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Large molecules as anti-adhesive compounds against pathogens

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Abstract

Anti-adhesive compounds are potential prophylactic tools in alternative treatment regimes against bacterial infection, as bacterial adhesion is commonly mediated by carbohydrate–protein interactions between surface adhesions of microorganisms and the host cell. The use of exogenous polyvalent, high-molecular carbohydrates and tannin-like plant-derived compounds should antagonize the adhesive interaction. A range of carbohydrates and carbohydrate- and proanthocyanidin-enriched plant extracts were screened for potential anti-adhesive effects against *Helicobacter pylori, Campylobacter jejuni, Porphyromonas gingivalis* and *Candida albicans* in different in-situ assays on primary tissue. The adhesion of *H. pylori* on human stomach tissue was effectively blocked by glucuronic acid-enriched polysaccharides from immature okra fruits (*Abelmoschus esculentus*). These compounds also had strong in-vitro effects against *C. jejuni* (inhibition up to 80%), but were ineffective in an in-vivo study in infected chicken broilers due to metabolism in the gastrointestinal system. Polysaccharides from *Glycyrrhizia glabra*, also enriched with glucuronic acid, showed strong anti-adhesive properties against *H. pylori* and *P. gingivalis* (inhibition 60–70%). *Pelargonium sidoides* extract, containing mainly polymeric proanthocyanidins, was effective against *H. pylori* in a dose-dependent manner. Due to the multifunctional adhesive strategy of *C. albicans*, no effective compounds were detected against this yeast. Structure–activity relationships are presented and the potential in-vivo use of carbohydrate-based anti-adhesives is discussed.

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Introduction

Treatment of bacterial infections in humans with antibiotic regimens is getting more and more problematic: the development and uncritical use of highly specific antimicrobial agents has dramatically increased the emergence and spread of multi-drug resistant bacteria and other pathogens. Antibiotic resistance is responsible for up to 60% of hospital-acquired infections in the USA (World Health Organization 2006). The screening and development of plant-derived antibiotics is increasing for several reasons. First, plant secondary products have in many cases been evolved as a sophisticated defence against microbials and these products therefore offer novel lead structures for further drug development. Second, the majority of plant defence mechanisms comprise mixtures of different classes of compounds acting simultaneously at different targets. Furthermore, understanding plant defence systems may lead to new strategies being devised for the human host. One of these principles is the blocking of host–parasite cell interactions by anti-adhesive compounds: many pathogens need a positive adhesion to host cells or host tissues as a pre-requisite for invasion and virulence. This adhesion is mediated by specific surface adhesins located on the outer cell wall or on fimbriae (Schmidt et al 2003). Interestingly, most of the receptor–ligand interactions necessary for a successful docking process are carbohydrate-mediated systems (Odenbreit 2005). *Helicobacter pylori* expresses several types of adhesins on its surface, each interacting with different classes of ligands. An extracellular isoform of the bacterial urease binds to acidic components of the mucins coating human gastric epithelial cells (Icatlo et al 1998, 2000), a second type interacts with tissue-associated sialic acids (e.g. Bennett & Roberts 2005), and a third type, a member of the family of heat-shock proteins, interacts with glycosphingolipids of the host tissue (e.g. Huesca et al 1996; Yamaguchi et al 1997). Protein–carbohydrate interactions are responsible for the adhesion of *H. pylori* to the

stomach. Only in the event of positive adhesion will symptomatic or asymptomatic infection occur. Non-adhesive bacteria can survive in the stomach, but without initiating virulence factors. From this point of view, the use of anti-adhesives based on carbohydrate-associated structures makes sense. Application of polyvalent carbohydrates (e.g. polysaccharides) would block the surface adhesins of *H. pylori*. The use of polyvalent and high-molecular sugar derivatives may also overcome the problems of in-vivo inefficacy, where low molecular carbohydrates show good in-vitro effects but no clinical efficacy in-vivo (a Phase II study using sialyllactose as an anti-adhesive compound against *H. pylori* failed to suppress or cure colonization in humans, in contrast to strong in-vitro anti-adhesive activity) (Parente et al 2003). A closely related bacterium with similar adhesion strategies is *Campylobacter* spp., increasingly recognized as a major source of food-borne enteritis in humans (Ketley 1997; Tauxe 2002; Samuel et al 2004). As *Campylobacter* strongly adheres to epithelial and connective tissue of the gastrointestinal system (Campbell et al 1987), the use of anti-adhesive compounds is increasingly being discussed, but so far no field data are available. Another microorganism using adhesion as the first step of infection is *Porphyromonas gingivalis*, a Gram-negative, anaerobic bacterium that is widely considered as a major pathogen in the development of destructive periodontal diseases such as chronic or aggressive periodontitis (Sandros et al 1993). The invasion and internalization of *P. gingivalis* into tissue starts with an adhesion to complementary surface factors of the target cells. Major adhesins located on the tip of its fimbriae attach the bacterial surface to epithelial cells. This binding can be inhibited by antibodies directed against fimbrial adhesion structures. Previous studies have clearly shown that the respective adhesins recognize glycosylated structures on the side of the host epithelium. Also, secreted gingipain peptides are found on the bacterial surface that have a strong capacity for adhesion (Chen & Duncan 2004). The fungus *Candida albicans* can only evoke clinical symptoms after adhering to surface receptors of the host cell, followed by absorption into tissue (for review see Hostetter 1994). Adhesion of *C. albicans* is much more complex than that of the aforementioned bacteria.

