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A PHARMACOVIGILANCE STUDY OF COMPARATIVE SAFETY ASSESSMENT OF BEDAQUILINE AND LEVOFLOXACIN, AMONG MULTI-DRUG RESISTANT TUBERCULOSIS PATIENTS IN GLOBAL MULTI-CENTRE TERTIARY CARE HOSPITALS, AND AN ANTI-TUBERCULAR MOLECULAR PHARMACOTHERAPEUTIC ANALYSIS OF BEDAQUILINE

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ABSTRACT

Introduction: Bedaquiline, a novel 1, 4 - diarylquinoline, inhibits mycobacterial adenosine triphosphate synthase, thereby inhibiting ATP generation, disrupting mycobacterial energy metabolism and replication of *M. tuberculosis*. Bedaquiline's initial bacteriostatic action is followed by a bactericidal effect after 5-7 days. Bedaquiline-based MDR-TB treatment regimens result in faster and more sustained disease resolution than bedaquiline-sparing MDR-TB treatment regimens. Levofloxacin, the S- or levorotatory isomer of racemic mixture of ofloxacin, is bactericidal to *M. tuberculosis*, MAC, *M. fortuitum*, and other atypical mycobacteria, with inhibitory effect on DNA gyrase, DNA topoisomerase IV and IL-1α, IL-6, IL-8. **Objectives:** The objective was to perform a pharmacovigilance study of comparative safety assessment of bedaquiline and levofloxacin, among multi-drug resistant tuberculosis patients in global multi-centre

tertiary care hospitals, and an anti-tubercular molecular pharmacotherapeutic analysis of bedaquiline. **Methods:** A multi-centre, prospective, comparative, randomised and single-blinded study of 100 multi-drug resistant tuberculosis patients, and a molecular pharmacological analytical study, were performed. For 24 – 48 weeks, Group A patients were prescribed anti-tubercular drug oral bedaquiline 400 mg once daily followed for 2 weeks followed by 200 mg thrice weekly for 22 weeks, and Group B patients were prescribed oral levofloxacin 750 mg once daily, as part of MDR-TB treatment regimens. The comparative anti-tubercular safety assessment was done by the monitoring of adverse drug reactions, like nausea, headache, diarrhoea, insomnia, dizziness, constipation, ECG QT prolongation, arthralgia, myalgia, among Group A patients, and adverse drug reactions, like arthralgia, chest pain, nausea, vomiting, diarrhoea, dizziness, headache, haemoptysis, among Group B patients, with Adverse Event Case Report Forms, on days 0, 30, 60, 90, 120, 150, 180, 210, 240, 260, 300, 330, 360, and on further follow-ups. The patient compliance and molecular pharmacological analyses of bedaquiline, were also performed. **Results:** All the 100 patients completed the treatment thoroughly. There were no dropout patients due to adverse effects, none was lost to follow-up and none of the patients withdrew voluntarily. The safety assessment showed that both in Group A and Group B patients, the occurrence of adverse effects were statistically non-significant. The molecular pharmacological analysis of bedaquiline depicted its efficienacy in the pharmacotherapeutic application among global multi-drug resistant and extensively drug-resistant tuberculosis patients. **Conclusions:** The patients' adherence to anti-tubercular treatment was very high. Both bedaquiline and levofloxacin, were safe and tolerable among multi-drug resistant tuberculosis patients. The molecular pharmacological analysis of bedaquiline elaborated its exceptional efficacy.

KEYWORDS: Pharmacovigilance, Diarylquinolines, Bedaquiline, Fluoroquinolones, Levofloxacin, Molecular Pharmacotherapeutics, Multi drug-resistant tuberculosis, Extensively drug-resistant tuberculosis.

INTRODUCTION

World Health Organisation estimated that over 480,000 cases of multidrug-resistant (MDR) tuberculosis occur every year globally, 9% of them being affected by extensively drug-resistant (XDR) strains of Mycobacterium tuberculosis. MDR, to at least rifampicin and isoniazid, is mainly acquired by alteration of the bacilli or by alteration of drug target through mutation or bacilli titration of the drug through overproduction of target. The treatment of

