Type: Poster Presentation

Final Abstract Number: 43.145
Session: Poster Session III
Date: Saturday, March 5, 2016
Time: 12:45-14:15
Room: Hall 3 (Posters & Exhibition)

Biological evaluation of a novel nitroimidazooxazole derivative, IIIM-MCD-019 against Mycobacterium tuberculosis and its in vivo efficacy

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Background: One of the nitroimidazo-oxazole derivatives, IIIM-MCD-019 discovered in-house was assessed for detailed biological activities against various strains of *Mycobacterium tuberculosis*. Further, *in-vitro* studies such as synergistic activity, intracellular MIC, time kill kinetics, cell cytotoxicity, microsomal stability and pharmacokinetics were performed. *In-vivo* efficacy of the compound was tested alone and in combination.

Methods & Materials: MIC was determined against $H_{37}Rv$, monoresistant as well multidrug resistant (MDR) isolates and streptomycin starved *M. tuberculosis* (ss18b). Synergistic studies were performed with rifampicin, isoniazid and ethambutol using microdilution checkerboard assay. Time kill of the compound was performed at MIC to 8X MIC against M. *tuberculosis* $H_{37}Rv$. Intracellular MIC was performed in macrophage J774 cell lines using RPMI-1640 media. Cell cytotoxicity of the compound was assessed on HepG-2 cell lines using glucose and galactose containing media to identify mitochondrial cytotoxicity. Microsomal stability of the compound was performed using rat liver microsomes. PK parameters were assessed in mice by standard protocols and *In-vivo* efficacy was assessed in intranasal model using Balb/c mice for 4 weeks.

Results: IIIM-MCD-019 exhibited an MIC ranged between 0.12-0.25 μg/ml for all strains except for NRP which is streptomycin starved. When combined with rifampicin, isoniazid and ethambutol individually, it showed synergistic effect with rifampicin and isoniazid and additive effect with ethambutol and it has intracellular MIC of 1μg/ml. Time kill studies shows that killing rate of this compound is comparable to the best in class drug candidate i.e. delamanid (OPC-67683). The compound did not exhibit cytotoxicity either in glucose or in galactose and found to be ≥ 99% stable in rat liver microsomes. Pharmacokinetic profile in terms of C_{max} & AUC_{0-t} also shows 1.5 times increase (C_{max} of 0.54 μg/ml and of 7.42 μg/ml* h) compared delamanid. In *in-vivo* model, the compound showed 1 log reduction in cfu wrt early control and better efficacy when combined with combination of rifmapicin and isoniazid.

Conclusion: IIIM-MCD-019 is a novel compound from nitroimidazo-oxazole scaffold and has a potent antiTB properties. The compound has shown better PK profile than the drug candidate, however further optimization of structure is required to achieve better *in-vivo* efficacy.

http://dx.doi.org/10.1016/j.ijid.2016.02.883

Type: Poster Presentation

Final Abstract Number: 43.147
Session: Poster Session III
Date: Saturday, March 5, 2016
Time: 12:45-14:15
Room: Hall 3 (Posters & Exhibition)

Isolation and identification of a novel Non-tuberculous Mycobacterium species of canine origin by multiple gene sequencing approach

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Background: Frequently isolated in clinical settings and environmental sources, members of the *Mycobacterium avium-intracellulare* complex (MAIC) comprising genetically distinct species and subtypes holds significant agents responsible for opportunistic infections. The work is focused on molecular identification of a novel *Mycobacterium* species within the MAIC isolated from an aged dog suffering from pulmonary infection in a rural area of Meghalaya, India.

Methods & Materials: The dog's nasal swabs were collected and after decontamination inoculated into Lowenstein Jensen Media. Since phenotypic characteristics were inconclusive and time consuming, molecular analysis was decisively adopted for the study. Genus confirmation was done on the basis of *hsp*65 gene amplification which was then subjected to PRA (PCR-Restriction Enzyme Pattern Analysis) using enzymes *Bst*EII and *Hae*III. To further speciate the isolate and determine its phylogenetic status, two important additional housekeeping genes *rpo*B, and 16S rRNA gene were included.

Results: At the end of three months a smooth, single, non-pigmented colony was observed showing strong acid fast bacilli and amplified the genus-specific hsp65 gene. PRA-*hsp65* profiles could only infer the isolate to be a Non-tuberculous Mycobacteria (NTM). The dog was instantly prescribed anti-tuberculosis drug and is currently undergoing therapy. BLAST analysis in NCBI exhibited no homology in the three genes with comparatively low similarity cut-offs. The 16S rRNA gene depicted 99% closeness to the reference strain of *M. yongonense*, *rpo*B gene showed 97% similarity to *M. Indicus pranii*, while non-MAIC member *M. genevense* was the closest hit for *hsp*65 with 97% similarity. All the three genes were individually subjected to phylogenetic analysis in MEGA6 taking together the reference ATCC sequences of MAIC and the closest BLAST hits which also presented similar ambiguous output with no conclusion on the isolate's species. Concatenation of the three sequences ultimately presented higher lucid discrimination and determined the isolate (HNP-1) as a novel entity within the MAIC (Figure 1).