This study investigates the ability of natural products and plant-derived extracts to influence microbial adhesion. High molecular weight products, especially polysaccharides (pectin, arabinogalactans, rhamnogalacturonans, fucans, heparins, xyloglucan) were investigated for their potential to block carbohydrate-mediated cell–cell interactions.

Materials and Methods

General experimentation procedure

Analytical grade reagents and chemicals were purchased from Sigma (Deisendorf, Germany) unless stated otherwise; anti-fibronectin Ab-3 and mouse monoclonal antibodies were obtained from Calbiochem (Darmstadt, Germany). Histological sections of human stomach tissue were provided by Professor G. Faller, University of Erlangen-Nürnberg (Germany). Sugar beet pectin and chitopentose were donated from Südzucker AG (Obrigheim, Germany), sialyllactose and fucosyllactose from Milupa AG (Friedrichsdorf, Germany), extracts from Taraxacum, Equisetum, Rubus and Urtica were from Frutarom-Flachsmann (Wädenswil, Switzerland). Characterization: Taraxacum extract, extractant water, herb/extract ratio 6:1, flavonoids (high-performance liquid chromatography) calculated as hyperosid 0.64%, SiO_2 0.64%; Urticae root extract, solvent ethanol 20% (v/v), herb/extract ratio 6:1; Rubi idaei extract, extractant water, herb/extract ratio 3:1. *Pelargonium sidoides* extract EPs 7630 was from Iso AG (Ettlingen, Germany). Polysaccharides from Malva sp., *Fucus vesiculosus* and *Calendula officinalis* were isolated and characterized according to Schmidgall et al (2000).

Culture conditions

H. pylori type I, strain G27, was kindly provided by Dr Beier (Lehrstuhl für Microbiologie, University of Würzburg, Germany) and cultured on Columbia agar base (Oxoid, Hampshire, UK) supplemented with 5% lysed horse blood (Oxoid) and *H. pylori* antibiotic selective supplement (Oxoid). *Campylobacter jejuni*, Vermicon 1, isolated from animal excrement, was provided by Schweizerisches Bundsamt für Veterinärmedizin BVET (Swiss Federal Veterinary Council), Bern, Switzerland. Bacteria were grown on Columbia agar base (Oxoid), supplemented with 5% lysed horse blood (Oxoid) and *Campylobacter* selective supplement (Oxoid). Plates were incubated for 48 h at 37°C under microaerophilic conditions in anaerobic jars with CampyPak (BBL, Becton-Dickinson, USA).

P. gingivalis, strain P4, was donated by Dr Beikler, University of Washington, Seattle (USA). Identification by polymerase chain reaction analysis was ensured prior to the experiments. Cultivation was in anaerobic jars with Anaero-Gen (Oxoid) for 7 days, 37°C. Medium: 15 g tryptic pepton, 5 g neutralized soy pepton, 5 g NaCl, 5 g yeast extract, 16 g agar, 0.5 g cysteine, 1 mL 1% vitamin K, 10 mL haemin solution (0.05%), sheep blood 50 mL, water 1 L.

Candida albicans, strain SC5314, was donated by Professor Ernst (University of Düsseldorf, Germany) and pre-cultivated on yeast extract/peptone/dextrose (YPD) agar plates at 30°C for 24–48 h, mass-cultivation in YPD liquid medium: pre-cultures with 5 mL volume until optical density at 600 nm of 0.1, then the inoculum was brought to the main culture. Cultivation was for 4 h until 10^6 cells mL^{-1}.

Adhesion test assay

Adhesion and anti-adhesion tests were performed according to Lengsfeld et al (2004a, b). Briefly, fluorescein isothiocyanate-labelled bacteria or yeast were pre-incubated with the test compounds (1 mg mL^{-1} unless otherwise stated). Deparaffinated tissue sections (obtained from animal organs, e.g. chicken colon, rat oesophagus, rat stomach as well as from human resections e.g. stomach tissues) were incubated with the bacteria at room temperature. Microorganisms adhering to epithelial tissue were counted under blinded conditions using fluorescent microscopy and evaluated against non-treated controls. The adhesion rate of the untreated control specimen (negative control) was considered as 100% adhesion and was expressed as +++++. Lower adhesion rates were indicated by

++++, +++, ++ and +, with complete absence of adhesion being expressed as –, as indicated by the positive control, sialyllactose. Results are the mean of three independent experiments. The Kruskal–Wallis test (non-parametric) was used for evaluation of test results using the SSPS program (SPSS Inc., Chicago, IL, USA). Test results were only analysed statistically if the adhesion score was better than +++.

Fluorescent area intensity was calculated by ImageJ (public domain software, NIH, USA), standardizing the fluorescent area of the negative control as 100%.

To exclude non-specific cell toxicity of test compounds against bacteria, a disk diffusion test was performed at 2.5 mg mL^{-1} of test compounds using BD Sensi-Disks (Becton-Dickinson, Heidelberg, Germany) and placed on agar plates.

