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Multi-targeted therapy for leprosy: Insilico strategy to overcome multi drug resistance and to improve therapeutic efficacy

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ABSTRACT

Leprosy remains a major public health problem, since single and multi-drug resistance has been reported worldwide over the last two decades. In the present study, we report the novel multi-targeted therapy for leprosy to overcome multi drug resistance and to improve therapeutic efficacy. If multiple enzymes of an essential metabolic pathway of a bacterium were targeted, then the therapy would become more effective and can prevent the occurrence of drug resistance. The MurC, MurD, MurE and MurF enzymes of peptidoglycan biosynthetic pathway were selected for multi targeted therapy. The conserved or class specific active site residues important for function or stability were predicted using evolutionary trace analysis and site directed mutagenesis studies. Ten such residues which were present in at least any three of the four Mur enzymes (MurC, MurD, MurE and MurF) were identified. Among the ten residues G125, K126, T127 and G293 (numbered based on their position in MurC) were found to be conserved in all the four Mur enzymes of the entire bacterial kingdom. In addition K143, T144, T166, G168, H234 and Y329 (numbered based on their position in MurE) were significant in binding substrates and/co-factors needed for the functional events in any three of the Mur enzymes. These are the probable residues for designing newer anti-leprosy drugs in an attempt to reduce drug resistance.

1. Introduction

Leprosy is a chronic infectious disease caused by slowly growing bacillus, *Mycobacterium leprae*. Though global efforts were taken to control leprosy by intensive chemotherapy, it is still a major health problem in several countries of Asia, Latin America, and Africa. *M. leprae* develops resistance since 1964 for Dapsone, 1976 for Rifampin, and 1996 for Ofloxacin (Nakata et al., 2011, 2012; Rocha Ada et al., 2012; Maeda et al., 2001).

To overcome the problem of drug-resistant *M. leprae* and to improve treatment efficacy, the World Health Organization (WHO) recommended multi drug therapy for leprosy in 1981 (WHO, 1995). Effective multi drug regimens are now used worldwide, and the infection in individuals is now curable. However, although the reported number of registered cases worldwide has declined in the last two decades, the reported number of new cases registered each year has remained the same. WHO receives the latest statistical data on the rates of prevalence and new case detection are for 1 case per 10,000 and per 100,000 populations respectively (WHO, 2011).

Today's modern drug discovery designs an antibiotic simply by focusing on targeting an essential protein or it tries to modify the antibiotic to overcome the acquired bacterial changes. This effort is daunting since the resistance mechanisms are generic, rapid and applicable to any protein target or modification that can be made to the antibiotic. Also available antibiotics tend to target a single enzyme for inhibition. This increases the likelihood that a key mutation in the active-site will render the antibiotic inactive with a minimal negative impact on the enzyme function. Hence what could be an effective alternative approach to overcome this drug resistance? The answer will be targeting multiple enzymes in an essential pathway with a broad-spectrum inhibitor. By targeting multiple enzymes, the probability of acquiring and propagating

Abbreviations: MurC, UDP-*N*-acetylmuramic acid:glycine ligase; MurD, UDP-*N* -acetylmuramoyl- glycine:D-glutamate ligase; MurE, UDP-*N*-acetylmuramoyl-glycyl-D-glutamate:meso-diaminopimelate ligase; MurF, UDP-*N*-acetylmuramoyl-glycyl-D-glutamyl-meso-2,6-diaminopimeloyl-D-Ala-D-Ala synthetase; MDT, multi drug therapy; MTT, multi targeted therapy; WHO, world health organization; UNAM, UDP-*N*-acetyl muramic acid; UMG, UDP-*N*-acetylmuramoyl- glycine; UMGG, UDP-*N*-acetylmuramoyl-glycyl-D-glutamate; meso-A_2pm, meso-diaminopimeleic acid; UMT, UDP-*N*-acetylmuramoyl tripeptide; UMPP, UDP-*N*-acetylmuramoyl pentapeptide; ATP, adenosine triphosphate; MSA, multiple sequence alignment; ETS, evolutionary trace server; ET, evolutionary trace; PIC, partition identity cut-off; RMSD, root mean square deviation; SMILES, simplified molecular input line entry system.

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resistance becomes negligible. Thus, the discovery of novel drug that target multiple enzymes of single essential pathway will inherently prolong the usefulness of existing antibiotics and provide new antimicrobial agent.

In this sense, we can use different multi-target computational (chem-bioinformatics or complex networks) strategies to speed up the discovery of new drugs and/or targets to halt multi-drug resistance. Some optional gateways on this direction are: (1) the use of multi-target Quantitative Structure–Activity Relationship (mt-QSAR) models without considering target structure (Prado-Prado et al., 2009b, 2010; Speck-Planche et al., 2010, 2011a,b; Marzaro et al., 2011; García et al., 2011), (2) using mt-QSAR models considering both durg and target structure (González-Díaz et al., 2011a,b; Concu et al., 2010), or use drug-target complex networks (González-Díaz et al., 2010; Prado-Prado et al., 2008, 2009a, 2011a,b; Viña et al., 2009; Rask-Andersen et al., 2011; Achenbach et al., 2011; Nussinov et al., 2011). Another alternative, without rely upon mt-QSAR or Systems biology methods is the use of drug-target docking strategies, but involving multiple targets as follows.

From our work on computational genome analyses of metabolic enzymes in *M. leprae* for drug target identification, we found eight enzymes that could be used as potential drug targets (Shanmugam and Natarajan, 2010). Interestingly, six of these eight enzymes belong to peptidoglycan biosynthetic pathway. Peptidoglycan, a component of the bacterial cell wall is a versatile material, rigid enough to provide a scaffold for bacteria to maintain their shape and protect them from osmotic pressure yet malleable enough for the bacteria to grow and expand (Hett and Rubin, 2008).

