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Therapeutic efficacy of liposomes containing 4-(5-pentadecyl-1,3,4-oxadiazol-2-yl)pyridine in a murine model of progressive pulmonary tuberculosis

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ABSTRACT

Background and objectives: Tuberculosis (TB) is one of the deadliest infectious diseases and comprises a global public health concern because co-infection with Human immunodeficiency virus (HIV) and, in particular, the continuous isolation of new Multidrug-resistant strains (MDR), rendering the discovery of novel anti-TB agents a strategic priority. One of the most effective first-line mycobactericidal drugs is Isoniazid (INH). Previously, we reported *in vitro* anti-mycobacterial activity against sensitive and MDR *Mycobacterium tuberculosis* strains of a new oxadiazole obtained from the hybridization of INH and palmitic acid. The present study evaluated the therapeutic potential of liposomes including Phosphatidylcholine (PC) and L-α Phosphatidic acid (PA) or PC and Cholesterol (Chol) containing 4-(5-pentadecyl-1,3,4-oxadiazol-2-yl)pyridine in BALB/c male mice infected by intratracheal (i.t.) route with drug-sensitive or MDR *M. tuberculosis*.
Methods: The lipophilic 4-(5-pentadecyl-1,3,4-oxadiazol-2-yl)pyridine was obtained to mix INH and palmitoyl chloride. The *in vivo* anti-TB effect of this oxadiazole derivative contained in two different liposomes was tested in BALB/c mice infected with a sensitive strain of *M. tuberculosis*, initiating treatment 2 months post-infection, by i.t. route, of 50 μg of oxadiazole derivative for 1 month. In a second stage, mice were infected with an MDR (resistant to first-line drugs) and treated with 150 μg of an oxadiazole derivative carried by PC + Chol liposomes for 2 months. The effect of the oxadiazole derivative *in vivo* was determined by the quantification of lung bacilli loads and histopathology.
Results: In comparison with control animals, drug-sensitive, strain-infected mice treated for 1 month with 50 μg of this oxadiazole derivative contained in the liposomes of PC + Chol showed a significant, 80% decrease of live bacilli in lungs, which correlated with the morphometric observation, and the group of MDR clinical isolate-infected mice treated with 150 μg of the oxadiazole derivative contained in the same type of liposome showed significantly lower lung bacillary loads than control mice, producing 90% of bacilli burden reduction after 2 months of treatment.

Abbreviations: anti-TB, anti-Tuberculosis; CFU, Colony-forming units; Chol, cholesterol; FDA, U.S. Federal Drug Administration; H&E, Hematoxylin and Eosin; HIV, human immunodeficiency virus; INH, isoniazid; MDR, multidrug-resistant; MIC, minimal inhibitory concentration; PA, L-α-Phosphatidic acid; PBS, phosphate-buffered saline solution; PC, L-α-Phosphatidylcholine; Pre-XDR, pre-extensively drug-resistant; SD, standard deviation; TB, tuberculosis; WHO, World Health Organization; XDR, eXtensively drug-resistant.

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Conclusion: These results confirm and extend the reported highly efficient anti-mycobacterial activity of this lipophilic oxidazole derivative when it is carried by liposomes in mice suffering from late progressive pulmonary TB induced by drug-sensitive, and most prominently by, MDR strains.

1. Introduction

Tuberculosis (TB) is an infectious disease that affects millions of persons each year, ranking in second place after the Human immunodeficiency virus (HIV) infection. Reports by the World Health Organization (WHO) indicate that there were 9.0 million new TB active cases and 1.5 million deaths during 2013 [1]. The epidemiological panorama of TB is aggravated with the emergence of Multidrug-resistant strains (MDR, resistant to at least the first-line drugs Isoniazid (INH) and Rifampin), eXtensively drug-resistant (XDR, resistant to Isoniazid, Rifampin, one fluoroquinolone, and one of three injectable, second-line drugs: Amikacin, Kanamycin, and Capreomycin), and pre-XDR strains (resistant to INH and Rifampin and either a fluoroquinolone or a second-line injectable agent, but not both) [2,3]. In addition, there are several problems with the currently available treatment for TB, such as non-adherence due to its long duration, complexity [4], adverse events [5], and the toxicity profiles of anti-retroviral and anti-TB drugs in patients co-infected with TB and HIV [6]. Thus, there is clearly an urgent need for potential new agents that should reduce the treatment duration, possess an acceptable tolerability profile, and be active against patients with MDR/XDR TB and HIV infection.

