophilic alkaloid moiety of the glycoalkaloid aligns itself parallel to cholesterol. These cholesterol-glycoalkaloid complexes are mobile and subsequently migrate within the membrane bilayer to aggregate into larger domains. The hydrophilic glyco-moiety of the glycoalkaloid, composed of a forked group containing three sugar molecules, protrudes out of the domain and away from the cell wall. Because of the bulk of the glycomoieties, glycoalkaloids compete for space in the intercellular fluid. Through formation of a curved surface, mutual interference between the glycogroups is minimized (Fig. 1) (5). Eventually, as the concentration of the glycoalkaloid in the cell wall increases suffi-

ciently, the pressure to form a curved surface causes the membrane bilayer to rupture, leading to disruption of barrier integrity.

Acute poisoning by potatoes is well documented. Indeed, potatoes with greater than 200  $\mu\text{g}/\text{kg}$  glycoalkaloids are considered poisonous (6). There is a paucity of data concerning long-term effects of repeated ingestion during long periods of time of small amounts of potato glycoalkaloids; nevertheless, there are indications that solanine and related compounds can accumulate in tissue (6).

The objective of this study was to determine if glycoalkaloids, at a concentration attainable in the human intestine following oral ingestion of potatoes, would disrupt intestinal epithelial barrier integrity and cause, or worsen, IBD.

## MATERIALS AND METHODS

### Materials

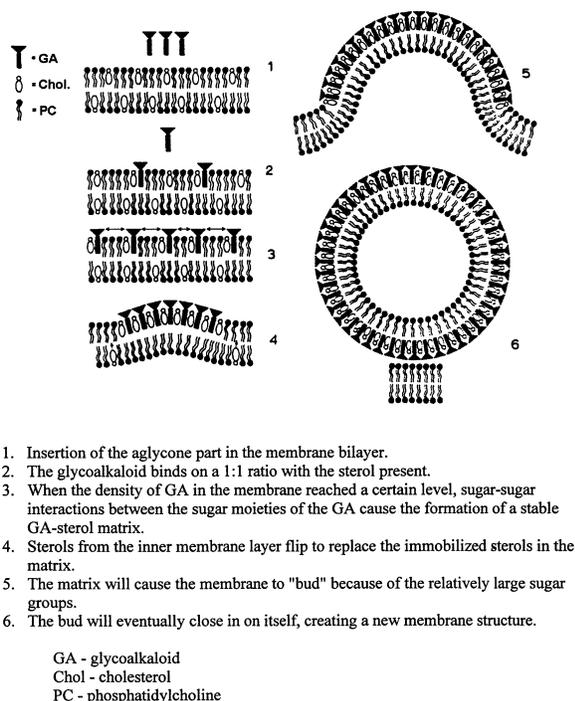
The radioisotope [ $^3\text{H}$ ] D-mannitol was obtained from New England Nuclear (Boston, MA, U.S.A.). Reagent-grade chemicals were obtained from either Sigma Chemical Company (St. Louis, MO, U.S.A.) or Fisher Scientific Canada (Napean, ON, Canada).

### Glycoalkaloids

Alpha-solanine and alpha-chaconine were obtained from Sigma Chemical Company (St. Louis, MO, USA). For the *in vitro* experiments, chaconine (0–20  $\mu\text{M}$ ), solanine (0–50  $\mu\text{M}$ ), and a 1:1 chaconine-to-solanine mixture (0–20  $\mu\text{M}$ ) were mixed daily in aqueous solution from stock aliquots frozen at  $-22^\circ\text{C}$ . A 1:1 ratio of chaconine-to-solanine is the approximate ratio found in potatoes used for human consumption. Furthermore, the amounts of glycoalkaloids used in these experiments are those shown to be attainable within the human gastrointestinal tract following consumption of potatoes (6). For the *in vitro* feeding experiments, 1:1 chaconine-to-solanine mixture 3 mg/kg/d was fed orally to mice via their drinking water. This quantity of chaconine-to-solanine (3 mg/kg per day) is equivalent to the amount that would be consumed by a human ingesting one plate of fried potato skins daily (6).

