Ibogaine reduces organ colonization in murine systemic and gastrointestinal *Candida albicans* infections

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In the present study the effect of the indole alkaloid ibogaine on the *in vitro* lipolytic activity and adherence to epithelial cells of *Candida albicans* was investigated. The substance was administered intraperitoneally at a dose of 5 mg kg⁻¹ day⁻¹ in mice with disseminated and gastrointestinal *C. albicans* infections. Ibogaine significantly decreased the rate of mortality and the number of *C. albicans* c.f.u. recovered from the kidney, liver and spleen. Ibogaine interfered with the early stages of both disseminated and gastrointestinal *C. albicans* infections but did not reduce the number of *C. albicans* c.f.u. in the organs at the late phase of infections. The development of a specific immune response was not influenced by ibogaine, since the delayed-type hypersensitivity reaction to *C. albicans* and the production of interferon (IFN)- γ were similar in control and ibogaine-treated mice. The combined use of amphotericin B plus ibogaine in the treatment of mice with gastrointestinal infection reduced organ colonization more strongly than each substance alone.

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INTRODUCTION

Candida albicans is an opportunistic fungal pathogen with a rising incidence of severe disseminated infections leading to fatal outcomes in humans (Fidel *et al.*, 1999; Komshian *et al.*, 1989; Pfaller, 1996). A number of factors have contributed to this, for example the wide use of immunosuppressive therapies and the increased cases of immune-deficiency diseases, such as AIDS (Shearer, 1998). The limited number of effective antifungals against *C. albicans* is further reduced by the appearance of drug-resistant species. The discovery of new compounds with different mechanisms of action is a challenge in antifungal drug research.

While immunity to *Candida* has been thoroughly investigated, the factors connected with the early penetration of the yeast into the host cells are less defined and exploited. Micro-organisms can damage host cell membranes through secretion of different proteolytic and lipolytic enzymes. It has been proven that *C. albicans* produces extracellular lipases, and since the characterization of the gene *LIP1* (Fu *et al.*, 1997) nine new members of a family encoding lipases have been identified (Hube *et al.*, 2000). An important observation is

that some of these *LIP* genes are expressed in infected organs during systemic *C. albicans* infection. These data suggest that lipases might be involved in the pathogenesis of the yeast.

Phospholipases belong to a heterogeneous group of enzymes capable of destroying cell membranes. Many investigations support the view that they act as pathogenic factors in protozoan (Saffer & Schwartzman, 1991), bacterial (Portnoy *et al.*, 1994; Songer, 1997; Titball, 1993) and fungal (Cox *et al.*, 2001; Leidich *et al.*, 1998) infections. Genes encoding phospholipase B and C have recently been cloned (Bennett *et al.*, 1998; Sugiyama *et al.*, 1999). According to a classification of phospholipases, based on the type of ester link that is cleaved, a single enzyme can express phospholipase B activity and also lysophospholipase and transacylase activity. If it is the case that all these activities are controlled by one gene product, it is difficult to correlate a certain biological effect with a single enzyme. *In vivo* data demonstrate that phospholipase-deficient *C. albicans* mutants are less invasive (Ghannoum, 2000; Mukherjee *et al.*, 2001). Targeted gene disruption is one of the most promising approaches to evaluate the contribution of different extracellular products to the virulence of *C. albicans*. Using this method, Leidich *et al.* (1998) showed that the disruption of a gene encoding a phospholipase B had no effect on yeast growth but significantly reduced its penetration into host cells and its virulence

Abbreviations: AmB, amphotericin B; DTH, delayed-type hypersensitivity reaction; IFN, interferon; i.v., intravenously.

in a murine model of disseminated candidiasis. Also, a *C. albicans* strain lacking a gene encoding a phosphatidylcholine-specific phospholipase D is less virulent in a model of oral infection (Hube *et al.*, 2001).

