

APPLIED RESEARCH

Completed studies

Determination of the binding ability of activated INH with AccD6 from *M. tuberculosis*

Experiments based on DNA micro array have provided information on the involvement of *accD6* (an important component of mycolic acid synthesis) with activation of INH. In order to understand the role of *accD6* gene, structural prediction of AccD6 protein and its docking with activated INH were undertaken. Homology modeling of AccD6 was performed using the software-MODELLER9v3, and docking of acetyl-CoA (substrate) and activated INH (inhibitor) was carried out by two softwares-GOLD 4.0.1 and AUTODOCK 4.0.5. The chosen template (2A7S) had a sequence identity of 43% for modeling AccD6 from *M. tuberculosis*. The generated model was subjected to validation by Ramachandran Plot using RAMPAGE and combinatorial extension method. The docking of acetyl-CoA with AccD6 (-63.82kcal/mol) was possible with GOLD, whereas the docking of isonicotinic acid was not feasible due to spatial inconsistency. The successful docking of both the substrate and inhibitor with AccD6 was performed with AUTODOCK displaying (acetyl-CoA) + 1536.92kcal/mol and (INADH not isonicotinic acid) -7.23kcal/mol as scores. Therefore, we have presented the model structure of AccD6 and made an attempt to predict a primary binding site for activated INH (INADH) in AccD6. The results suggest that AccD6 could also be the target for activated INH in addition to InhA and KasA.

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Field evaluation of concentrated method for improvement of ZN staining techniques

Background

For over a century, the diagnosis of PTB is being confirmed by detection of AFB in direct smears of sputum made on glass slides. Good laboratory practices have to be followed while making direct smears from the mucopurulent portion of sputum on glass slides in order to avoid laboratory acquired TB infection. There is a felt need to develop a new technique to stain the AFB, which would be both simple and non-hazardous. Recently, it has been shown that the deposit of sputum sample, obtained after decontamination with 4% sodium hydroxide and concentrated by centrifugation, can be stained with 1% carbol-fuchsin in its container. The smear can be made subsequently on a glass slide, decolourised and counter-stained by the procedure followed in the Ziehl Neelsen (ZN) method.

Aim

- To stain the sputum sample in its container with phenol ammonium sulphate carbol-fuchsin with subsequent decolourisation and counter-staining of its smear on glass slide for detection of AFB

Methods

A total of 560 sputum samples collected from patients attending a TB clinic were selected. Direct smears were made and stained by the conventional ZN staining method. The samples were then treated with 1-2 ml of phenol ammonium sulphate basic fuchsin solution and left at room temperature for 90 minutes. Later, smears (sediment smear) were made, air dried, and decolourized and counterstained using 25% sulphuric acid and 0.1% methylene blue. The sediment and direct smears were coded and read, and the results were compared.

Results

The yield of AFB positive smears was similar in direct and sediment smears and the difference was not significant (table 12).

Table 12: Comparison of sediment smears with direct smears

Direct smears		Sediment smears*					Total
		Negative	1+	2+	3+	Scanty	
Sediment smears	Negative	473	0	1	6	0	480
	1+	7	12	9	6	3	37
	2+	2	6	5	4	1	18
	3+	2	5	1	12	3	23
	Scanty	0	0	0	2	0	2
Total		484	23	16	30	7	560

* Negative = no AFB in 100 fields; scanty = 1- 9 AFB in 100 fields; 1+ = 10 – 99. AFB in 100 fields; 2+ = 1 to 9 AFB per field in at least 50 fields; 3+ = more than 10 AFB per field in at least 20 fields.

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Profile of sputum samples submitted for acid-fast bacilli microscopy in a semi-urban TB clinic, Tamil Nadu, India

Background

The profile of sputum samples submitted by PTB suspects in a TB clinic during a 1 year period, and its influence on smear results was studied.