Results

Adhesive and anti-adhesive compounds against *H. pylori*

To develop a model permitting the study of potential anti-adhesive test compounds, the adhesion of *H. pylori* against human stomach epithelia was investigated. This test system quantifies the specific adhesion of fluorescein isothiocyanate-labelled *H. pylori*, a microorganism with a strong adhesive tendency towards human stomach epithelia. Sections from human stomach were incubated with the fluorescein isothiocyanate-labelled *H. pylori* and the epithelial adhesion evaluated after removal of non-adherent bacteria by thorough washing. Bacteria not pre-treated with test compounds served as a negative control, indicating the maximal adhesion level, while sialyllactose, a powerful inhibitor of *H. pylori* adhesion, was used as a positive control (Falk et al 1993). In a broad screening, plant-derived extracts, polysaccharide-enriched extracts, as well as various oligosaccharides and polysaccharides were tested. Table 1 summarizes the results. Although most test compounds were inactive, some anti-adhesive preparations and compounds that increased adhesion (e.g. zinc salts) were identified. High anti-adhesive activity (dose-dependent) was shown by polysaccharides from immature okra fruits (*Abelmoschus esculentus*), blackcurrant seeds (*Ribes nigrum)* (Lengsfeld et al 2004a, b) and from liquorice, the root of *Glyccyhriza glabra*. In addition to polysaccharide-enriched extracts, the root extracts from *P. sidoides* were also found to have anti-adhesive activity. These root extracts have been used therapeutically for the treatment of respiratory diseases. The main constituents of this extract are polymeric proanthocyanidins that exhibit slight astringent effects, probably responsible for the interaction with surface adhesins (Kayser & Kolodziej 1997).

Figure 1 shows representative fluorescent microphotographs taken from human stomach mucosa after incubation. The effect of pre-treatment of *H. pylori* with liquorice polysaccharides and *P. sidoides* extract was examined microscopically with regard to adhesion to the human stomach mucosa (Figure 1). To exclude non-specific cell toxicity of the test compounds against *H. pylori*, a disk diffusion test was performed with the active test compounds and extracts over the same concentration range used for the adhesion assays (data not shown). None of the compounds exhibited bacteriostatic or bactericidal properties, indicating that the anti-adhesive effects observed were due to a reduced binding of the test organisms and not due to increased lethality.

Adhesive and anti-adhesive effects against *C. jejuni*

Campylobacter spp. is mainly transferred from alternate hosts (e.g. chicken) to human intestinal tissue. The removal of *C. jejuni* from the alternate host by anti-adhesive agents is therefore desirable. Investigations were performed with histological sections from tissues of different functional regions in the gastrointestinal tract, taken from freshly killed *Campylobacter*-free chicken. Adhesion rates varied between different intestinal sections: only basal adhesion was seen on gastric tissue, marginal adhesion was present in the duodenal section, but strong and stable adhesion occurred on sections from jejunum. *C. jejuni* had no affinity for tissue material from ileum and caecum, but bound strongly to colonic tissue (C. Lengsfeld, unpublished observations).

In contrast to the epithelia-specific adhesion of *H. pylori*, binding of *C. jejuni* was not limited to a distinct histological layer but was found at the mucous membranes as well as at the muscle and connective tissue layers (Figure 2). In addition to the affinity towards mucosal surface receptors, the adhesion of *C. jejuni* can probably also be mediated by collagen fibres within the connective tissue (Barot et al 1983; Campbell et al 1987). Only polysaccharides from *A. esculentus* inhibited the adhesion; other glycans from the range of compounds screened were found to be inactive (Table 2).

Adhesive and anti-adhesive effects against *P. gingivalis*

P. gingivalis is a major pathogen in the development of destructive periodontal diseases such as chronic or aggressive periodontitis, exhibiting strong adhesion to erythrocytes, epithelial cells and collagen fibres (Sandros et al 1993). The respective fimbrial adhesins are quite complex systems, derived mainly from the gingipain series that act as both adhesins and digestive proteases against the host cell. For adhesion tests, sections from rat oesophageal tissue were used to assess the adhesion of *P. gingivalis* directed against epithelial and connective tissue. Table 3 shows that liquorice root extract from *G. glabra* and okra polysaccharides from *A. esculentus* clearly inhibited *P. gingivalis* adhesion in a concentration-dependent manner (Table 3; Figure 3). The test compounds did not exhibit direct cytotoxic effects against *P. gingivalis* (data not shown). Figure 3 shows some examples of microphotographic determinations using okra polysaccharides, indicating strong anti-adhesive effects.

