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DOD/AMB: *IN VIVO* ACTIVITY OF A NOVEL AMB FORMULATION WITH SYNTHETIC CATIONIC BILAYER FRAGMENTS

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ABSTRACT

The ability of the versatile dioctadecyldimethylammonium bromide (DODAB), a bilayer-forming synthetic lipid previously shown to solubilize Amphotericin B (AMB), inspired this evaluation of *in vivo* activity of the DODAB/AMB formulation (DOD/AMB) against systemic candidiasis in a mouse model from survival and tissue burden experiments. AMB was simply added to a DODAB powder dispersion in water previously obtained by sonication with tip at concentrations ≤0.1 and 10 mg/mL, respectively, organic solvents completely absent. AMB aggregation state was evaluated from UV-visible light absorption and dynamic light scattering for aggregate sizing. AMB was stabilized by the DODAB bilayer fragments in its monomeric form, causing disappearance of large water insoluble drug aggregates. From survival and tissue burden experiments, DOD/AMB efficacy was equivalent to the one exhibited by Fungizone (DOC/AMB) —— 100 and 70% survival respectively, at 0.4 mg/kg/day given i.p. for 10 days (P>0.05) ——, regarding elimination of *Candida* colonization in spleen and kidneys. In summary, DOD/AMB, was effective for treating systemic candidiasis in a mouse model.

Key words: novel lipossome formulation, sistemic candidiasis, sinthetic bylayer, anti fungal lipossomes.

INTRODUCTION

Opportunistic infections caused by fungi are one of the leading causes of death in immunocompromised individuals. For the treatment of disseminated mycotic infections, only a limited spectrum of antimycotic agents is available. Amphotericin B (AMB) is the therapy of choice for most invasive *Candida* infections. Unfortunately, AMB nefrotoxicity is high and has been related to AMB occurrence as large aggregates both in water solution and in the lipid membrane. The low AMB solubility in water and in many organic solvents is a problem not easily solved. In order to formulate AMB, liposomes, surfactants, oil-in-water emulsions, cochleates, etc, have been used and certainly improved the therapeutic index of the drug, but the lipid-based formulations are expensive. Dioctadecyldimethylammonium bromide (DODAB) is a synthetic and unexpensive cationic lipid which assembles in water solution forming bilayer vesicles or bilayer fragments (1,3) depending on the dispersion method. The versatility of DODAB as interface agent has been associated with numerous applications, such as immunoadjuvant activity (3), interaction with biomoleculas such as prokariotic (3), eukaryotic cells (3); nucleic acid (3) and synthetic polymers as latex (3). Recently, solubilization of AMB by nanosized, synthetic and charged bilayer fragments was descrited (1). In this field, the increased interest for study novel formulations, alternative to higher toxic

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Fungizone® (AMB comercial, DOC/AMB), has inspired to study the *in vivo* activity of a DOD/AMB formulation.

MATERIALS AND METHODS

AMB solubilization on DODAB solution bilayer fragments (SBF) was done in absence of any organic solvent using AMB 0.05 mg in 10 mg/mL of DODAB bilayer fragments obtained by sonication with tip (1). The physicochemical characterization of DOD/AMB formulation, prior to parenteral administration, was done by UV-visible spectra and the size distribution for DOD/AMB formulations (mean zeta-average diameter, Dz), was determined by using a ZetaPlus Zeta-Potential Analyzer. All spectra and size distributions were obtained at room temperature (25ºC) just after mixing AMB and DODAB SBF or at 24 h, 72 h, 7 days, and 15 days thereafter. Afterwards, the in vitro susceptibility study (2) and an *in vivo* activity were evaluated by % survival, tissue burden study and hystopatologic studies, in a female *swiss webster* mice model of systemic candidiasis. DOC/AMB was used throughout for comparison.

Mouse model systemic candidiasis was developed in accordance to standard procedures. The Infection was developed by injecting 0.1mL of 1 x 10^6 CFU/mouse of *C. albicans* HU 168, intravenously (i.v.) through the tail vein.

Statistics

Differences in survival after 35 days of observation were assessed by Kaplan-Meier analysis folowed by the Wilcoxon test. Comparisons of colony counts among different treatment groups were permormed by the Kruskal-Wallis test. A *P* value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Chemical and colloidal stability for monomeric AMB in synthetic bilayer fragments (SBF) - In complete absence of any organic solvent, the 10 mg/mL DODAB dispersion was efficient to solubilize AMB at concentrations lower than 0.1 mg/mL (Fig. 1).

In vitro and *in vivo* activity of the DOD/AMB formulation - The minimum lethal concentration (MLC) of DOD/AMB against *C. albicans* ATCC 90028 and pathogenic *C. albicans* HU168 were determined as 0.125 e 0.250 mg/mL, respectively. *In vivo*, survival percentiles for mice groups submitted to the DOD/AMB treatment given i.p. were similar to those obtained for equivalent AMB concentrations delivered by the DOC/AMB formulation. 100% survival was achieved at 0.4 mg/kg/day given i.p. for 10 days in contrast to the 70% survival obtained with the same AMB dose given as DOC/AMB (P<0.05) (Fig. 2). Tissue burden studies were conducted in parallell with the survival studies. The tissue burden experiments showed that DOD/AMB was as efficient as DOC/AMB to reduce CFU/g tissue in spleen (Fig. 3A) and kidneys (Fig. 3B).