Aim

- To study the 'type', 'quantity' and 'quality' of sputum specimens submitted by the PTB suspects attending a TB clinic and their influence on AFB smear results

Methods

Sputum specimens were collected from patients attending the TB clinic at Poonamallee. During the 12 month period, from November 2007 to October 2008, laboratory technicians trained at the National Reference Laboratory (NRL) recorded the 'quality', 'quantity' and 'type' of sputum submitted by patients. The laboratory technicians examined and reported the results of sputum smears stained by the hot ZN method. Sputum samples from all smear positive patients were analysed to know the influence of sputum profile on AFB smear results.

Results

Distribution of smear results of 579 samples from 221 sputum smear-positive patients revealed that 352 (60.8%) samples were saliva, 364 (62.9%) contained

more than 2 but less than 4ml of sputum. The yield of AFB positives was, similar for mucoid (200/219 - 91.3%) and saliva (326/352 - 92.6%), and higher in samples with more than 4ml quantity (178/180 – 99.4%), and in morning samples (178/180 – 99.4%).

Conclusion

The yield of AFB positive smears in sputum smear positive patients was similar in mucoid and salivary samples, high in samples containing >4ml volume, and in samples collected in the morning. The 'type' and 'quantity' rather than the 'quality' of sputum play a significant role in detection of AFB positive smears.

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Age, nutritional status and CYP2B6 G516T polymorphism influence nevirapine blood levels in HIV-infected children on generic anti-retroviral treatment

(Collaboration with BJ Wadia Hospital for Children, Mumbai, Govt. Hospital of Thoracic Medicine, Chennai and Govt. Rajaji Hospital, Madurai)

Background

Most ART programs in resource limited countries use NVP based generic fixed dose combinations (FDC) for treatment, because of affordability, ease of administration and lack of teratogenicity. In India, antiretroviral drugs for pediatric use were made available by the NACO at the Government ART centres only from November, 2006; prior to that, adult formulations were used. These specially formulated pediatric generic drugs are available as FDC, consisting of stavudine (d4T) with 3TC and NVP formulated in two different ratios (6:30:50 & 10:40:70 mg respectively). It is however, important to ensure that children receive adequate doses, and plasma concentrations of antiretroviral drugs are maintained within the therapeutic range.

Some of the factors known to influence NVP drug levels include age, co-administration of other drugs and pharmacogenetic variability. It has been suggested that in general, adult FDCs, while suitable for older children, are not suitable for very young children. Not many studies have specifically examined

the bio-availability of pediatric FDCs and none have been conducted in Indian children. Further, the relationship of drug levels with moderate and severe grades of underweight and stunting has not been well studied.

Aim

- To examine the influence of age, sex, drug dose, nutritional status and CYP2B6 G516T polymorphism on blood levels of NVP in HIV-infected children treated with generic antiretroviral drugs

Methods

This was a multi-centric study conducted at four sites in India. Ninety four HIV-infected children, aged 6 months to 14 years, receiving generic NVP-based FDCs from the out-patient clinics at the Government Rajaji Hospital, Madurai, B.J.Wadia Hospital, Mumbai, Government Hospital of Thoracic Medicine, Tambaram and Kilpauk Medical College and Hospital, Chennai were recruited. Trough and 2-hr NVP plasma concentrations were determined by high performance liquid chromatography (HPLC) and genotyping of CYP2B6 G516T polymorphism by direct sequencing.

Results

The trough and two-hr NVP concentrations in the different groups of children are given in table 13. Stunted children had significantly lower 2-hr NVP concentration compared to non-stunted ($p < 0.05$). NVP levels were significantly higher in TT compared to GG and GT CYP2B6 genotypes ($p < 0.01$). Children below three years had a 3.2 times (95% CI: 1.07 – 9.45) higher odds of having sub-therapeutic NVP levels.

Conclusions

A combination of factors such as young age, CYP2B6 GG/GT genotype and stunting could result in sub-therapeutic NVP levels in children. A substantial proportion of children had levels below the generally accepted lower therapeutic limit of 3.0µg/ml and this was more pronounced in the younger children. This is a matter of concern and is likely to play a role in long-term viral control. The study findings suggest that higher dose recommendations may be required for malnourished (stunted) children and those below three years of age.