Adhesive and anti-adhesive effects against *C. albicans*

C. albicans adhesion is characterized as a multifunctional system with a variety of surface adhesins forming either lectin-like, (non-)covalent protein–protein or protein–lipid interactions with specific ligands on the host cell and several non-specific systems binding to collagen, laminin or fibronectin

Table 1 Effect of a 2-h pre-treatment with different test compounds on the adhesion of fluorescein isothiocyanate-labelled *Helicobacter pylori* to sections of human gastric mucosa

Compound	Reference or structural features	Origin	Test concentration (mg mL^{-1})	Adhesion of *H. pylori*	Remarks
Negative control				+++++	Maximum adhesion
Positive control (3′-sialylactose)	Defined structure	Milk	1	− ($P < 0.01$)	No adhesion
Okra aqueous extract	Lengsfeld et al (2004b) Acidic rhamnogalacturonan	*Abelmoschus esculentus*	1, 0.1, 0.01	+, ++, +++ (all $P < 0.01$)	Highly active
Blackcurrant seed polysaccharides	Lengsfeld et al (2004a) Arabinogalactan	*Ribes nigrum*	1, 0.1	++, +++ ($P < 0.01$)	Active
Sugar beet pectin	Hensel & Meier (1999) Schmidgall & Hensel (2002)	*Beta vulgaris*	1, 2	++++	Not active
Liquorice root polysaccharides	Wittschier et al (2006) Pectic polymers	*Glycyrrhiza glabra*	1, 0.1	++ ($P < 0.01$)	Active
Malva flowers polysaccharides	Schmidgall et al (2000) Pectic polymers	*Malva sylvestris*	1	+++++	Not active
Mistletoe polysaccharides	Edlund et al (2000) Arabinogalactans	*Viscum album*	1.5	+++++	Not active
Fucus polysaccharides	Schmidgall et al (2000) Fucan	*Fucus vesiculosus*	1	+++++	Not active
Kiwi fruit polysaccharides	Deters et al (2005a) Pectic acidic rhamnogalacturonan	*Actinida deliciosa*	1	++++	Not active
Kaki polysaccharides	Deters et al (2005b) Pectic acidic rhamnogalacturonan	*Diospyros kaki*	1	++++	Not active
Guar polysaccharides	Dea & Morrison (1975) Glucose/mannose 1:2	*Cyamopsis tetragonoloba*	1	+++++	Not active
Calendula flower polysaccharides	Schmidgall et al (2000) Fructan	*Calendula officinalis*	1	+++	Slightly active
Glucosamin pentamer $(GluNH_2)_5$	Defined structure	Deacetylated Chitopentose	1	+++++	Not active
Fucosyllactose	Defined structure		1	+++++	Not active
Fucoidan	Sulfated fucan	*Fucus vesiculosus*	1	++ ($P < 0.01$)	Active
Sialyllactotetrose	Defined structure	Human milk	1	+ ($P < 0.01$)	Highly active
Mucin, type II	Podolsky (1985)	Porcine	2	+++++	Not active
Mucin, type II	Podolsky (1985)	Porcine	2	+++	Slightly active
Fetuin	Defined structure	Beef	1	+++++	Not active
N-Acetylneuraminic acid	Defined structure		1	+++++	Not active
Heparin Na	Defined structure		346 U mL^{-1}	+++++	Not active
Heparin Ca	Defined structure		250 U mL^{-1}	+ (no statistics available)	Highly active
Transferrin	Defined structure	Human	1	+++++	Not active
Pelargonium sidoides extract EPs® 7630	Kolodziej (2000)	*Pelargonium sidoides*	10, 1, 0,1, 0.01	− ($P < 0.01$), + ($P < 0.05$), ++ ($P < 0.05$) +++ ($P < 0.05$)	Highly active
Zinc sulphate	Defined structure		2	++++++	Increased proliferation
Zinc histidine	Defined structure		1	++++++	Increased proliferation

P values are only given for significant measurements.

