

APPLIED RESEARCH

Completed studies

Studies on sputum transported in cetyl pyridinium chloride for detection of *M. tuberculosis*

Background

Drug resistance surveys require transportation of sputum samples collected from pulmonary TB patients in cetyl pyridinium chloride (CPC) solution to the reference laboratory for bacteriological investigations. It was observed that smears from sputum transported in powder CPC (P-CPC) yielded less AFB.

Aim

- To elucidate the reasons for the reduction of AFB positivity in sputum transported with P-CPC

Specific objectives

- To detect and demonstrate any cell wall changes after exposure to CPC using transmission electron microscopy (TEM) and phage adsorption assay
- To estimate the proportion of metabolically active cells of *M. tuberculosis* after exposure to CPC using fluorescein diacetate-ethidium bromide (FDA-EB) vital staining method and viable count
- To determine changes in mycolic acid content of tubercle bacilli exposed to CPC using high performance liquid chromatography (HPLC).

Results and conclusion

Damage to cell wall after exposure to CPC was demonstrated by both TEM and phage adsorption assay. FDA/EB stain showed upto 90% viable cells (green) prior to CPC exposure and upto 70% non-viable cells (red-orange) after CPC exposure. Viable count was not altered significantly after exposure to CPC. HPLC analysis revealed marked reduction in mycolic acid content. The liquid as well as P-CPC were found to affect the sensitivity of AFB microscopy.

[Contact person: Dr.N.Selvakumar (E-Mail ID: selvakumarn@trcchennai.in)]

Effect of rifampicin, isoniazid, pyrazinamide and ethambutol on the steady state pharmacokinetics of moxifloxacin

Background

Moxifloxacin (MFX) is reported to have promising antimycobacterial activity, and has a potential to shorten TB treatment. MFX undergoes phase II metabolism by means of sulphate and glucuronide conjugation. RMP, a potent inducer of cytochrome P-450 isoenzymes also induces the phase II glucuronidation pathway.

Aim

- To study the influence of RMP & INH on the steady state pharmacokinetics of MFX individually

Methods

The study was performed in 12 healthy adults who were not suffering from any illness. Each subject was investigated on two occasions; a cross-over design was employed in which each subject served as his control. A baseline pharmacokinetic study of MFX (400 mg once daily) was conducted (Occasion 1) and repeated after one week of daily MFX with either RMP (450/600 mg) or INH (300 mg). Each drug group had six subjects. During both occasions, serial blood samples were collected pre-dosing and at 1, 2, 4, 6, 8 and 12 hrs after drug administration. Plasma MFX concentrations were determined by a validated HPLC method.

Results

Plasma exposure of MFX was significantly lower when combined with RMP, ($p < 0.05$). This was accompanied by a significant increase in the plasma clearance of MFX ($p < 0.01$). Peak concentration of MFX was lower when combined with RMP than when given alone (4.25 vs. 6.36 $\mu\text{g/ml}$); the difference was not statistically significant ($p = 0.074$). Although no significant differences in mean values of pharmacokinetic parameters of MFX when given alone and when combined with INH were observed, the AUC_{0-12} values MFX in four subjects showed a decrease when combined with INH.

Conclusion

This study done in a small sample has shown that concomitant RMP administration caused a 39% mean decrease in the MFX exposure and a 46% mean increase in the oral clearance of MFX; the MFX-INH interaction is unclear. The clinical relevance of these interactions needs further investigations.