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Table 13: Plasma NVP concentrations [median, (Q1, Q3)] in the different groups of children

Groups	N	Trough concentration (µg/ml)	N	2-hr concentration (µg/ml)
Sex				
Female	41	3.57 (2.27, 5.24)	39	4.98 (3.10, 8.12)
Male	47	3.71 (2.39, 5.24)	48	5.96 (4.17, 7.89)
Dose				
<300 mg/sq.m/day	40	3.32 (2.34, 4.66)	40	5.29 (3.03, 6.21)
≥300 mg/sq.m/day	48	4.10 (2.34, 6.09)	47	6.16 (4.30, 9.50)
Drug formulations				
d4T : 3TC : NVP				
6 : 30 : 50	24	3.47 (2.12, 4.52)	22	5.19 (3.40, 6.49)
10 : 40 : 70	25	3.33 (2.32, 4.53)	27	5.33 (3.00, 8.59)
30 : 150 : 200	39	4.50 (2.58, 6.12)	38	6.06 (4.06, 10.09)
Height-for-age Z score				
Stunted (< - 2 HAZ)	55	3.55 (2.39, 4.81)	55	5.29 (3.24, 6.87)*
Non – stunted	33	3.91 (2.32, 6.43)	32	6.08 (4.98, 10.90)
Weight-for-age Z score				
Underweight (< - 2 WAZ)	51	3.71 (2.42, 5.24)	53	5.61 (3.88, 7.77)
Normal weight	37	3.20 (2.27, 5.28)	34	5.63 (3.26, 8.09)
Age				
≤ 3 years	17	2.52 (1.75, 3.80)*	14	4.21 (2.59, 5.81)*
3 years	71	3.98 (2.47, 5.53)	73	5.74 (3.99, 8.35)

* denotes p<0.05

Phages and phage lysin to control overgrowth of normal flora in processed sputum specimens grown in liquid medium

Background

Rapid diagnosis of TB is essential to control the spread of TB especially in MDR-TB patients and in those co-infected with TB and HIV. The overgrowth of normal flora escaping the action of sputum processing chemical such as 4% sodium hydroxide is a major problem in rapid broth-based detection systems, affecting the sensitivity of any rapid assay. Use of phagebiotics to overcome this problem has been established and it forms a novel, bio-friendly approach to tackle non-mycobacterial contaminants.

Aim

- To strengthen phagebiotics by use of phage lysin for complete and effective control of normal flora in sputum specimens and to evaluate the same in retrieval of *M. tuberculosis* by luciferase reporter phage (LRP) assay

Methods

Crude lysin was prepared from phage host mixture using standard procedures. One hundred and twenty sputum samples processed using 4% sodium hydroxide were collected and divided in to four aliquots after inoculating on to blood agar plates (Stage I). Nutrient broth was added to one part (Stage II) that served as control. To the other three parts, phagebiotics (Stage III), phagebiotics-lysostaphin (Stage IV) and phagebiotics-lysin (Stage V) were added, randomized, incubated at 37°C for 18-24 hrs and inoculated on blood agar plates. The effect of lysin on retrieval of *M. tuberculosis* up to 3 days by LRP assay was tested using the standard protocol.

Results

The phagebiotics supplemented with lysin arrested the growth of surviving normal flora in more number of samples (112) when compared to Stage II (12), Stage III (70) and Stage IV (81); the difference was statically significant (table

14). Lysin did not show any inhibitory activity on *M. tuberculosis* H₃₇Rv and clinical strains of *M. tuberculosis*.

Conclusion: Phagebiotics supplemented with lysin can be used to control the overgrowth of normal flora in liquid media without compromising on the viability of *M. tuberculosis*.