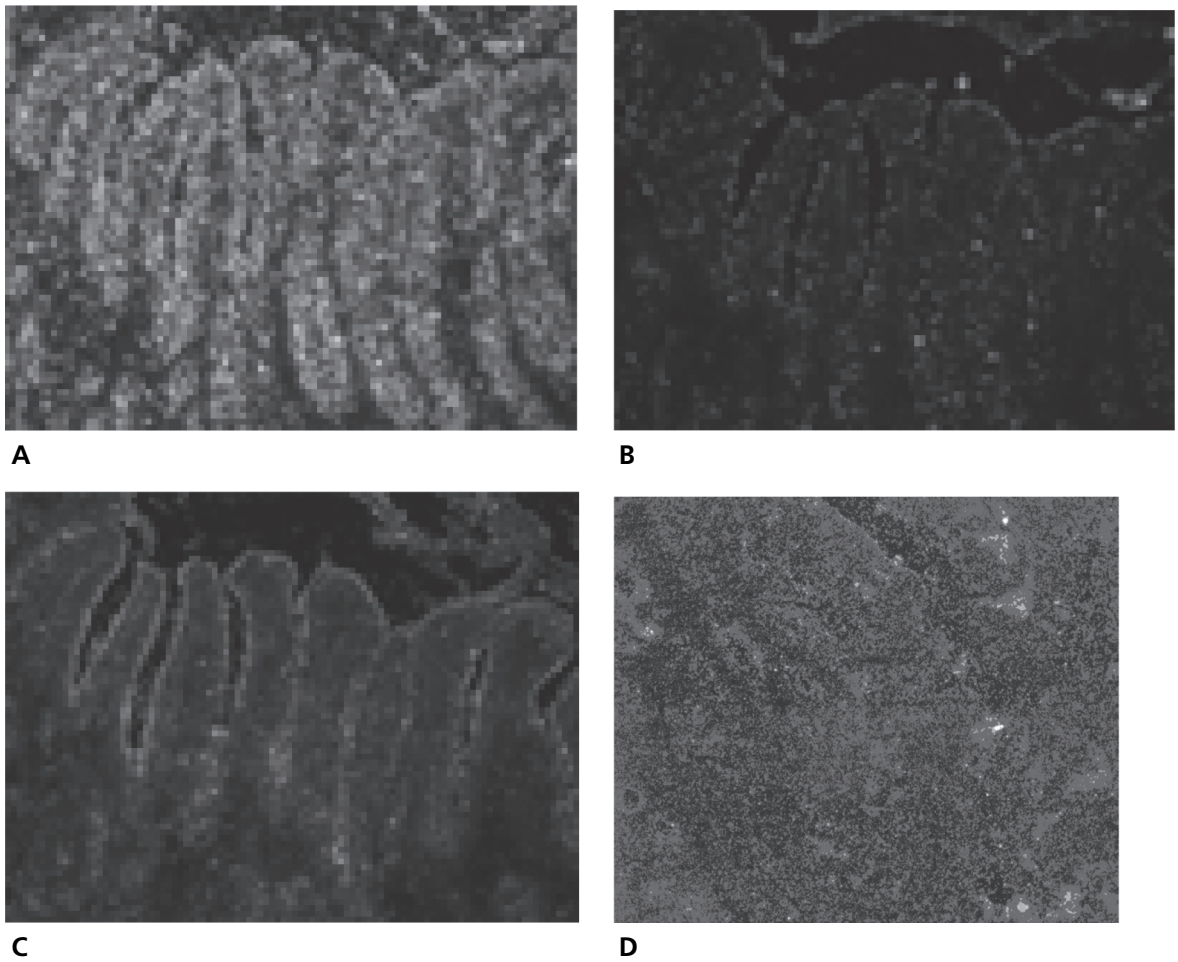

Figure 1 Fluorescent microscopy of representative in-situ experiments with fluorescein isothiocyanate-labelled *Helicobacter pylori* on histological sections of human gastric mucosa after incubation of tissue with labelled bacteria. Magnification ×200. A. complete adhesion (+++++) of non-treated bacteria (negative control), fluorescent area intensity standardized as 100%; B. positive control sialyllactose (–), fluorescent area intensity 0%; C. liquorice root polysaccharides (++), fluorescent area intensity 57%; D. *Pelargonium sidoides* root extract 1 mg mL^{-1} (+), fluorescent area intensity 33% (photographs are equalized for brightness).

(Chaffin 2002). This highly adaptable strategy enables *C. albicans* to adhere very effectively to many types of cell and tissue; adherence is also mediated by hydrophobic or electrostatic biofilm formation (e.g. Nikawa et al 1989; Klotz 1994; Masuoka & Hazen 1997). Tissue sections from different organs (human stomach, human buccal tissue, rat buccal tissue, rat vaginal tissue) were used for the development of an adhesion assay to determine *C. albicans* adhesion in the presence of different test compounds (Table 4). It was evident that the multifunctional adhesion strategy of *C. albicans* adhesion could not be blocked effectively by the carbohydrate- and polysaccharide-enriched extracts studied here. However, a moderate anti-adhesive effect was observed for purified galactomannan after pre-treatment of tissue sections instead of the fungal cells. On the contrary, pre-treatment of *Candida* with galactomannan did not inhibit its adhesion to the tissue sections. Apart from galactomannan, none of the polysaccharides tested exhibited any significant anti-adhesive effects against *C. albicans*. The blocking of hexose-containing structures (e.g. mannans) on the epithelial surface using concanavalin A (Jin et al 2005) inhibited *Candida* adhesion almost completely, but not in the case of the respective pre-treatment of fungal cells. This indicates the necessity of α-1,4-mannose residues on the surface of epithelial cells for adhesion of *C. albicans* to tissue (Sandin 1987).

To investigate the influence of plant extracts with ethnotraditional uses against skin diseases, several aqueous preparations from different plants were screened for anti-adhesive properties. An extract from raspberry leaves (*Rubus idaeus* L.) decreased adhesion against stomach tissue slightly (but not significantly) and may be worth considering as a candidate for further development.

Discussion

Adhesion to host cells and tissues is for many pathogens a pre-requisite for internalization and clinical infection.