Targeting lipolytic enzymes might represent a novel approach in antimicrobial therapy, including therapy for *C. albicans* infections (Andriole, 1999). The application of lipase and phospholipase inhibitors can influence the early phase of *C. albicans* penetration and colonization of host tissues. Some beta-blocker-like structures have inhibitory activity on phospholipases and correlate with a protective effect during *C. albicans* infections (Hanel *et al.*, 1995).

In a preliminary screen of a series of compounds, we observed an inhibitory effect of the indole alkaloid ibogaine on the activity of several lipases. In this context, some indoline alkaloids, analogues of β -carboline, have been shown to possess antifungal activity *in vitro* equal to that of amphotericin B (AmB) but with low cytotoxicity (Kariba *et al.*, 2002). The antifungal or antilipase activity of ibogaine has not been investigated. Thus, in the present study we decided to study the influence of ibogaine on *C. albicans* lipase activity, cell adherence and on disseminated and gastrointestinal *C. albicans* infections in relation to organ colonization and specific immune responses.

METHODS

Reagents. *Candida rugosa* lipase (Crl) was purchased from Nippon Fats and Oil and further purified by ion exchange chromatography. *Thermomyces lanuginosa* lipase (TLL), *Fusarium oxysporum* lipase (Fol), *Candida antarctica* lipase A (CALA), *C. antarctica* lipase B (CALB) and *Rhizomucor miehei* lipase (Rml) were expressed in *Aspergillus oryzae* and purified at Novozymes. All lipases used for this study were more than 95% pure as determined by SDS-PAGE. Ibogaine and AmB were obtained from Sigma.

Evaluation of lipase activity. *p*-Nitrophenyl-butyrate was used as a substrate and the release of *p*-nitrophenol was measured spectrophotometrically at 405 nm using a microplate reader. As each lipase has different activity on *p*-nitrophenyl-butyrate as a substrate, a pilot experiment was carried out to find the optimal amount of each enzyme to give linear kinetics for *p*-nitrophenol formation and to give an OD₄₀₅ of 1 after a 5 min incubation. Aliquots of 100 μ l of each enzyme, diluted in 50 mM borate (pH 8), were pipetted into a 96-well microplate and the reaction was started by adding 100 μ l of the substrate. *p*-Nitrophenyl-butyrate was prepared as 100 mM stock solution in 2-propanol and diluted 1:100 in 50 mM borate (pH 8) just before use. The final concentration in the microtitre well was 0.5 mM of the substrate.

Assay of lipase inhibition. Ibogaine dissolved in DMSO at a concentration of 1 mg ml⁻¹ was pipetted into the microtitre wells and 100 μ l of the optimal amount of each lipase was added. After incubation for 5 min 100 μ l of the substrate was added. The reaction was followed by measuring *p*-nitrophenol produced after 5 min as described above. During the assay, the plate was constantly agitated. Blanks, using the same amount of DMSO without ibogaine, were also assayed.

Determination of the reversibility of the inhibition. All the enzymes were dissolved in 1 ml 50 mM HEPES (pH 7.0 at 22 °C) to a concentration of 3.5×10^{-6} M, and 10 μ l of ibogaine dissolved in DMSO to a concentration of 100 mM was added and the samples were

incubated for 30 min at room temperature. All the enzymes were then diluted at least 750-fold in water and residual enzymic activity was assayed using the pH-stat assay with tributyrine emulsified with gum-arabic as a substrate.

In vitro candidacidal activity. The antifungal activity of ibogaine on *C. albicans* was tested by a diffusion method. Ibogaine at a concentration range from 0.05 to 1.0 mg ml⁻¹ was pipetted into 20 mm wells in Sabouraud agar inoculated with 1×10^5 *Candida* cells ml⁻¹. After 48 h at 37 °C the zone of inhibition was measured. AmB was used as a positive control.

Extracellular phospholipase activity. Extracellular phospholipase activity was determined by the method of Price *et al.* (1982). Ten microlitres of *C. albicans* suspension (1×10^5 cells ml⁻¹) was plated on Sabouraud agar containing 5 mM CaCl₂ and 8% Bacto Egg Yolk (Difco) for 48 h at 30 °C. Phospholipase secretion produced a distinct opaque zone around the colonies.