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Table 14: Growth of normal flora surviving after processing of sputum samples

Growth on blood agar	Number of samples				
	Stage I	Stage II	Stage III	Stage IV	Stage V
Confluent growth, mixed	0	105	5	3	0
<i>Bacillus sp</i>	0	0	24	23	3
<i>Staphylococcus sp</i>	26	3	21	13	5
No growth	94	12	70	81	112
Total number of samples	120				

Stage I: Soon after processing of sputum samples

Stage II: Grown overnight in nutrient broth

Stage III: Grown overnight in phagebiotics

Stage IV: Grown overnight in phagebiotics supplemented with lysostaphin

Stage V: Grown overnight in phagebiotics supplemented with lysin

Isolation of active compounds from essential oils against *M. tuberculosis* using bioassay guided fractionation

(Funded by Council of Scientific and Industrial Research, New Delhi)

Background

Search for novel and more effective anti-TB agents is important in order to tackle MDR strains of *M. tuberculosis*. Plants are of diverse chemical nature and form a valuable source of new antimycobacterial drugs or a lead compound from which new drugs may be developed.

Aims

- To isolate the active fraction against *M. tuberculosis* from cinnamon oil using bioassay guided fractionation method
- To determine the minimum inhibitory concentration (MIC) of the isolated active compound against clinical isolates of *M. tuberculosis*

Methods

Based on antimycobacterial screening by LRP assay, cinnamon oil was chosen to be the most promising oil, and was subjected to further fractionation and phytochemical analysis. The oil was fractionated by high vacuum distillation and purified using different chromatographic techniques. Based on bioassay guided fractionation, one active molecule was identified, and spectral analysis was performed to elucidate the structure. The MIC was determined for the active molecule against drug sensitive and drug-resistant clinical isolates of *M. tuberculosis*.

Results

Four fractions were obtained from cinnamon oil of which fraction III showed high activity (96.11%) against *M. tuberculosis* even at a low concentration of 50 µg/ml (table 15). Based on spectral analysis, the active fraction was identified as *trans*-cinnamaldehyde. The MIC value of *trans*-cinnamaldehyde ranged from 5-100µg/ml for drug sensitive and drug-resistant clinical isolates of *M. tuberculosis*.

Conclusion

The results suggest that cinnamaldehyde has good antimycobacterial activity. It is worth pursuing further studies using this compound to evaluate its potential as a potent anti-TB agent.

Table 15: Percentage reduction in relative light units (RLU) by different fractions of cinnamon oil against *M. tuberculosis*

Fractions	Concentration	
	50 µg/ml	100 µg/ml
I	31.15 ± 1.56 ^d	42.37 ± 3.29 ^c
II	55.41 ± 2.77 ^b	58.30 ± 0.33 ^b
III	96.11 ± 0.36 ^a	98.78 ± 0.03 ^a
IV	48.96 ± 1.14 ^c	61.38 ± 1.17 ^b

Values represent mean % reduction in RLU ±SD of two replicates

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Lytic efficiency of mycobacteriophages

Background

The emergence of resistance to mycobacteria by currently available antimicrobial agents prompted interest in searching for alternatives to conventional drugs. One possible option is to use bacteriophages. They are often highly specific and are nontoxic to animals and plants. An attempt was made to determine their efficiency in killing the host bacteria. The performance of five phages namely, D29, TM4, I3, Che7, Che11 was tested in killing clinical strains of *M. tuberculosis*.

Aims

- To determine the efficiency of five lytic mycobacteriophages in killing clinical strains of *M. tuberculosis*
- To evaluate the lytic efficiency of D29 and TM4 based on codon usage analysis

Materials and methods

Suspensions were made from 10 clinical strains of *M. tuberculosis* in Middlebrook 7H9 liquid medium supplemented with bovine serum albumin and divided into 6 aliquots of 1.5 ml each. 300 µl of high titre phages namely, D29, TM4, I3, Che7, Che11 was added to each of the aliquots. A negative control was included. After 3, 6, 24 and 48 hrs of incubation, 250 µl of the mixture was removed and added to 50 µl of phAE129. RLUs were measured using a cuvette luminometer after three hrs of incubation. Codon usage of TM4 and D29 was analyzed using CodonW. Blast and Pfam were used for database search and domain analysis. ClustalW was used for multiple sequence alignment was used.

Results and conclusion

D29 phage was found to be most effective in killing all the 10 clinical strains tested. The Chennai phage Che7 was able to kill 8 of the 10 mycobacterial strains while TM4 was least effective.