MDR / XDR – TB is unfortunately long, expensive, producing further resistance, with increased occurence of adverse events, and the success rate largely unsatisfactory (<20% among cases with resistance patterns beyond XDR), mostly due to the insufficient number of active drugs during both intensive and continuation phases.[1, 2, 3, 4, 5] Ofloxacin, the racemic mixture, and levofloxacin, the S- or levorotatory isomer of ofloxacin, are bactericidal to M. tuberculosis, MAC, M. fortuitum, and other atypical mycobacteria, with their inhibitory effect on DNA gyrase, DNA topoisomerase IV and pro-inflammatory cytokines interleukins: IL-1α, IL-6, IL-8 and tumour necrosis factor α and with their superinducing effect on IL-2. Bedaquiline, a novel 1, 4 - diarylquinoline, inhibits gamma subunit or sububnit c of mycobacterial adenosine triphosphate synthase, thereby inhibiting ATP generation, disrupting mycobacterial energy metabolism and replication of *Mycobacterium tuberculosis*. The initial bacteriostatic action is followed by a bactericidal effect after 5-7 days. Bedaquiline-based MDR-TB treatment regimens result in faster and more sustained disease resolution than bedaquiline-sparing MDR-TB treatment regimens.[5,6,7,8,9,10]

According to the structure activity relationship studies of quinolones as antitubercular agents, the β-keto carboxylic acid moiety is required for hydrogen bonding interactions with DNA bases, and therefore it is essential for their anti-tubercular activity. The substituent at N-1 and C-8 positions should be relatively small and lipophilic to enhance the activity. Fluorine at C-6 is the best substituent, and it improves cell penetration and gyrase affinity. Substituents at the C-7 position are very essential and attribute to the physicochemical properties, bioavailability, lipophilicity and safety. Mycobacteria have lipid rich cell wall, and lipophilicity is an important consideration in the design and activity of newer antitubercular agents. Several research studies revealed that increasing the lipophilicity of the side chain at C-7, improves the anti-TB activity. The methoxy group at C-8 was found to enhance the lipophilicity and decrease the possibility of the development of resistance to quinolones as in moxifloxacin. Based on this concept, several fluoroquinolone derivatives had been synthesized and evaluated for their anti-tubercular activities against different TB strains. A series of N-4-piperazinyl ciprofloxacin-cephalosporin conjugates was synthesized, and these conjugates were evaluated for their in vitro antitubercular activity. A group of 1-aryl fluoroquinolones was synthesized, and these compounds were tested for their in vitro antitubercular activity against M. tuberculosis, which exhibited 98% growth inhibition. A new fluoroquinolone bearing an aromatic moiety at C-7 and an alkyl group at N-1 was synthesized and the compound was tested in vitro against M. tuberculosis H37Rv, which was

subsequently found to be effective against M. tuberculosis H37Rv. Similarly, novel fluoroquinolone derivative containing an oxime functional moiety was synthesized and the compound was evaluated against M. tuberculosis H37Rv. The results revealed that this compound has considerable anti-tubercular activity. Moreover, a series of N-4-piperazinyl derivatives of ciprofloxacin was synthesized, and these compounds were screened for their in vitro anti-tubercular activity, which exhibited activity against M. tuberculosis H37Rv strain. Independently, a novel dihydro artemisinin-fluoroquinolone conjugate experienced remarkable in vitro activity against M. tuberculosis H37Rv strain. A novel C-7 fluoroquinolone derivative containing an 3-alkoxyimino-4-(cyclopropylanimo) methylpyrrolidine moiety was designed, synthesized and tested against M. tuberculosis H37Rv ATCC 27294 strain and MDR M. tuberculosis 6133 clinical isolate. Results revealed that this compound has shown remarkable activity against MTB H37Rv ATCC 27294 and MDRMTB 6133 clinical isolate. A new ciprofloxacin-pallidum complex was synthesized, and its antitubercular activity was evaluated. It exhibited good antitubercular activity. A novel ciprofloxacin derivative demonstrated remarkable improvement in lipophilicity, when a substituted benzyl moiety was introduced to the N-4-piperazine ring. The antimycobacterial results revealed that the compound has good in vitro activity against M. tuberculosis H37Rv ATCC 27294. A novel ciprofloxacin derivative was synthesized and evaluated for its antimycobacterial in vitro and in vivo activity against M. tuberculosis H37Rv, multi-drug resistant M. tuberculosis and Mycobacterium smegmatis, and this compound was found to be effective in vitro against M. tuberculosis H37Rv and multi-drug-resistant M. tuberculosis. A new C-7 fluoroquinolone derivative with enhanced lipophilicity was synthesized by the introduction of N-alkyl-1,3-propanediamine at C-7, and this compound displayed activity against M. tuberculosis.[11]

OBJECTIVE

The objective was to perform a pharmacovigilance study of comparative safety assessment of bedaquiline and levofloxacin, among multi-drug resistant tuberculosis patients in global multi-centre tertiary care hospitals, and an anti-tubercular molecular pharmacotherapeutic analysis of bedaquiline.

