Tuberculosis 115 (2019) 108–112

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: www.elsevier.com/locate/tube

JVA, an isoniazid analogue, is a bioactive compound against a clinical isolate of the *Mycobacterium avium* complex

Júlia S. Fahel[a], Rafael P. Vieira[a], Fábio V. Marinho[b], Viviane C. Santos[a], João Vitor de Assis[c], Patrícia P. Corsetti[a], Rafaela S. Ferreira[a], Mauro V. de Almeida[c], Sergio C. Oliveira[a,d,*]

[a] Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[b] Programa de Pós-graduação em Genética, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[c] Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Juiz de Fora, Juiz de Fora, Brazil
[d] Instituto Nacional de Ciência e Tecnologia em Doenças Tropicais (INCT-DT), CNPq MCT, Salvador, BA, Brazil

ARTICLE INFO

Keywords:
Mycobacterium avium complex
Isoniazid analogue
Macrophage

ABSTRACT

Bacteria belonging to *Mycobacterium avium* complex are organisms of low pathogenicity that infect immunosuppressed individuals. Infection is treated with an antimicrobial macrolide, Clarithromycin (CAM) or Azitromycin, associated with Ethambutol and Rifabutin during 12 months. Regimen long duration and side effects hinder patient's commitment to treatment favoring emergence of antibiotic resistance. In this present study, we evaluated the activity of JVA, an Isoniazid (INH) derivative, against *M. avium* 2447, a clinical isolate. We demonstrated that JVA reduces *M. avium* 2447 growth in macrophages, more efficiently than CAM and INH. In order to explore JVA mechanism of action, we investigated compound properties and performed pH-dependent stability studies. Our results suggest an enhanced ability of JVA to cross biological membranes. Furthermore, we suggest that in acidic conditions of macrophages' phagosomes, where mycobacteria replicate, JVA would be promptly hydrolyzed to INH, delivering the adduct INH-nicotinamide adenine dinucleotide and thus inhibiting *M. avium* 2447 growth.

1. Introduction

The genus *Mycobacterium* comprises tuberculous mycobacteria, such as *M. tuberculosis,* and nontuberculous mycobacteria (NTM), such as members of the *Mycobacterium avium* complex (MAC). MAC bacteria display low pathogenicity and are ubiquitously present in soil and aquatic environments. Its clinical relevance was not recognized by the health community until the 90s when AIDS pandemic favored the outcome of several opportunistic infections by these bacteria in HIV positive patients [1]. Today we know that MAC species infect immunosuppressed patients, not only in the context of HIV infection, but also in the case of patients undertaking immunosuppressive therapies. Additionally, they are the most frequent causes of pulmonary disease by NTM [2,3]. Treatment consists of 12-month therapy with Clarithromycin (CAM) or Azitromycin, associated with Ethambutol and Rifabutin [3]. The long duration and side effects of this regimen hinder patient's commitment to treatment favoring the emergence of antibiotic resistance [4]. Studies aiming the development of new drug candidates elucidating their mechanisms of action are relevant endeavors to circumvent the resistance issue.

In the present study, we evaluated the antimicrobial action of *E-N* [2]-3,7-dimethyl-2-*E*,6-octadienylidenyl (JVA) against a MAC clinical isolate, the *M. avium* strain 2447 and investigated its possible mechanism of action. Previous studies with this compound have shown promising activities against *M. tuberculosis,* stimulating new investigations concerning other relevant *Mycobacterium* spp [5]. JVA is a hydrazone, a derivative obtained from the reaction between Isoniazid (INH) and geranial. The mechanism of action of JVA has not been investigated up to date. However, it is already clinically demonstrated that its precursor compound, INH, which is an efficient tuberculostatic agent, acts primarily through inhibition of InhA, a 2-trans-enoyl-acyl carrier protein. InhA is an enzyme involved in the biosynthesis of mycolic and fatty acids, essential components of the cell wall of *Mycobacterium* spp [6]. Once INH is activated, an INH-nicotinamide adenine dinucleotide (NAD) adduct is formed, a derivative responsible for the non-covalent inhibition of InhA. This inhibition and NAD-dependence of InhA have been extensively characterized for *M. tuberculosis,* with binding mode elucidation through X-ray crystallography studies [6–9].