### Animals

Homozygous IL-10 gene-deficient mice generated on a 129 Sv/Ev background (DNAX Research Institute, Palo Alto, CA, U.S.A.) and 129 Sv/Ev controls (Jackson



**FIG. 1.** Proposed mechanism of membrane disruption by glycoalkaloids. From: Keukens EA, de Vrije T, van den Bloom, et al. Molecular basis of glycoalkaloid induced membrane disruption. *Biochim Biophys Acta* 1995;1240:216–28. Reprinted with permission from Elsevier Science.

Laboratories, Tacoma, WA, U.S.A.) were maintained in a colony at the University of Alberta, housed behind a barrier under specific pathogen-free conditions. All supplies for the facility were autoclaved. Nonautoclavable supplies were sprayed with disinfectant and introduced through a high efficiency particulate air (HEPA)-filtered air lock. Mice were housed in microisolator cages provided with tight-fitting lids containing a spun polyester fiber filter. Tests on sentinel Balb/c mice (bacterial cultures, parasitological examinations, serological tracking profiles, and histological stains negative for known murine viral and bacterial pathogens) indicated that the barrier was intact. However, these mice did colonize their gastrointestinal lumen with normal enteric flora. Interleukin-10 gene-deficient mice used in this experiment were 12 weeks of age, by which time they had developed maximal enterocolitis; however, the segments of small intestine used in the Ussing chamber experiments were histologically normal.

#### T84 Cell Culture Monolayers

T84 monolayers were used to assess the ability of solanine and chaconine to disrupt a cultured epithelial barrier. Cells at passages 30 to 34 were grown as monolayers in a 1:1 mixture of Dulbecco-Vogt modified Eagle's medium and Ham's F-12 medium supplemented with 15 mmol/L Na<sup>+</sup>-HEPES buffer, pH 7.5, 14 mmol/L NaHCO<sub>3</sub>, and 5% new-born calf serum. For subculture, a cell suspension was obtained from confluent monolayers by exposing the monolayers to 0.25% trypsin and 0.9-mmol/L ethylenediaminetetraacetic acid in Ca<sup>+</sup>-free and Mg<sup>+</sup>-free phosphate-buffered saline. Cells were seeded at a density of 1 × 10<sup>6</sup> cells/1.13 cm<sup>2</sup> polycarbonate tissue culture-treated filter and maintained at 37°C in a 5% CO<sub>2</sub> atmosphere. Cultures were refed twice weekly with fresh media.

To qualitatively determine whether the T84 cells had reached confluence, formed tight junctions, and established cell polarity, the electrical conductance and spontaneous potential across the T84 monolayer were determined using an EVOM volt meter and an STX-2 electrode set (World Precision Instruments, Sarasota, FL, U.S.A.). The change in electrical conductance on apical exposure to glycoalkaloids was used to determine disruption of monolayer barrier integrity.

#### Intestinal Barrier Integrity Measurements

For electrophysiology and permeability studies, small intestine from control or IL-10 gene-deficient mice was mounted in Ussing chambers and bathed on both sides

with a bicarbonate-Ringer solution containing 17.5 mmol/L glucose circulated with 5% CO<sub>2</sub>/95% O<sub>2</sub> (pH 7.4) at 37°C. The spontaneous transepithelial potential difference (PD) and short circuit (I<sub>sc</sub>) were determined, and the transepithelial conductance was calculated from PD and I<sub>sc</sub> according to Ohm Law (7). Unidirectional fluxes of mannitol (serosal to mucosal) were measured by adding 5 μCi [<sup>3</sup>H] D-mannitol into the serosal chamber. Exactly 1 mmol/L mannitol was present on both mucosal and serosal surfaces. After a 10-min equilibration period, two 10-min mannitol fluxes were determined. Aliquots were counted in a scintillation counter and expressed as Nmol flux/cm<sup>2</sup> per hour.

#### Intestinal Histological Assessment

The ileum and colon in their entirety were harvested and fixed in 10% phosphate-buffered formalin. These samples were paraffin-embedded, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E) for light microscopic examination. The slides were reviewed in a blinded manner by two pathologists (J.S.D. and L.D.J.), and were assigned a histologic score for intestinal inflammation using a modification of the scoring scheme described by Savarymuttu et al. (8), as previously detailed (9). Briefly, histological grades (ranging from 0–10) represent the numerical sum of four scoring criteria: mucosal ulceration, epithelial hyperplasia, lamina propria mononuclear infiltration, and lamina propria neutrophilic infiltration.