METHODS

Ethical approval

At first, the Institutional Ethics Committee clearance and approval was taken. The study was conducted in accordance with the ethical principles of Declaration of Helsinki and Good

Clinical Practices contained within the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH-E6), and in compliance with the global regulatory requirements. The patients who were included in the study were assured confidentiality, and a written informed consent was obtained from each patient.

Study design

It was a global, multi-centre, prospective, comparative, randomised and single-blinded study, and an molecular pharmacological analytical study.

Study population

The study population consisted of 100 multi drug-resistant tuberculosis patients.

Selection criteria of the study population

Inclusion criteria

(i) patients of any gender, (ii) patients within 18 and 55 years, (iii) patients presenting with multi drug-resistant tuberculosis with a baseline drug susceptibility testing result confirming MDR-TB (sample collected either before starting MDR-TB treatment or $\leq$ 1 month after commencement), (iv) WHO definitions, criteria and categorisations for tuberculosis, (v) co-operative and conscious patients, (vi) patients willing to undergo all pre and post- treatment investigations and willing to complete the entire course of treatment, (vii) patients who have given consent and are willing to go for a follow-up, (viii) patients not taking any previous anti-tubercular drug, (ix) patients not taking any concomitant medication.

Exclusion criteria

(i) uncooperative or unconscious patients, (ii) patients below 18 and above 55 years, (iii) patients presenting with any category other than multi drug-resistant tuberculosis, (iv) patients with a history of hypersensitivity to any of the study drugs, (v) patients with high risk diseases or co-morbidities, (vi) cardiac, renal or any other associated complications or co-morbidities, (vii) any chronic disease intervening with the study data, (viii) immunocompromised patients, (ix) patients suffering from gastrointestinal diseases like peptic ulcer, regional enteritis and ulcerative colitis, (x) pregnant or lactating women (women of child bearing potential are required to have a negative urine pregnancy test result and to agree to use an effective form of contraception for the duration of study), (xi) children or very old patients, (xii) other associated medical illness or disorders having impact on study results, (xiii) female patients using hormonal contraceptives.

Study period

The study period, comprising of the periods for the research study and the compilation of the study literature, was 1.5 years, from July, 2014 to January, 2015; and October, 2020 to December, 2021.

Place of study

The research study and the compilation of the study literature was done in the Departments of Pharmacology, Clinical Pharmacology, Molecular Pharmacology, Rational Pharmacotherapeutics, Pharmacovigilance, Pharmacogenomics, Pathology, Clinical Pathology, Molecular Diagnostics, Internal Medicine, Tuberculosis, Chest Diseases and Respiratory Medicine, Cardiology, Clinical Research, in global multi-centre tertiary care hospitals: Dr. Moumita Hazra's Polyclinic And Diagnostic Centre, Hazra Nursing Home, Rama Medical College Hospital and Research Centre, Rama University, Mamata Medical College and Hospitals, J. J. M. Medical College and Hospitals, and GIOSTAR Institute of Regenerative Medicine Institutes, Hospitals and Laboratories.

Study procedure

In this study, 100 global multi-drug resistant tuberculosis patients were randomly allocated into Group A (bedaquiline therapy) = 50 patients and Group B (levofloxacin therapy) = 50 patients. The study patients were single-blinded, regarding the allotted drug therapy being administered. For 24 – 48 weeks, Group A patients were prescribed anti-tubercular drug oral bedaquiline 400 mg once daily followed for 2 weeks followed by 200 mg thrice weekly for 22 weeks, and Group B patients were prescribed oral levofloxacin 750 mg once daily, as part of MDR-TB treatment regimens, recommended by WHO, The American Thoracic Society, U.S. Centers for Disease Control and Prevention, European Respiratory Society, Infectious Diseases Society of America and similar associations, ratified by Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) methodology.[12, 13] From the 100 multi drug-resistant tuberculous patients, thorough patients' history with complete examination details, before and after the administration of the study drugs therapy were obtained with the study proforma, thoroughly analysed and and the following details were recorded : the patients' participation assessment and adherence to treatment (including patients who completed the study thoroughly), patients who were dropout patients due to adverse effects, lost to follow-up patients, and patients who withdrew voluntarily; the demographic characteristics, including age, gender, race, duration of symptoms of

