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Original Article

Evaluation of anti-bacterial activity of Rifapentine, Clarithromycin, Minocycline, Moxifloxacin, Ofloxacin and their combinations in Murine Model of Rifampicin Resistant Leprosy

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Leprosy, a debilitating disease of the skin and peripheral nerves is caused by *Mycobacterium leprae* (*M. leprae*) and is treated by multidrug therapy (MDT) comprising of Dapsone, Rifampicin and Clofazimine. Resistance to any of these drugs poses a threat to the current disease control strategies. With the emergence of Rifampicin resistance in leprosy, it is important that alternative drugs need to be tested to develop a treatment strategy to combat drug resistant leprosy. In the current study, we have investigated WHO MDT, Rifapentine, Clarithromycin, Minocycline, Moxifloxacin, Ofloxacin and their combinations in intermittent and daily dose regimens in rifampicin resistant strains of *M. leprae* through mouse foot pad experiments in order to determine the loss in viability of *M. leprae* in response to these drugs and their combinations. Our findings suggest that WHO MDT is still the best combination in Rifampicin resistance cases. Combination of Moxifloxacin with Minocycline and Clarithromycin may also be taken up for clinical trials in cases with Rifampicin resistant leprosy. Rifapentine and Moxifloxacin can be effective alternative drugs to replace Rifampicin where required either in daily dose shorter duration regimens or intermittent dose longer regimen to treat resistant strains.

Key words : Multidrug Therapy, *Mycobacterium leprae*, Rifampicin Resistant Leprosy, Bactericidal Drugs

Introduction

Leprosy caused by *Mycobacterium leprae* (*M. leprae*) is treated with WHO regimen of Multi-drug Therapy (MDT) containing Dapsone, Rifampicin and Clofazimine; however, new case detection rate remains steady. While the modes of entry, point of exit, demonstration of attenuation of bacterial activity with treatment and complete cure in leprosy are unclear, emergence of drug resistant strains poses a much greater threat of resurgence of the disease with no appropriate treatment strategies to combat the same.

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Cure rates are difficult to estimate as there is no infallible test for residual disease and histo-pathological changes resolve after varying periods of time. The diagnosis of relapse or detection of persistent bacilli becomes difficult due to non-availability of techniques in the field/patient settings. Drug resistance is one of the major causes of relapse in leprosy.

The most common drug resistance reported in leprosy is to Dapsone. This was found to be due to mutations at positions 157, 158 and 164 in *folP1* gene of *M. leprae*, resulting in changes in codon positions 53 and 55 of dihydropteroate synthase (DHPS).

Drug resistance to rifampicin has been found to be emerging due to various mutations in the rpoB gene of *M. leprae* in the rifampicin resistance determining region (RRDR). This gene encodes the beta subunit of RNA polymerase on which rifampicin acts. However it has been found that rifampicin resistance demonstrated by the mouse foot pad assay has not always been confirmed by mutations in the *rpoB* gene. Maeda et al suggested other mechanisms such as acquired changes in membrane permeability and efflux pump functioning. In a recent study, Singh et al have identified new SNPs in the multi-drug resistant Airaku-3 stain where the mechanism of resistance to rifampicin has not yet been found.

In 2013, Williams et al reported a case of multidrug resistant leprosy, resistant to dapsone and rifampicin, who showed clinical response (clearing of skin lesions) to daily Dapsone, Clofazimine and Rifampicin for 44 months, but relapsed after 6 years. This illustrates that the Rifampicin-Dapsone-Clofazimine may work in a setting of drug resistance, if the strain of *M. leprae* is resistant to only one of the drugs, where the other drugs compensate for the resistant one and shows a temporary response, but does not kill the bacilli completely.

Quinolones are the third group of drugs to which *M. leprae* has become resistant. The molecular mechanism involves mutations of the *gyrA* gene which results in *M. leprae* resistant to quinolones. As most of the mutations lie between amino acid 67 and 106 of the *gyrA* gene, this region is denoted as quinolone resistance determining region (QRDR).