Table 9: Steady state pharmacokinetics of MFX alone and in combination with RMP or INH

Healthy subjects	Mean (95% Confidence intervals)				
	C _{max}	T _{max}	AUC ₍₀₋₁₂₎	Cl	t _{1/2}
MFX (n = 6)	6.36 (4.79, 7.93)	3.0 (2.12, 3.88)	51.75 (40.98, 62.52)	4.86 (3.99, 5.73)	8.49 (6.53, 10.45)
MFX + RMP	4.25 (3.87, 4.63)	2.0 (1.12, 2.88)	31.43* (27.53, 35.33)	8.96* (7.65, 10.27)	6.71 (5.55, 7.87)
MFX (n = 6)	6.19 (4.71, 7.67)	2.33 (1.24, 3.42)	50.09 (36.64, 63.54)	5.27 (3.93, 6.61)	8.25 (7.66, 8.84)
MFX + INH	5.06 (4.48, 5.64)	1.83 (0.89, 2.77)	37.97 (33.14, 42.80)	6.32 (5.25, 7.39)	8.61 (7.77, 9.45)

* denotes p < 0.05

C_{max} – Peak concentration; T_{max} – Time to attain C_{max}; AUC₍₀₋₁₂₎ – Exposure; Cl – Clearance; t_{1/2} – Half-life

[Contact person: Dr.Geetha Ramachandran (E-Mail ID: geethar@trcchennai.in)]

Single dose pharmacokinetics of lamivudine in healthy volunteers: comparison of blood and urine kinetics

Background

Lamivudine (3TC) forms an important component of highly active antiretroviral therapy (HAART) that is used to treat HIV-infected individuals in India. It is present in all fixed dose combination (FDC) pills, has a short elimination half-life,

and hence estimation of the drug in blood or urine could be useful in monitoring patient adherence to ART.

Aim

- To study the single dose pharmacokinetics of 3TC in healthy subjects, and also to assess the correlation between plasma and urine kinetics of 3TC

Methods

Twelve healthy adult males meeting the study criteria were recruited to the study. The study was carried out at the Pharmacology Ward in Madras Medical College, Chennai. They were administered 3TC (150mg) under supervision and blood samples were collected pre-dosing and at 1, 2, 4, 6, 8, 12 and 24 hrs after drug administration. They were instructed to make complete urine collections excreted up to 24 hrs after drug administration. 3TC concentrations in plasma and urine were estimated by HPLC according to validated methods.

Results

The peak concentration of 3TC in plasma was achieved at about one hour, suggesting rapid and almost complete absorption. The concentration of 3TC at 24 hours was undetectable in all the 12 study subjects. The correlation between plasma exposure (0 to infinity) and percent dose of 3TC excreted in urine between 0 to 24 hrs was highly significant ($p < 0.001$; $r = 0.96$).

Conclusions

Urine 3TC was highly correlated with plasma exposure, which suggests that urine 3TC estimations can be used to obtain information on the bioavailability of the drug. Thus invasive blood collections can be replaced by simple, non-invasive urine collections. The study has also demonstrated the usefulness of plasma 3TC in predicting ART adherence.

[Contact person: Dr.Geetha Ramachandran (E-Mail ID: geethar@trcchennai.in)]

Comparison of HPLC and spectrophotometric methods for estimation of antiretroviral drug content in pharmaceutical products

Background

Although the growth in antiretroviral availability is encouraging, it must be accompanied by independent quality-control studies to check for adherence of amount of active ingredient in the tablet/capsule to the stated content. The most common method used for analysis of antiretroviral drugs is by HPLC. However, many laboratories in resource-constrained settings may not afford to have HPLC equipment, which also requires skilled personnel to operate it. An alternative is to carry out drug estimations by spectrophotometry which is relatively cheap and simple.

Aim

- To compare the content of NVP, 3TC and stavudine (d4T) in single tablets/capsules estimated by HPLC and spectrophotometric methods

Methods

Twenty tablets/capsules each of NVP, 3TC and d4T were analysed for their drug content by HPLC and spectrophotometric methods. The tablets/capsule contents were crushed into a fine powder and suitably diluted in methanol to yield a concentration of 1.0mg/ml for each drug. Further dilutions were made using milli-Q water to obtain a final concentration of 25µg/ml. The stock solution (1mg/ml) of each drug was prepared by dissolving the pure powder in methanol and these solutions were stored at -20°C.