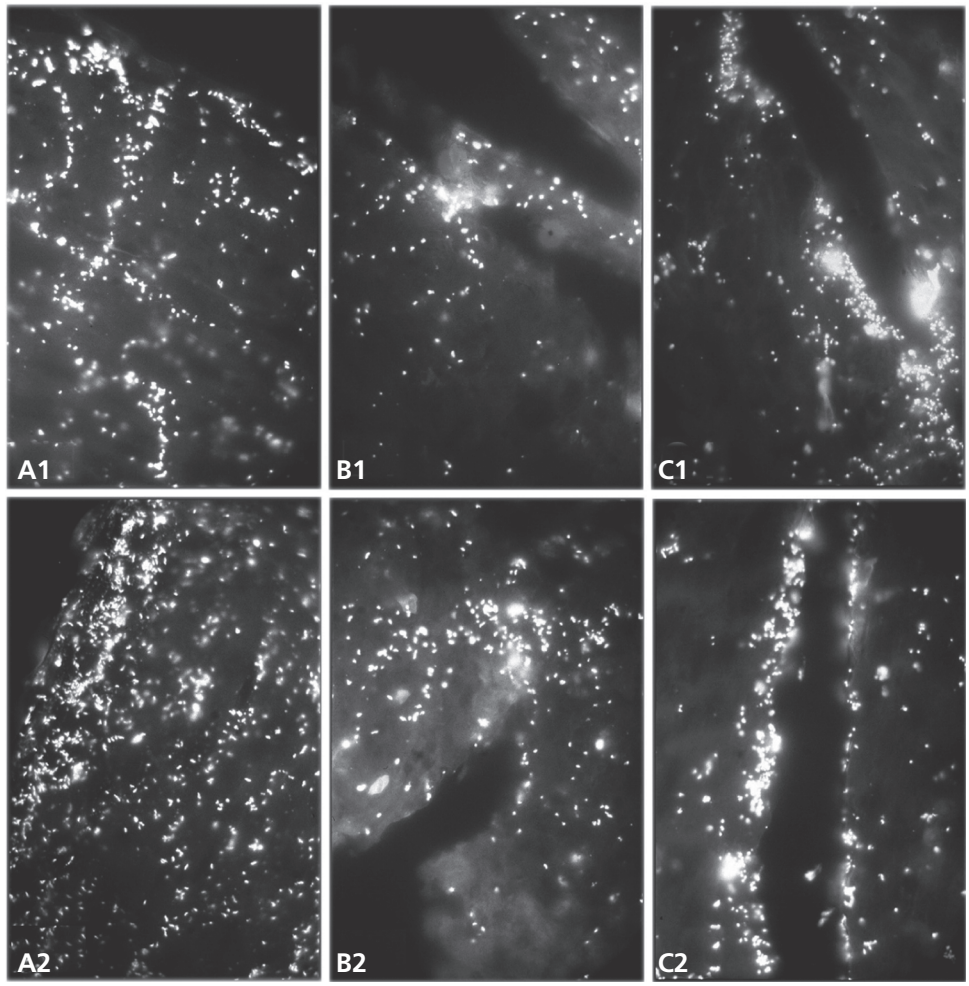

Figure 2 Fluorescent microscopy of representative in-situ experiments with fluorescein isothiocyanate-labelled *Campylobacter jejuni* on ileum and colon from chicken. Magnification ×200. A1and A2: connective tissue area from jejunum section; B1 and B2: mucosal tissue area from jejunum; C1and C2: mucosal tissue area from colon. Adhesion of fluorescein isothiocyanate-labelled *C. jejuni* is obvious for colonic and jejunum tissue.

Table 2 Effect of a 2-h pre-treatment with different test compounds on the adhesion of fluorescein isothiocyanate-labelled *Campylobacter jejuni* to sections of colonic mucosa from chicken

Compound	Reference or structural features	Origin	Test concentration (mg mL^{-1})	Result against *C. jejuni* adhesion	Remarks
Negative control				+++++	Maximum adhesion
Okra aqueous extract	Lengsfeld et al (2004b) Acidic rhamnogalacturonan	*Abelmoschus esculentus*	1	+ ($P < 0.01$)	Highly active
Blackcurrant seed polysaccharides	Lengsfeld et al (2004a) Arabinogalactan	*Ribes nigrum*	2	+++++	Not active
Sugar beet pectin	Hensel & Meier (1999) Schmidgall & Hensel (2002) Pectin	*Beta vulgaris*	2	+++++	Not active

P values are only given for significant measurements.

Although anti-adhesive drugs against bacterial diseases are not available on the pharmaceutical market, the use of anti-adhesive molecules may provide a powerful prophylatic tool against infections. Given the increasing bacterial resistance to antibiotics, preventing the invasion of microorganisms could avoid many clinical problems and reduce the cost of therapy.

Table 3 Effect of different test compounds on the adhesion of fluorescein isothiocyanate-labelled *Porphyromonas gingivalis* to sections of rat oesophageal tissue

Compound	Reference or structural features	Origin	Test concentration (mg mL^{-1})	Result against *P. gingivalis* adhesion	Remarks
Negative control				+++++	Maximum adhesion
Okra aqueous extract	Lengsfeld et al (2004b) Acidic rhamnogalacturonan	*Abelmoschus esculentus*	1, 0.1	−, + ($P < 0.01$)	Highly active
Liquorice root polysaccharides	Lengsfeld et al (2004a) Arabinogalactan	*Glycyhhrizia glabra*	1, 0.1	+, ++ ($P < 0.01$)	Active

P values are only given for significant measurements.