In the current study, we have investigated the efficacy of WHO MDT, Rifapentine (RPT), Clarithromycin (CLARI), Minocycline (MINO), Moxifloxacin (MOXI), Ofloxacin (OFLO) and their combinations in intermittent and daily dose regimens in rifampicin resistant strain of *M. leprae* through mouse foot pad experiments, determining the loss in viability of *M. leprae* in response to these drugs and their combinations. The alternative drugs CLARI, RPT, MINO MOXI and OFLO were chosen based on the earlier reports in murine models (Single-lesion Multicentre Trial Group 1997, Ji and Grosset 2000, Ji et al 1991) which proved as effective alternatives to MDT in leprosy. Other clinical trials performed with different patient settings as well as mice model experiments testing bacteriostatic and bact-ericidal effects of alternate drugs to WHO MDT, were also taken into consideration. (Colston et al 1978, Grosset et al 1990, Ji et al 1994 & 1998, Pattyn and Saerens 1974).

Methodology

The study was conducted in accordance with the ethical guidelines of “Indian Council of Medical Research” and was approved by the Institutional Ethical Committee of Schieffelin Institute of Health-Research & Leprosy Centre and the Animal Ethical Committee.

1. **Selection of patient with leprosy relapse**

   The Rifampicin resistant strain was obtained from a patient who was diagnosed clinically as a leprosy relapse. This patient was

diagnosed as a case of relapse with new skin lesions and bacteriological index of 3+ at the ear lobes and at skin lesions, three years after completing MB MDT at SIH-R&LC Karigiri. Informed consent for participation was sought from the chosen patient and biopsy was procured from the site of active lesion.

2. **Confirmation of Rifampicin Resistance by Mouse Foot pad and Molecular Methods**

   a. **Mouse Foot Pad Technique:** Bacilli were extracted from the skin biopsy by manual homogenization (Shepard 1960) and resistance to rifampicin in varying concentrations was determined using technique described by Levy and Ji, 2006.

   b. **Molecular Technique:** DNA was extracted from the skin biopsy using DNeasy Blood and Tissue Kit (Cat No: 69506, Qiagen Inc. USA) and primers corresponding to the rifampicin resistance determining region (RRDR) of *M. leprae* were used to amplify *rpoB* gene through PCR as described earlier by Matsuoka (2010). The results indicated that *rpoB* gene region of *M. leprae* showed a mutation at cod on position 441 where *Asp* was replaced by *Tyr*. This mutation was reported earlier (Maeda et al 2001) and was identified to demonstrate a strong pattern of rifampicin resistance in leprosy.

3. **Mice used in the Experiments:**

   i. **Normal Mice:** Cross bred albino (CBA mice) were used to multiply the identified strains of *M. leprae* to prepare sufficient inoculum for the experiments.

   ii. **Immunocompromised (Thymectomized Irradiated (TR)) Mice:** The mice were thymectomized at 6-8 weeks and then subjected to radiation of 900 rads after a further 3 weeks. The mice were then inducted into the experiments after a further period of 6 weeks.

4. **Preparation of Initial Inoculum:**

   M. leprae strains were extracted from the biopsy tissues of relapse patient using manual homogenization protocol in normal saline and injected into foot pads of 5 TR mice. Nine months later the TR mice were euthanized and *M. leprae* was extracted from the hind footpads. Suspensions were then pooled and an inoculum prepared to yield 2.5×10^6 bacilli per ml with a solid ratio of 1%.

5. **Inoculation of TR Mice and Induction into treatment schedules:**

   i. **Inoculation into experimental TR mice:** The inoculum thus prepared above was then diluted to $1 \text{ X } 10^5$ bacilli per 0.03ml and was injected into hind foot pads of 108 TR mice. The number of mice was calculated based on the treatment regimens in Table 1 taking into account 10% possibility of failure to develop swollen footpads and 20% mortality rate during experiments. The mice were grown on normal diet for 9 months for the footpads to be swollen with *M. leprae*.

   ii. **Treatment Schedules:**

   After the end of 9 months, 93 mice survived with swollen foot pads out of the 108 TR mice (86.11%). The count was estimated in a representative set of two mice and was identified to be on an average of 3×10^6 bacilli per each hind foot pad. These mice were then allocated into the treatment groups as shown in Table 1. The treatment schedule was divided into intermittent

and daily dose with a control group. The intermittent and the daily dose regimens were followed as per the earlier reports (Ji B et al 1996).

a. Intermittent Dosage:

In the intermittent dose, single drugs Rifapentine (RPT) and Moxifloxacin (MOXI) and drug combinations Clarithromycin, Moxifloxacin, Minocycline (CMM) and Clarithromycin, Ofloxacin, Minocycline (COM) were administered as per the dosage shown in Table 1. The group with WHO MB MDT served as positive control and the control group which was untreated served as a negative control. The dosage was administered for 24 weeks (once every 4 weeks).

b. Daily Dosage

The daily dose regimens included administration of a single bactericidal drugs (RPT or MOXI) as well as drug combinations – CLARI + MOXI + MINO (CMM) and CLARI + OFLO + MINO (COM). Untreated mice were used as controls. The treatment schedules therefore were for a maximum period of 6 days in daily dose regimen.

iii. Harvest post treatment: At the end of designated period of treatment, the mice were sacrificed and hind foot pads were harvested for *M. leprae* from each group under the intermittent and daily regimens. The bacterial counts were enumerated and solid ratios estimated. A solid bacillus is defined as the organism that was stained adequately by Ziehl Nielsen staining and whose length is approximately four times its width. The solid ratio was estimated in all suspensions (Ridley 1960).

6. Sub-inoculations:

The bacterial harvests mentioned in the above section were serially diluted 10-fold to obtain 10^2, 10^3, 10^4 and $>10^5$ bacilli/ml (undiluted) suspensions. These dilution was sub-inoculated into 2 normal mice and 1 TR Mice except for undiluted suspensions which were inoculated into 2 normal and 2 TR mice as mentioned in the Table 1. The mice were maintained on normal diet for 12 months.

At the end of 12 months *M. leprae* were harvested from the hind foot pads of sub inoculated mice, enumerated (Levy and Ji 2006) and the proportion % of viable bacilli were counted based on Spearman and Karber calculations (Shepard 1982). The p value for statistical significance was calculated using *z* test of proportions.

7. Statistical Methods and Assessment of treatment efficacy:

The proportional bactericidal technique was employed in the establishment of efficacy of drugs and drug regimens based on the dosage (Colston et al 1978). The bactericidal activity in each of the regimens was estimated by measuring and comparing the proportion of viable organisms in each groups using Spearman and Karber calculations (Shepard 1982). The calculations employ a median infective dose (ID50) and the percentage of viable *M. leprae* organisms remaining after treatment was derived from the equation: % viable *M. leprae* = 0.69/50% infectious dose. A two tailed p value of <0.05 was considered statistically significant.

Results

A pre-treatment bacterial load in the experimental TR Mice (n=93) at the end of 9 months

Table 1 : Schedule of Experiments : Rifampicin Resistant Strain

Groups	Drugs* (mg/kg/dose)	No of Mice	Duration of treatment	Frequency of harvests**	Number of Mice in sub inoculations***			
					At each Harvest#		Total	
	Intermittent Dosage:				**N**	**TR**	**N**	**TR**
1	MOXI (150) + CLARI (100)+ MINO (50) (CMM)	9	24 Wks.	W4, W12, W24	8	5	24	15
2	CLARI (100) + MINO (50) + OFLOX (150) (COM)	9	24 Wks.	W4, W12, W24	8	5	24	15
3	WHO MDT†	9	24 Wks.	W4, W12, W24	8	5	24	15
4	RPT (10)	9	24 Wks.	W4, W12, W24	8	5	24	15
5	MOXI (150)	9	24 Wks.	W4, W12, W24	8	5	24	15
	Daily Dosage:				**N**	**TR**	**N**	**TR**
6	RPT (10)	6	6 Days	D1,D3,D6	8	5	24	15
7	MOXI (150)	6	6 Days	D1,D3,D6	8	5	24	15
8	MOXI (150) + CLARI (50)+ MINO (25) (CMMD)	10	8 Wks.	D1, D3, W4,W8	8	5	32	20
9	CLARI (50) + MINO (25) + OFLOX (150) (COMD)	6	6 Days	D1,D3,D6	8	5	24	15
	Untreated Control:				**N**	**TR**	**N**	**TR**
10	Control Mice	20	24 Wks.	D0,D1,D3,D6,	-	2	-	16
	on Normal Diet			W4,W8,W12,				
				W24				