#### Statistical Analysis

Statistical analysis was performed using the statistical software SigmaStat (SPSS, Chicago, IL, U.S.A.). Differences between means were evaluated using analysis of variance or paired *t* tests where appropriate. A *p* value of < 0.05 was considered statistically significant.

## RESULTS

#### Effect of Glycoalkaloids on Cultured Epithelial Barrier Integrity

To determine the effects of glycoalkaloids on the epithelial barrier of cultured epithelial monolayers, chaconine (0–20 μM), solanine (0–50 μM), and a 1:1 chaconine-to-solanine mixture (0–20 μM) was exposed to T84 cultured epithelial monolayers for varying intervals of time (Figs. 2–4), and change in monolayer electrical resistance was measured. The concentrations of glycoalkaloids used in this experiment have been shown attainable

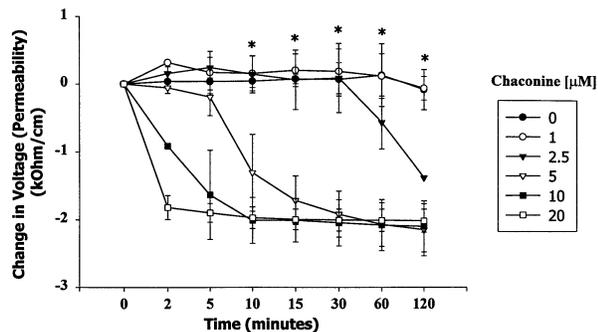
within the gastrointestinal tract following consumption of potatoes (6). Glycoalkaloid exposure resulted in disruption of the T84 monolayer epithelial barrier integrity in a dose-dependent fashion with the injury caused by chaconine-to-solanine mixture (Fig. 4) greater than chaconine alone (Fig. 2) and greater than solanine alone (Fig. 3).

### Effect of Glycoalkaloids on In Vitro Mammalian Intestinal Barrier Integrity

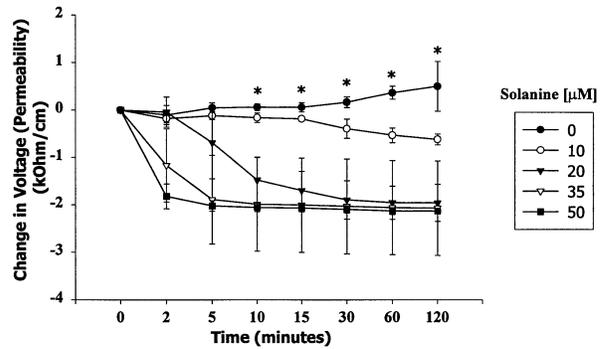
Having demonstrated that physiological concentrations of in vitro glycoalkaloids were able to disrupt the epithelial barrier in cultured intestinal monolayers, we proceeded to determine if a similar effect would be seen if glycoalkaloids were exposed to intact sheets of mammalian intestine. We examined this effect on intestine from normal control mice and on intestine from IL-10 gene-deficient mice with genetically engineered IBD. Since a 1:1 mixture of chaconine-to-solanine occurs naturally in potatoes, we used this mixture for the subsequent experiments. A 1:1 chaconine-to-solanine physiologic mixture (5  $\mu\text{M}$ ) was thus exposed to sheets of murine intestine from control and genetically engineered mice with IBD while mounted in vitro in Ussing chambers. As determined by transmural mannitol flux, the 5  $\mu\text{M}$  chaconine-to-solanine mixture did not affect the permeability of small intestine from normal control mice, but did cause significant additional intestinal barrier disruption in intestine from IL-10 gene-deficient mice with IBD ( $p < 0.01$ ) (Fig. 5).

### Effect of Glycoalkaloids on In Vivo Mammalian Intestinal Injury

Finally, to determine if the in vitro intestinal barrier disruption would translate into intestinal injury in vivo, a



**FIG. 2.** Effect of  $\alpha$ -chaconine on T84 epithelial monolayer barrier integrity. The  $\alpha$ -chaconine disrupted the epithelial T84 monolayer barrier integrity and permeabilized the membrane in a concentration-dependent fashion. Values lower than zero represent decreased barrier integrity.  $n = 6$  monolayers with quadruplicate determinations. Values represent mean  $\pm$  SEM. \* $p < 0.005$ ; 5  $\mu\text{M}$  relative to control (0  $\mu\text{M}$ ).