Extracellular lipase activity. Yeast cells were washed with PBS and adjusted to 5×10^6 cells ml⁻¹ in different growth media in the presence or absence of 250 μ g ibogaine ml⁻¹. The flasks were shaken at 37 °C for 24 h. The lipolytic activity in filtrated culture supernatants was determined by a plate assay on olive oil agar that contained 0.7% yeast nitrogen base (YNB; Difco) (w/v), 2% olive oil (v/v), 0.001% rhodamine B (w/v) and 2% agar (w/v). The following liquid media were used for *C. albicans* growth: minimal (YNB without glucose and amino acids), yeast peptone glucose (YPG; Difco), 0.67% (w/v) YNB containing 2.5% (v/v) Tween 20, Tween 40 or Tween 80 (Sigma).

Adherence assay. Late-exponential-phase yeast cells were harvested, washed twice in 0.9% NaCl and resuspended to 1×10^2 cells ml⁻¹ in Hanks' balanced salt solution (HBSS). One millilitre of this suspension was added to an HT-29 epithelial cell line monolayer in six-well tissue-culture plates. Following incubation for 15 min at 37 °C in the absence (taken as 100% adherence) or presence of different ibogaine concentrations, the nonadherent yeast cells were aspirated and each well was rinsed twice with HBSS. Next, the wells were overlaid with Sabouraud agar and the number of adherent *C. albicans* was quantified by colony counting (repeated in triplicate). Dose-response curves were drawn by plotting the inhibition of adherence against dilutions of the substance. From this the concentration giving 50% inhibition of adherence was determined.

***C. albicans* systemic and gastrointestinal infections.** Male and female BALB/c mice (Iffa-Credo, Charles River, L'Arbresle, France) were kept on a standard commercial pellet diet with water *ad libitum*. The animal use protocols were approved by the Animal Care Commission at the Institute of Microbiology according to international laws.

A virulent clinical isolate of *C. albicans*, 562 (Institute of Infectious and Parasitic Diseases, Sofia, Bulgaria), was used in the study. Prior to inoculation, the yeast was grown for 48 h on Sabouraud broth (Difco) at 37 °C. After methylene-blue staining no hyphae were observed. A standard curve was drawn by plotting the OD₆₂₀ against the concentration of the yeast, determined by counting the c.f.u. of different suspensions.

For lethal disseminated infection, 1×10^6 cells in 0.1 ml sterile pyrogen-free PBS was injected into the tail vein of the mice.

To induce gastrointestinal infection, mice were given water containing 1 mg amoxicillin ml⁻¹ (Yamanouchi B.V. Netherlands) and 0.1 mg amikacin ml⁻¹ (Amikin, Bristol Lab., USA) for 7 days before inoculation. Mice were infected by an oral administration of 1×10^7 cells of *C. albicans* in a volume of 0.1 ml with a gastric gavage. Ibogaine and AmB were dissolved in sterile distilled water and injected intraperitoneally at a dose of 0.5 mg kg⁻¹ in a volume of 0.2 ml.

At different intervals, the mice were killed and various organs were removed aseptically, weighed and homogenized in a glass tissue grinder with 5 ml of saline. Tenfold serial dilutions were made of each homogenate and plated on Sabouraud agar, and the colonies were counted after 24 h.

Delayed type hypersensitivity (DTH) reaction. Mice were inoculated intravenously with 1×10^6 *C. albicans* cells. Five animals from each group were injected at day 20 of infection with 1×10^7 glutaraldehyde-inactivated *Candida* cells (treated overnight at 4 °C with 2 % glutaraldehyde) into the hind paw. After 24 h the footpad thickness of the injected paw was measured and the difference with the control paw, injected with saline, was calculated in millimetres. A control group of non-infected mice was injected in the same way with inactivated yeast cells.