*Moxi = Moxifloxacin, Mino = Minocycline, Clari= Clarithromycin, Oflox= Ofloxacin, RPT= Rifapentine,
**W= Week, D= Day.
***N = normal mice; TR =Thymectomized radiated mice
† =RMP at 10 mg/kg of body weight once every 4 weeks plus 0.01% DDS (Dapsone) 1 0.005% CLO (Clofazimine) in daily diet.
# = At each harvest, the inoculum was diluted into 10^2, 10^3, 10^4, and undiluted (>10^5) and injected into 2 normal mice and 1 TR mice per dilution totaling to 8 normal mice and 5 TR mice (one additional TR mice was inoculated with >10^5).

and before induction in the treatment groups was 6.47±0.05 (mean Log_{10}). This increased to 6.69 ± 0.24 at the end of week 4 and decreased to 6.45± 0.31 at the end of 24 weeks in all the treatment groups. The solid ratios were in the range of 1-3%.

Hence, there was no perceptible change indicated in the bacterial populations with both intermittent and daily dose regimens at 4 weeks or at 24 weeks when compared to the pre-treatment levels. (Tables 2 & 3). This indicates that the infection is well established and follow-up

Table 2 : Initial Harvest Counts from normal mice in the intermittent regimen before titration and subinoculation (Mean log_{10} ± Standard Deviation)

Drugs	Harvest	Week 4		Week 12		Week 24	
	Weeks	A1*	A2	A1	A2	A1	A2
CMM	W4 W12 W24	6.28 + 0.01	6.55 + 0.15	6.22 + 0.11	6.37 + 0.12	6.70 + 0.10	6.39 + 0.00
COM	W4 W12 W24	6.35 + 0.72	6.14 + 0.0	6.79 + 0.27	6.65 + 0.10	6.62 + 0.10	6.77 + 0.26
RPT	W4 W12 W24	6.69 + 0.14	6.30 + 0.11	6.86 + 0.14	6.06 + 0.02	6.79 + 0.08	6.78 + 0.21
MOXI	W4 W12 W24	6.76 + 0.23	6.62 + 0.02	6.71 + 0.03	6.40 + 0.17	6.02 + 0.07	6.28 + 0.07
WHO MDT	W4 W12 W24	6.87 + 0.01	6.72 + 0.17	6.53 + 0.18	6.45 + 0.12	6.19 + 0.15	6.11 + 0.00
CONTROL	W4 W12 W24	6.88 + 0.09	6.51 + 0.19	6.64 + 0.0	6.25 + 0.03	6.71 + 0.09	6.26 + 0.08

*A1, A2 indicate Mouse1 and Mouse 2 respectively.

Table 3 : Initial Harvest Counts from normal mice in the daily dose regimen before titration and subinoculation (Mean log_{10} ± Standard Deviation)

Drugs	Harvest	Day 0		Day 1		Day 3		Day 6		Week 4		Week 8	
	Days	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
Control	D0 D1	6.50+	6.41+	6.51+	6.85+	6.71+	6.57+	6.54+	6.90+			6.32+	
	D3 D6	0.03	0.08	0.04	0.12	0.02	0.13	0.07	0.03			0.03	
RPT	D1 D3			6.46+	6.71+	6.52+	6.51+	6.70+	6.69+				
	D6			0.02	0.01	0.18	0.02	0.03	0.10				
MOXI	D1 D3			7.48+	6.87+	6.72+	6.71+	6.68+	5.84+				
	D6			0.68	0.14	0.22	0.11	0.01	0.20				
CMM	D1 D6			6.87+	6.64+	NA**		6.16+	6.64+	6.72+	6.19+	7.10+	6.82+
	W4 W8			0.01	0.10			0.02	0.14	0.02	0.01	0.14	0.20
COM	D1 D3			6.45+	6.87+	6.72+	6.68+	6.88+	6.65+				
	D6			0.53	0.09	0.01	0.15	0.00	0.02				

** NA = Not able to enumerate the suspension

observations in sub-inoculations are feasible. These observations were in concordance with the similar studies reported earlier (Ji et al 1996).

1. **Intermittent dose:**

   a. **Intermittent Dose - Normal Mice:**

   (Table 4)

   At the end of 4 weeks, RPT showed antibacterial activity with 68.37% proportion killed. The rest of the drugs, combinations and WHO MDT did not show bactericidal activity at the end of 4 weeks.