* Corresponding author. Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Pampulha, Belo Horizonte, Minas Gerais, Brazil.
E-mail address: scozeus@icb.ufmg.br (S.C. Oliveira).

https://doi.org/10.1016/j.tube.2019.03.002
Received 26 October 2018; Received in revised form 1 March 2019; Accepted 4 March 2019

To the best of our knowledge, no available crystal structure or computational study elucidated the interaction between the adduct and InhA of *M. avium*.

Herein, we demonstrated that JVA reduces *M. avium* 2447 growth in macrophages, more efficiently than CAM, an antibiotic commonly used in clinic, and INH, the compound from which JVA derives. In order to explore a mechanism of action, we describe the first study on the physicochemical properties and pH-dependent stability of JVA correlated to the molecular modeling and antibacterial evaluations. We suggest that JVA may act as a precursor, delivering the adduct INH-NAD inside macrophages and thus inhibiting bacterial growth.

2. Material and methods

2.1. Bacterial strain

The bacterial strain used in this study was *M. avium* 2447, originally isolated from a patient with acquired immunodeficiency syndrome [10], kindly donated by Prof. Rui Appelberg from Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Porto, Portugal. All the experiments were performed in a Biosafety level 2 laboratory.

2.2. Mice

C57BL/6 mice were housed in a specific pathogen–free animal facility at Universidade Federal de Minas Gerais. Experiments were performed according to protocols approved by the Institutional Animal Ethics Committee (CETEA#45/2018).

2.3. Preparation of JVA, INH and CAM solutions

JVA was prepared as described previously [5]. JVA, INH (Sigma-Aldrich, St. Louis, MO, USA) and CAM (Sigma-Aldrich, St. Louis, MO, USA) were firstly dissolved in 500 μL of DMSO at 0.2 M. These solutions were diluted to a working solution of 50% DMSO in PBS at 1000 μM.

2.4. Evaluation of bacterial growth in the presence of JVA, INH and CAM

JVA, INH and CAM were added to Difco Middlebrock 7H9 Broth (BD, Franklin lakes, NJ, USA) supplemented with BBL™ Middlebrook ADC Enrichment (BD, Franklin lakes, NJ, USA), 0.05% Tween-80 and 0.2% glycerol (7H9 complete medium) at 0.1, 1 and 10 μM. The final concentration of DMSO in all medium aliquots was 0.5%. *M. avium* 2447 was inoculated at 1×10^6 CFU/mL. OD_{600} was measure on days 0, 2, 4, 6 and 10. At day 10, a sample of each medium aliquot was collected, serially diluted and plated for CFU counting.

2.5. Determination of minimum inhibitory concentration (MIC)

JVA and INH MICs were determined using 10-point 2-fold broth macrodilution. *M. avium* 2447 1×10^6 CFU/mL was co-incubated with JVA or INH at 320, 160, 80, 40, 20, 10, 5, 2.5, 1.25 and 0.625 μM. Bacteria was also incubated with DMSO only as control. Cultures were incubated in a shaker at 37 °C and 200 rpm. At day 7, serial dilutions were plated in Difco Middlebrock 7H10 Agar Base Broth (BD, Franklin lakes, NJ, USA) supplemented with 10% oleic acid albumin dextrose catalase (OADC) and 0.2% glycerol. Plates were incubated at 37 °C for CFU counting 7 days later. The lowest concentration that prevented 100% of *M. avium* 2447 growth observed in absence of compounds was defined as the MIC.

2.6. In vitro infection of bone marrow-derived macrophages (BMMs) with M. avium 2447 followed by treatment with JVA, INH and CAM

Macrophages were derived from the bone marrow of C57BL/6 mice as described previously [11]. After differentiation, BMMs were washed with saline and medium (DMEM, 10% FBS, 1% HEPES) was added to the cells. Cells were then infected with *M. avium* 2447 MOI 5:1 during 4 h. After infection, cells were washed with saline and incubated with JVA, INH or CAM at 0.1, 1 and 10 μM. At time zero, days 2 and 4, cells were washed with saline and incubated in 10% saponin at 37 °C during 20 min. Lysates were serially diluted and plated in 7H10 Agar Base Broth supplemented with 10% OADC. Plates were incubated at 37 °C during 7 days and CFU counting was performed.

2.7. JVA properties

Physical, physicochemical and electronic properties of JVA, INH and CAM were obtained by using the softwares Chemicalize (https://chemicalize.com/) and Alogps (http://www.vcclab.org/lab/alogps/).