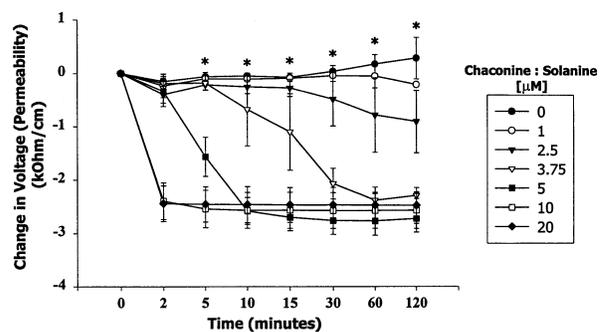


**FIG. 3.** Effect of  $\alpha$ -solanine on T84 epithelial monolayer barrier integrity.  $\alpha$ -Solanine disrupted the epithelial T84 monolayer barrier integrity and permeabilized the membrane in a concentration-dependent fashion. Values lower than zero represent decreased barrier integrity.  $n = 6$  monolayers with quadruplicate determinations. Values represent mean  $\pm$  SEM.

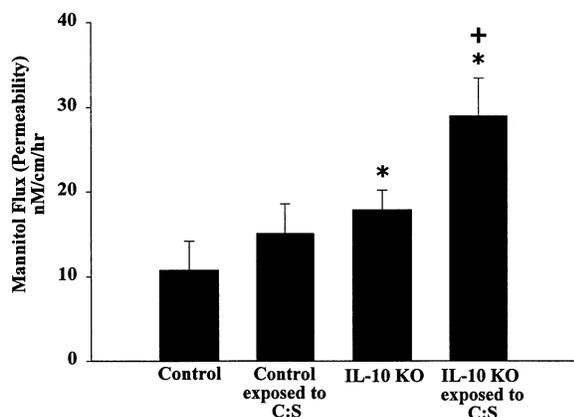
\*  $p < 0.005$ ; 20  $\mu\text{M}$  relative to control 0  $\mu\text{M}$ .

1:1 chaconine-to-solanine mixture (3 mg/kg/d) was fed to mice orally via their drinking water for 21 days and histologic injury was examined. This amount of daily oral chaconine and solanine fed to mice represents the daily amount that would be consumed in a human diet supplemented with one plate of fried potato skins daily over a similar time interval (6). Furthermore, derived from 3 mg/kg/d, the concentration of 1:1 chaconine-to-solanine ingested daily by the mice in their drinking water was 6.9  $\mu\text{M}$  and similar to that utilized in the in vitro barrier disruption study above.

Oral administration of the chaconine-to-solanine mixture did not adversely affect intestine from control animals; nevertheless, it did markedly accentuate the intestinal histologic injury seen in IL-10 gene-deficient mice with IBD ( $p < 0.01$ ) (Fig. 6).



**FIG. 4.** Effect of  $\alpha$ -chaconine/ $\alpha$ -solanine (1:1) on T84 epithelial monolayer barrier integrity. The  $\alpha$ -chaconine/ $\alpha$ -solanine, in a 1:1 mixture, disrupted the epithelial T84 monolayer barrier integrity and permeabilized the membrane in a dose-dependent fashion. Values lower than zero represent decreased barrier integrity.  $n = 6$  monolayers with quadruplicate determinations. Values represent mean  $\pm$  SEM.  $p < 0.005$ ; 5  $\mu\text{M}$  relative to control (0  $\mu\text{M}$ ).



**FIG. 5.** Effect of  $\alpha$ -chaconine/ $\alpha$ -solanine mixture on in vitro small intestinal barrier integrity. The  $\alpha$ -chaconine/ $\alpha$ -solanine (1:1, 5  $\mu$ M) mixture exposed to intestine mounted in Ussing chambers aggravated small intestinal epithelial barrier disruption in mice deficient in the IL-10 gene. IL-10 KO, IL-10 knock-out mice. C:S, chaconine/solanine (1:1, 5  $\mu$ M).  $n = 6$  animals with duplicate determinations. Values represent mean  $\pm$  SEM. \* $p < 0.05$  relative to respective controls. † $p < 0.01$  relative to IL-10 KO.