Cytokine assay. At day 8 of intravenous (i.v.) infection, cell suspensions were prepared from freshly removed spleens and erythrocytes were depleted using lysis buffer (0.15 M NH_4Cl , 10 mM KHCO_3 , 0.1 mM EDTA, pH 7.2). Splenocytes (5×10^6 cells ml^{-1}) were incubated in 24-well Falcon plates (Becton Dickinson) in RPMI 1640 media (Gibco-BRL), containing 10 % fetal bovine serum (Sigma), 100 U penicillin ml^{-1} , 100 μg streptomycin ml^{-1} (Sigma), 2 mM L-glutamine, 25 mM β -mercaptoethanol and 10 mM HEPES. Cytokine concentrations were determined in the supernatants after 3 days of culture in the presence or absence of 1×10^7 glutaraldehyde-inactivated *C. albicans* cells. Quantitative ELISA for interferon (IFN)- γ was performed using paired mAbs according to manufacturer's recommendations (Euroclone). The minimum detectable concentration was less than 15 $\mu\text{g ml}^{-1}$.

Statistical analysis. The statistical difference in the survival rate or c.f.u. was examined by Wilcoxon test or by Student's *t*-test, respectively.

RESULTS

In vitro ibogaine activity

In the present study, the indole alkaloid agent ibogaine was used (Fig. 1). First, we evaluated the effect of ibogaine on several fungal lipolytic enzymes such as *F. oxysporum* lipase (Fol), *C. rugosa* lipase (Crl) and the *R. miehei* lipase (Rml). Ibogaine showed high levels of inhibition at a concentration range from 10^{-4} M to 10^{-6} M (Fig. 2). Its mechanism of action appeared to be reversible, since the enzyme activity was restored after ibogaine removal.

The plate diffusion assay showed that ibogaine had no candidacidal effect on *C. albicans* up to a concentration of 500 $\mu\text{g ml}^{-1}$. We established that the strain produced extracellular lipase or phospholipases, sufficient for detection on egg yolk agar. The preincubation of *C. albicans* with 100–1000 $\mu\text{g ibogaine ml}^{-1}$ for 24 h before seeding on yolk agar resulted in a less-marked opaque zone at concentrations above 500 $\mu\text{g ml}^{-1}$ (not shown).

In the next experiments it was shown that the yeast expressed lipolytic activity when grown in YPG medium or in a medium containing Tween 40 as a sole carbon source (Fig. 3). Such activity was not detected in a minimal medium or in YNB with Tween 20 or Tween 80. Yeast cells were grown for 24 h and at different intervals the supernatants were collected, the pH was measured and the cells were counted. The lipolytic activity increased slowly during the first 6 h of

Fig. 1. Ibogaine.

Fig. 2. Inhibition of selected lipases by ibogaine. Effect of ibogaine on the activity of (a) *T. lanuginosa*, (b) *F. oxysporum*, (c) *C. rugosa*, (d) *C. antarctica* A, (e) *C. antarctica* B and (f) *R. miehei* lipases.

growth as no substantial difference in cell number or pH was observed. Therefore, the data in Fig. 3 represent the effect of ibogaine after 6 h of cultivation. It is evident that the substance inhibited the lipolytic activity of the yeast in both YPG medium and in YNB with Tween 40.

When ibogaine was tested in adherence assays, the substance caused a 50 % inhibition of *C. albicans* attachment to the HT-29 epithelial cell line at a concentration of 52.0 ± 0.4 $\mu\text{g ml}^{-1}$.

In vivo ibogaine activity

Ibogaine was administered intraperitoneally at a dose of 5 mg kg^{-1} for the first 3 days of i.v. *C. albicans* infection. The rate of mortality was significantly decreased in the ibogaine-treated group (Fig. 4). The number of *C. albicans* c.f.u. recovered from the kidneys, liver and spleen was reduced as a

Fig. 3. Detection of lipolytic activity in the culture supernatants after 6 h growth of *C. albicans* in the presence or absence of 250 µg ibogaine ml⁻¹. Panels: YPG medium as a negative control (1); lipase of *C. rugosa* (250 µg ml⁻¹) as a positive control (2); YPG medium inoculated with *C. albicans* in the absence (3) or presence (4) of ibogaine; YNB containing Tween 40 inoculated with the yeast in the absence (5) or in the presence (6) of ibogaine.

result of ibogaine administration at day 4 and 10 of infection (Table 1). At day 28 of infection ibogaine-treated and nontreated mice showed similar kidney colonization, while in both groups liver contamination was low and the spleen was free of the yeast. Ibogaine-treatment did not affect brain colonization.

