Effect of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine

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See Commentaries pages 1314, 1315

Diets containing genetically modified (GM) potatoes expressing the lectin *Galanthus nivalis* agglutinin (GNA) had variable effects on different parts of the rat gastrointestinal tract. Some effects, such as the proliferation of the gastric mucosa, were mainly due to the expression of the GNA transgene. However, other parts of the construct or the genetic transformation (or both) could also have contributed to the overall biological effects of the GNA-GM potatoes, particularly on the small intestine and caecum.

Genetically modified (GM) plant products are becoming increasingly common in the human food-chain, yet in contrast to the general acceptance of the need for the biological testing of novel foods and feedstuffs, few studies have been carried out on the possible effects of GM products on the mammalian ut mucosa. GM potatoes expressing a snowdrop lectin (*Galanthus nivalis* agglutinin [GNA]) under the CaMV35s promoter have been developed to increase insect and nematode resistance.[1] GNA was selected for insertion into potatoes because the initial effect of this mannose-specific lectin on the rat small bowel has been shown to be minimal,[2] and because its binding to mannose present on the epithelial surface of rat jejunal villi is demonstrable only after feeding for 10 days. We compared the histological indices of the gut of rats fed potato diets containing GM potatoes, non-GM potatoes, or non-GM potatoes supplemented with GNA, to find out whether GNA gene insertion had affected the nutritional and physiological impact of potatoes on the mammalian gut.

ELISA analysis confirmed that the expression level of GNA in raw GM potatoes was 25·4 μg/g dry matter; the concentration was decreased to 4·9 μg/g after boiling for 1 h. Six rats were randomly allocated to each group, and were fed diets containing either raw or boiled GNA-GM potatoes, parent potatoes (Desiree), or parent-line potatoes supplemented with 25·4 μg/g GNA for 10 days. All potato diets were isocaloric and contained an average of 6% protein. Histological samples of stomach, jejunum, ileum, caecum, and colon were taken 10 days after the start of feeding. The samples, each 2 cm in length, were opened along the antimesenteric border. The serosal surface was allowed to adhere to card for 3 min and was then fixed in 10% neutral buffered formalin for 18 h at 20°C. Paraffin sections (4 μm) were stained with haematoxylin and eosin, and mucosal thickness (stomach) or crypt length (jejunum, ileum, caecum, and colon) was measured by video-image analysis. Intraepithelial lymphocytes are equally distributed in all parts of the small intestine, and are known to increase when non-specific intestinal damage occurs. Thus, to assess potential damage, intraepithelial lymphocytes were counted in eight jejunal villi from each of the six rats fed diets containing GNA-GM potatoes or parent potatoes, both raw and boiled. No such measurements were made for the group fed parent potatoes spiked with GNA because dietary GNA or other lectins do not induce lymphocyte infiltration. GNA binding to the jejunum and ileum was measured by elution with 0·1 mol/L mannose, followed by ELISA.

	Mean (SD) crypt length (μm) and difference between treatments*						Statistical analysis (p)†			Interaction (p)†	
	Parent	Parent *vs* parent+GNA (p)	Parent+GNA	Parent+GNA *vs* GNA-GM (p)	GNA-GM	Parent *vs* GNA-GM (p)	Effect of GNA	Effect of cooking	Effect of trans-formation	GNA×cook	Trans×cook
Stomach											
Boiled	294 (46)	0·29	347 (42)	0·37	339 (36)	0·02	0·001	0·052	0·868	0·917	0·543
Raw	261 (32)	0·03	312 (32)	0·98	323 (54)	0·07					
p	0·18		0·94		0·35						
Jejunum											
Boiled	75 (19)	0·72	78 (17)	0·97	78 (12)	0·71	0·029	0·171	0·041	0·035	0·037
Raw	57 (8)	0·14	64 (11)	0·01	90 (20)	<0·01					
p	0·06		0·09		0·24						
Ileum											
Boiled	59 (8)	0·20	55 (7)	0·12	63 (13)	0·43	0·221	0·001	0·106	0·209	0·942
Raw	71 (9)	0·24	79 (13)	0·43	87 (25)	0·15					
p	0·02		<0·01		0·06						
Caecum											
Boiled	95 (19)	0·90	98 (21)	0·04	70 (15)	0·05	0·033	0·001	0·566	0·497	0·021
Raw	132 (19)	0·02	104 (17)	0·25	119 (25)	0·35					
p	<0·01		0·55		<0·01						
Colon											
Boiled	146 (15)	0·02	177 (24)	0·02	139 (24)	0·65	0·878	0·002	0·181	0·231	0·001
Raw	192 (34)	0·04	148 (25)	<0·01	215 (34)	0·28					
p	0·02		0·07		<0·01						

Data are the means of six animals calculated from five observations for each. GNA×cook=interaction between GNA and cooking; Trans×cook=interaction between transformation and cooking.
* By Student's *t* test. †By multivariate analysis with Tukey's test.