## DISCUSSION

The lumen of the intestine holds bacteria, bacterial products, and bacterial antigens capable of initiating and sustaining inflammation. The normal intestinal epithelium provides a barrier relatively impermeable to these luminal constituents, and controlled luminal antigens uptake occurs through intestinal immune and epithelial cells. In IBD the intestinal epithelial barrier is disrupted; likely both as a primary event, under genetic control, and later as a secondary event following release of proinflammatory cytokines. Any process that further disrupts the epithelial barrier integrity also could serve to initiate or aggravate mucosal inflammation.

Glycoalkaloids are natural steroidal toxins occurring in cultivated potatoes (*Solanum tuberosum*) (10–12). Breeding for a reduction in the levels of the glycoalkaloids has enhanced the commercial success of the potato. Despite the fact that levels of glycoalkaloids are much lower in modern potatoes than in wild progenitors, if the potato were to be introduced today as a novel food it is likely that its use would not be approved because of the presence of these toxic compounds. Indeed, toxicological effects of potatoes have been well described in humans, ranging from gastrointestinal disturbances to hemolysis and neurotoxic effects (13,14).

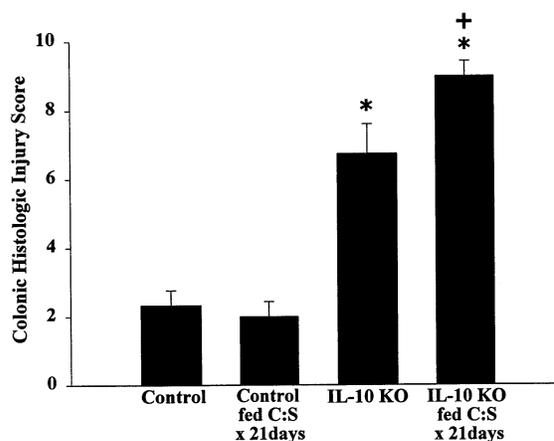
The two glycoalkaloids in potatoes are alpha-solanine and alpha-chaconine, accounting for over 95% of the glycoalkaloids present. They consist of a nonpolar lipophilic six-ring steroid aglycon nucleus with a nitrogen atom connecting the fifth and sixth ring, and a polar

water-soluble trisaccharide sugar moiety at the 3-OH position. Solanine and chaconine share the same aglycone, namely solanidine, but differ in their trisaccharide component (15).

Both solanine and chaconine inhibit acetylcholine esterase (16) and can interfere with membrane function and structure (17–21), leading to leakage of cells contents and proteins.

In our study, glycoalkaloids permeabilized epithelial membranes and disrupted epithelial barrier integrity in both cell culture monolayers and in sheets of mammalian intestine mounted in Ussing chambers. In T84 epithelial monolayers, this effect was concentration dependent and demonstrated selectivity with solanine being less disruptive at any given concentration than chaconine (Figs. 2 and 3). Furthermore, the membrane disruptive effect of a one-to-one mixture of solanine and chaconine was additive (Fig. 4). These concentration-dependent and synergistic effects of glycoalkaloids in the T84 epithelial monolayers are similar to those previously reported in Caco-2, IEC-6, and IEC-18 epithelial cells, liposome membranes, and erythrocyte ghosts (5,22–27).

The mechanism of action of glycoalkaloids on the epithelial barrier appears to be related to their high affinity for cholesterol and their ability to insert into the cholesterol-containing membranes. Indeed, disruption of the membrane is linearly correlated with the cholesterol content of the membrane (22). Since intestinal epithelial membranes are approximately one-third cholesterol, gly-



**FIG. 6.** Effect of  $\alpha$ -chaconine/ $\alpha$ -solanine on in vivo colonic histologic injury. The  $\alpha$ -chaconine/ $\alpha$ -solanine mixture fed orally (3 mg/kg per day) for 21 days aggravated colonic intestinal histologic injury in the colons of mice deficient in the IL-10 gene. Colonic injury scores: 0 = minimal injury, 10 = maximal injury. IL-10 KO, IL-10 knock-out mice. C:S; chaconine/solanine 3 mg/kg per day orally.  $n = 6$  animals. Values represent mean  $\pm$  SEM. \* $p < 0.05$  relative to respective controls. † $p < 0.01$  relative to IL-10 KO.

coalkaloids would be avidly attracted and inserted into them, thereby compromising the epithelial barrier (Fig. 1).