tuberculosis, severity of tuberculosis symptoms, present controller medications, the patients' present and past history, smoking history, respiratory history including respiratory infection and immunological history, chronic obstructive pulmonary disease, history of MDR-TB contacts, past TB treatment history, defined as new cases (≤ 1 month of antituberculosis treatment), previously treated cases (first and second line anti-tuberculosis drugs), presence of cavities on chest radiograph, sputum smear microscopy results (negative, low [scanty or 1+] and high bacillary load [2+ or 3+]), and drug susceptibility testing results, cardiac history, history of co-morbidities, family history, personal history, socio-economic history, reproductive history, concomitant medication history, surgical history, the symptomatic effect of treatment on tuberculosis. Details of complete general physical examination, including body mass index, pulse rate, respiratory rate, oxygen saturation, and systemic examination, including oto-rhino-laryngo-tracheal, respiratory and cardio-pulmonary examinations, were recorded. The WHO definitions of treatment outcomes requiring at least five consecutive negative culture results during the final 12 months of treatment were to be classified as cured, and either 2 positive results among the five cultures recorded in the final 12 months, one positive in any one of the final three cultures, or a clinical decision, was to be considered, to continue or discontinue treatment depending on the treatment success or failure respectively. Favourable outcome was defined as a combination of cured and treatment completed, and unfavourable outcome as a combination of death and failure. Multi drug-resistance was defined as resistance to at least rifampicin and isoniazid, that had been detected at baseline.

The comparative anti-tubercular pharmacotherapeutic occurrence of adverse effects, due to oral bedaquiline therapy and oral levofloxacin therapy was thoroughly analysed, with adequate consideration of causality assessment, conducted by the safety assessment done by the monitoring of adverse drug reactions, like nausea, headache, diarrhoea, insomnia, dizziness, constipation, ECG QT prolongation, arthralgia, myalgia, among Group A patients, and adverse drug reactions, like arthralgia, chest pain, nausea, vomiting, diarrhoea, dizziness, headache, haemoptysis, among Group B patients, with Adverse Event Case Report Forms, on days 0, 30, 60, 90, 120, 150, 180, 210, 240, 260, 300, 330, 360 and on further follow-ups. The analysis of different attributes of patient compliance was also performed.

The molecular pharmacological basis of the anti-tubercular pharmacotherapeutic drug bedaquiline, was also thoroughly analysed from wide ranged molecular pharmacological research, review and case presentation study literature to derive the anti-microbial rationale

of the clinical pharmacotherapeutic use of this diarylquinoline bedaquiline, among multi-drug resistant tuberculous patients.

Statistical analysis

The statistical analyses were made by unpaired 't' test, Chi-Square test, one way ANOVA test, two sample Z – test, test of significance with p values, with subsequent tabular representations.

RESULTS

All the 100 patients completed the treatment thoroughly. There were no dropout patients due to adverse effects, none was lost to follow-up and none of the patients withdrew voluntarily. The patients' adherence to treatment was very high. The demographic characteristics for bedaquiline and levofloxacin, were comparable.

Table 1: Group A: The occurrence of adverse effects with bedaquiline therapy.

Adverse effects	Number of patient occurrence	Z-value	p-value
Nausea	1	-	Non-significant
Headache	0	-	Non-significant
Diarrhoea	0	-	Non-significant
Insomnia	0	-	Non-significant
Dizziness	0	-	Non-significant
Constipation	0	-	Non-significant
ECG QT Prolongation	0	-	Non-significant
Arthralgia	0	-	Non-significant
Myalgia	1	-	Non-significant

Table 2: Group B: The occurrence of adverse effects with levofloxacin therapy.

Adverse effects	Number of patient occurrence	Z-value	p-value
Arthralgia	0	-	Non-significant
Chest pain	0	-	Non-significant
Nausea	0	-	Non-significant
Vomiting	0	-	Non-significant
Diarrhoea	0	-	Non-significant
Dizziness	0	-	Non-significant
Headache	0	-	Non-significant
Haemoptysis	0	-	Non-significant

Adverse effects were negligible in either group. Thus, the safety assessment showed that both in Group A (Table 1) and Group B (Table 2) patients, the occurrence of adverse effects were

statistically non-significant. Tolerability was good for both bedaquiline and levofloxacin, among multi-drug resistant tuberculosis patients.