2.8. JVA stability

JVA solutions (100 μM, 12.5% DMSO in buffer) were prepared in citrate 0.1 M (pH 4.5 and 5.5) and phosphate 0.2 M (pH 6.5 and 7.4) buffers. Electronic spectra between 270 and 450 nm were obtained at 0 h and 24 h, at 37 °C, using a Varioskan microplate reader (Thermo Fischer, Waltham, MA, USA).

2.9. Protein comparative modeling

A tridimensional model for *M. avium* InhA was obtained by homology modeling to investigate the binding mode of JVA-derived adduct (INH-NAD) with the enzyme according to procedures previously described [9,12–17].

2.10. Statistical analysis

Statistically significant differences were evaluated by Student *t*-test or one-way ANOVA followed by Dunnet post test using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA). P values < 0.05 were considered significant.

3. Results

3.1. Comparison of the effect of JVA, INH and CAM in M. avium 2447 growth in liquid culture medium

Our first question was whether JVA had a direct effect in *M. avium* 2447 growth. To answer it, bacteria were co-incubated during 10 days with JVA at 0.1, 1 or 10 μM. INH and CAM were used as controls. OD_{600} was measured at days 0, 2, 4, 6 and 10. Additionally, the number of bacteria was quantified, at day 10, by CFU counting (Fig. 1 A, B and C).

Considering incubation with JVA, there was a significant delay in *M. avium* 2447 growth when the compound was used at 10 μM in comparison to control medium (Fig. 1A). In this condition, the values of OD_{600} began to rise after day 4 and they remained below control values throughout the experiment. No major differences were noticed with JVA 0.1 and 1 μM, except for a significant reduction on bacterial growth after day 6 at JVA 1 μM.

Similarly, INH at 10 μM also induced a delay in *M. avium* 2447 growth during the 10 days of culture (Fig. 1B). OD_{600} values began to rise after day 4 and they remained below control values. INH 1 μM affected *M. avium* 2447 growth after day 6 and INH 0.1 μM did not affected bacteria growth.

On the other side, CAM displayed a more striking effect in *M. avium* 2447 growth (Fig. 1C). All three concentrations induced significantly lower values of OD_{600} throughout the 10 days of culture as compared to control. CAM 0.1 μM induced a significant delay in bacterial growth and, at 1 μM, OD_{600} values began to rise only at day 10. No bacterial growth was detected when CAM was used at 10 μM.