The present article extends the above observations by demonstrating similar effects of glycoalkaloids on sheets of mammalian intestine mounted in Ussing chambers. Using a 5  $\mu$ M concentration of chaconine-to-solanine [1:1] mixture that would be achieved within the lumen of humans following the ingestion of potatoes, we demonstrated that normal small intestine from IL-10 gene-deficient mice (a species known to spontaneously develop an enterocolitis similar to human Crohn's disease), when exposed to this mixture of chaconine-to-solanine, developed a marked epithelial barrier disruption as evidenced by an increase in transmural mannitol flux (Fig. 5).

While the small intestine from normal control mice was not affected by the 5  $\mu$ M physiologic concentration of chaconine-to-solanine, pharmacologic concentrations (20  $\mu$ M) of chaconine-to-solanine mixture were capable of disrupting the epithelial barrier in control animals (data not shown). Implying, that intestine from IL-10 gene-deficient mice has a lower threshold to glycoalkaloid-induced intestinal barrier disruption than does control intestine. Whether this propensity for epithelial barrier disruption in the small intestine of mice genetically engineered to develop IBD occurred due to alterations in the intestinal bilayer cholesterol composition, glycoalkaloid binding affinity, or another cause remains to be determined.

In vivo feeding of the chaconine-to-solanine mixture for 21 days, at 3 mg/kg per day, an amount that humans would consume with one plate of fried potato skins daily, led to results that paralleled those seen in the in vitro Ussing chamber studies (Fig. 5). That is, the chaconine-to-solanine mixture did not affect control intestine but did significantly worsen colonic histological injury in IL-10 gene-deficient mice (Fig. 6). Once again, the reason that control intestine was not perturbed by this physiologic level of chaconine-to-solanine, while intestine from IL-10 gene-deficient mice was, remains to be determined.

Levels of available glycoalkaloids vary in different types of potatoes, in different parts of the plant, being highest within 1.5 mm of peel and with the method of food preparation. Freshly dug potato tubers may contain between 9 and 400 mg/kg of chaconine and solanine (6,28). In general chaconine and solanine occur in potatoes in a 1:1 mixture. Glycoalkaloids available to humans via oral intake will be between 0.075 and 0.15 (boiled peeled potatoes), 0.3 and 0.4 (baked jacket potato), 0.4 and 2.5 (potato chips), and 2.0 and 5.1 (fried potato skins) mg/kg body mass per day (6).

While consumption of potatoes per capita has not

changed, what has changed in recent years is the way in which potatoes are prepared for consumption. Specifically, mechanical slicing and frying of potatoes is more prevalent in developed countries, where, coincidentally, the prevalence of IBD is highest. Mechanical damage to potato tissue increases the concentration of glycoalkaloids available for consumption. In addition, frying potatoes at high temperatures does not inactivate but instead serves to preserve and concentrate glycoalkaloids within the potato, leaving them available for ingestion and delivery to the intestine. In this way the exposure of the small and large intestine to glycoalkaloids from mechanically prepared and commercially fried potatoes exceeds the exposure from an equivalent intake of potatoes boiled in water. Indeed, on boiling peeled potatoes in tap water (i.e., dilute acid), glycoalkaloids are readily hydrolyzed, yielding sugars and solanidine, both completely inactive (5).

It is thus intriguing to hypothesize that consumption of concentrated glycoalkaloids from mechanically prepared and commercially fried potatoes, in a genetically predisposed human host, is sufficient to disrupt the intestinal epithelial barrier and subsequently initiate or sustain luminal antigen presentation and development of IBD. Indeed, the prevalence of IBD in the world is closely aligned to developing countries, where the preparation and consumption of fried potatoes, a process known to concentrate glycoalkaloids, is common.

## CONCLUSION

In conclusion, glycoalkaloids, normally available while eating potatoes, embed themselves and disrupt epithelial barrier integrity in a dose-dependent fashion in both cell culture models and in sheets of mammalian intestine. In addition, IL-10 gene-deficient mice, animals with the genetic predisposition to develop IBD, demonstrated a greater degree of small intestinal epithelial barrier disruption and inflammation when their epithelium was exposed to the potato glycoalkaloids chaconine and solanine.

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