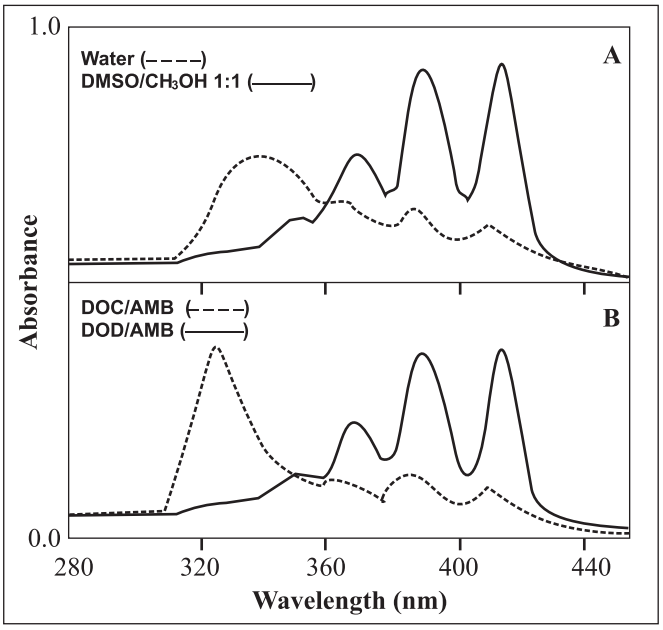

Figure 1. Optical absorption spectra of AMB. In (A), AMB is in DMSO:methanol 1:1 (———) or in water (– – – –). In (B), AMB is in DOD/AMB (———) or in DOC/AMB (– – – –). Final AMB and DODAB concentrations are 7 μg/mL and 1.4 mg/mL, respectively.

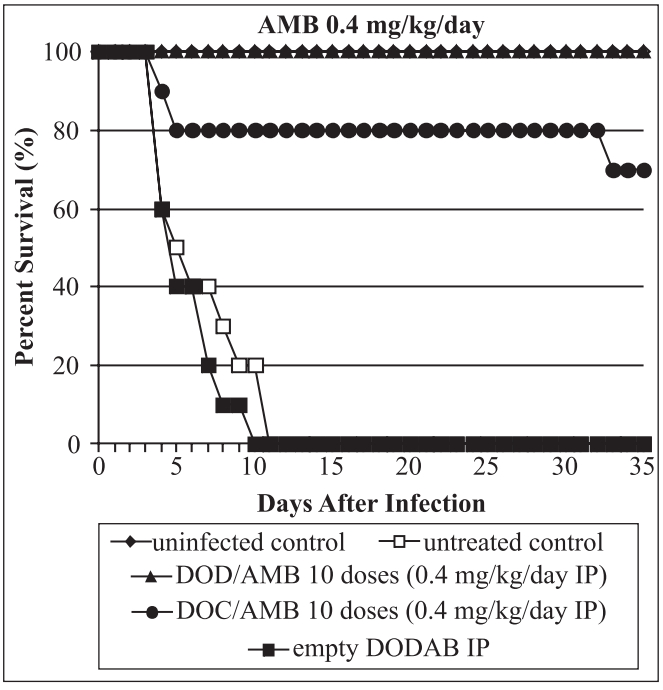

Figure 2. Survival of mice infected with *C. albicans* and treated either with: DOD/AMB at 0.4 mg AMB/kg/day for 10 days i.p. (▲); DOC/AMB at 0.4 mg AMB/kg/day for 10 days i.p. (●); or with empty DODAB SBF i.p. (■), compared to the untreated (□) and uninfected control (◇).