Calibration curves containing known concentrations of each drug were prepared fresh individually and set up on each day by making suitable dilutions in water from the stock solution. The concentration curve was constructed for each drug using a set of calibration standards (12.5, 25 and 50µg/ml for NVP and 3TC and 6.25, 12.5 and 25µg/ml for d4T) that were prepared in water and run along with the unknown solutions with each run by HPLC and spectrophotometric methods.

HPLC method

Analysis was performed using C₁₈ column. The mobile phase consisted of phosphate buffer and acetonitrile in different compositions for the three drugs. The UV detector was set at 260 nm. The retention times of 3TC, d4T and NVP were 3, 3.9 and 6.7 minutes respectively. The active ingredient in each tablet/capsule was calculated from the height of the peak that was obtained on the chromatogram and compared with that obtained for the corresponding standard solutions of known drug concentration. Appropriate dilution factors were employed to calculate the drug content.

Spectrophotometric method

The instrument was set at 300, 285 and 270nm for NVP, 3TC and d4T respectively. The drug content in the unknown solutions was calculated based on the absorbance values of known standard solutions.

Prior to undertaking the drug assays, calibration standards of NVP, 3TC and d4T were run on six consecutive days by HPLC and spectrophotometric methods. The inter-day variability for each drug concentration was calculated and the linearity of the calibration standards was verified using estimates of correlation coefficient (r).

Results

The calibration curve of NVP, 3TC and d4T from six individual experiments for standard concentrations ranging from 12.5 to 50µg/ml for NVP and 3TC and 6.25 to 25µg/ml for d4T showed a linear relationship between peak height and drug concentration in the case of HPLC and absorbance and concentration in the case of spectrophotometric method. The NVP, 3TC and d4T content from 20 tablets are given in table 10. The percent variation between these methods ranged from 0.45 to 4.49% for NVP, 0 to 4.98% for 3TC and 0.35 to 8.73% for d4T.

Conclusions

The study suggests that the spectrophotometric method is as accurate as the HPLC method for estimation of NVP, 3TC and d4T in tablet/capsule. Hence laboratories that do not have HPLC equipment can also undertake these drug estimations using a spectrophotometer.

Table 10: NVP, 3TC and d4T content in tablets (mg)

	NVP (200mg)		3TC (150mg)		d4T (30mg)	
	HPLC	Spectrophotometer	HPLC	Spectrophotometer	HPLC	Spectrophotometer
Mean of 20 tablets	208.9 (197.1 – 229.6)	208.1 (190.7 – 223.5)	151.2 (130.6 – 170.2)	151.9 (136.1 – 169.7)	28.6 (25.3 – 30.7)	28.6 (25.6 – 30.6)
% variation	2.5 (0.45 – 4.49)		1.4 (0 – 4.98)		3.1 (0.35-8.73)	

Range is given in parentheses

[Contact person: Dr.Geetha Ramachandran (E-Mail ID: geethar@trcchennai.in)]

Improved diagnostic luciferase reporter phage assay for detecting active and non-replicating tubercle bacilli

Background

There is no simple, direct bacteriological tool available till date for the diagnosis of latent TB infection. Among the various alternative diagnostic tests being evaluated, tests based on mycobacteriophages have shown promise. The use of temperate mycobacteriophage Che12 and a temperature sensitive phage mutant, phAE159 (TM4 based) resulted in the development of the reporter phage constructs expressing FFlux driven by promoters of genes highly expressed during dormancy such as isocitrate lyase and alpha crystallin protein. TM4 based constructs expressing Fflux driven by alpha crystallin protein promoter exhibited detectable luciferase activity in dormant as well as in actively growing *M. tuberculosis* cells. Hence the present approach was aimed to evaluate the performance of the constructs in sputum samples.

Aim

- To evaluate the luciferase reporters in sputum samples for the development of a rapid diagnostic method for TB

Methods

Sensitivity of the constructs

M. tuberculosis H₃₇Rv along with two clinical isolates (one of them is an MDR strain) were titrated with the luciferase reporter phage (LRP) constructs to determine the minimal number of bacilli required to produce detectable light.

