Gastric colonization with *Candida albicans*

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Received 24 July 1992; accepted in revised form 23 November 1992

Abstract. We adapted a rat model of gastrointestinal candidiasis for studies of in vivo gastric colonization with *Candida albicans*. Whereas normal rats cleared a single intragastric inoculum of $5 \times 10^6$ *C. albicans* from the stomach within 4 hours, rats pretreated with chloramphenicol and gentamicin achieved stable gastric colonization for at least 5 days after administration of this inoculum. We next used this model to study host modifications hypothesized to alter gastric colonization. A first group received dilute HCl 4 hr before yeast inoculation, to induce acute superficial gastric erosions; another group was treated with glucocorticosteroid beginning 12 days before yeast inoculation; and another group received famotidine therapy beginning 3 days before yeast inoculation, to neutralize gastric acidity. Recovery of yeasts from stomachs was significantly different from the control group only in rats treated with steroids; greater colonization was found in the rats so treated. In a final group of experiments, we attempted to inhibit in vivo gastric colonization with yeasts by preincubation of yeasts in vitro with a polyclonal antiserum raised in rabbits against heat-killed *C. albicans*. We were not able to demonstrate inhibition of gastric colonization by preincubation with this antiserum in this model system.

Key words: Adherence, *Candida albicans*, Candidiasis, Gastrointestinal candidiasis, Mycology

Introduction

*Candida albicans* and related species frequently inhabit the gastrointestinal (GI) tract of normal humans [1], and most infections are endogenously acquired from this reservoir [2]. In patients with diabetes mellitus, malignant diseases, or the acquired immunodeficiency syndrome (AIDS), or who are treated with broad-spectrum antibacterials, glucocorticosteroids, or cytotoxic/immunosuppressive therapy, symptomatic oral or esophageal infections may develop from GI proliferation of *Candida* [3, 4]. *Candida* colonization and infection of the GI tract are also important as the portal of entry for the majority of episodes of hematogenously disseminated candidiasis in neutropenic patients [3, 5–7]. Thus further understanding of factors affecting GI candidiasis and exploration of possible new approaches to prevention and treatment are critical.

Previous studies have reported the development of a rat model of GI candidiasis [6, 8, 9]. It has been demonstrated that the primary site of colonization in this model is the junction of the squamous epithelium – lined and the glandular epithelium – lined portions of the stomach. In this study, we more carefully examine factors potentially enhancing gastric colonization with *C.*
albicans and examine the effect of preincubating C. albicans in polyclonal antiserum on subsequent establishment of gastric colonization in this rat model.

Materials and methods

Organism and culture techniques. C. albicans strain B311 [10] was grown in yeast-phase by overnight incubation in modified Sabouraud's glucose broth (Difco) on an orbital shaker at 37 °C, harvested by centrifugation, washed three times in phosphate-buffered saline (PBS), enumerated in a hemacytometer, and a suspension of $5 \times 10^6$ yeast per ml prepared in sterile water for intragastric (IG) inoculation to rats. The final suspension was quantitatively cultured by standard pour-plate technique in modified Sabouraud's glucose agar (Difco) for confirmation of the inoculum concentration.

For quantitative gastric cultures, rats were killed by intraperitoneal injection of ketamine and xylazine. At necropsy, the peritoneal cavity was entered aseptically, the stomach dissected free of supporting tissues, the distal esophagus and pylorus suture-ligated, and the stomach removed. The stomach and its contents were then weighed. Next, multiple incisions were made through the stomach, and the stomach and gastric contents were homogenized in PBS for 3 min in a Tekmar® stomacher-type tissue homogenizer. Quantitative cultures were then performed by standard pour-plate technique in modified Sabouraud's glucose agar. After 48 hr incubation at 37 °C, colonies were enumerated and log$_{10}$ colony forming units (cfu) of yeast per stomach calculated. Because the minimum detectable level of colonization was 10 cfu/stomach, animals with no growth at this dilution were assigned 1.0 log$_{10}$ cfu yeast per stomach.

Animal acquisition and maintenance. Female, 6 week old, Sprague-Dawley rats were obtained. Rats were caged in pairs and fed rodent chow and water ad libitum, except as otherwise indicated. All animals were food deprived but provided with water for the 16 hr before yeast inoculation.

Experimental design. Experiment 1. Four groups of 3 rats each received IG inoculation with $5 \times 10^6$ C. albicans [9] and underwent necropsy for quantitative gastric culture at 1, 4, and 8 hr after inoculation. These rats were deprived of food throughout the experiment. Other groups of 3 rats each received antibiotic treatment before IG inoculation: 100 mg of chloramphenicol sodium succinate by gastric gavage 48 hr before IG inoculation with yeast and 25 mg of gentamicin sulfate by gastric gavage 24 hr before IG inoculation [8]. Three rats were sacrificed for quantitative gastric culture at 1, 4, 8, 16, 24, 36, 48, 72, 96, and 120 hr after IG with challenge $5 \times 10^6$ C. albicans. Animals were deprived of food for 8 hr before death.

Experiment 2. A control group of 40 rats received only the antibiotics and yeast inoculation detailed above. A second group (10 rats) additionally received, 4 hr before IG inoculation with yeast, a single gastric gavage of 1 ml 0.6 N HCl to produce acute gastric erosions [11]. A third group (21 rats) received 1 mg of cortisone acetate subcutaneously daily beginning 12 days before inoculation with yeast [8]. A fourth group (22 rats) received 10 mg/kg of the H$_2$-receptor antihistamine famotidine by gastric gavage every 12 hr beginning 72 hr before IG inoculation with yeast and continuing throughout the experiment. This dose and schedule of famotidine treatment was chosen to achieve maximal inhibition of gastric acid secretion without altering gastric emptying [12, 13]. All animals in this experiment were sacrificed for quantitative gastric cultures at 96 hr after IG inoculation with Candida, after 8 hr of food deprivation. Data were analyzed by a one-factor analysis of variance followed by comparisons by the Tukey studentized range method performed with BMDP Program 7D (BMDP Statistical Software, Los Angeles, CA).