

Bacterial translocation in the rat model of lectin induced diarrhoea

R Shoda, D Mahalanabis, M A Wahed, M J Albert

Abstract

Red kidney beans were fed to weanling Long-Evans rats to cause diarrhoea (mean (SD) faecal wet weight: 2.66 (0.73) g/day in six rats fed beans *v* 1.12 (0.47) g/day in six control rats, $p < 0.01$) and increased faecal energy loss (4.87 (0.41) *v* 2.14 (0.23) kcal/day, $p < 0.01$). In addition, the rats fed beans had heavier small intestines (80.6 (4.6) *v* 51.9 (8.4) g/kg body weight, $p < 0.01$), heavier mesenteric lymph nodes (0.72 (0.27) *v* 0.08 (0.08) g/kg body weight, $p < 0.05$), and translocation of indigenous intestinal bacteria, *Citrobacter Spp* and *Escherichia coli*, to the mesenteric lymph nodes. (Translocation positive, that is, > 100 colonies per g of nodal tissue: 75% *v* 0%, $p < 0.005$.) These data suggest that diarrhoea induced by red kidney beans is a suitable model for studies of an important cause of persistent diarrhoea – that is, systemic complications. This rat model of lectin induced diarrhoea with translocation of intraluminal enteric bacteria into mesenteric lymph nodes should be useful in understanding the well known septicaemic complications associated with prolonged diarrhoea in infants and small children and in studies on factors that may modify or prevent bacterial translocation.

(Gut 1995; 36: 379–381)

Keywords: rat model, persistent diarrhoea, bacterial translocation.

Persistent diarrhoea in children is a serious health problem in developing countries and is associated with a high mortality.¹ It is frequently found with severe protein-energy malnutrition^{2,3} that requires prolonged empirical nutritional management.⁴ The pathogenesis of persistent diarrhoea has not been defined but it is believed to be multifactorial.⁵ In some cases intestinal bacterial overgrowth^{6,7} has been identified. Previously, Banwell *et al* observed⁸ that giving rats a diet rich in lectin caused a malabsorptive state secondary to intestinal bacterial overgrowth. We decided to explore further this animal model in the light of recent evidence linking persisting childhood diarrhoea to bacterial contamination of the upper intestine.⁶

Methods

Twelve weanling Long-Evans rats weighing 60–70 g and aged 28 days were used. We

modified the previously described experimental model, obtaining red kidney beans from a local market as a lectin source and basic rat chow from the Government Flour Mill (Dhaka, Bangladesh).

The beans were ground with chow mixed in a ratio of 40:60 as the study diet. For a control diet, ground red kidney beans were autoclaved at 120°C with 1.5 atmospheres for 20 minutes, a procedure known to abolish lectin activity.⁹ For both control and study diets, calorie densities were 4 kcal/g of diet and protein concentrations were 22–24% of total energy.

Rats were individually housed in metabolic cages and fasted for 24 hours before the experiment. A pilot study had shown that these rats ate less lectin-containing study diet than control diet. Therefore, six rats in the lectin fed group were pair fed with six controls for the experimental period. The first three days of the study were considered as an equilibrium period. From the fourth to the 10th experimental day, body weight and food consumption were measured, stool was weighed and collected for calorimetry, and faecal energy was measured by an automatic adiabatic bomb calorimeter (Autobomb/Gallenkamp, England).

After completing the 10 day metabolic experiment, rats were fasted for 18 hours. Under light ether anaesthesia, using sterile procedures, mesenteric lymph nodes were first collected through a mid-line incision. Then a 10 cm segment of the jejunum below the ligament of Treitz and a 10 cm segment of the ileum proximal to the ileocecal sphincter were collected from each rat. The lumen of each resected segment was rinsed gently with 20 ml of sterile PBS (pH 7.2) which was also collected. Mesenteric lymph nodes, rinsed jejunum and ileum, and PBS washouts were cultured for aerobic bacteria quantitatively using an established technique.¹⁰ Liver, spleen, total small intestine, and mesenteric lymph nodes were weighed and the wet weight of these organs was expressed in g/kg of body weight.