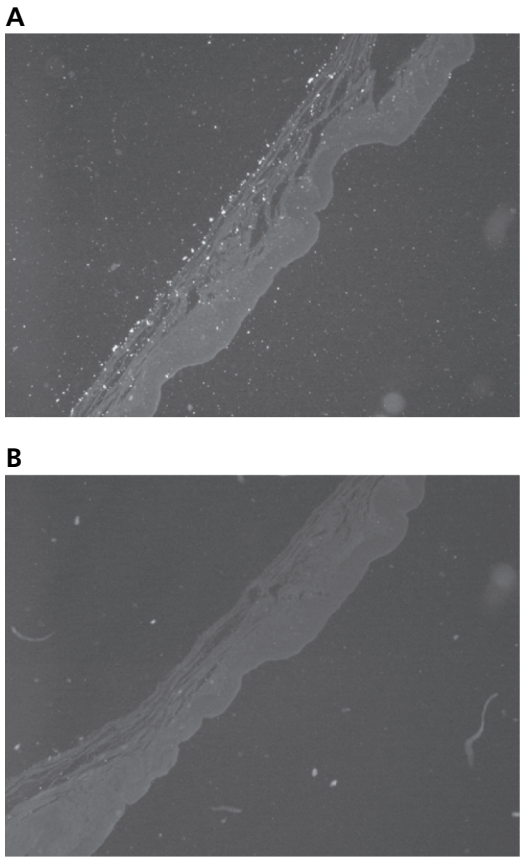

Figure 3 Fluorescent microscopy of representative in-situ experiments with fluorescein isothiocyanate-labelled *Porphyromonas gingivalis* on oesophageal tissue of the rat. Magnification ×250. A. Complete adhesion (+++++) of non-treated bacteria (negative control); B. pretreatment of *P. gingivalis* with liquorice root polysaccharides.

Adhesion in many cases is a multifactorial interaction of different surface adhesins with complementary receptor structures on the host surface. Therefore, the use of single distinct low molecular anti-adhesives is not likely to be as efficient as the application of mixtures of different specific anti-adhesives or the use of polyvalent molecules such as crude plant extracts or polysaccharides. In this study, it was found that binding of microorganims could not be blocked efficiently by a single anti-adhesive compound (sialyllactose being an exception) because non-antagonized adhesins are still able to undergo interaction with the host cell. Particularly in the case of anti-adhesion experiments with *C. albicans*, it was evident that crude mixtures were more efficient than isolated compounds, probably because they interact with many different adhesins. The use of plant extracts as multi-component systems could be a reasonable approach for further anti-adhesion development. Because many adhesive processes are mediated by carbohydrate–carbohydrate or carbohydrate–protein interactions, the use of antagonistic saccharides, especially polysaccharides, was shown to be a successful way of inhibiting adhesive interactions. In our experiments, blocking by anti-adhesive polysaccharides was in many cases particularly effective when acidic polymers were present. This is supported by the observation that polysaccharides from immature okra fruits (*A. esculentus*) containing highly acidic polymers with a high glucuronic acid content (Lengsfeld 2004b) are strong inhibitors of the adhesion of *H. pylori, P. gingivalis* and *C. albicans*. This leads to the hypothesis that an electrostatic interaction occurs, based on the negatively charged polymers with the respective adhesins. Future development strategies will focus on such polyvalent and strongly acid polymers.

Ex-situ experiments with *C. jejuni* showed that okra and blackcurrant polysaccharides produced good in-vitro anti-adhesion effects. The use of chicken tissue as a test model for *C. jejuni* adhesion assays requires particular discussion with respect to several points. Chickens are one of the major sources of human infection because of faecal contamination of poultry meat during production processes (Adkin et al 2006). Additionally, *Campylobacter* infections can also be found within the muscle and liver tissue of the birds, providing a source of infection without faecal contamination (Barot et al 1983; Young et al 1999). This means that *Campylobacter* can invade the liver either via the gall system or by becoming internalized through the intestinal epithelium via adhesive and invasive processes. Most of the *Campylobacter* load in the chicken intestinal system persists in a non-adhering state within the faecal mass. Investigations in this study showed that colonization of intestinal surfaces is possible on jejunal and colonic tissues. Adhesion probably leads to internalization of the microbes into the deeper tissue as the initial crucial step of infection (Russel & Blake 1994).

Table 4 Effect of pre-treatment of either yeast or tissue with different test compounds on the adhesion of fluorescein isothiocyanate-labelled *Candida albicans*