A comparison of CFU counting in samples collected at day 10

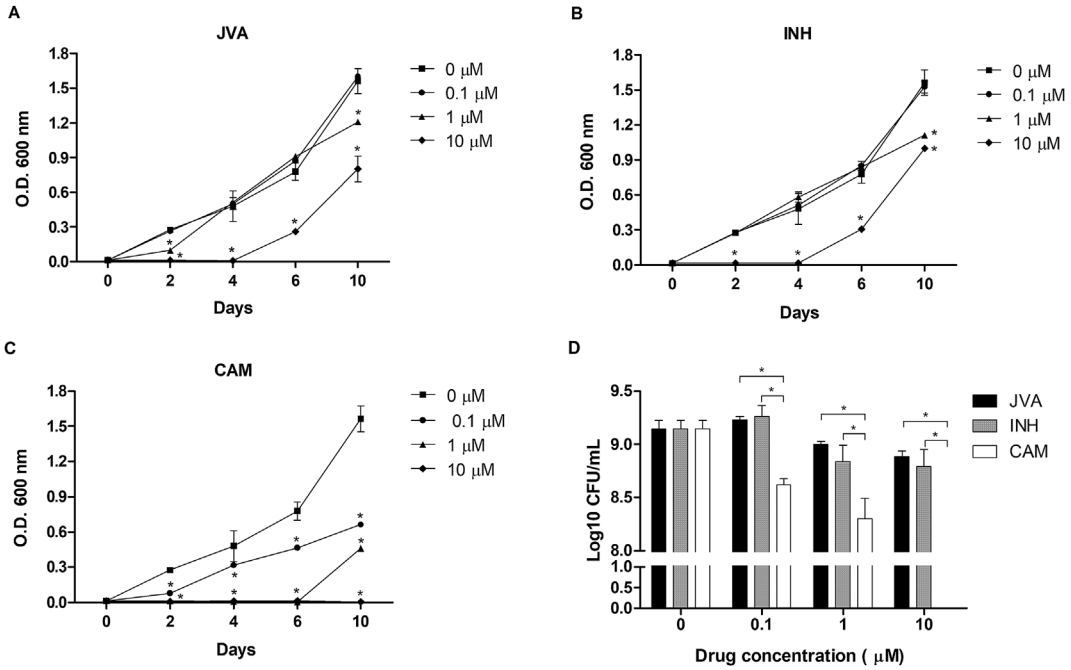

Fig. 1. *M. avium* growth in liquid culture in the presence of JVA, CAM and INH. *M. avium* 2447 was grown during 10 days in the presence of JVA (A), INH (B) or CAM (C) in three concentrations: 0.1, 1 or 10 μM. At days 0, 2, 4, 6 and 10, OD_{600} was measured. CFU counting was performed at day 10 (D). Data were expressed as mean ± SD. *p < 0.05.

showed that CAM reduced bacterial growth more efficiently than JVA and INH (Fig. 1D). This reduction was observed in all concentrations tested. No bacteria were detected within CAM 10 μM and no significant differences were found between JVA and INH regardless of compound concentration.

Additionally, minimum inhibitory concentration (MIC) values for JVA and INH were determined using broth macrodilution. *M. avium* 2447 was co-incubated with JVA or INH at decreasing concentrations and, at day 7, the number of bacteria was evaluated by CFU counting. MIC values for JVA and INH were 87.8 μg/mL and 43.9 μg/mL, respectively, which corresponds to 320 μM.

3.2. JVA is more efficient than CAM and INH in reducing M. avium 2447 growth in macrophages

Our next question was whether treatment with JVA would affect *M. avium* 2447 growth inside macrophages since these are mycobacteria preferential host cells [18]. To address this issue, macrophages derived from mice bone marrow were infected with *M. avium* 2447 and treated with JVA, INH and CAM at 0.1, 1 or 10 μM. After 2 and 4 days of incubation, cells were lysed for CFU counting.

Regarding CFU counting from cells at day 2, none of the tested compounds had an effect in *M. avium* 2447 growth inside macrophages at 0.1 μM (Fig. 2A). In contrast, at 1 and 10 μM, the treatment with JVA significantly reduced *M. avium* 2447 replication in macrophages when compared to INH or CAM. No difference was detected between CFU counting from macrophages treated with INH or CAM. Regarding day 4, similarly, none of the compounds affected *M. avium* 2447 growth when tested at 0.1 μM (Fig. 2B). However, at 1 and 10 μM, JVA induced significant reduction of *M. avium* 2447 replication intracellularly when compared to INH or CAM.

3.3. JVA properties and stability studies

Physical, physicochemical and electronic properties of JVA, INH and CAM were calculated to investigate the mechanism of action of JVA (Fig. S1, Tables S1 and S2). Isoelectric points and pKa values of compounds suggest the prevalence of neutral forms of both INH and JVA at physiological conditions (pH 7.4). Alternatively, a protonated form of CAM is the prevalent one at pH 7.4 (Fig. S1 and Table S1). Regarding octanol-water partition coefficient values, outcomes from software and experimental values suggest the same trend on significant higher hydrophobicity of JVA in comparison to INH. In comparison to CAM, JVA showed similar hydrophobicity behaviour (Table S2). Taken together, these results suggest an enhanced ability of JVA to cross biological membranes.

To evaluate JVA stability at different pHs, electronic spectra in aqueous solution were obtained at UV–visible range (Fig. 3). Results of these measurements at pH 4.5, 5.5, 6.5 and 7.4 showed a decrease of 88, 85, 40 and 1%, respectively, of the absorbance after 24 h, based on peaks at 300 nm which were attributed to JVA [19,20]. These data clearly indicate a trend of significant decrease on absorbance as pH values are decreased, in accordance to previous studies [19,20]. Therefore, considering acidic conditions of macrophages' phagosomes (pH 5.5), JVA would be promptly hydrolyzed to INH, being available to form the adduct INH-NAD, subsequently.