Based on codon usage analysis, the genome of TM4 was identified to have high codon usage bias, revealing that the genes of TM4 have better translational efficiency than D29. This better translational efficiency is expected to quicken the lysis process also probably qualifying it to be a better lytic phage. However, the lytic performance of TM4 was poor in all the strains tested in the present study.

In order to understand the reasons for the poor performance of TM4 contradictory to the prediction by the whole genome analysis, sequence analysis of holin and lysin genes was done. It was found that the observed low lytic activity of TM4 may be due to the following reasons:

- ◆ The presence of highly charged N-terminal region which may act as antiholin
- ◆ The number of effective codons (Nc value) of TM4 lysin genes are lower than D29. However, the holin genes of D29 have lower Nc values than TM4. These results suggest that holin of D29 has better translational efficiency

- ◆ TM4 encodes no tRNA genes, whereas D29 has five tRNA genes that match with the highly used codons
- ◆ The residues surrounding the active site in TM4 lysin B is different from the well conserved residues of D29. Aspartic acid and histidine in D29 are replaced by glycine and alanine respectively in TM4

The holin gene of TM4 functioning as antiholin, its low translational efficiency and the lack of tRNA genes may be the reasons for D29 outperforming TM4 in lytic activity. Though codon usage analysis of whole genome indicates the overall expression level, further analysis of specific genes is required to understand their performance level.

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Ongoing studies

Antimycobacterial compounds from marine actinomycetes

(Collaboration with Indian Institute of Technology, Chennai and Periyar University, Salem)

(Funded by Department of Science & Technology, New Delhi)

Background

There is an urgent need to discover novel bioactive compounds to fight against drug-resistant *M. tuberculosis*. Actinomycetes are biotechnologically valuable prokaryotes which are a major source of antibiotics.

Aim

- To screen marine actinomycetes for antimycobacterial activity

Methods

Actinomycetes strains isolated from different marine ecosystems were screened for antimycobacterial and antibacterial activity. The CFs and mycelial methanol extracts of actinomycetes strains were tested against *M. tuberculosis* H₃₇Rv, a drug-resistant and a drug sensitive clinical isolate of *M. tuberculosis* by LRP assay. CFs of selected actinomycetes strains were extracted using different

solvents and tested for antimycobacterial activity. Ethyl acetate extract of the potential strain was purified by thin layer chromatography and bioassay guided fractionation. Spectral analysis of the active fraction was carried out at the Indian Institute of Technology, Chennai. Antibacterial activity was also studied by cross streak method and agar well diffusion method against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*.

Results

A total of 55 actinomycete strains were selected from different marine ecosystems, several of which exhibited antimycobacterial activity (table 16). Based on the results, 8 potential actinomycete strains were selected for further investigations. In bioassay guided fractionation, fraction II of ethyl acetate extract of R2 strain showed antimycobacterial activity. A number of actinomycete strains also showed activity against *S. aureus*, *B. subtilis*, *E. coli*, *S. typhin* and *P. aeruginose* by both cross streak and agar diffusion methods (Figs. 1 & 2).

Conclusion

Marine actinomycetes are potential sources for antimycobacterial compounds, since, 40 out of 55 strains tested inhibited *M. tuberculosis*. Purification, characterization and structure elucidation of the active compound from potential actinomycete strains are in progress.

Table 16: Number of actinomycete CFs and mycelial methanol extracts showing different range of antimycobacterial activity in LRP assay

Ranges of % RLU reduction	Culture filtrates			Methanol extracts		
	H ₃₇ Rv	SHRE-sensitive MTB	SHRE-resistant MTB	H ₃₇ Rv	SHRE-sensitive MTB	SHRE-resistant MTB
>50%	40	30	26	21	22	24
>50-75%	19	8	4	10	6	9
>75-90%	10	8	3	8	8	7
>90%	11	14	19	3	7	8
<50%	15	25	29	34	33	31