Each variable in the lectin fed group was compared with that in the control group by Student's *t* test. The proportion of lymph nodes from which positive bacterial cultures were obtained were analysed by Fisher's exact test.

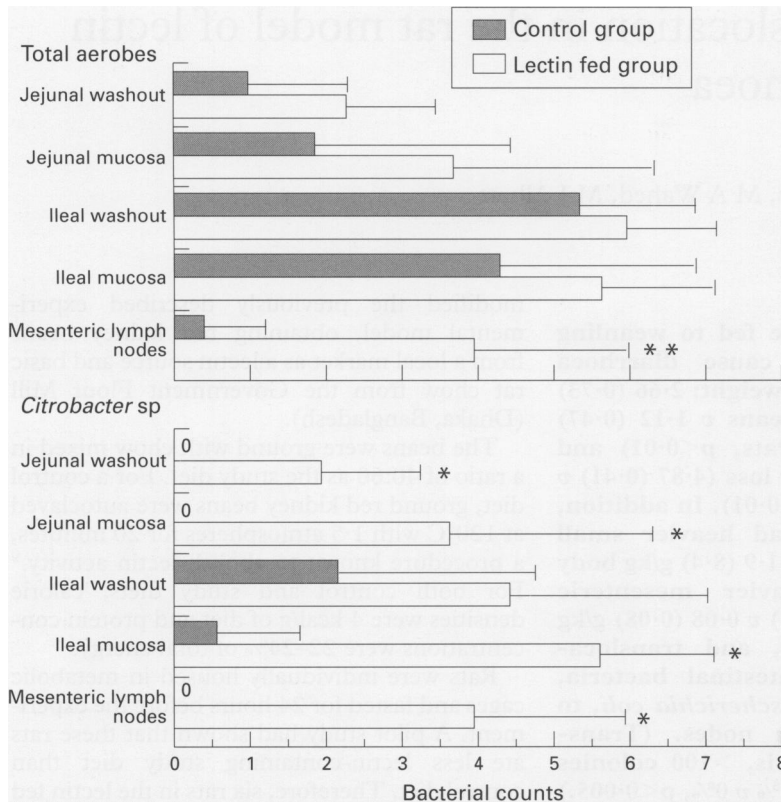
Results

All values are mean (SD) unless otherwise stated. Both the lectin fed and the pair fed control rats lost body weight of a similar magnitude (–13.5 (5.0) *v* –13.4 (4.4) g/10

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Accepted for publication
15 July 1994



Bacterial colony counts in lectin fed (n=4) and control rats (n=5). Numbers of aerobic bacterial colonies cultured in each material are expressed as mean (SD) in logarithm (lg or /ml). Values in the lectin fed and control groups are compared by Student's t test. Two rats in the lectin fed group and one in the control group died on the last experimental day. *p<0.01, **p<0.05.

days, NS) and consumed similar amounts of food (2.7 (1.5) v 2.7 (1.5) g/day/rat). Rats in the lectin fed group had a significantly (p<0.01) heavier total faecal wet weight (2.66 (0.73) v 1.12 (0.47) g/day) and dry faecal weight (1.07 (0.10) v 0.49 (0.05) g/day) than control rats. Daily energy loss in faeces was also significantly (p<0.01) higher in the lectin fed group (4.87 (0.41) kcal/day) than in the control group (2.14 (0.23) kcal/day). In the lectin fed group, stool consistency was soft or mushy in contrast to near pellets in the control group. Livers (37.7 (6.6) v 32.0 (5.1) g/kg body weight) and spleens (1.73 (0.37) v 2.26 (0.53) g/kg body weight) from the lectin fed group were not significantly heavier than controls. The wet weight of the small intestine was greater (p<0.01) in the lectin fed group (80.6 (4.6) v 51.9 (8.4) g/kg body weight). Mesenteric lymph nodes were also heavier in the lectin fed group (0.72 (0.27) v 0.08 (0.08) g/kg body weight, p<0.05).

