Inhibition of Diarrhea by Immune Egg: A Castor Oil Mouse Model

Henry I. Jacoby
George Moore
Gary Wnorowski

ABSTRACT. Powdered immune egg was given by oral bolus doses for two days in order to evaluate the potential for inhibition of castor oil-induced diarrhea in the mouse. Castor oil was administered after the second dose of powdered immune egg and the response was quantified by grading the consistency of the stool at 2, 4 and 6 hours. A dose

Henry I. Jacoby, PhD, received his PhD in Pharmacology from the University of Michigan in 1962. His area of interests include the effects of drugs on the gastrointestinal tract, drugs having analgesic activity and evaluation of ancillary pharmacology. He has worked in discovery research at Merck and Johnson & Johnson. After leaving Johnson & Johnson he became a principal in Discovery Research Consultants and recently was named Director of Pharmacology at Product Safety Labs. He has published more than 40 scientific papers as well as being the author of several book chapters.

George Moore, BS, received his BS degree from Towson State College. He has over 28 years of experience in toxicology and medical device testing. Prior to joining Product Safety Labs he held positions as Laboratory Manager of Toxicology at Biosearch, Inc., where he was responsible for evaluations of cosmetics, pesticides and industrial chemicals. He has also held several positions at Huntindon Research Center. He is considered an expert in the evaluation of a wide variety of medical device materials. He is presently Senior Study Director at Product Safety Labs.

Gary Wnorowski, BA, MBA, received his BA in Biology from Rutgers University and has been employed in various positions at Product Safety Labs since that time. He is presently Laboratory Director. He has extensive experience in general toxicology, inhalation toxicology and aerosol research. He has recently received his MBA from Monmouth University.

Address correspondence to: Henry I. Jacoby, PhD, Discovery Research Consultants, 4119 Ocean Avenue, Brigantine, NJ 08203.

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related inhibitory effect was seen when doses of 1, 2 and 4 grams/kg were administered. Although the mechanism of inhibitory activity is unclear, powdered immune egg may be useful for the therapy of acute and chronic diarrheal or inflammatory diseases in animals and humans.
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INTRODUCTION

Diarrhea is a common condition, which at very least is life disrupting and can be life threatening. Acute diarrhea can be produced by a variety of pathological organisms, functional disruptions of intestinal function and as a drug-related side effect. Current treatment consists of antibiotic treatment of the causative organisms or pharmacological intervention in pathophysiologic function.

Antidiarrheal drugs reduce the symptoms of diarrhea (loose stool consistency, frequency of defecation and excessive stool weight) by action(s) on intestinal transit, mucosal transport or luminal contents.[1] Opioids such as loperamide are the most useful antidiarrheal agents and act by a combination of inhibition of intestinal transit, pro-absorptive and anti-secretory effects. Other useful pharmacological therapies include use of alpha-adrenergic agonists such as clonidine and somatostatin analogues. These drugs may modify mucosal transport in addition to slowing transit but have limited clinical utility due to nonspecificity of action. Adsorbents such as Kaopectate®, bismuth preparations such as Pepto-Bismol® and stool texture modifiers are used frequently as over the counter medication, but their efficacy, other than that of bismuth salts, is largely unproven.

A safe and effective anti-diarrheal agent would be very useful in therapy of acute and chronic diarrheal conditions. Immune egg (IE) powder is obtained from eggs produced by a special vaccination protocol using a multivalent vaccine of killed enteric human pathogens. The resulting immune eggs contain high concentrations of antibodies and immunomodulatory compounds that are passively transferred into the eggs. In pre-clinical studies immune eggs have been shown to have

anti-inflammatory benefit[2-5] and clinical effectiveness has been shown in inflammatory conditions.[6] Some of these studies, as well as anecdotal reports, suggest that immune egg products might be useful in preventing or treating acute and chronic diarrheal disease and may even be useful in inflammatory bowel disease.

The purpose of this study is to identify, characterize and document the preventive actions of immune egg products in an experimental model of diarrhea in mice. In this mouse model diarrhea is induced by oral administration of castor oil. This model is a standard test for anti-diarrheal agents for human use and was used for the discovery of the two most utilized anti-diarrheal agents, loperamide and diphenoxylate.[7]

MATERIALS AND METHODS

Male mice weighing 25-30 grams were obtained from Ace Laboratories (Boyertown, PA). They were quarantined for a minimum of five days and then randomized into groups of 10 and placed in plastic boxes with bedding. Treatments were all administered by gavage to fed mice on two consecutive days between the same hour. Mice were given 4.0 grams/kg p.o. of either powdered immune or powdered control egg in 20 mL/kg distilled water. Spray-dried immune egg powder (IE) was provided by DCV, Inc., Wilmington, DE. It was obtained from eggs of chickens that have been repeatedly vaccinated with large amounts of killed enteric pathogens of human origin. Spray-dried table eggs were used as the control.

One hour after treatment on day two of gavage, 0.3 mL of castor oil was administered by gavage. Mice were then placed in individual wire-bottomed cages and fecal output observed and recorded at 2, 4 and 6 hours after castor oil administration. A positive or negative response was used to assess the presence of diarrhea. The number of normal, soft or loose stools was also recorded. Stools were rated, either normal or diarrheal at each of these time periods. Results are expressed as the number of mice with diarrheal stools.

DATA ANALYSIS

Results were analyzed using an all or none criterion, with ano-genital staining and soft stools counting as diarrhea. Results are provided

as percentage of mice with diarrhea at each time and the cumulative number of positives over the 6 hour period. Percent inhibition was calculated by comparison to the appropriate control. Statistical analysis was done using the chi-square test.