The molecular pharmacological analysis of bedaquiline, as deduced from the evidence-based medical databases, regarding the systematic functional synchrony in the anti-mycobacterial pharmacodynamic and pharmacotherapeutic response mechanisms, established that the clinical pharmacotherapeutic application of bedaquiline is very beneficial among multi-drug resistant and extensively drug resistant tuberculosis patients, most significantly, because of its novel two-step sequential amplification of the anti-mycobacterial activity, that is, an initial bacteriostatic action, followed by a bactericidal effect after 5-7 days, while maintaining its exceptional efficacy in multi-drug resistant and extensively drug resistant tuberculosis patients.

DISCUSSION

With the advent of quinolones, and later the fluorinated 4-quinolones, the fluoroquinolones, the medical world has certainly taken long strides in treating enormous number of maladies.[14]

Fluoroquinolones are chemical derivatives of quinoline, the prodrome of chloroquine. Fluoroquinolones, a family of 6-fluoro-7-piperazinyl-4-quinolones, are broad spectrum synthetic antimicrobial agents derived from quinolones with the addition of a fluorine atom attached to the central ring.[15]

Substitution at C-7 or its N-4-piperazinyl moiety was found to affect potency, bioavailability, and physicochemical properties. Also, it can increase the affinity towards mammalian topoisomerases that may shift quinolones from antibacterial to anticancer candidates. Moreover, the presence of DNA topoisomerases in both eukaryotic and prokaryotic cells makes them excellent targets for chemotherapeutic intervention in antibacterial and anticancer therapies.[11]

Fluoroquinolones are quite significantly efficacious for their bactericidal inhibitory effect on:

i. DNA gyrase, caused by the binding of fluoroquinolones to the A subunits (gyr A), thus inhibiting the replication and transcription of bacterial DNA, responsible for the proper functioning of the cell, and the subsequent change of conformity of DNA gyrase molecule

caused by the binding of fluoroquinolones to the DNA binding groove between A (gyr A) and B (gyr B) subunits;

ii. Par C subunits (par C) and Par E subunits (par E) of DNA topoisomerase IV, thus inhibiting decatenation and relaxation of DNA and segregation of replicating chromosomes or plasmids in bacteria;

iii. Pro-inflammatory cytokines, like interleukins : IL-1α, IL-6, IL-8, and tumour necrosis factor α, leading to attenuation of inflammatory response and exhibiting multiple immunomodulatory actions.[14,16]

Fluoroquinolones also have superinducing effect on interleukin IL-2[14]

First-generation quinolones (e.g., nalidixic acid) achieve minimal serum levels. Second-generation quinolones (e.g., ciprofloxacin) have increased gram-negative and systemic activity. Third-generation quinolones (e.g., levofloxacin) have expanded activity against gram-positive bacteria and atypical pathogens. Fourth-generation quinolones (eg., trovafloxacin) have significant activity against anaerobes. Fifth-generation quinolones (eg., aravofloxacin) have activity against multi-resistant pathogens.[14]

They are characterized by advantageous pharmacokinetic properties; higher concentrations in the lungs; and an excellent safety profile comparable to other antibiotics used to treat respiratory infections, such as macrolides and β-lactams.

The newer fluoroquinolones have broad-spectrum bactericidal activity, excellent oral bioavailability, good tissue penetration and favourable safety and tolerability profiles.[14]

Fluoroquinolones are active against *Haemophilus influenzae, Moraxella catarrhalis, Mycoplasma* species, *Chlamydia* species, *Chlamydophila* species, *Legionella* species, *Enterobacteriaceae, Pseudomonas aeruginosa* (particularly ciprofloxacin), *Mycobacterium tuberculosis,* some atypical mycobacteria, some methicillin-sensitive staphylococci, *Campylobacter* species, *Salmonellae, Shigellae,* Vibrios, *Yersinia enterocolitica, Chlamydia trachomatis, Legionella,* and are also indicated in anthrax prophylaxis and meningococcal prophylaxis. The dual inhibitory activity of fluoroquinolones against the bacterial replication enzymes, DNA gyrase and topoisomerase IV, protects them from the development of resistance. A mutant prevention concentration (MPC) of an antibiotic for a particular organism can be defined, at which the selection of resistant mutants during treatment is suppressed. For MTB, the MPC90 (MPC for 90% of strains) for fluoroquinolones have been