Evaluation of these constructs in sputum deposits

Evaluation of the lytic construct phAE129 in 50 sputum deposits, temperate construct phAETRC16 in 36 samples and the construct from *ts* mutant phAETRC201 in 18 sputum samples were carried out. The relative light units were measured at 4, 24 and 72 hrs after the second infection.

Results and conclusion

When *M. tuberculosis* strains were titrated with the LRP constructs, phAETRC201 was more sensitive than other constructs. It was able to produce readable RLU with 81 viable cells of *M. tuberculosis* H₃₇Rv, while earlier constructs required 10⁴ viable organisms. Constructs phAETRC16 and phAETRC201, when tested in patients' samples, diagnosed almost all the positives detected by the conventional method. In addition, 17 more positives had been detected by LRP assay.

[Contact person: Dr.Vanaja Kumar (E-Mail ID: vanajakumar@trcchennai.in)]

Impact of antiretroviral treatment on nutritional and immunological status of HIV-infected children

Background

HIV infection is associated with growth retardation, immune deficiency and alteration in cytokine milieu.

Aim

- To investigate the impact of ART on growth (BMI and plasma leptin) and immune response (CD4+ T cell counts, cytokine production, apoptosis and nitric oxide production) in HIV-infected children

Methods

The study population comprised of 11 HIV seropositive children starting on ART and 12 HIV-positive children who did not require ART. Both groups were followed up for 6 months. Height and weight were measured at each visit. CD4+ and CD8+ T-cells counts were analyzed by flow cytometry. Plasma levels of IFN- γ , IL-2, IL-10, IL-8, IL-6, IL-4, IL-5, IL-1, TNF- α and TNF- β were evaluated using a bead based flow cytometric assay. Plasma leptin, nitrate, nitrite and cell death were evaluated using commercially available ELISA kits. Statistical package SPSS (version 13.0) was used to analyze the data.

Results

The mean age of the children on ART was 5 years and that of the ART naïve children was 7 years. Following 6 months of ART, there was a significant elevation in body weight (13 vs. 15 kg), CD4+ T-cells percentage (13 vs. 24%) and plasma leptin levels (0.8 vs. 3.1ng/l). This was associated with a decline in plasma nitrate (0.8 vs. 0.4 mM/l), plasma nitrite (3.3 vs. 1.3 mM/l), IFN- γ (865 \pm 386 vs. 240 \pm 90 pg/ml), IL-6 (31 \pm 17 vs. 5 \pm 3 pg/ml) and apoptotic index (13 \pm 5 vs. 7 \pm 3) after 6 months of ART (table 11). On the other hand, there was no significant difference in any of the above parameters in children who did not receive ART, except an increase in weight.

Conclusion

The findings of the study imply that HIV associated immune activation and concomitant inflammation can be reduced by ART and this may have long term beneficial effects.

Table 11: Nutritional and immunological markers in HIV-positive children on ART and not on ART at baseline and during follow-up

Parameters	HIV-positive children on ART		HIV-positive children not on ART	
	Baseline	Follow-up (at 6 months of ART)	Baseline	Follow-up (at 6 months of ART)
Weight (kg)	13 ± 1	16 ± 1	17 ± 2	19 ± 2
BMI	14 ± 1	16 ± 1	14 ± 0	15 ± 0
Plasma leptin (ng/ml)	0.9 ± 0.3	3.2 ± 0.9 *	1.8 ± 0.7	2.3 ± 0.4
CD4 cell counts (cells/cu.mm)	650 ± 152	1347 ± 332 *	1395 ± 224	1200 ± 200
IFN-γ (pg/ml)	865 ± 386	240 ± 90 *	312 ± 75	333 ± 93
Apoptotic index	13 ± 5	7 ± 3	7 ± 5	3 ± 2
IL-6 (pg/ml)	31 ± 17	5 ± 3 *	92 ± 6	94 ± 8
Nitrate (mM/L)	0.8 ± 0.2	0.4 ± 0.1 *	0.4 ± 0.1	0.4 ± 0
Nitrite (mM/L)	3.3 ± 0.6	1.3 ± 0.1*	1.33 ± 0.1	1.2 ± 0.1

*p < 0.05 vs. baseline

[Contact person: Dr.Soumya Swaminathan (E-Mail ID: soumyas@trcchennai.in)]

Loss of CD27 expression on CD8+ T lymphocytes in HIV-infected children

Background

Cytotoxic CD8+ T lymphocyte responses play a significant role in controlling viral infections. CD27 is a marker that has been utilized to distinguish memory (CD27+) from effector (CD27-) subsets of CD8+ T-cells.