   At the end of 12 weeks, in addition to RPT, MOXI and WHO MDT also showed bactericidal effect. The bactericidal effect of RPT reduced from the effect at the end of 4 weeks.

   At the end of 24 weeks, all drug combinations and individual drugs showed antibacterial activity with WHO MDT showing highest bactericidal

Table 4 : Rifampicin Resistant Strain - Intermittent Dosage

Regimen	Proportion % of Viable *M. leprae*			Proportion of viable *M. leprae* killed in %		
	4th week	12th week	24th week	4th week	12th week	24th week
Normal Mice:						
COM	2.18197	2.18197	0.69000	0	0	68.37
CMM	2.18197	2.18197	0.38802	0	0	82.21
RPT	0.69000	1.22701	1.22701	68.37	43.76	43.76
MOXI	2.18197	0.69000	0.00690	0	68.37	99.68*
WHO MDT	2.18197	1.22701	0.00218	0	43.76	99.90*
TR Mice:						
COM	2.18197	2.18197	0.06900	0	96.83	96.83
CMM	2.18197	0.2182	0.02182	0	89.99	98.99
RPT	0.02181	0.02181	0.06900	98.99	98.99	96.83
MOXI	2.18197	2.18197	0.03880	0	96.83	98.22
WHO MDT	2.18197	2.18197	0.00690	0	96.83	99.68

*p<0.05 (Statistically Significant differences) (Z Test of Proportions)

activity (99.90%) followed by MOXI (99.68%) and CMM (82.21%). At this point, the bactericidal effect of both MOXI and WHO MDT are statistically significant (p<0.05) among the intermittent group when compared to COM, CMM and RPT in normal mice.

b. Intermittent Dose - TR Mice: (Table 4)

At the end of 4 weeks, Rifapentine showed bactericidal activity (98.99%). Moxifloxacin, CMM, COM and WHO MDT did not show bactericidal activity.

At the end of 12 weeks, all combinations and individual drugs showed bactericidal activity with CMM (98.99%) showing the highest bactericidal effect.

At the end of 24 weeks, all combinations and individual drugs showed bactericidal activity with WHO MDT (99.68%) having a superior effect. At the end of 24 weeks, WHO MDT, CMM and MOXI demonstrated 98-99% bacterial killing in TR mice. However, the statistical analysis revealed that there is no significant difference that exists across various drug combinations and individual drugs at the end of 24 weeks.

With the intermittent dose among single drugs Moxifloxacin had the best bactericidal activity comparable to WHO MDT both in normal and TR mice. Among drug combinations WHO MDT had the best bactericidal activity both in normal and TR mice. In both normal and TR mice, as an alternative to WHO MDT the next best available combination is CMM followed by COM.

2. Daily dose

a. Daily dose - Normal Mice: (Table 5)

At the end of Day 1, Moxifloxacin showed antibacterial activity with 98.22% proportion killed. The rest of the

Table 5 : Rifampicin Resistant Strain - Daily Dosage

Regimen (dose	Proportion % of Viable *M. leprae*					Proportion of viable *M. leprae* killed				
[mg/kg])	Day 1	Day 3	Day 6	Wk 4	Wk 8	Day 1	Day 3	Day 6	Wk 4	Wk 8
Normal Mice:										
CMM	2.18197	2.18197	0.02182	0.00022	0.00022	0	0	98.99	99.98	99.98
COM	2.18197	2.18197	0.02182	-	-	0	0	98.99	-	-
RPT	2.18197	2.18197	0.02182	-	-	0	0	98.99	-	-
MOXI	0.03880	2.18197	0.02182	-	-	98.22*	ND	98.99		-
TR Mice:										
CMMD	0.6900	0.2182	0.00022	0.00039	0.00039	68.37	89.99	99.99	99.99	99.99
COMD	0.6900	0.00022	0.2182	-	-	68.37	99.99	89.99	-	-
RPT	0.6900	0.00022	0.06900	-	-	68.37	99.99	96.83	-	-
MOXI	0.00690	0.2182	0.06900	-	-	99.68*	89.99	96.83	-	-

*p<0.05 (Statistically Significant differences) (Z Test of Proportions), ND= Data not available

drugs, combinations did not show bactericidal activity.