At day 21 of infection the DTH reaction to *C. albicans* was determined. The data show that ibogaine-treated and non-

Fig. 4. Effect of ibogaine treatment on the survival of mice intravenously inoculated with 1×10^6 c.f.u. of *C. albicans*. Mice were injected intraperitoneally with 5 mg ibogaine kg⁻¹ from day 0 to day 3 of infection. Control mice, solid line; ibogaine-treated mice, dashed line. Each group consisted of 10 animals, and data are representative of three independent experiments. * $P < 0.001$, Wilcoxon test.

treated animals developed significant swelling 24 h after injection of the inactivated yeast into the paw. The swelling measured was 4.7 ± 0.3 mm in the control group and 5.6 ± 0.2 mm for treated mice. Eight days after i.v. infection, spleen cells were isolated from ibogaine-treated and nontreated mice, and stimulated *in vitro* with inactivated *C. albicans*. After 48 h of growth the concentration of IFN- γ in the supernatants was assayed. No difference was observed in the cytokine secretion by splenocytes from ibogaine-treated and nontreated groups (300 ± 20 pg ml⁻¹ and 280 ± 25 pg ml⁻¹, respectively).

To induce gastrointestinal infection, mice were given two antibiotics orally to reduce the number of intestinal microflora and then were inoculated orally with 1×10^8 *C. albicans* cells. In preliminary experiments a comparison was made with mice not receiving antibiotics. It was established that 2.6×10^6 c.f.u. g⁻¹ (day 5 of inoculation), 2.2×10^6 c.f.u. g⁻¹ (day 10) and 1.6×10^6 c.f.u. g⁻¹ (day 15) *C. albicans* were isolated from the faeces of animals fed with antibiotics. In the faeces of *C. albicans*-infected mice without antibiotic treatment the yeast was not detected after day 5 ($< 2 \times 10^2$

Table 1. Distribution of *C. albicans* in different organs after intravenous infection

Mice were inoculated intravenously with 5×10^6 c.f.u. of *C. albicans* and treated with ibogaine intraperitoneally at a dose of 5 mg kg⁻¹ from day 0 to day 3 of infection. At the indicated days various organs (five mice per point) were removed, homogenized and plated for determination of c.f.u. per gram of tissue. Data were similar in three experiments and are represented as mean $\pm$ SD. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Days of infection	Kidneys (log c.f.u. g ⁻¹)		Liver (log c.f.u. g ⁻¹)		Spleen (log c.f.u. g ⁻¹)		Brain (log c.f.u. g ⁻¹)	
	Control	Ibogaine-treated	Control	Ibogaine-treated	Control	Ibogaine-treated	Control	Ibogaine-treated
4	5.2 $\pm$ 0.5	3.8 $\pm$ 0.4**	7.0 $\pm$ 1.0	4.8 $\pm$ 0.5**	5.2 $\pm$ 0.8	3.4 $\pm$ 0.6**	< 2.0	< 2.0
10	6.4 $\pm$ 0.6	5.4 $\pm$ 0.8*	4.4 $\pm$ 0.4	3.0 $\pm$ 0.5**	4.0 $\pm$ 0.5	< 2.0***	3.0 $\pm$ 0.4	2.4 $\pm$ 0.2
28	4.0 $\pm$ 0.6	3.8 $\pm$ 0.4	2.0 $\pm$ 0.5	2.2 $\pm$ 0.4	< 2.0	< 2.0	2.8 $\pm$ 0.5	< 2.0*

c.f.u. g⁻¹). Two days after the final ibogaine-treatment (at a dose of 5 mg kg⁻¹ from day 0 to day 3 of infection), fewer c.f.u. of *C. albicans* were counted in the stomach of this group than in nontreated mice (Fig. 5). At day 20 of infection no difference was observed between ibogaine-treated and nontreated animals. At that time point the mice stopped receiving the antibiotics and in a similar manner the stomach colonization decreased in both groups until day 35. This was probably due to the recovery of the commensal microflora, since mice maintained on antibiotics showed higher stomach colonization (data not shown).