Compound	Reference or structural features	Origin	Test concentration (mg mL^{-1})	Result against *C. albicans* adhesion	Remarks
Negative control				+++++	Maximum adhesion
Xyloglucan, pre-treatment, *C. albicans*, human stomach	Hensel & Meier (1999)	*Pisum sativum*	1	+++	Moderately active
Fucoidan, pre-treatment, *C. albicans*, human stomach	Hensel & Meier (1999)	*Fucus vesiculosus*	1	+++++	Not active
Fucus polysaccharides, pre-treatment, *C. albicans*, human stomach	Hensel & Meier (1999)	*Fucus vesiculosus*	1	+++++	Not active
Glucomannan, pre-treatment, *C. albicans*, human stomach	Defined compound	*Lillium testaceum*	1	+++	Moderately active
Dextran, pre-treatment, *C. albicans*, human stomach	Defined compound	*Leuconostoc* spp.	1	+++++	Not active
Sugar beet pectin, pre-treatment, *C. albicans*, human stomach	Hensel & Meier (1999)	*Beta vulgaris*	1	++++	Not active
Galactomannan, pre-treatment, human stomach epithelia	Defined compound		0.01 to 1	++ to +++	Dose dependently active
Galactomannan, pre-treatment, human buccal tissue	Defined compound		1	+++	Slightly active
Galactomannan, pre-treatment, rat buccal epithelia	Defined compound		1	+++++	Not active
Galactomannan, pre-treatment, rat vaginal tissue	Defined compound		1	++++	Not active
Galactomannan, pre-treatment, *C. albicans*, human stomach	Defined compound		1	++++	Not active
Mannose, pre-treatment, *C. albicans*, human stomach	Defined compound		1	+++++	Not active
Mannosamin, pre-treatment, *C. albicans*, human stomach	Defined compound		1	+++	Slightly active
Methyl-D-mannose, pre-treatment, *C. albicans*, human stomach	Defined compound		1	+++++	Not active
Anti-fibronectin antibody, pre-treatment, *C. albicans*, human stomach		Calbiochem	1 to 100 μg	++ to ++++++	1 μg: anti-adhesive, <10 μg: increased adhesion
Concanavalin A, pre-treatment, *C. albicans*, human stomach		*Canavalia ensiformis*	0.5	+++++	Not active
Concanavalin A, pre-treatment, human stomach		*Canavalia ensiformis*	0.5	− ($P < 0.01$)	Complete adhesion block
Taraxacum officinalis extract, pre-treatment, *C. albicans*, human stomach		H_2O dry extract	1	+++++	Not active
Taraxacum officinalis extract, pre-treatment, human stomach		H_2O dry extract	1	+++++	Not active
Urtica extract, pre-treatment *C. albicans*, human stomach		EtOH dry extract	1	+++++	Not active
Urtica spp. extract, pre-treatment, epithelia		EtOH dry extract	1	++++++	Increase of adhesion
Rubi idaei extract, pre-treatment, *C. albicans*, human stomach		H_2O dry extract	1	+++	Slightly active
Rubi idaei extract, pre-treatment, human stomach		H_2O dry extract	1	++ ($P < 0.12$)	Slightly active
Equisetum spp. extract, pre-treatment, *C. albicans*, human stomach		H_2O dry extract	1	+++++	Not active
Equisetum spp. extract, pre-treatment, human stomach		H_2O dry extract	1	+++	Slightly active

P values are only given for significant measurements.

Regarding potential structure–activity relationships, it dose not seem essential that a certain type of carbohydrate backbone is active, but it is strongly indicated that anti-adhesion is mainly due to highly charged polymers, with glucuronic acid as the main uronic acid. This can be clearly seen when inactive pectin-like compounds (e.g. sugar beet pectin) are compared with other similar active polymers (e.g. okra polysaccharides and liquorice polysaccharides), which are mainly dominated by glucuronic acid.

Anti-adhesives could theoretically be used as prophylactics, for example as dietary or daily nutritional supplements. Their use could only be prophylactic because with all the active compounds an interaction with bacterial surface receptors was observed. A dissociation of bacteria already in the state of adherence with the host tissue seems unlikely. Prophylactic use as a diet or functional food could only be successful if the active compounds are not degraded in the gastrointestinal system. Because many of our active compounds were carbohydrate-based molecules, it follows that the application of such anti-adhesives for prophylactic use within the gastrointestinal tract must be critically regarded. Local treatment (e.g. mouthwash products) against *P. gingivalis* would overcome the problem of degradation and seems to have greater practical potential. The negative results of the in-vivo studies presented here are in accord with other clinical trials (Sharon & Ofek 2000). For example, an anti-adhesive pentasaccharide effective under in-vitro conditions against *Streptococcus* spp. and *Haemophilus influenzae*, failed in a Phase II clinical trial with over 500 patients (Neose Technologies 1999). A similar failure in clinical development is reported for the use of sialyllactose against *H. pylori* infection (Parente et al 2003). If the instability of saccharides in the gastrointestinal tract is the reason for the negative results observed in clinical studies, then the topical use of compounds for local treatment of infections would overcome this problem. For this reason, the inhibition of *P. gingivalis* was investigated. *P. gingivalis* is a strongly adherent bacterium to buccal and oesophageal tissue and one of the major risk factors for development of gingivitis and parodontitis. The investigations allowed the assessment of adherent bacteria where application of a topical anti-adhesive may have therapeutic benefits. Extracts from okra fruits and liquorice roots produced good anti-adhesive effects against *P. gingivalis* in the ex-situ assay. Future intentions are to construct a pre-clinical trial to investigate if these extracts can be used locally as oral hygiene products. For further pharmaceutical development additional practical problems have to be overcome, particularly the development of suitable delivery systems and evaluation of effective in-vivo concentrations, receptor redundancy or valency.

Conclusion

This study focused on the screening of natural products, especially carbohydrates from different origins, against the adhesion of different pathogenic microorganisms to host tissues. The results clearly indicate that under in-vitro/ex-situ conditions, certain polysaccharides can interact with the surface adhesions of the bacteria (not of the host cell), leading to reduced adhesion. High glucuronic acid containing rhamnogalacturonans from *A. esculentus* are seen as a promising polymer group of anti-adhesives. More research has to be done to establish distinct structure–activity relationships in order to overcome the problem of heterogeneous polymers and to reduce the activity to a single, defined oligosaccharide. Local treatment with anti-adhesives against *P. gingivalis* could lead to a potential new class of oral hygiene products. Research on anti-adhesive compounds is worth continuing in order to develop new agents against bacterial diseases. Anti-adhesives would not be a silver bullet, but if stability problems and the problem of getting the compounds to the host target organ could be solved they may offer a potent new tool in prophylactic pharmaceutical development.

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