RLU – Relative light units; S – streptomycin; H – isoniazid; R – rifampicin;
E – ethambutol; MTB – *M. tuberculosis*

Fig.1: Antibacterial activity of marine actinomycetes by cross streak method

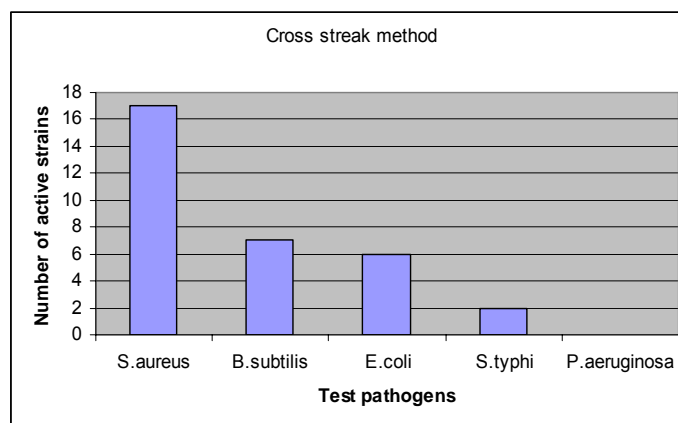
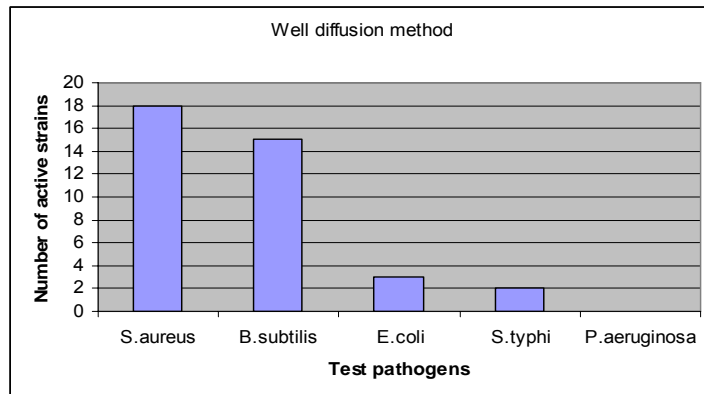


Fig. 2: Antibacterial activity of marine actinomycetes by well diffusion method



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Efficacy of slide culture in rapid detection of *M. tuberculosis* in sputum specimens processed by modified Petroff's method

Background

Rapid diagnosis of TB is vital for control and prevention of spread of the disease in the community. Assays aimed at field level applications should be simple, rapid, reliable not requiring expensive instruments. Slide culture technique is simple with high potential for rapid diagnosis and detection of drug-resistant strains directly from sputum specimens.

Aim

- To standardize slide culture technique for rapid detection of *M. tuberculosis* in sputum specimens processed by modified Petroff's method

Method

Fifty smear positive sputum specimens were included in the pilot study. The specimens were randomized and processed by modified Petroff's method. From the final pellet, two LJ slopes were inoculated and incubated. From the remaining deposit, triplicate smears each covering an approximate area of 0.05 cm² were made on one end of specially prepared sterile slides using a standard 5 mm twisted wire loop. The smears were air dried and gently heat fixed by

passing the smear thrice over the flame. Two smears were placed back to back in a universal container with 4 ml of Kirschner's liquid medium and incubated. After 7 days, the slides were removed, de-contaminated using Cidex, air dried and heat fixed. All three smears including the deposit smear were stained by auramine phenol. Growth on the incubated smears was compared with the deposit smear and graded. Results were compared with those obtained by the conventional culture method.

Results

Among the 50 samples that were included, three cultures were contaminated and were excluded. Among the remaining 47 samples, 40 were culture positive and 7 were culture negative. Among the 40 culture positives, 38 were positive by slide culture. Among the 7 culture negatives, 2 were negative by slide culture.

Conclusion

Slide culture technique shows promise in rapid detection of *M. tuberculosis* in sputum specimens. However, further work with larger number of samples has to be done to eliminate false results.

