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Therapeutic efficacy of chitosan against invasive candidiasis in mice

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Abstract The prevalence of antibiotic resistance has resulted in the need for new approaches to be developed to combat the previously easily treatable infections. This work aims to evaluate the antifungal and antioxidant effects of the chitosan, as a new alternative or complementary anti-fungal drug, alone or in combination with amphotericin B against a pathogenic *Candida albicans* in mice. Eighty neutropenic infected mice were randomly assigned into four main groups (20 mice/group). The 1st group was treated with saline, neutropenic infected (NI group) (IPC group, invasive pulmonary candidiasis), the 2nd group was treated with chitosan (ED_{50}) (CE group), the 3rd group was treated with amphotericin B (150 mg/kg) (AMB group) and the 4th group was treated with chitosan plus amphotericin B (CE + AMB group). Treatment was started at 24 h after fungal inoculation and was administered for 3 consecutive days. All the previous treatments demonstrated notable growth inhibition against a *C. albicans* isolate as indicated by measuring the mean diameter of the inhibition zone. Compared with IPC group, CE, AMB, and AMB + CE-treated animals had 73%, 87%, and 90% reduction in fungal burden, respectively. Furthermore, treatment with CE and/or AMB for 24 and 72 h significantly decreased MDA, SOD, CAT and NO levels and increased GSH and in the lung tissues as compared with the infected untreated group. In conclusion, CE treatment, with the combination of antifungal therapy, can alleviate oxidative stress and lung injury associated with IPC in neutropenic mice.

Introduction

Candida albicans (*C. albicans*) is an opportunistic pathogen that causes superficial and systemic infections (Selvaraj et al., 2014). *C. albicans* is a pathogenic yeast, which forms a range of polarized and expanded cell shapes (Canonico et al., 2014). It is the most common human fungal pathogen and causes significant morbidity and mortality worldwide (Noble and Johnson, 2005; Kaufman et al., 2014). It is a dimorphic yeast capable of producing alternate morphological forms (yeast or mycelium) in response to environmental changes (Manavathu et al., 1996). It exists as a commensal organism in healthy individuals by colonizing several niches of the human body which includes skin, mucosal surfaces, oral

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cavity, vagina, and gastrointestinal tract (Larriba et al., 2000). An altered balance between the host immunity and this opportunistic fungus, as in the case of immunocompromised patients, is one of the leading causes of candidiasis in humans (Bodey, 1993). After entering the blood stream, the yeast cells can infect all internal organs and may cause life-threatening septicemia (Karkowska-Kuleta et al., 2009). Candidiasis can develop as superficial candidiasis (skin and mucosa) which occurs in healthy individuals, or invasive candidiasis which is seen in cancer patients, AIDS patients, and immunocompromised individuals following transplantation (Larriba et al., 2000).

Invasive candidiasis remains a challenging complication, which frequently occurs in patients with one or more underlying diseases or surgical interventions. In recent point prevalence studies, a candidaemia incidence of 6.9 per 1000 ICU patients was reported, and 7.5% of ICU patients received antifungal therapy (Kett et al., 2011; Azoulay et al., 2012). Candidaemia increases mortality rates in the range of 20–49% (Gudlaugsson et al., 2003; Arendrup et al., 2011), but still there are many open management questions. Pulmonary candida infections may present as the manifestations of disseminated candidiasis spread by hematogenous route or as a primary bronchial or pulmonary process from the airways (Odds, 1988).

Highly reactive oxygen metabolites are one of the primary effector mechanisms used by the host immune system to control or clear microbial infections (Youseff et al., 2012). Reactive oxygen species (ROS) are essential components of the defensive mechanism against fungus infection (Ibrahim-Granet et al., 2003; Philippe et al., 2003). Initial host defenses against fungal invaders rely on the responses of innate immune cells, particularly macrophages, neutrophils and other phagocytic cells. These phagocytes generate potent reactive oxygen and nitrogen species (ROS and RNS), which are toxic to most fungal pathogens, causing damage to DNA, proteins and lipids (Bogdan et al., 2000; Youseff et al., 2012). To protect against damage, cells contain a number of defense mechanisms including endogenous well-characterized antioxidant enzymes, such as catalase, superoxide dismutase, nitric oxide and low molecular weight antioxidant, such as glutathione (GSH) (Mates et al., 1999). Indeed, ROS induce programmed cell death in *C. albicans* (Phillips et al., 2003).