At the end of Day 3, none of the drug combinations or drugs had bactericidal effect including Moxifloxacin.

At the end of day 6, all drug combinations and individual drugs showed equal bactericidal activity. Statistical analysis revealed that there is no significant difference between the bactericidal activities of various drug combinations at the end 6 days of treatment.

At the end of 4th week and 8th week, the bactericidal effect of CMM increased from day 6 and the effect was sustained into the 4th and 8th week.

b. Daily Dose - TR Mice: (Table 5)

At the end of day 1, all drug combinations and single drugs showed antibacterial activity with Moxifloxacin showing significant (99.68%) bactericidal effect.

At the end of day 3, antibacterial activity of all combinations and single drugs increased from day 1, except for Moxifloxacin where it decreased (89.99%).

At the end of day 6, all combinations and single drugs showed bactericidal activity with CMM being the highest (99.99%). Statistical analysis revealed that there is no significant difference between the bactericidal activities of various drugs and combinations at the end of 6 days of treatment.

At the end of 4th and 8th week, the bactericidal effect of CMM at day 6, continued to be sustained into the 4th week and 8th week.

With daily dose regimen as far as single drugs are concerned Rifapentine and Moxifloxacin have shown bactericidal activity at Day 3 and Day 6

with both having the same degree of effect in normal and TR mice. With daily dose drug combinations, CMM had the best bactericidal activity both in normal and TR mice when compared to COM.

3. **Comparison of daily dose with inter-mittent dose – Normal mice (Table 6)**

   At the end of 4 weeks, when daily dose regimen of CMM was compared to inter-mittent dose of CMM, COM and WHO MDT, daily dose CMM showed bactericidal activity (99.98%) whereas intermittent dose of CMM or COM or WHO MDT did not show any activity. The bactericidal effect of daily dose CMM (99.98%) continued into the 8th week.

   At the end of 12 weeks, CMM and COM did not show any bactericidal activity. WHO MDT showed weak bactericidal activity (43.27%).

   At the end of 24 weeks, WHO MDT (99.90%), intermittent dose of CMM (82.21%) and COM (68.37%) showed bactericidal activity. WHO MDT showed the best bactericidal activity.

   The bactericidal activity of daily dose of CMM at the end of 8 weeks (99.98%) is higher than the bactericidal effect of intermittent regimens of CMM, COM and WHO MDT at the end of 24 weeks. This comparison was statistically significant (p<0.05).

In normal mice, daily dose activity of CMM showed early onset of bactericidal activity which sustained into the 8th week and probably would have sustained the effect into the 24th week if continued, being comparable to WHO MDT.

4. **Comparison of daily dose with intermittent dose – Tr mice (Table 7)**

   At the end of 4 weeks, when daily dose of CMM was compared to intermittent dose of CMM, COM and WHO MDT, daily dose CMM showed bactericidal activity (99.98%) while other combinations including WHO MDT not showing bactericidal activity. The bactericidal activity of daily dose CMM continued to the end of 8th week (99.98%).

Table 6 : Comparison of Daily Dose with Intermittent Dose - Normal Mice

Regimen	4th week	8th week	12th week	24th week
CMM	98.99(D)*	99.98(D)*	-	-
	0 (I)	-	0(I)	82.21(I)
COM	0 (I)	-	0(I)	68.37(I)
WHO MDT	0 (I)	-	43.27(I)	99.90(I)*

*p<0.05 (Statistically Significant differences) (Z Test of Proportions)

Table 7 : Comparison of Daily Dose with Intermittent Dose - TR Mice

Regimen	4th week	8th week	12th week	24th week
CMM	98.99(D)*	99.98(D)*	-	-
	0 (I)	-	89.99(I)	98.99(I)
COM	0 (I)	-	96.83(I)	96.83(I)
WHO MDT	0 (I)	-	96.83(I)	99.68(I)

*p<0.05 (Statistically Significant differences) (Z Test of Proportions)

At the end of 12 weeks, intermittent dose of CMM (89.99%), COM (96.83%) and WHO MDT (96.83%) showed bactericidal activity.

At the end of 24 weeks, WHO MDT, CMM and COM showed bactericidal activity with WHO MDT (99.90%) being the best.