3.4. Protein comparative modeling and active site analysis

Since our results suggested that JVA may form the adduct INH-NAD, it is plausible to consider that the molecular target in *M. avium* is the same as in *M. tuberculosis*, the InhA enzyme. The active sites of *M. avium* InhA model and *M. tuberculosis* InhA crystal (PDB 1ZID) are very similar. Thus, the well-established pattern of ligand-protein interactions between INH-NAD adduct and *M. tuberculosis* InhA was transferred to *M. avium* InhA model. Slight differences (Fig. S2) between these proteins are summarized in Table S3. Based on measured distances between the closest atoms from ligand and receptor, four of the five different residues (F97S; H265S; Y127D and M130L) are too distant to interact with the adduct, so alterations might not impact substantially in protein-ligand associations. The only residue that interacts with the adduct, Gly96, is altered to Ala96 in *M. avium* InhA. However, since this residue is in contact with the adduct by hydrogen bonds established by a backbone interaction, alteration of Gly96 to Ala96 does not interfere

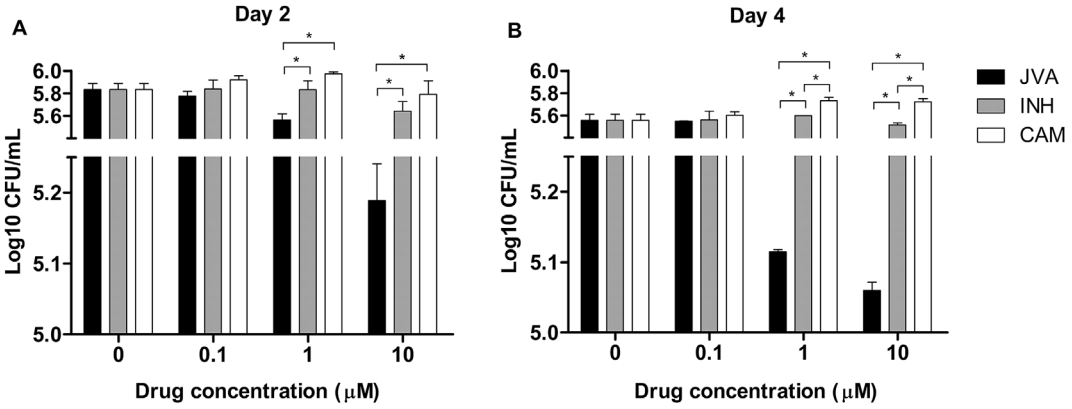

Fig. 2. *M. avium* 2447 growth in macrophages derived from mice bone marrow and treatment with JVA, INH and CAM. Macrophages derived from the bone marrow of C57BL/6 mice were infected with *M. avium* 2447 and treated with JVA, INH and CAM at three concentrations: 0.1, 1 or 10 μM. After 2 and 4 days of incubation, cells were lysed for CFU counting. Data were expressed as mean ± SD. *p < 0.05.

with this binding. Despite these differences, no considerable conformational changes were predicted in our model. The active site analysis of *M. avium* InhA model indicates that JVA should act through the same mechanism against *M. avium* and *M. tuberculosis*.

4. Discussion

This present study aimed to evaluate the antimicrobial activity of JVA against a clinical isolate of the *Mycobacterium avium* complex, *M. avium* strain 2447, investigating its possible mechanism of action by computational and stability studies. The antimicrobial activity was investigated using two different approaches. *M. avium* 2447 was grown in liquid medium or in macrophages in the presence of JVA. Additionally, CAM, an antibiotic commonly used to treat *M. avium* infection, and INH, the compound from which JVA derives, were used as controls. We demonstrated that JVA delays *M. avium* 2447 growth in liquid medium. However, its antimicrobial effect was not as strong as CAM, which completely abrogates bacterial growth in this condition. A different scenario is seen when *M. avium* 2447 is inside macrophages. In this context, JVA is significantly more efficient in killing the bacteria when compared to CAM or INH.

JVA is an INH-derivative and, according to pH-dependent stability studies, it has been suggested as a potential INH's precursor, subsequently forming INH-NAD, a well-known InhA inhibitor adduct [6,7,9,21]. Therefore, the computational studies were performed with the adduct, evaluating ligand-protein interactions based on a suitable modeled target (*M. avium* InhA). The computational studies suggested high similarity between *M. avium* and *M. tuberculosis* InhAs, regarding structural features and likely also their affinity to INH-NAD adduct and analogous inhibitors.

JVA susceptibility to hydrolysis was crucial in the present study. The culture media used for *M. avium* 2447 presented pH 6.5 just after preparation. The pH value of macrophages' phagosomes, where the mycobacteria is located upon infection, is originally 5.5 and reduced to 4.5 after 24 h [22,23]. In addition, evaluation of stability at physiological pH value (7.4) is an important feature of a bioactive compound.