Amphotericin B (AMB) is a polyene antifungal antibiotic by-product of the actinomycete bacterium *Streptomyces nodosus*. In spite of AMB's proven track record in the management of serious systemic fungal infections, its well-known side effects and toxicity will sometimes require discontinuation of therapy despite a life-threatening systemic fungal infection. The principal acute toxicity of AMB is nephrotoxicity (Geo vigila and Baskaran, 2011). Clinical manifestations of AMB nephrotoxicity include renal insufficient hypokalemia, hypomagnesaemia, metabolic academia, and polyuria due to nephrogenic diabetes insipidus (Laniado-Laborín and Cabrales-Vargas, 2009). There have been an increasing number of reports of clinically significant amphotericin B (AMB) resistance in fungal pathogens, including *C. albicans* (Sterling and Merz, 1998). Since many of the currently available drugs have undesirable side effects and are ineffective against *C. albicans* infection, there is now a greater interest in the next generation of antifungal agents. Many people worldwide, including those in developed countries, turn to complementary or alternative medicine. Products from freshwater and marine sources have recently become attractive as nutraceutical and functional foods and as a source material for the development of drugs (Koyama et al., 2006).

Chitosan is a linear polysaccharide composed of randomly distributed β-(1–4) linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). It is made by deacetylation of chitin, the primary polysaccharide component of crustacean shells with the alkali sodium hydroxide (Shahidi and Synowiecki, 1991). Chitosan can be used to produce value-added products because it is rich in protein, carotenoids and chitin (Lertsutthiwong et al., 2002). This polysaccharide was found to be non-toxic, biocompatible and biodegradable (Arvanitoyannis et al., 1998). Chitosan has several applications being employed either alone or in blends with other natural polymers (starch, gelatin and alginates) in the food and pharmaceutical industries mainly due to its high biodegradability and antimicrobial properties (Hague et al., 2005). Microbiological activity of chitosan has been detected for many bacteria, filamentous fungi and yeasts (Hirano and Nagao, 1989). Data in the literature have the tendency to characterize chitosan as bacteriostatic rather than bactericidal (Coma et al., 2002), although the exact mechanism is not fully understood and several other factors may contribute to the antibacterial action (Raafat et al., 2008). Three models have been proposed, the most acceptable being the interaction between positively charged chitin/chitosan molecules and negatively charged microbial cell membranes. In this model the interaction is mediated by the electrostatic forces between the protonated NH^{+3} groups and the negative residues (Tsai and Su, 1999), presumably by competing with Ca^{+2} for electronegative sites on the membrane surface (Young and Kauss, 1983). Since such mechanism is based on electrostatic interaction, it suggests that the greater the number of cationized amines, the higher will be the antimicrobial activity (Yalpani et al., 2002; Másson et al., 2008). This suggests that chitosan has higher activity than that found for chitin and this has been confirmed experimentally (Tsai and Su, 1999; Másson et al., 2008).

To improve the suboptimal therapy for many fungal infections, the efficacy of some drug combinations has been examined. Several studies involving combinations of amphotericin B with other antimicrobial agents have been reported. Such combinations were expected to be synergistic because amphotericin B facilitated the entry of the second agent into the fungal cell (Jit Sud and Feingold, 1983).

Therefore, this study aims to evaluate the antifungal and antioxidant effects of the chitosan, as a new alternative or complementary anti-fungal drug, alone or in combination with amphotericin B against a pathogenic *C. albicans* in mice.

Materials and methods

Chemicals, media, and drugs

Chitosan (CAT No. 50494) and Sabouraud Dextrose Agar (Product No. S 3181) were purchased from Sigma–Aldrich (St Louis, MO, USA). Cyclophosphamide (Endoxan), and Amphotericin B (supplied as Fungizone; E.R. Squibb & Sons, Princeton, NJ) were purchased. All other chemicals were purchased from local standard companies and were of reagent grade or better.