The bactericidal activity of daily dose of CMM at the end of 8 weeks is higher than the bactericidal effect of intermittent regimens of CMM, COM and WHO MDT at the end of 24 weeks.

Daily dose CMM indicated statistically significant (p<0.05) bacterial killing at 4th week when compared to WHO MDT. The effect of daily dose CMM at 8 weeks was comparable to WHO MDT at 12 and 24 weeks.

As in normal mice, in TR mice also Daily dose CMM showed early onset of bactericidal activity which sustained into the 8th week and probably would have continued into the 24th week being comparable to WHO MDT.

Discussion

In this study, nine regimens of single drugs and drug combinations both in intermittent dose and daily dose and WHO MDT were tested in normal and TR mice infected with Rifampicin resistant strain. Two single drugs, RPT and MOXI were tested for individual efficacy against the resistant organism. Two drug regimes, CLARI/MOXI/MINO (CMM) and CLARI/OFLO/MINO (COM) were tested against WHO MDT for efficacy against rifampicin resistant strain.

Single Drugs

Intermittent dose and daily dose of Moxifloxacin exhibited bactericidal effect in normal and TR mice in Rifampicin resistant strain. The bactericidal results of daily and intermittent Moxifloxacin regimes are comparable with WHO MDT at different time intervals.

Intermittent dose Rifapentine showed better bactericidal effect in TR mice than normal mice. This effect was less than that of Moxifloxacin. Daily dose Rifapentine showed bactericidal effects comparable to Moxifloxacin both in normal and TR mice. From these results it seems that Moxifloxacin is the drug of choice if Rifampicin has to be replaced in the present WHO MB MDT.

Drug Combinations

Clarithromycin, Ofloxacin and Minocycline (COM) in normal mice in intermittent dose showed no bactericidal effect at the end of 4th and 12th week. At the end of 24th week the bactericidal effect of COM (68.37%) was not very good. Even in TR mice, COM did not fare well when compared to CMM or WHO MDT. With daily dose, even though the bactericidal effect of COM was comparable with CMM and WHO MDT in normal mice, in TR mice it was less. Based on these results it seems that COM may not be considered as an effective drug combination for rifampicin resistant cases.

The intermittent dose of Clarithromycin, Moxifloxacin and Minocycline (CMM) in normal mice showed no bactericidal effect at the end of 4th and 12th week. Even at the end of 24th week the bactericidal effect of CMM (82.21%) was not comparable with WHO MDT in normal mice. However in TR mice, intermittent dose CMM bactericidal effect was comparable to WHO MDT at 24th week. With daily dose CMM, bactericidal effect of CMM on 6th day, 4th week and 8th week was very good in normal mice and TR mice. When daily dose and intermittent dose were compared, the bactericidal activity of daily dose of CMM at the end of 8 weeks is higher than the bactericidal effect of intermittent regimens of CMM, COM at the end of 24 weeks and comparable to WHO MDT in both normal and TR mice.

The results show that among the drug combinations tested, WHO MDT is still the best combination to be used in Rifampicin resistance

cases. If for some reason WHO MDT has to be replaced in Rifampicin resistance cases, CMM may be the choice of combination preferably as a daily dose regimen.

It is paradoxical that WHO MDT is still the best drug combination in the presence of resistance to Rifampicin. One of the explanations could be that in the presence of Rifampicin resistance, DDS and Clofazimine compensate and treatment with WHO MDT shows clinical response. Even in MFP studies DDS and Clofazimine combination have been shown to be effective antibacterial drugs in earlier studies.

As per the present clinical protocol in rifampicin resistance proven cases a further trial of WHO MDT is tried. If this further trial of WHO MB MDT in patients with Rifampicin resistance proven by molecular methods does not show clinical response, the recommendation will be to treat the patient with daily or intermittent CMM combination because merely replacing Rifampicin in WHO MDT with Moxifloxacin alone may not cover for co-existing DDS resistance.

In the field setting where relapses are diagnosed on the basis of clinical criteria alone with no smear or molecular biology support it is prudent to start the patient on MB MDT because a number of patients would not have completed MDT and treatment history is not reliable. If no clinical improvement is seen then a combination of CMM can be started preferably daily dose.

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