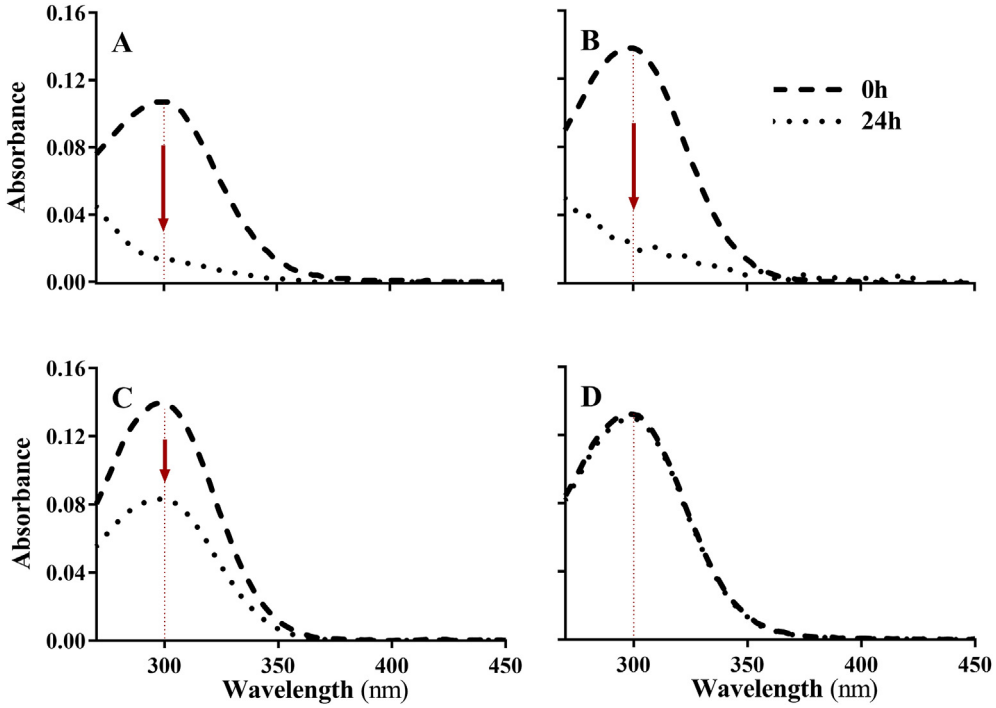

Fig. 3. Electronic spectra of JVA (100 μM) in citrate (pH 4.5 (A) and 5.5 (B)) and phosphate (pH 6.5(C) and 7.4(D)) buffers. Black dashed line, 0 h and black dotted line, 24 h. Red dotted line, 300 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Results on pH-sensitivity (Fig. 3) confirmed the previously observed acidic susceptibility of JVA to degradation [19,20]. The lower the pH, the more intense is the phenomena, indicated by a pronounced difference between the values of absorbance at 300 nm, measured at 0 h and 24 h of incubation. In case of pH 4.5, this event is even faster, an indication of immediate degradation of JVA at that condition.

JVA displays more lipophilic behaviour in comparison to INH at *M. avium* 2447 culture media (pH 6.5) and physiological conditions (pH 7.4). This is suggested by theoretical calculations of physicochemical properties, pKa values and theoretical octanol-water partition coefficient ($LogK_{ow}$ or LogP), and confirmed by experimental values previously determined [5]. The high hydrophobicity of JVA and the maintenance of the neutral form at pH 7.4 might be the crucial points for its biological activity, making it a suitable probe to cross membranes, delivering INH at its site of action.

In comparison to CAM, JVA markedly displayed higher activity against *M. avium* 2447 in macrophages, which might be due to the presence of neutral form (major species) of compounds at pH 6.5–7.4. Simultaneously, CAM displays higher percentage of protonated form at same conditions. Thus, neutral forms of JVA and INH are suitable to cross macrophage membranes in comparison to ionized CAM. In case of BMMs, these pKa and $LogK_{ow}$ effects are probably significant to allow the diffusion of compounds to phagosomes, compartments where *Mycobacterium* spp. are usually located. The possible difficulty for CAM to reach these sites due to predominance of ionized species in media also helps to explain the low activity of the control against *M. avium* 2447 on this *in vitro* cell assay in comparison to JVA and INH. Once internalized in macrophages, JVA might be hydrolyzed in low pH values (4.5–5.5), forming isoniazid and following to subsequent InhA inhibition. The hydrophobic profile of JVA might explain its higher activity in comparison to INH.

