Polyphenols Reduce Gastritis Induced by *Helicobacter pylori* Infection or VacA Toxin Administration in Mice

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Received 11 August 2005/Returned for modification 30 December 2005/Accepted 12 April 2006

***Helicobacter pylori* colonizes the human gastric mucosa, causing inflammation that leads to atrophic gastritis, and it can cause peptic ulcer and gastric cancer. We show that polyphenol administration to mice experimentally infected by *H. pylori* or treated with VacA toxin can limit gastric epithelium damage, an effect that may be linked to VacA inhibition.**

Helicobacter pylori chronically infects the gastric mucosa of >50% of the human population, causing gastritis. The infection can lead to the development of peptic ulcer (27) and gastric mucosa-associated lymphoid tissue lymphoma (6) and increases the risk of gastric cancer in humans (8, 11, 31). It has been proven that *H. pylori* infection can cause gastric cancer in animals (9, 33). In both humans and animals, the gastric pathology depends on the virulence of the *H. pylori* infecting strain and on the genetic background of the host (4, 10, 22). In humans, lifestyle is also relevant (13, 26). The current antibiotic-based therapies are generally effective but can fail due to antibiotic resistance or lack of patient compliance. Thus, there is a continuous effort to develop new tools to fight against this pathogen. In particular, plant extracts or plant-derived substances have been investigated for anti-*H. pylori* activity in vitro (1, 2, 14, 15, 18, 20, 24) and in vivo (12, 19, 28).

H. pylori expresses several factors that allow host stomach colonization and can play a role in pathogenesis. One of the most important factors is the vacuolating cytotoxin VacA (3, 29). We have already reported that polyphenols inhibit VacA-induced ion/urea conduction and cell vacuolation (30). Also, we have observed the ability of dealcoholized wine and green tea to reduce gastritis in mice experimentally infected by *H. pylori* (unpublished data). Hop bract extract has been recently reported to exert anti-VacA activity (35). Here, we investigated whether pure polyphenols could influence gastric colonization or gastritis in mice and/or counteract the effects of VacA in vivo.

Animal experiments were done in compliance with current law. Data were evaluated by one-tailed Mann-Whitney U test, with *P* values of <0.05 considered significant.

H. pylori strain SPM 326 type I, expressing the s1/m1 VacA isoform, was used to intragastrically infect specific-pathogen-free CD1 mice (Charles River, Calco, Italy), a well-established *H. pylori* infection model (17, 32). Noninfected controls received saline only. Starting 24 h before the infection, the animals had free access to drinking water containing 1% glucose

and a mixture of 1.5 mg/ml each of tannic acid (average molecular weight, 1,701.20) and *n*-propyl gallate (Sigma-Aldrich, Milan, Italy) (TA+NPG). TA and NPG were selected because of their previously shown anti-VacA activity in vitro (30), their water solubility, and the relative abundance of tannins and gallates in common beverages, such as wine and tea. Also, TA is a GRAS (generally regarded as safe) food additive. Based on previous results obtained with wine (unpublished data), the polyphenol concentration chosen was close to that of the total extractable polyphenols from red wine, which can reach 2 to 3 mg/ml. Infected controls received 1% glucose. The daily water intake was about 5 ml/mouse.

Two, 4, and 8 weeks after infection, the animals were euthanized, and their stomachs were removed and cut along the greater and lesser curvature, obtaining two equivalent parts. The gastric mucosa was scraped from each half-stomach and layered on selective plates, and *H. pylori* CFU were counted after culturing. Polyphenol-treated mice exhibited bacterial colonization only marginally lower than infected controls (Table 1). Thus, in our model, TA+NPG was unable to significantly reduce the infection, although the possibility that it could show greater efficacy under different experimental conditions cannot be excluded. The remaining half-stomachs were formalin fixed and paraffin embedded, and 4-μm-thick sections were stained with hematoxylin-eosin (HE) and observed under light microscopy. Antrum, corpus, and fundus gastritis was blindly graded in each sample according to the updated Sydney system (5) and scored from 0 to 3, corresponding to no, mild, moderate, or severe gastritis, respectively. Gastric inflammation was found mainly in the corpus and fundus. TA+NPG-treated mice showed gastritis scores significantly lower than those of the corresponding infected controls at each time point