Aim

- To characterize effector CD8+ T-cells in HIV-infected children

Methods

Seventy eight HIV-infected children (age ranging from 1.5-13 years) were recruited from the HIV-TB clinic of the TRC. CD27 expression on the CD4+ and CD8+ T-cell subsets was analyzed by dual colour flow cytometry. HIV-1 plasma viral load was measured using the fully automated COBAS Amplicor HIV-1 monitor in 39 children. Intracellular IFN- γ production by HIV-1 p24 stimulated PBMC was also determined by flow cytometry.

Results

The mean percentages of CD4+ and CD8+ T-cells were 14.8 and 49.1% respectively. The mean viral load was 430,940 copies per ml of plasma (range 2020–750,000). The percentage of CD27+CD8+ cells was significantly higher than CD27-CD8+ cells (32 ± 1.7 vs. 16 ± 1.2 , $p<0.001$) (Fig.2). Plasma viral load showed a positive correlation with the percentage of CD27-CD8+ T-cells ($r=0.362$, $p<0.05$) (Fig.3) and a negative correlation with percentage of CD27+CD4+ T-cells. CD27-CD8+ T-cells produced higher amounts of IFN- γ than CD27+CD8+ T-cells, in spite of the former being present at lower numbers than the latter.

Conclusion

The lower proportion of CD27-CD8+ cells in this cohort is suggestive of impaired maturation of CD8+ T-cells in chronically infected HIV-positive children, resulting in inefficient control of viral replication. Positive correlation between the proportion of CD27-CD8+ T-cells and plasma viral load indicates that the maturation of CD8+ T-cells is partially dependent upon antigenic stimulation. The effector CD8+ T-cell population was found to be responsible for most of the IFN- γ production.