In conclusion, JVA hydrolytic process and physicochemical properties indicate that its higher efficiency in killing *M. avium* 2447 in macrophages might be attributed to an intracellular delivery of INH. This mechanism of action turns JVA into an interesting INH-derivative that deserves further evaluation as a promising bioactive compound.

Acknowledgements

This study was funded by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (# 302660/2015-1), Comissão de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Grant AUXPE 3379/2013 and CAPES/COFECUB 868/15) and Fundação de Amparo à Pesquisa em Minas Gerais (FAPEMIG) (Rede Mineira de Imunobiológicos # 00140-16). RSF acknowledges L'Oréal-UNESCO-ABC "For Women in Science Award, Category Chemistry, Brazil, 2017" and L'Oréal-UNESCO "For Women in Science International Rising Talents 2018 Award". RPV acknowledges post-doctoral fellowships from CAPES (Edital PVE A118/2013 and Edital Biocomputacional AUXPE 3379/2013). FVM acknowledges post-doctoral fellowship from Pós-Graduação em Genética – PNPD/CAPES.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tube.2019.03.002.

References

[1] Horsburgh Jr. CR. Mycobacterium avium complex infection in the acquired immunodeficiency syndrome. N Engl J Med 1991;324:1332–8. https://doi.org/10.1056/NEJM199105093241906.

[2] Hoefsloot W, van Ingen J, Andrejak C, Angeby K, Bauriaud R, Bemer P, Beylis N, Boeree MJ, Cacho J, Chihota V, Chimara E, Churchyard G, Cias R, Daza R, Daley CL, Dekhuijzen PN, Domingo D, Drobniewski F, Esteban J, Fauville-Dufaux M, Folkvardsen DB, Gibbons N, Gomez-Mampaso E, Gonzalez R, Hoffmann H, Hsueh PR, Indra A, Jagielski T, Jamieson F, Jankovic M, Jong E, Keane J, Koh WJ, Lange B, Leao S, Macedo R, Mannsaker T, Marras TK, Maugein J, Milburn HJ, Mlinko T, Morcillo N, Morimoto K, Papaventsis D, Palenque E, Paez-Pena M, Piersimoni C, Polanova M, Rastogi N, Richter E, Ruiz-Serrano MJ, Silva A, da Silva MP, Simsek H, van Soolingen D, Szabo N, Thomson R, Tortola Fernandez T, Tortoli E, Totten SE, Tyrrell G, Vasankari T, Villar M, Walkiewicz R, Winthrop KL, Wagner D. Nontuberculous Mycobacteria Network European Trials G. The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: an NTM-NET collaborative study. Eur Respir J 2013;42:1604–13. https://doi.org/10.1183/09031936.00149212.

[3] Henkle E, Winthrop KL. Nontuberculous mycobacteria infections in immunosuppressed hosts. Clin Chest Med 2015;36:91–9. https://doi.org/10.1016/j.ccm.2014.11.002.

[4] Kadota T, Matsui H, Hirose T, Suzuki J, Saito M, Akaba T, Kobayashi K, Akashi S, Kawashima M, Tamura A, Nagai H, Akagawa S, Kobayashi N, Ohta K. Analysis of drug treatment outcome in clarithromycin-resistant Mycobacterium avium complex lung disease. BMC Infect Dis 2016;16:31. https://doi.org/10.1186/s12879-016-1384-7.

[5] de Faria TJ, Roman M, de Souza NM, De Vecchi R, de Assis JV, dos Santos AL, Bechtold IH, Winter N, Soares MJ, Silva LP, De Almeida MV, Bafica A. An isoniazid analogue promotes Mycobacterium tuberculosis-nanoparticle interactions and enhances bacterial killing by macrophages. Antimicrob Agents Chemother 2012;56:2259–67. https://doi.org/10.1128/AAC.05993-11.

[6] Chollet A, Mourey L, Lherbet C, Delbot A, Julien S, Baltas M, Bernadou J, Pratviel G, Maveyraud L, Bernardes-Genisson V. Crystal structure of the enoyl-ACP reductase of Mycobacterium tuberculosis (InhA) in the apo-form and in complex with the active metabolite of isoniazid pre-formed by a biomimetic approach. J Struct Biol 2015;190:328–37. https://doi.org/10.1016/j.jsb.2015.04.008.

