Elevated Fecal Candida Counts in Patients with Antibiotic-Associated Diarrhea: Role of Soluble Fecal Substances

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To assess the role of soluble fecal substances in the elevation of fecal Candida counts in patients with antibiotic-associated diarrhea (AAD), we investigated the growth of Candida albicans in vitro in serially diluted stool fluids from patients with AAD and healthy subjects. There were significantly higher Candida albicans counts in stool fluids diluted 1:10 from AAD patients than in healthy subjects and the phosphate-buffered saline growth control, which may be due to reduced soluble Candida inhibitors and increased availability of growth factors and nutrients.

Elevated fecal Candida counts have been reported in patients with antibiotic-associated diarrhea (AAD) (3, 7, 8). It has recently been demonstrated that elevated Candida counts in stool are a result of antibiotic treatment of diarrhea per se rather than a cause of AAD (12). In studies where the effect of broad-spectrum antibiotics on the composition of the intestinal microflora of humans was investigated, an increase in yeasts and a decrease in anaerobes were observed (3, 5, 6). The indigenous microflora forms a dense layer of mucus gel that outcompetes yeasts for adhesion sites and produces inhibitory substances that reduce Candida albicans adhesion. It further suppresses Candida growth by providing competition for growth-limiting nutrients and by producing inhibitors that limit substrate availability (1). Whereas the role of attachment of Candida to mucosal cells in raising intestinal Candida counts has been described, the influence of stool fluids containing growth-supporting or -inhibiting factors that control intestinal Candida counts has not been investigated in humans.

Fifteen stools from patients with AAD and 15 stools from healthy subjects (not receiving antibiotics and not experiencing diarrhea) that had been collected during a previous study were investigated (12). Stool samples were diluted 1:10 with phosphate-buffered saline (PBS) and thoroughly homogenized. To remove microorganisms and solid parts, the suspensions were filtered (Rotilabo 0.2 μm; Carl Roth, Karlsruhe, Germany) and again diluted 1:50 and 1:100 with PBS. Ninety microliters of every diluted stool fluid was transferred to u-shaped wells containing the stool fluid dilutions, yielding a final concentration of 10^3 cells/ml. For growth control, 10 μl of the Candida albicans suspension was pipetted into 15 u-shaped wells containing 90 μl of PBS to obtain a final concentration of 10^5 cells/ml. The microtiter plates were incubated overnight at 37°C. After incubation, the Candida cell density in every dilution was calculated with a Neubauer counting chamber. All tests were performed twice. Differences between groups were calculated by using an unpaired t test (Jandel SigmaStat version 2.0). Differences with a P value of less than 0.05 were considered significant.

Mean Candida albicans counts for AAD patients, healthy subjects, and the PBS growth controls are shown in Table 1. Candida counts were significantly higher (P < 0.001) in stool fluids diluted 1:10 from AAD patients than in those from healthy subjects. Candida counts were also significantly higher in AAD patients than in the PBS growth control (P < 0.001). In stool fluids from healthy subjects, Candida counts were not significantly different from those in the PBS growth control.

Our experiments differ from others in which the growth-supporting or -inhibitory properties of a single bacterial species to control Candida counts have been investigated. Whereas a single bacterial species cannot be expected to reflect the interactions of a complex indigenous microflora, the stool fluids used in our study and obtained from AAD patients and healthy subjects contain the soluble environment of the assumed 400 to 500 distinct and indigenous bacterial species (4, 9). In our study, in stool fluids diluted 1:10 from AAD patients, Candida counts reached significantly higher levels than in those from healthy subjects and the PBS growth control, indicating that Candida growth was enhanced. In higher dilutions (1:50 and 1:100) of stool fluids from AAD patients, Candida counts were comparable to those in healthy subjects and the PBS growth control (Table 1), indicating that the growth-enhancing effect diminished.
Continuous-flow (CF) culture models have been used in vitro to study the effects of the indigenous intestinal microflora on Candida growth. CF models have been shown to provide an environment (pH, short-chain fatty acids [SCFA], anaerobic and facultative anaerobic bacteria, Enterobacteriaceae, H,S) for Candida similar to that of the large intestine or feces (9). It was found that Candida was unable to multiply in sterilized CF culture filtrates, regardless of whether the cultures were incubated aerobically or anaerobically (9). Since the addition of glucose, carbon or nitrogen sources, vitamins, and trace elements or changes in the pH did not reverse the impaired growth, the presence of inhibitory substances has been suggested (10). In an adhesion assay, large numbers of Candida organisms attached to intestinal mucosa from antimicrobial-treated hamsters. Interestingly, the ability of C. albicans to attach to intestinal mucosa from antimicrobial-treated hamsters was reduced when the association assay was performed with filtered stool fluids from untreated animals (11). The results of these experiments also suggested that inhibitory mechanisms were operating to inhibit the association of Candida with intestinal mucosa. Bacterial metabolism of carbohydrates and dehydroxylation of primary bile acids result in production of SCFA and secondary bile acids (SBA), which have been demonstrated to act as Candida inhibitor substances that reduce Candida mucosal attachment (7, 9, 11). SCFA further inhibited the growth or caused a prolonge lag phase in in vitro CF experiments that caused Candida organisms to have a long doubling time (9, 10). During antibiotic therapy, major changes in the composition of the protective intestinal microflora leading to the disappearance of bacterial populations occur and this disturbance decreases the production of SCFA and SBA (2, 3, 5, 6).

In our study, Candida albicans counts in stool fluids from healthy subjects were comparable to those in PBS growth controls. One could therefore assume that stool fluid from healthy subjects does not suppress Candida growth. In the CF model, it was shown that a difference between the culture effluent resembling a normal intestinal environment and the control medium occurred after growth for at least 24 h and was not apparent after 12 h (9). Since we performed overnight cultures, the short incubation time might explain why there was no difference between stool fluids from healthy subjects and those from PBS controls in our study.

In summary, Candida albicans grows better in stool fluids from AAD patients than in stool fluids from healthy subjects, obviously due to reduced soluble Candida inhibitor factors (SCFA and SBA) and increased availability of growth and nutrient factors physiologically consumed by the indigenous microflora.

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TABLE 1. Candida albicans counts in stool fluid dilutions and PBS growth control

| Dilution | AAD patients | Healthy subjects | PBS |
|----------|--------------|-----------------|-----|
| 1:10     | 1.97 ± 0.25abc | 1.45 ± 0.42 | 1.37 ± 0.02 |
| 1:50     | 1.36 ± 0.38 | 1.46 ± 0.39 | |
| 1:100    | 1.44 ± 0.29 | 1.26 ± 0.35 | |

*a* Significantly higher than value for healthy subjects (P < 0.001; t test).

*b* Significantly higher than value for PBS (P < 0.001; t test).