Fig.2: Percentage CD4 and CD8 T-cell subsets in HIV-infected children

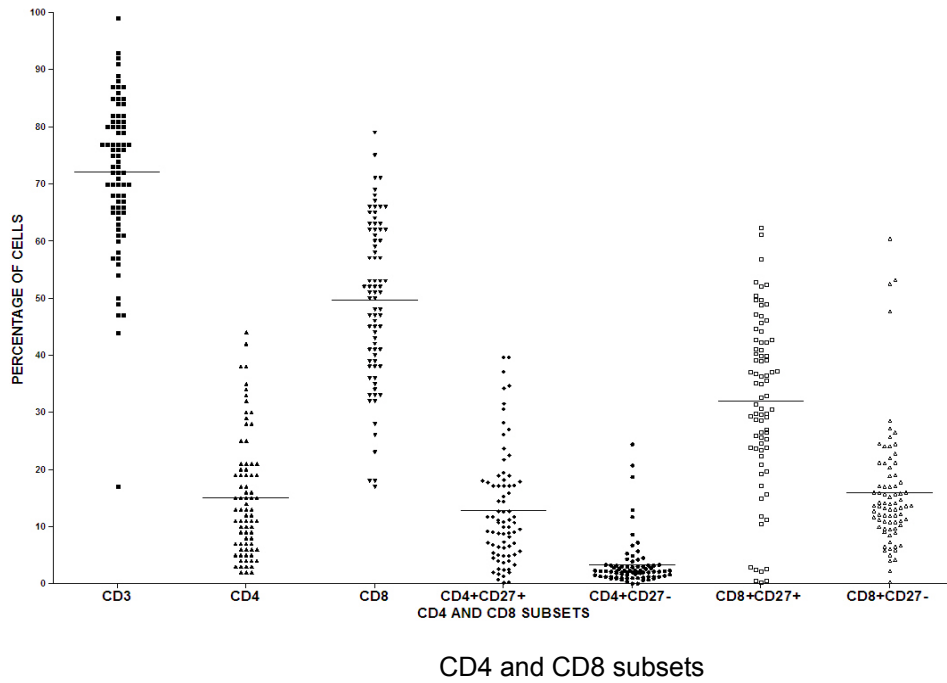
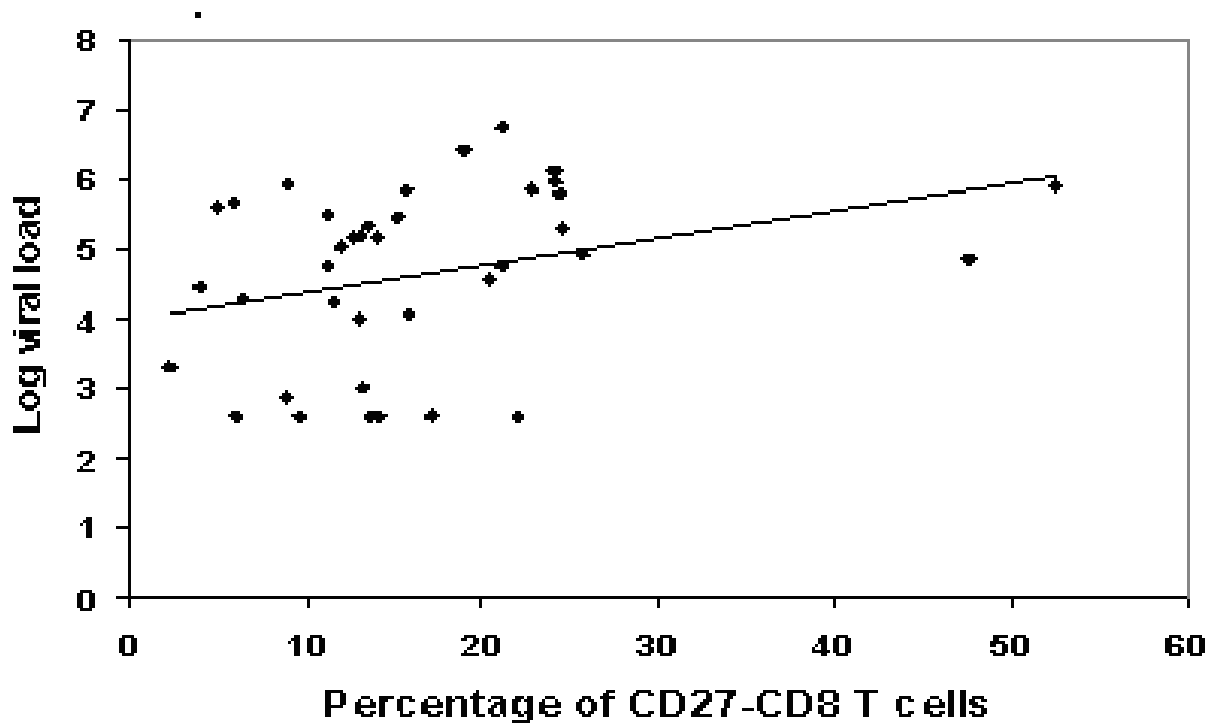


Fig.3: Correlation between CD27-CD8 T-cells and plasma viral load in HIV-infected children



[Contact person: Dr.Soumya Swaminathan (E-Mail ID: soumyas@trchennai.in)]

CD4/CD8 ratio as a surrogate marker for HIV infection in infancy

Background

It is estimated that 25-30% of HIV-infected infants will progress rapidly to AIDS in the first year of life. In the first 18 months of life, serologic tests for HIV infection do not differentiate between exposure and infection, due to maternally acquired antibodies. Virologic tests like DNA- or RNA-PCR are confirmatory but difficult to perform in resource-constrained settings.