found to be ciprofloxacin > levofloxacin > gatifloxacin > moxifloxacin respectively. So, gatifloxacin and moxifloxacin are less likely to provoke the development of resistance. Several studies have recommended that levofloxacin is the first-choice fluoroquinolone for MDR-TB. Ofloxacin is also effective for MDR-TB, being the racemic mixture of the S- or levorotatory isomer of ofloxacin: levofloxacin. The Complementary List of the Model Essential Medicines List currently lists three fluoroquinolones for the treatment of MDR-TB: ciprofloxacin, ofloxacin and levofloxacin.[17]

Fluoroquinolones, like ofloxacin, levofloxacin, ciprofloxacin and moxifloxacin, are relatively new potent oral bactericidal drugs for TB, that have gained prominence as well tolerated alternatives to first line anti-tubercular drugs. They are active against *Mycobacterium avium* complex, *M. fortuitum* and some other atypical mycobacteria as well. Moxifloxacin is the most active fluoroquinolone against *M. tuberculosis*, while levofloxacin is more active than ofloxacin and ciprofloxacin. On the other hand, ciprofloxacin is more active than levofloxacin against atypical mycobacteria. The fluoroquinolones penetrate cells and kill mycobacteria lodged inside macrophages as well. Though ciprofloxacin was initially used in tuberculosis, it is not favoured now because of its extensive use in other bacterial infections and chances of resistance. The primary indication of fluoroquinolones is for the treatment of drug resistant tuberculosis. They have also been tried in first line regimens for new cases. Substitution of ethambutol with moxifloxacin to accompany rifampicin, isoniazid and pyrazinamide in the four drug regimen has been found to enhance the rate of bacillary killing and cause faster sputum conversion. In contrast, ciprofloxacin, ofloxacin and levofloxacin did not enhance the sterilizing ability of rifampicin and isoniazid, and were no better than ethambutol. Thus, addition of moxifloxacin to rifampicin, isoniazid and pyrazinamide regimen holds the possibility of reducing the duration of treatments of tuberculosis from 6 months with rifampicin, isoniazid, pyrazinamide and ethambutol used currently. However, experience with moxifloxacin in the treatment of tuberculosis is still limited, and it is not routinely used. Fluoroquinolones are a key component of all regimens for multi drug-resistant tuberculosis, except when the bacilli are found to be resistant to them. The Revised National Tuberculosis Control Programme of India have included ofloxacin or levofloxacin in the standardized regimen for multi drug-resistant tuberculosis. If used alone, mycobacterial resistance to ofloxacin, levofloxacin and ciprofloxacin develops rapidly by the mutation of DNA gyrase gene. Experimental data indicates that the resistance against moxifloxacin is slow to develop.[18]

Fluoroquinolones have early bactericidal activity (EBA), which is the decline in colony-forming units in sputum over the first two days of treatment, reflecting rapid killing of metabolically active organisms, an important factor in interrupting transmission, over days 2-7.

Experimental studies have demonstrated that levofloxacin exerts antioxidative and NO regulatory effects in an animal model of H1N1 influenza virus induced lung injury, and significantly improves survival. In particular, levofloxacin exhibited scavenging actions against neutrophil-derived hydroxyl radicals and suppressed NO production, leading to decreased markers of oxidative stress and NO metabolites in the lungs of H1N1 influenza virus infected animals. A recent in silico study demonstrated that the fluoroquinolones, ciprofloxacin and moxifloxacin, exert strong capacity for binding to SARS-CoV-2 main protease (Mpro), indicating that fluoroquinolones may inhibit SARS-CoV-2 replication. Furthermore, fluoroquinolones may bind to the Mpro active site more strongly than chloroquine and nelfinavir, a protease inhibitor antiretroviral drug used in the treatment of the AIDS.[15]

Ofloxacin has more potent gram-positive activity; separation of the more active S- or levo rotatory isomer yields levofloxacin, which has even better anti-microbial activity. Bioavailability of both of these drugs is excellent, such that intravenous and oral doses are the same; levofloxacin is dosed once daily as opposed to twice daily dosing for ofloxacin.[19]