Table 1: **Effect of raw and cooked parent, parent+GNA, and GNA+GM potatoes on histological indices of rat gut**

	Raw potato		Boiled potato	
	Parent+GNA	GNA-GM	Parent+GNA	GNA-GM
GNA intake (μg)	30	29	15	5·6
Mean (SD) bound GNA (μg)				
Jejunum	0·47 (0·28)	0·37 (0·27)	0·25 (0·21)	0·05 (0·04)
Ileum	0·28 (0·15)	0·44 (0·25)	0·17 (0·08)	0·07 (0·02)
Remainder	5·04 (2·67)	2·23 (0·63)	0·78 (0·35)	0·20 (0·17)
Total	5·79 (2·71)	3·04 (0·60)	1·20 (0·49)	0·32 (0·17)

On the morning of day 10, rats were given 1·5 g allocated diet and were killed 2 h later. After dissection, oesophagus, pylorus, and ileocaecal junction were clipped, and small intestine was washed thoroughly with saline. Small intestine was cut into three segments: jejunum (first 20 cm), ileum (last 20 cm), and remainder. Tissues were homogenised with phosphate-buffered saline containing 0·1 mol/L mannose, and solutions were used for determination of GNA content by competitive ELISA.

Table 2: **GNA binding to the jejunum and ileum of rats given diets containing GNA-GM potatoes or parent potato diets spiked with GNA**

The presence of GNA in the diets, irrespective of whether originating from GNA-GM potatoes or from parent-potato diets supplemented with GNA, was associated with significantly greater mucosal thickness of the stomach when compared with parent-potato diets (table 1). This effect was observed with both raw and boiled potatoes. Crypt length in the jejunum of rats fed on raw GNA-GM potato diets was significantly greater than in those given parent-line or parent-line plus GNA potato diets. However, the increase in jejunal crypt length was not seen in rats fed boiled GNA-GM potatoes (table 1). GNA had no significant effects on the ileum, but rats fed boiled potatoes had shorter ileal crypts than rats given respective raw potato diets. Rats fed boiled GNA-GM potatoes had significantly thinner caecal mucosae than rats given boiled parent potatoes, with or without GNA supplementation (table 1). Intraepithelial lymphocyte counts per 48 villi were 7·6 (SD 2·7) in rats fed on boiled parent potatoes, compared with 10·3 (3·3) in rats fed boiled transgenic potatoes ($p<0·01$). With raw potato diets, the intraepithelial lymphocyte counts were again significantly different: 5·3 (2·0) and 9·3 (2·6) in parent and GM potatoes, respectively ($p<0·01$). Peyer's patches appeared normal in all rats. GNA binding in the jejunum and ileum was about the same, irrespective of whether spiked GNA potatoes or GM potatoes were fed (table 2). Measurement of GNA binding by immunocytochemistry also showed a similar pattern.[2]

We suggest that the promotion of jejunal growth was the result of the transformation of the potato with the GNA gene, since the jejunum of rats was shown to be stimulated only by GM potatoes but not by dietary GNA (table 1), in agreement with a previous study in which the dietary GNA concentration was 1000-fold higher than the one used in this study.[2] Thus, we propose that the unexpected proliferative effect was caused by either the expression of other genes of the construct, or by some form of positioning effect in the potato genome caused by GNA gene insertion. Because caecal thickness was similar in rats given boiled parent potatoes in the presence or absence of spiked GNA, we suggest that the decrease in caecal mucosal thickness seen in rats fed boiled GM-potato diets was the consequence of the transfer of the GNA gene into the potato. Caecal mucosal thickness in rats given raw potato diets was significantly higher than in those given the corresponding boiled potatoes. Thus, the main effect of boiling was to decrease mucosal thickness; this binding was fully in line with expectations. The raw parent-line potato diets supplemented with GNA were associated with a significantly thinner caecal mucosa than that of rats given parent-line potato diets. A similar trend was also observed in rats fed raw GNA-GM potatoes, but the difference did not reach significance (table 1).

As expected, colonic crypt lengths were generally higher in rats given raw potato diets than in those given boiled potatoes, except for animals fed GNA-supplemented raw or boiled potato diets, between which there was no significant difference. Feeding rats on diets containing GM potatoes, irrespective of whether raw or boiled, had no significant effect on colonic crypt length compared with that in animals fed the corresponding parent-line potatoes (table 1). Rats fed on GNA-supplemented parent potatoes had significantly shorter colonic crypt lengths than those fed on parent potatoes of GNA-GM potatoes; the reason for this finding is not clear.

In conclusion, the stimulatory effect of GNA-GM potatoes on the stomach was mainly due to the expression of the GNA transgene in the potato. By contrast, the potent proliferative effect of raw GNA-GM potatoes on the jejunum, and the antiproliferative effect of boiled transgenic potatoes on the caecum can be attributed only partly to GNA gene expression. Other parts of the GM construct, or the transformation, could have contributed to the overall effects. Once bound, GNA is internalised by endocytosis;[2] some other component of the construct in the GNA-GM potato or its expressed gene product might also be able to penetrate and affect the rat mucosal cells in a similar manner. The growth-promoting effect of raw GNA-GM potatoes in the jejunum, evident as crypt hyperplasia, is probably due to a direct stimulatory effect on crypt cells; the increase in T lymphocyte infiltration may be important in the elimination of damaged enterocytes.[3] The possibility that a plant vector in common use in some GM plants can affect the mucosa of the gastrointestinal tract and exert powerful biological effects may also apply to GM plants containing similar constructs, particularly those containing lectins, such as soya beans or any plants expressing lectin genes or transgenes.

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Differential binding of the insecticidal lectin GNA to human blood cells

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Evidence of snowdrop lectin binding to human white cells supports the need for greater understanding of the possible health consequences of incorporating plant lectins into the food chain.

There is interest in the possible use of lectins to protect food plants from attack by insects. Many of these carbohydrate-binding proteins agglutinate vertebrate red blood cells. The lectin peanut agglutinin (PNA) also binds to the Thomsen-Friedenreich antigen on the surfaces of some human colon cells. After eating peanuts, PNA has been detected in the