RESULTS

Data are presented as percentage of mice showing diarrhea and as percent inhibition compared to control. The results from the initial study comparing immune egg to control egg are in Table 1. Immune egg produced a statistically significant inhibition of castor oil induced diarrhea. Four grams/kg of IE significantly blocked castor oil induced diarrhea at the 4 hour observation period when compared to normal egg. No diarrhea was noted at the 2 hour period in any group. A significant inhibition in the cumulative occurrence of diarrhea over the entire 6 hours was found in immune egg-treated group.

Three doses of IE were then compared to distilled water in the second study. All groups were dosed on two consecutive days. Results are presented in Table 2. There was a minimal occurrence of diarrhea noted in the first observation period. However, significant diarrhea

TABLE 1. Castor Oil Induced Diarrhea–Effect of Immune Egg Protein (IE) Compared to Control Egg

Treatment	Dose g/kg PO	# mice with diarrhea/# of mice tested 2 hr	%	4 hr	%	6 hr	%	0-6 hr	%	% Inh
Conrol Egg	4	0/10	0	9/10	90	3/10	30	12/30	40	—
IE	4	0/10	0	2/10*	20	2/10	20	4/30*	13	68

* Statistically significant difference from control egg group $p < 0.05$.

TABLE 2. Castor Oil Induced Diarrhea–Effect of Immune Egg Protein (IE) Compared to Vehicle

Treatment	Dose g/kg PO	# mice with diarrhea/# of mice tested 2 hr	%	4 hr	%	6 hr	%	0-6 hr	%	% Inh
Vehicle	0	1/10	10	10/10	100	10/10	100	21/30	70	—
IE	1	1/10	10	6/10	60	6/10	60	13/30	43	38
	2	1/10	10	7/10	70	4/10*	40	12/30	40	43
	4	0/10	0	4/10*	40	2/10*	20	8/30*	27	62

* Statistically significant difference from vehicle control $p < 0.05$.

was noted in the control treated mice at the 4 and 6 hour observation period. IE at 1 gram/kg did not show significant anti-diarrheal activity at any time period. IE, 2 grams/kg showed significant activity at the 6 hour period but not at 2 or the cumulative 0-6 hour period. IE, 4 grams per kg had significant effects at all time periods in which diarrhea was noted in the control period. Percent inhibition for castor oil diarrhea over the 0-6 hour period was dose related with 38% inhibition with 1 gram/kg, 43% with 2 grams/kg and 62% with 4 grams/kg. An estimated 50% effective dose would be between 2 and 4 grams/kg.

DISCUSSION

The mechanism of the diarrheogenic activity of castor oil is complex. Castor oil must first be metabolized to ricinoleic acid in the lumen of the intestinal tract. Ricinoleic acid then produces a marked increase in net secretion of fluid and electrolytes in the intestine resulting in diarrhea. The mechanism of action of castor oil has been studied recently by Mascolo and colleagues who found an activation of Platelet Activating Factor (PAF) in duodenal tissue by nitric oxide (NO) released in response to castor oil.[8]

Castor oil (2 ml/rat orally) increased PAF production in the rat duodenum 3 hours after challenge and NG-nitroarginine methyl ester (a non-selective inhibitor of nitric acid synthase), but not its D-isomer, enhanced the amount of PAF formed by duodenal tissue.[8,9] These effects were reduced by a PAF antagonist.[10] Mascolo and colleagues also suggests that castor oil-induced diarrhea in rats involves the L-arginine nitric oxide pathway.[8] This is further supported by the effect of isosorbide dinitrate and isosorbide-5-mononitrate. When administered to castor oil-treated rats, these two nitric oxide-generating agents prevented in a dose-dependent fashion the inhibitory effect of NG-nitroarginine methyl ester in a time course of PAF formation by gastrointestinal tissue in rats after castor oil challenge.[10] When castor oil was administered by gavage to rats, the duodenum and jejunum, but not the stomach, produced large amounts of platelet activating factor 3-7 hours after oil challenge with a peak at 3 hours.

Inhibition of castor oil induced diarrhea has been used as the animal model for the development of antidiarrheal agents such as diphenoxylate and loperamide and has been shown to be a good predictor of anti-diarrheal activity in humans.[7]

The present study indicates that acute dosing with egg obtained from chickens immunized against a variety of pathogens which include strains of *Escherichia coli, Shigella, Staphylococcus* and *Salmonella* can inhibit castor oil induced diarrhea. The mechanism by which it is acting is unclear at present, but could be related to interference with PAF formation or receptor interaction.

Immune egg has been shown to have anti-inflammatory activity in humans[6] and animals[2,3,5] can lessen the occurrence of indomethacin induced intestinal lesions,[4] and reduce cardiovascular risk.[11,12] All the pathways involved in these activities may have relationship to PAF levels. Although the underlying explanation for these events are not understood, there are clearly unique agents present in immune eggs that are not present in the same amounts in normal eggs.

There is a need for a safe effective anti-diarrheal and gastrointestinal anti-inflammatory agents. Diarrhea is common in cancer patients and may interfere with cancer treatment.[13] Chemotherapy, radiation therapy, surgery, graft-versus-host disease (GVHD), bone marrow transplantation, or infection may induce diarrhea. Bacterial pathogens may also produce diarrhea with a spectrum of effects ranging from severe tissue damage to a lack of perceptible damage. Enterotoxigenic *Escherichia coli,* which cause acute and severe diarrhea, does so by producing potent toxins, but these toxins act by altering the biological activity in epithelial cells.[14] This study shows that long periods of pretreatment are not needed for showing activity and that it is effective when given orally in a bolus dose. Thus it is possible that immune egg may be a safe and effective therapy for alleviation of acute or chronic diarrhea irrespective of cause and may be a valuable addition to therapy in inflammatory bowel diseases such as Crohn's disease and ulcerative colitis.

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