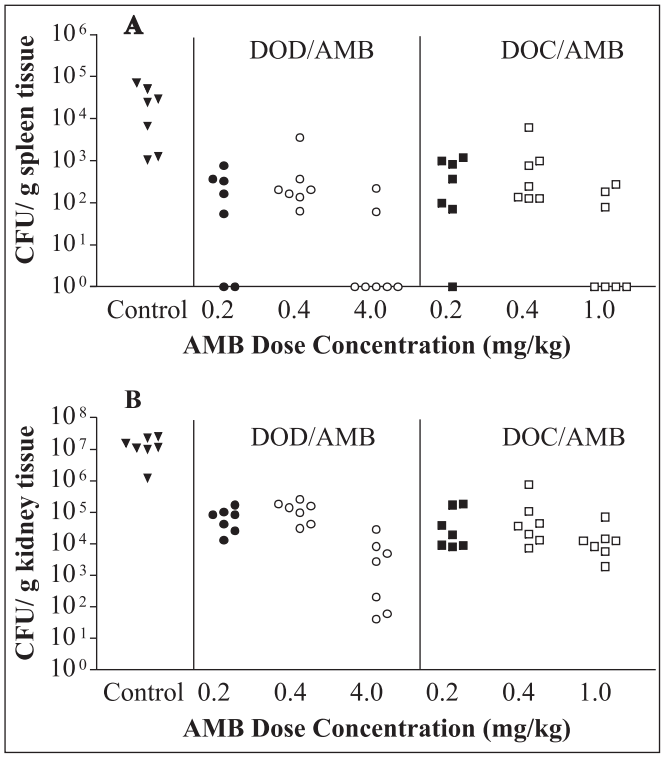

Figure 3. (A) Spleen tissue burden of mice infected with *C. albicans* and treated with either: DOD/AMB i.v. (●), at 0.2 mg AMB/kg/day for 4 days, or i.p. (○), at 0.4, or 4.0 mg AMB/kg/day for 7 days; DOC/AMB i.v. (■), at 0.2 mg AMB/kg/day for 4 days, or i.p. (□), at 0.4, or 1.0 mg AMB/kg/day for 7 days, compared to untreated controls (▼). **(B)** Kidney tissue burden for the same groups of mice. The effect of treatment was determined at day 4 for i.v. group or, at day 8 for i.p. group.

For all groups, CFU found in the kidneys and spleen were reduced significantly ($P<0.05$).

Bilayer fragments conveniently offering a very large area of hydrophobic nanosurfaces suitable to solubilize hydrophobic substances or drugs (Fig. 4). Monomeric state for AMB in the DOD/AMB is observed (Fig 1). Whereas the size of DOD/AMB aggregates decreased as a function of time, the contrary occurred for DOC/AMB at 25ºC (Data not shown), therefore, colloid stability for the new formulation is also better than that exhibited by DOC/AMB. The main DOD/AMB drawback is related to its limited capacity to carry the monomeric form of the drug: 10 mg/mL DODAB dispersion was efficient to solubilize AMB over a drug concentration range that had to be smaller than 0.1 mg/mL. Therefore, if the DOD/AMB eventualy comes to be sucessful also for use in humans, the treatment will possibly have to be prolonged to administer AMB small doses daily distributed over a larger number of days.

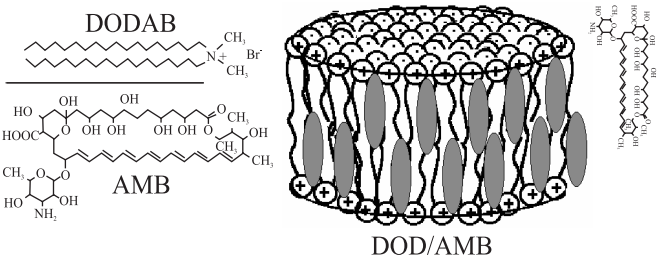

Figure 4. Schematic representation of physical state of AMB in dispersed synthetic cationic bilayer fragments. Amphotericin B in its monomeric form in the DOD/AMB aqueous formulation (1).

CONCLUSIONS

DOD/AMB formulation is a monomeric, colloidally stable, organic-solvent-free, and unexpensive lipid-based formulation with *in vitro* and *in vivo* activity against *Candida albicans.*

ACKNOWLEDGMENTS

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RESUMO

DOD/AMB: Atividade *in vivo* de uma nova formulação com fragmentos sintéticos de bicamadas catiônicas

A habilidade do brometo de dioctadecildimetilamônio (DOBAB), em formar bicamada de lipídio sintético e a demonstração prévia do forte poder solubilizante de anfotericina B (ANB) , incentivou-nos a realizar a avaliação da atividade de DODAB/AMB *in vivo* contra candidíase sistêmica em modelo de camundongos para verificar a sua sobrevida bem como a recuperação das leveduras de *C. albicans* dos órgãos colonizados (baço e rins). O AMB foi simplesmente adicionado à DODAB em pó previamente disperso em água e sonicado com auxílio de ponteiras, nas concentrações de ≤0,1e 10 mg/mL, respectivamente, assegurando-se a completa ausência de solventes orgânicos nesta formulação. O estado de agregação do AMB foi avaliado por meio do espectro de absorção da luz UV-visível e a distribuição de tamanhos das formulações estudadas, determinadas por meio de analisador *ZetaPlus* Brookhaven Instruments Corporation, Holtsville, NY) equipado com um laser de 570 nm e *dynamic light scattering* (90ºC) para a medição dos tamanhos (Grabowski & Morrison, 1983). AMB foi estabilizada na bicamada de DODAB em forma monomérica, eliminando-se os grandes agregados de AMB insolúveis em água. Tanto a sobrevida dos animais como os experimentos

com recuperação das leveduras dos órgãos colonizados (baço e rins) mostraram eficácia equivalente à demonstrada por Fungizona (DOC/AMB) – a sobrevida foi de 100 e 70% respectivamente nas concentrações de 0.4 mg/kg/dia via i.p. por 10 dias (P>0.05), em relação a eliminação da colonização de *C. albicans* dos rins e baço. Em resumo, DOD/AMB foi efetivo no tratamento de candidíase sistêmica em modelo animal.

Palavras-chave: nova formulação lipossomal, candidíase sistêmica, bicamada sintética, lipossoma anti fúgica.

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