Drugs such as ofloxacin have been second-line anti-TB agents for many years, but they are limited by the rapid development of resistance. Adding C8 halogen and C8 methoxy groups markedly reduces the propensity for drug resistance. Of the C8 methoxy quinolones, moxifloxacin is being studied to replace either isoniazid or ethambutol. Fluoroquinolone microbial kill is best explained by AUC0-24/MIC ratio. This time for the emergence of resistance harmonizes well with the speed of resistance emergence in patients.[19]

In documented drug resistance, therapy should be based on the evidence of susceptibility, and should include:

1. At least three drugs to which the pathogen is susceptible, with at least one of the injectable anti-TB agents,
2. In the case of MDR-TB, use of four to six medications for better outcome,
3. At least 18 months of therapy.

The addition to the regimen of a fluoroquinolone and the surgical resection of the main lesions have been associated with improved outcome.

In the patients of disseminated *M. avium* complex, a potential fourth drug includes levofloxacin, for definitive and suppressive therapy. Patients should be treated on suppressive therapy until all three of the following criteria are met:

1. Therapy duration of at least 12 months,
2. CD4 count > 100 / mm^3 for at least 6 months
3. Asymptomatic for MAC infection

In *M. kansasii*, *M. fortuitum* complex, *M. malmoense*, fluoroquinolones have been prescribed as alternative anti-tubercular agents and in *M. haemophilum*, quinolones have been prescribed as a first-line therapy.[19]

Fluoroquinolones are active against gram-negative and gram-positive bacteria, anaerobes, mycobacteria and atypical pathogens. Respiratory fluoroquinolones, levofloxacin and moxifloxacin, constitute fist line therapeutic agents for the management of severe community-acquired pneumonia, according to the treatment guidelines.

Hybridization of different pharmacophores from various bioactive substances into a single molecule is the potential weapon to prevent the drug resistance since this strategy can provide new leads with complementary activities and/or multiple pharmacological targets. Fluoroquinolone and isatin are common pharmacophores, and their derivatives possess various biological activities. Obviously, hybridization of these two pharmacophores into one molecule may result in novel candidates with broader spectrum, higher efficiency, lower toxicity as well as multiple mechanisms of action. Therefore, fluoroquinolone-isatin hybrids have the potential for clinical deployment in the control and eradication of various diseases. Fluoroquinolone-isatin hybrids are potential anti-bacterial, anti-tubercular, anti-viral and anti-cancer agents. Their structure-activity relationship paves the way for the further rational development of this kind of hybrids.[20]

WHO recommendations state that the shorter regimen for MDR-TB would improve the adherence, and its “relatively” low cost would ensure sustainability; which are extremely important in resource-limited settings and in resourceful countries.[21]

This study would certainly facilitate a wider, better and thorough analysis of the complete cycle of the multi-dimensional disease of multi-drug resistant tuberculosis and its treatment patterns, while also focusing on the intricacies of safety assessment of bedaquiline and levofloxacin, patient compliance to anti-mycobacterial drugs and the molecular pharmacological analytical elaborations about the pharmacotherapeutic rationale of bedaquiline among multi-drug resistant and extensively drug-resistant tuberculosis.[22]

Bedaquiline has also delineated its therapeutic potentials on non-small cell lung cancer (NSCLC), being one of the newer drug candidates and drug delivery systems that have limited adverse effects with significant anti-cancer efficacy. Bedaquiline, an FDA-approved anti-tuberculosis drug, has previously shown excellent anti-cancer efficacy. However, poor aqueous solubility limits its delivery via the lungs. In a research study project, an inhalable BQ-loaded cubosome (BQLC) nanocarriers was developed against NSCLC. The BQLC were prepared using a solvent evaporation technique with the cubosomal nanocarriers exhibiting a particle size of 150.2 ± 5.1 nm, zeta potential of (+) 35.4 ± 2.3 mV, and encapsulation efficiency of 51.85 ± 4.83%. The solid state characterization (DSC and XRD) confirmed drug encapsulation and in an amorphous form within the cubosomes. The BQLC nanocarriers showed excellent aerodynamic properties after nebulization (MMAD of 4.21 ± 0.53 μm and FPF > 75%). The BQLC displayed enhanced cellular internalization and cytotoxicity with a ~ 3-fold reduction in IC50 compared to free BQ in NSCLC (A549) cells, after 48 hours treatment. The BQLC suppressed cell proliferation via apoptotic pathway, further inhibited colony formation, and cancer metastasis *in vitro*. Additionally, 3D-tumor simulation studies established the anti-cancer efficacy of cubosomal nanocarriers as compared to free BQ. This suggested that BQLC may be a promising NSCLC therapy due to excellent aerosolization performance and enhanced anti-cancer activity.[23]