Both mucosal specimens and luminal lavage fluid contained higher mean bacterial counts in the jejunum and ileum of the lectin fed group than the controls, but the difference did not

Proportion of rats with positive bacterial culture* in the mesenteric lymph nodes

	Lectin fed group	Control group	p value†
All aerobes	75% (3/4)	0% (0/5)	<0.05
<i>Escherichia coli</i>	25% (1/4)	0% (0/5)	NS
<i>Citrobacter freundii</i>	75% (3/4)	0% (0/5)	<0.05

*More than 100 colony forming units/g nodal tissue. †Fisher's exact, two tailed.

reach statistical significance (Figure, Table). Mesenteric lymph nodes from the lectin fed group, however, had substantially larger numbers of viable bacteria than did the controls.

No statistical differences in *Escherichia coli* counts were noted in jejunal and ileal mucosa and washouts from the study group and control specimens but culture positivity of the mesenteric lymph nodes tended to be higher in the lectin fed group than in the control group. Jejunal mucosa and washout and ileal mucosa in the lectin fed group had significantly (p<0.01) higher numbers of *Citrobacter* Spp than did controls. Counts of this species in the mesenteric lymph nodes of the lectin fed group were significantly (p<0.05) higher than those of the control group (3.91 (2.02) v 0 colony counts in logarithm/g of wet tissue).

Discussion

The limitations of extrapolating data from animal models to human disease should be considered in interpreting these results. In the present study, lectin in locally grown red kidney beans (phytohemagglutinin) induced diarrhoea, as evidenced by a significantly heavier faecal weight with an appreciable change in stool consistency, in Long-Evans rats. An experimental period of 10 days was chosen, considering the life span of rats, as against two weeks, which is the minimum duration used to define persistent diarrhoea in children.¹ The diarrhoea was associated with intraluminal bacterial overgrowth and increased bacterial counts associated with the intestinal mucosa of certain indigenous intestinal bacteria. The rats fed raw beans also lost more faecal energy, which suggests malabsorption. These results are consistent with previously published data.⁸ In spite of excessive faecal energy losses, the study group did not lose more body weight than controls, however, perhaps because of their increased accumulation of intestinal content.

A major new finding was the translocation of indigenous intestinal bacteria to the mesenteric lymph nodes in the bean fed rats. This observation was not attributable to some unspecific response to dietary deprivation and weight loss since pair fed controls showed no such translocation. Presumably, bacterial translocation is an antecedent of septicaemia¹¹ attributed to faecal organisms. This process has been suspected in the septicaemia that complicates adult respiratory distress syndrome in patients with major burns, trauma,^{12 13} and hypovolaemic shock.¹⁴ Septicaemia is common too in patients with severe or prolonged diarrhoea¹⁵ and malnutrition.¹⁶ In the present study, *Citrobacter freundii* was the bacterium that translocated into the lymphatics. *Citrobacter* has been shown to produce heat stable enterotoxin which is very closely related to that of enterotoxigenic *E coli*,¹⁷ and has been reported to have a tendency to be associated with bacteraemia.¹⁸ Over the past five years, six cases of *C freundii* bacteraemia have been found in this research centre. Three

were associated with other enteric bacteria suggesting translocation of indigenous intestinal flora into the systemic circulation (unpublished data).

Although animal models for watery and invasive diarrhoea have been used to develop therapeutic modalities,^{19 20} there have been no animal models for persistent diarrhoea in children, which still needs prolonged empirical nutritional management. Furthermore, because persistent diarrhoea is multifactorial a single animal model is unlikely to be suitable for studying all the causes. Our preliminary data suggest that diarrhoea induced by red kidney beans in rats may be a suitable model with which to pursue further studies of at least one important mechanism of persistent diarrhoea and its complications. Translocation of bacteria may be a good indicator of both intestinal mucosal damage and a predisposing factor for septicaemia.

This research was supported by the United States Agency for International Development (USAID) under grant no DPE-5986-A-00-1009-00) with the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B).

The ICDDR,B is supported by countries and agencies which share its concern for the health problems of developing countries. We thank all the staff at the animal house, biochemistry and nutrition laboratory and to Mr K Alam (Microbiology Lab) of the ICDDR,B for their excellent technical assistance; Dr J R Hamilton, McGill University, Canada for editing the manuscript. Dr Shoda would like to thank Drs T Wagatsuma, N Umeda, and D Habte for making it possible for him to work at the ICDDR,B.

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