TABLE 1. Effect of polyphenols on *H. pylori* gastric colonization

Wk	No. of animals per group	Geometric mean CFU/stomach for group indicated		<i>P</i> value
		TA+NPG	Infected control	
2	6	1.0×10^3	1.6×10^3	0.24
4	12	1.5×10^3	1.8×10^3	0.49
8	12	2.9×10^3	4.6×10^3	0.33

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FIG. 1. Gastritis score of (A) *H. pylori*-infected or (B) *VacA*-treated mice with or without TA+NPG treatment as indicated. Mean of antrum, corpus, and fundus scores of each group. Data at the 2-week time point resulted from two independent experiments. *, $P < 0.01$. Bars, 1 standard error.

(Fig. 1A). When the localization of *H. pylori* was assessed for gastric sections by immunohistochemistry using a mouse monoclonal antibody to VacA (23), immunostaining was evident mainly in the corpus and fundus of infected controls and was generally associated with typical *H. pylori* morphology. TA+NPG-treated mice showed slightly weaker immunostaining, with similar localization (not shown).

The observation that polyphenol treatment did not significantly affect bacterial colonization while significantly decreasing gastritis suggested that these effects may be linked to VacA inhibition. We tested this hypothesis by administering native s1/m1-type VacA, purified as previously described (16, 21), to BALB/c mice (Charles River). While stable colonization by SPM326 is difficult to achieve for this mouse strain (32), treatment with VacA has been described to produce in a short time evident damage of the gastric epithelium (29). On day 0, mice started to drink the TA+NPG mixture (1.5 mg/ml each) or 1% glucose, and then, on days 1 and 3, they received intragastrically 50 μ g of VacA, a dose previously selected to produce statistically significant gastric damage. Mice were sacrificed on day 5. Gastritis was evaluated with HE-stained gastric sections as described above. VacA-treated mice presented the expected gastric inflammation, mainly in the corpus and fundus, while treatment with TA+NPG significantly reduced gastritis to the extent that results were almost indistinguishable from those for the control group, which received saline (Fig. 1B and 2).

Remarkably, although TA+NPG significantly reduced gastritis in both *H. pylori*-infected and VacA-treated mice, the effect seemed more evident in the latter case (compare Fig. 1 A and B). Indeed, upon *H. pylori* infection, bacterial factors other than VacA contribute to the gastric inflammation, which VacA inhibitors can therefore counteract only partially; conversely, upon VacA treatment, the maximum effect of VacA inhibitors is, obviously, expected.

The hypothesis that VacA is the predominant in vivo target of TA+NPG can explain the failure of these compounds to significantly alter *H. pylori* colonization in mice, given the abil-

FIG. 2. Representative images of HE-stained sections of gastric corpus mucosa of VacA-treated mice in the presence (a, b) or in the absence (c, d) of TA+NPG, in comparison with saline-treated controls (e, f). VacA controls show a typical loss of mucosal architecture, with areas of cellular debris and inflammatory cells admixture (arrowheads); these alterations are practically undetectable in the presence of TA+NPG treatment. Bars, 50 μ m.

ity of VacA-deficient strains to experimentally infect mammals (7, 34), albeit at a higher infecting dose than Vac-positive strains (25). VacA thus emerges as one of the possible molecular targets for the suggested protective effects of plant-derived foods and beverages on the gastric mucosa of *H. pylori*-infected individuals.

In conclusion, we have shown that, in mice, a mixture of tannic acid and *n*-propyl gallate can limit the gastric mucosa deterioration induced by *H. pylori* infection or VacA administration, suggesting that VacA inhibition plays a role in this protective activity. Thus, it could be realistically proposed that polyphenols or polyphenol-rich beverages and foods may contribute to limit some of the aspects of the pathological outcome of *H. pylori* infection.

The possible relevance to humans of the protective activity exerted by polyphenols ought to be further investigated.

F.T. was supported by a University of Padova Young Investigator Grant.

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