Targeting ATP5F1C with bedaquiline, prevents aggressive cancer cell behaviours, including spontaneous metastasis. Originally, it was thought that bedaquiline only affected the mycobacterial ATP-synthase, but more recent studies have indicated that bedaquiline also potently inhibits the yeast and human mitochondrial ATP-synthase. High-resolution cryo-EM studies have shown that bedaquiline binds directly to the c-ring of the six transmembrane proteins (ATP5G1/G2/G3), that form the F0 subunit of the mitochondrial ATP synthase. In turn, the c-ring of the F0 subunit is directly connected to the F1 subunit, via the gamma-subunit (ATP5F1C). The gamma subunit (ATP5F1C), which forms the rotary shaft of the

mitochondrial ATP-synthase, is critically involved in torque transmission, ultimately providing the necessary mechanochemical energy for ATP-synthesis. As bedaquiline-binding significantly alters the 3D conformation of the c-ring, it was speculated that this conformational change would induce the degradation of ATP5F1C. In accordance with this hypothesis, it was observed that bedaquiline induced the down-regulation of ATP5F1C protein expression, with concomitant mitochondrial ATP depletion, in a time- and concentration-dependent manner. Furthermore, ATP depletion induced by bedaquiline treatment was indeed sufficient to effectively block spontaneous metastasis *in vivo*, without showing significant toxicity in non-tumorigenic human cells (MCF10A) *in vitro* or chicken embryos *in vivo*. As a consequence, it was concluded that the gamma-subunit of the mitochondrial ATP-synthase (ATP5F1C) is a new therapeutic target, for mitigating aggressive cancer cell behaviours, including spontaneous metastasis.[24]

In another study, bedaquiline loaded binary solid dispersion (BSD) and ternary solid dispersion (TSD) were prepared with the aid of solvent evaporation technique wherein poloxamer 188 and tocopheryl polyethylene glycol 1000 succinate were used as dispersing matrix. The prepared BSD and TSD were characterised by dynamic light scattering, attenuated total reflectance-Fourier transformed infrared spectroscopy (ATR-FTIR), differential scanning calorimetry (DSC), hot stage microscopy, powder X-ray diffraction (PXRD) and evaluated for enhancement in saturation solubility, *in vitro* dissolution performance and permeability through rat intestine. BSD and TSD showed 5.68-fold and 7.46-fold increment in saturation solubility, respectively. *In vitro* dissolution data showed about 99.8 ± 1.48% bedaquiline release from BSD within 15 minutes and 98.68 ± 1.98% bedaquiline release from TSD within 30 minutes, which were significantly higher than that of bedaquiline plain drug and their respective physical mixtures at respective time points. BSD was unable to sustain the parachute effect of bedaquiline in dissolution medium and showed decreased concentration, whereas TSD maintained the same throughout the experimental period. Permeability of bedaquiline from BSD and TSD was found to be 1.86-fold and 3.53-fold, respectively, when compared to bedaquiline plain drug. TSD was found to be stable in accelerated condition, for the period of 3 months when evaluated by PXRD, FTIR, DSC and *in vitro* dissolution study. Therefore, solid dispersions of bedaquiline can be accepted as promising alternative formulation approach to enhance biopharmaceutical performance of bedaquiline, in terms of solubility, dissolution rate and permeability for treatment of tuberculosis and may benefit the patients with MDR-TB for the emergency treatment with

bedaquiline, which could ultimately lead to increase in bioavailability, increased bactericidal effect and reduced chances of resistance development.[25]

This global pharmaco-epidemiological and pharmaco-molecular study would remain a milestone in the development of newer, quicker, better, safer and more precise anti-tubercular diagnostic and therapeutic agents, thus causing an enhancement of respiratory health, during these times, and times to come.

CONCLUSIONS

The patients' adherence to anti-tubercular treatment was very high. Both bedaquiline and levofloxacin, were safe and tolerable among multi-drug resistant tuberculosis patients. The molecular pharmacological analysis of bedaquiline depicted its efficiency in the pharmacotherapeutic application among global multi-drug resistant and extensively drug-resistant tuberculosis patients.

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