[7] Dias MV, Vasconcelos IB, Prado AM, Fadel V, Basso LA, de Azevedo Jr. WF, Santos DS. Crystallographic studies on the binding of isonicotinyl-NAD adduct to wild-type and isoniazid resistant 2-trans-enoyl-ACP (CoA) reductase from Mycobacterium tuberculosis. J Struct Biol 2007;159:369–80. https://doi.org/10.1016/j.jsb.2007.04.009.

[8] Rozwarski DA, Vilcheze C, Sugantino M, Bittman R, Sacchettini JC. Crystal structure of the Mycobacterium tuberculosis enoyl-ACP reductase, InhA, in complex with NAD+ and a C16 fatty acyl substrate. J Biol Chem 1999;274:15582–9.

[9] Rozwarski DA, Grant GA, Barton DH, Jacobs Jr. WR, Sacchettini JC. Modification of the NADH of the isoniazid target (InhA) from Mycobacterium tuberculosis. Science 1998;279:98–102.

[10] Appelberg R, Castro AG, Pedrosa J, Minoprio P. Role of interleukin-6 in the induction of protective T cells during mycobacterial infections in mice. Immunology 1994;82:361–4.

[11] Marinho FV, Fahel JS, Scanga CA, Gomes MT, Guimaraes G, Carvalho GR, Morales SV, Bafica A, Oliveira SC. Lack of IL-1 receptor-associated kinase-4 leads to defective Th1 cell responses and renders mice susceptible to mycobacterial infection. J Immunol 2016;197:1852–63. https://doi.org/10.4049/jimmunol.1502157.

[12] Labo M, Gusberti L, Rossi ED, Speziale P, Riccardi G. Determination of a 15437 bp nucleotide sequence around the inhA gene of Mycobacterium avium and similarity analysis of the products of putative ORFs. Microbiology 1998;144(Pt 3):807–14. https://doi.org/10.1099/00221287-144-3-807.

[13] Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990;215:403–10. https://doi.org/10.1016/S0022-2836(05)80360-2.

[14] Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Soding J, Thompson JD, Higgins DG. Fast, scalable generation of high-quality protein multiple sequence alignments using clustal omega. Mol Syst Biol 2011;7:539. https://doi.org/10.1038/msb.2011.75.

[15] Sali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. J Mol Biol 1993;234:779–815. https://doi.org/10.1006/jmbi.1993.1626.

[16] Benkert P, Tosatto SC, Schomburg D. QMEAN: a comprehensive scoring function for model quality assessment. Proteins 2008;71:261–77. https://doi.org/10.1002/prot.21715.

[17] Lovell SC, Davis IW, Arendall 3rd WB, de Bakker PI, Word JM, Prisant MG, Richardson JS, Richardson DC. Structure validation by calpha geometry: phi,psi and Cbeta deviation. Proteins 2003;50:437–50. https://doi.org/10.1002/prot.10286.

[18] Neyrolles O, Wolschendorf F, Mitra A, Niederweis M. Mycobacteria, metals, and the macrophage. Immunol Rev 2015;264:249–63. https://doi.org/10.1111/imr.12265.

[19] Peres LB. Obtenção de nanopartículas de PLLA e nanopartículas blenda PLLA/PMMA e PLLA/PS para incorporação de um análogo da isoniazida. Florianópolis, Brazil: Federal University of Santa Catarina; 2012.

[20] Roman M. Estudo farmacocinético de um análogo lipofílico da isoniazida, denominado JVA, administrado em camundongos. Florianópolis, Brazil: Federal University of Santa Catarina; 2012.

[21] Vilcheze C, Wang F, Arai M, Hazbon MH, Colangeli R, Kremer L, Weisbrod TR, Alland D, Sacchettini JC, Jacobs Jr. WR. Transfer of a point mutation in Mycobacterium tuberculosis inhA resolves the target of isoniazid. Nat Med 2006;12:1027–9. https://doi.org/10.1038/nm1466.

[22] Danelishvili L, Bermudez LE. Mycobacterium avium MAV_2941 mimics phosphoinositol-3-kinase to interfere with macrophage phagosome maturation. Microb Infect 2015;17:628–37. https://doi.org/10.1016/j.micinf.2015.05.005.

[23] Oh YK, Straubinger RM. Intracellular fate of Mycobacterium avium: use of dual-label spectrofluorometry to investigate the influence of bacterial viability and opsonization on phagosomal pH and phagosome-lysosome interaction. Infect Immun 1996;64:319–25.