In recent years, there has been enhanced activity in the research and development of novel drugs for TB. Several compounds are now under development, while others are being investigated in an effort to discover new molecules for target-based treatment of TB [7–9]. At the time of this writing, there are currently at least 21 drugs at different stages of preclinical or clinical evaluation [10–13]. Moreover, after >40 years without a new anti-TB drug, the mycobacterial selective (ATP)-synthase inhibitor Bedaquiline was approved by the U.S. Federal Drug Administration (FDA) at the end of 2012 [14], and several new targets are being identified and validated for their practical usefulness [15–19].

One of the most effective first-line mycobactericidal drugs is INH (Fig. 1). Several analogues bearing the structure of isonicotinic acid, the central scaffold of INH, have been synthesized and tested as anti-mycobacterials [20]. The conversion has been reported of INH to 1,3,4-oxadiazolone derivatives [21]. The compound 1,3,4-oxadiazole (Fig. 1) is a heterocyclic scaffold containing one oxygen atom and two nitrogen atoms in a five-member ring [22,23]. Compounds containing the 1,3,4-oxadiazole core have a broad pharmacological activity spectrum including anti-diabetic [24], anti-hypertensive [25], anti-inflammatory [26], analgesic [27], anti-convulsant [28], anti-cancer [29], anti-bacterial [30], anti-fungal [31], and anti-viral properties [32]. In addition, 1,3,4-oxadiazole has become an important construction motif for the development of new drugs, such as Raltegravir®, an anti-retroviral, and Zibotenatan®, an anti-cancer drug [22]. In previous studies, we designed diverse 4-(5-substituted-1,3,4-oxadiazol-2-yl)pyridine using INH as central scaffold and several short- and long-chain fatty acids. Diverse compounds were synthesized and tested *in vitro,* determining their anti-mycobacterial activity in several sensitive and MDR *Mycobacterium tuberculosis* strains, as well as their cytotoxicity against the Vero cell line, and primary cultures of human peripheral blood mononuclear cells. Our results showed that the high-lipophilic derivative obtained from the hybridization' of INH and palmitic acid exhibited highest *in vitro* selective antibiotic bioactivity in the low micromolar range [33]. The influence of lipophilic substituents on anti-TB activity coincides with the results reported for different chemical entities, such as 1,5-diphenylpyrrole and 1,4-dihydropyridines derivatives against MDR [34–36], and pentacyclo-undecane-derived cyclic tetra-amines on XDR strains of *M. tuberculosis* [37].

The present study had the aim of evaluating the therapeutic potential of liposomes containing 4-(5-pentadecyl-1,3,4-oxadiazol-2-yl)pyridine (Fig. 1) in a murine model of progressive pulmonary TB produced by drug-sensitive and MDR-TB strains. Due to the highly lipophilic activity of this compound, it exhibited very low solubility and it was necessary to deliver it in liposomes.

2. Materials and methods

2.1. Chemical material

The lipophilic derivative of 1,3,4-oxadiazole was obtained as described previously [33]. Briefly, the mixture of INH (0.0036 mol) and the 1.1 equiv of palmitoyl chloride in 10 mL of dimethylformamide was heated to reflux for 3–4.5 h. Thin layer chromatography was used to monitor the reaction. After cooling, the mixture was neutralized with saturated $NaHCO_3$ solution and the precipitate formed was filtered by suction. The crude product was purified by recrystallization from adequate solvent and the compound 4-(5-pentadecyl-1,3,4-oxadiazol-2-yl)pyridine was identified by spectroscopic (^1H– and ^{13}C Nuclear Magnetic Resonance) and spectrometric data (Mass Spectrometry) [33]. All reagents and laboratory materials were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Liposome preparation

The phospholipids L-α-Phosphatidylcholine (PC) and L-α-Phosphatidic acid (PA), both from egg yolk and Cholesterol (Chol) (all

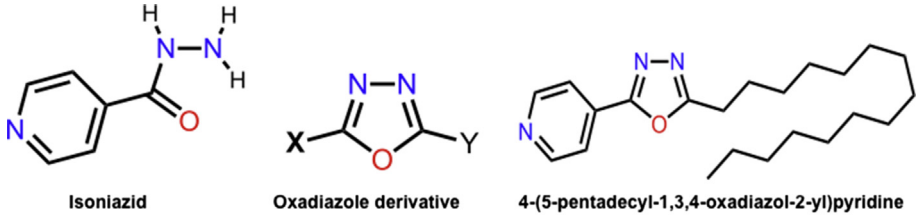

Fig. 1. Chemical structures of Isoniazid (INH), 1,3,4-oxadiazole and 4-(5-pentadecyl-1,3,4-oxadiazol-2-yl)pyridine.

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