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Characterization of ethionamide resistance in naïve and treated TB patients from south India

Background

Ethionamide (ETH) is an efficacious, relatively non-toxic, second line anti-TB drug acting on the fatty acid synthesis of cell wall components of *M. tuberculosis*. Though ETH is a structural analogue of INH, only minimal cross resistance to these drugs is observed among clinical isolates. Although the activation of INH and ETH differs, the putative final metabolites for both drugs are very similar, and they share the same cellular target, namely inhA. Overproduction of the drug target also appears to lead to resistance to INH and ETH. ethA encodes a protein that belongs to the flavin containing monooxygenase family catalyzing the activation of ETH. ethR encodes a repressor belonging to the TetR/CamR family

of transcriptional regulators and negatively regulates the expression of ethA. The genetic locus, *inhA* has been known to be associated with resistance of *M. tuberculosis* to INH and ETH. Co-resistance to INH and ETH is not only mediated by dominant mutations in the target gene *inhA*, encoding an enoyl-ACP reductase, but also by recessive mutations in *ndh*, encoding a type II NADH dehydrogenase. Studies have shown no association between *katG* mutation and the level of ETH resistance, but mutations within the *ethA* and *inhA* structural genes were associated with relatively high levels of ETH resistance.

Rationale of the study

Researchers have carried out studies pertaining to ETH resistance and ETH and INH cross-resistance across the globe. But similar studies have not been carried out in a TB endemic setting like India. Clinical studies carried out at TRC have shown differences in the *in vitro* and *in vivo* activities of ETH. Screening TB patients for the presence of mutations / polymorphisms in the genes associated with ETH resistance might throw light on the level of primary resistance or possible acquired resistance within the geographical locale.

Aims

- To characterize the phenotypic and genotypic resistance pattern of ETH resistance among TB patients from south India
- To study the mechanism of cross resistance between INH and ETH by analyzing the genes conferring resistance to the drugs

Preliminary work

Standardization of phenotypic drug susceptibility testing for ETH was carried out on solid as well as automated liquid culture systems. Initial Phase I standardization was performed using *M. tuberculosis* H₃₇Rv and a panel of 15 *M. tuberculosis* strains isolated from naïve patients. Seventy *M. tuberculosis* strains (MDR and non-MDR) were included in phase II standardization. The results of the above standardization experiments and the retrospective analysis (n=407) for ETH suggest that MIC method is comparable with the proportion sensitivity

method which is the gold standard. Comparison of DST in solid medium with that of automated liquid culture systems will help in identifying the method that can best demarcate a sensitive strain from that of a resistant one.

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Monitoring plasma nevirapine and efavirenz in HIV-TB patients undergoing anti-TB and anti-retroviral treatment

Background

Concomitant RMP - based antitubercular therapy is known to significantly reduce NVP concentrations to sub-therapeutic levels in a significant proportion of patients, while in the case of EFV, the impact is to a relatively lesser extent. A few studies have observed that NVP-based ART has failed to yield satisfying clinical outcomes, compared to EFV-containing regimen in HIV-TB patients receiving treatment for both infections. However, it is not clear whether sub-therapeutic blood levels of NVP contribute to poor treatment outcomes in HIV-TB co-infected patients. In an ongoing controlled clinical trial at the Centre, two different ART regimens along with RMP-containing ATT are being evaluated in patients with HIV-1 and TB. The two regimens have either EFV or NVP with didanosine and lamivudine.

Aim

- To study the trough levels of EFV and NVP at different time points (during and after completion of ATT) and correlate with treatment outcome (viral load and CD4 cell count measurements)

Methods

The study is being carried out in patients who are recruited into the ongoing controlled clinical trial. The trough levels of NVP and EFV are being studied at months 1, 4, 6 and 12 after start of ART (months 1 & 4 while receiving ART & ATT and months 6 & 12 while receiving only ART). At these time points, a sample of blood (3 ml) is collected at predosing. Plasma NVP and EFV are estimated by HPLC according to validated methods.

So far, 64 and 105 patients receiving NVP and EFV respectively have been recruited into this study. The study is in progress.

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