In the next experiments ibogaine treatment was continued until day 5 of infection at a dose of 5 mg kg⁻¹. Additionally, a group of mice was treated for 5 days with a suboptimal dose of 0.5 mg AmB kg⁻¹ and a group was injected with 0.5 mg AmB kg⁻¹ plus 5 mg ibogaine kg⁻¹. Two weeks later the colonization of stomach and kidneys was examined (Table 2). There was a significant reduction in the number of c.f.u. in both organs in mice injected with ibogaine or with AmB plus ibogaine. The combined treatment was more effective than the ibogaine administration alone, statistically significant in respect to kidney colonization.

DISCUSSION

In humans who develop systemic yeast infections it is supposed that the source of the micro-organism is the digestive tract, where *C. albicans* is a part of the normal flora (Wenzel, 1995). One possible approach to limit yeast penetration into the host cells is through the inhibition of fungal enzymes engaged in the destruction of cell surface membranes. Since our *in vitro* data showed that ibogaine is capable of inhibiting several lipases and also of preventing the adherence of *C. albicans* to epithelial cells, we theorized that this substance may have beneficial effects during *in vivo* infections.

C. albicans expressed lipolytic activity not only in a medium containing Tween 40 as a sole source of carbon but also in a

Fig. 5. Outgrowth of *C. albicans* in the stomach. For the induction and maintenance of gastrointestinal infection, mice were fed with antibiotics from day -7 to day 20. At different time points after inoculation with 1×10^7 cells five animals from ibogaine-treated (○) and nontreated (●) groups were killed to assess the viable *C. albicans* cells. Data were similar for three experiments and represent means $\pm$ SD. * $P < 0.05$, Student's *t*-test.

Table 2. Contamination of stomach and kidneys after intragastric inoculation

Ten days after last treatment (day 15 of infection) organs were removed and assessed for yeast growth. Values are mean $\pm$ SD from five mice per group. Experiments were performed three times. * $P < 0.05$ vs control; ** $P < 0.05$ vs ibogaine-treated group; *** $P < 0.001$.

Group	Viable <i>Candida</i> cells [log c.f.u. (g tissue ⁻¹)]	
	Stomach	Kidney
Control†	6.8 $\pm$ 0.5	4.3 $\pm$ 0.8
AmB‡	6.4 $\pm$ 0.8	4.9 $\pm$ 0.8
Ibogaine§	4.6 $\pm$ 0.6***	3.2 $\pm$ 0.6*
AmB+Ibogaine	3.9 $\pm$ 0.7***	2.3 $\pm$ 0.7**

†BALB/c mice inoculated intragastrically with 1×10^8 viable *C. albicans* cells after being fed with amoxicillin and amikacin for 7 days.

‡Mice treated intraperitoneally with 0.5 mg AmB kg⁻¹ from day 0 to day 5 of infection.

§Mice treated intraperitoneally with 5 mg ibogaine kg⁻¹ from day 0 to day 5 of infection.

||Mice treated intraperitoneally with 0.5 mg AmB kg⁻¹ plus 5 mg ibogaine kg⁻¹ from day 0 to day 5 of infection.

medium containing glucose. Different lipase or phospholipase genes are expressed under various environmental conditions. For example, *LIP1*, a gene that encodes a lipase, is expressed when *C. albicans* is grown in media containing Tweens or triglycerides, while in the presence of carbohydrates the gene expression is inhibited (Fu *et al.*, 1997). Conversely, a *PLB* gene encoding phospholipase B is most highly expressed in a medium with a high glucose concentration. Ibogaine inhibited the lipolytic activity of the yeast grown in both YPG and YNB/Tween 40 media. The substance also tended to suppress the lipase or phospholipase secretion as detected on egg yolk agar. At the same time, it should be taken into account that egg yolk contains substrates for both phospholipases and lipases (Fu *et al.*, 1997). In addition, *C. albicans* was shown to secrete an extracellular esterase in a medium containing Tween 80 as the sole carbon source (Tsuboi *et al.*, 1996).