The MurC, MurD, MurE and MurF enzymes involved in the cytoplasmic steps of the peptidoglycan biosynthetic pathway share similar active-site characteristics. The MurC, MurD, MurE and MurF enzymes are responsible for ligation of glycine (Mahapatra et al., 2000), D-glutamic acid, meso-diaminopimelic acid and D-Ala-D-Ala onto the D-lactoyl group of UDP-MurNAc during the elongation of the peptidoglycan chain (Barreteau et al., 2008). Further, each of these four Mur enzymes binds the product of the previous Mur enzyme and follows same mechanism of action; there is a significant overlap in the sequence and structure in active-site region (Barreteau et al., 2008).

The peptidoglycan in *M. leprae* is unique in nature. The structure of peptidoglycan in *M. leprae* differs from other bacterial species (Brennan and Besra, 1997). The unique features include,

- Muramic acid residues of *M. leprae* peptidoglycan are exclusively acetylated (Mahapatra et al., 2008).
- First amino acid in the pentapeptide side chain of the peptidoglycan is glycine instead of L-Alanine (Mahapatra et al., 2000).
- Direct cross-linkage between meso-diaminopimelic acid residues (Mahapatra et al., 2008).
- Un-cross-linked peptide side chains of *M. leprae* consist of tetra- and tripeptides. In some cases, additional glycine residues are also observed (Mahapatra et al., 2008).

Due to massive genome decay in *M. leprae*, the organism has never been successfully grown on an artificial cell culture media. It is always derived from host tissue. This may be the reason for the unusual peptidoglycan structure in this species. This was confirmed when *Escherichia coli* and *Salmonella* cells are grown in human epithelial cells, changes in the chemical composition of the peptidoglycan are observed (Hirschfield et al., 1990).

These unique structural features in the peptidoglycan structure and the substrate specificity of MurC and MurE ligases towards glycine and meso-diaminopimeleic acid suggested that peptidoglycan is a rich source of potential drug targets. MurC, MurD, MurE and MurF enzymes associated with the unique features of the peptidoglycan structure are selected as the drug targets for multi targeted therapy. Emergence of resistance would then be unlikely requiring mutations in each of the genes encoding the Mur enzymes. The schematic diagram representing the formation of pentapeptide in the cytoplasmic steps of peptidoglycan biosynthesis is given in Fig. 1.

As the 3D-structure of these target enzymes from *M. leprae* are necessary for the multi-targeted therapy, we developed the 3D-structure of these enzymes using homology modeling and predicted the amino acid residues important for binding its various substrates and co-factors. The quality of the developed model, the docking parameters and the binding residues were reported in our recent publications (Shanmugam and Natarajan, 2012a,b).

In this paper we focus on novel multi-targeted therapy for leprosy to overcome multi drug resistance and to improve therapeutic efficacy. The earlier developed models of MurC, MurD, MurE and MurF enzymes and their corresponding multiple sequence alignment files were subjected to evolutionary trace analysis and followed by site directed mutagenesis studies. The evolutionary trace (ET) method (Lichtarge et al., 1996) exploits phylogenetic tree-based sequence comparisons along with crystal structure information to detect functional sites in proteins (Chakravarty et al., 2005; Lichtarge and Sowa, 2002; Mihalek et al., 2003; Sowa et al., 2001). We applied this ET method for predicting the functional residues in Mur enzymes to overcome single and multiple drug resistant strains of *M. leprae*. The site directed mutagenesis studies used to predict the evolutionarily conserved residues which were structural and/or functional determinants. Such residues present in at least three of the four Mur target enzymes were predicted and reported here as suitable residues for multi-targeted therapy.

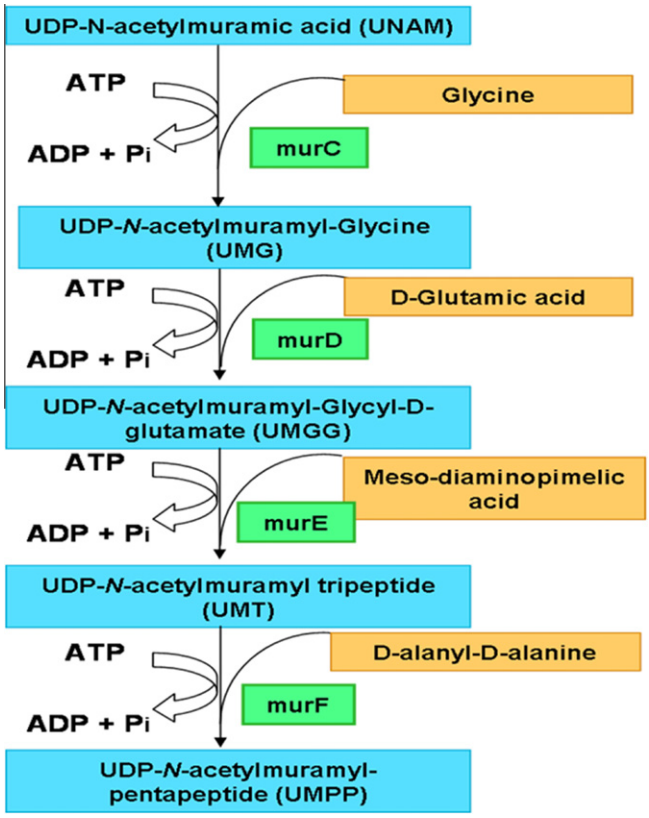

Fig. 1. Role of Mur enzymes* in the cytoplasmic steps of Peptidoglycan biosynthesis. *Enzymes selected for multi targeted therapy.