Ibogaine is a naturally occurring compound with anti-addictive properties (Mash *et al.*, 2000). A few investigations *in vivo* have demonstrated that ibogaine is rapidly transformed to 12-hydroxyibogaine (noribogaine), which persists in the bloodstream for at least 1 day (Baumann *et al.*, 2001a; Mash *et al.*, 1995). Both forms cause various effects within the nervous system through binding to kappa opioid receptors and to serotonin uptake sites (Glick *et al.*, 2000, 2001). Ibogaine at high doses can cause a degeneration of cerebellum and tremor as side effects (Baumann *et al.*, 2001b; Molinari *et al.*, 1996). In clinical studies ibogaine has been administered in drug-dependent persons in single or triple

doses (400–800 mg), which is equivalent to a mean dose above 80 mg kg⁻¹ (Glick *et al.*, 2001). At a dose of 40 mg kg⁻¹ this compound has been investigated in rat models (Hough *et al.*, 2000). We have applied ibogaine at a low dose of 5 mg kg⁻¹ in order to avoid possible adverse effects.

We showed here that ibogaine is capable of diminishing the mortality in mice of systemic *C. albicans* infections by reducing the organ colonization. At the late stage of infection (28 days) in the control and in ibogaine-treated groups, the kidney colonization tended to decrease. The lack of a direct anticandidacidal effect could have been because in the early phase ibogaine prevented colonization through candidastatic mechanisms, which were not sufficient for the eradication of the pathogen. Also, the action of the compound might differ in various tissues. Data from the literature show that after intraperitoneal administration of ibogaine in rats (40 mg kg⁻¹) it is accumulated mainly in adipose tissue and is detected to a lesser extent in the brain, liver and kidneys (Hough *et al.*, 1996). Usually in experimental models of gastrointestinal *C. albicans* infection, a translocation of the yeast to internal organs is achieved by treatment with immunosuppressive agents. In the present experiments, such dissemination was induced by the use of a high inoculation dose in addition to a prolonged antibiotic application. The results show that ibogaine administered in the first 5 days of infection effectively decreased stomach colonization and prevented the yeast migration into the kidneys.

In vitro data have demonstrated that ibogaine suppresses T-cell, B-cell and natural-killer-cell functions in a dose-dependent manner but has no effect on macrophages (House *et al.*, 1995). We tried to determine whether its administration in systemic infections might influence the specific anti-*Candida* responses. Immunocompetent individuals express positive DTH reactions to *C. albicans* in correlation with a decreased progression of systemic infection (Fidel, 2004). IFN- γ is one of the major cytokines playing a decisive role for the early protective response to the fungus and underlying the development of DTH response (O'Garra *et al.*, 1997). Data suggest that under the scheme used, ibogaine does not affect the late immune responses, at least in systemic infections. The DTH response to *C. albicans* was not significantly influenced in ibogaine-treated mice, neither was IFN- γ production changed. These results do not exclude the fact that other cellular or humoral anti-infectious mechanisms might be affected.

AmB is the most frequently used antibiotic for severe invasive fungal infections, despite its renal toxicity. It is desirable to use AmB at lower doses but without the risk that the pathogen might invade further into the organs. This goal might be achieved by a combination of agents with different mechanisms of action. In the present study we administered AmB at a suboptimal dose together with ibogaine. The results show that the combination decreased the stomach and kidney colonization by *C. albicans* more effectively than each substance alone. A combination of classical antibiotics and lipase inhibitors appears to be promising because candida-

cidal activity can be supported by suppression of fungus penetration into the host cells. Further investigations are needed to confirm the correlation between the antilipase activity of ibogaine and its beneficial effect in *C. albicans* infection.

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