Aim

- To evaluate whether CD4 count, CD4% or CD4/CD8 ratio could serve as surrogate markers for HIV infection in infants below 18 months of age

Methods

The study included 273 infants with a mean age of 5.96 (range 0.2-18) months (139 females, 134 males). They were all born to HIV-positive mothers and were referred to the TRC for diagnosis. Informed consent was obtained from the parent. DNA-PCR test was performed using the Roche Amplicor HIV-1 DNA test (version 1.5) qualitative kit and CD4 and CD8 counts determined by two-colour flow cytometry (BD FACS Calibur).

Results

Fifty five infants were DNA-PCR positive while 218 were negative. There were significant differences in mean CD4%, CD4 count, and CD4/CD8 ratio between HIV-infected and uninfected infants (table 12). The mean CD4% and counts of PCR positive infants were significantly lower than those of the PCR negative infants. The CD4/CD8 ratio was <1.0 in all but 6 infected children and in 14 of the 218 uninfected children (sensitivity 89%, specificity 93.5%).

Conclusion

Our findings suggest that the CD4/CD8 ratio may be used as a surrogate marker of HIV infection in an infant born to a HIV-positive woman as CD4/CD8 counting facilities are more widely available than virological assays, in resource – poor settings. This would help in identifying infants for cotrimoxazole prophylaxis and/or ART.

Table 12: CD4+ & CD8+ T-cell subsets (mean \pm SD) in HIV-positive & HIV-negative infants

Patients	CD4%	CD4 cell count (cells/cu.mm)	CD8%	CD8 cell count (cells/cu.mm)	CD4/CD8 ratio
HIV-positive (n=55)	19 \pm 13*	1328 \pm 1209*	48 \pm 18*	3064 \pm 2408*	0.51 \pm 0.45*
HIV-negative (n=218)	43 \pm 12	3188 \pm 1500	22 \pm 8	1663 \pm 896	2.2 \pm 1.2

*P value < 0.01

[Contact person: Dr.Soumya Swaminathan (E-Mail ID: soumyas@trchennai.in)]

Ongoing studies

Antimycobacterial activity of actinomycetes isolated from less explored ecosystems

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

Background

The worldwide problem caused by TB and the lack of new drugs necessitate the search for novel drugs to control MDR-TB. Actinomycetes have long been considered as a source of high value metabolites especially antibiotics.

Aim

- To screen actinomycetes isolated from less explored ecosystems for antimycobacterial activity

Methods

Actinomycete strains isolated from less explored ecosystems like desert, marine, alkaline and forest soil samples were screened for antimycobacterial and antibacterial activity. The culture filtrates of fermented actinomycete strains were tested against *M. tuberculosis* H₃₇Rv, MDR isolate and a drug-sensitive clinical isolate of *M. tuberculosis* by LRP assay. Ethyl acetate extracts of fermentation broth and methanol extracts of mycelium prepared from actinomycete strains were tested for antimycobacterial activity. Antibacterial activity was also studied by cross streak and disc diffusion methods against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*. Ethyl acetate and methanol extracts were tested against the bacterial strains by disc diffusion method.

Results

In LRP assay, out of 53 actinomycetes CFs tested, 17 showed antimycobacterial activity. Three out of 5 ethyl acetate extracts, 8 out of 30 methanol extracts and 1 out of 3 acetone extract showed antimycobacterial activity.

Conclusion

The ecosystems explored are potential sources for antagonistic actinomycetes as 17 out of 53 strains tested could inhibit *M. tuberculosis*.

The study is in progress.

[Contact person: Dr.Vanaja Kumar (E-Mail ID: vanajakumar@trcchennai.in)]

Novel phage based assay for rapid detection of tubercle bacilli in pulmonary specimens for application in field conditions

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 and not require expensive instruments. Phages exhibit high level of host specificity, which make them ideal tools in specific diagnosis.

Aim

- To construct *lacZ* expressing mycobacteriophages and evaluate their role in rapid diagnosis of TB from pulmonary samples for application in field conditions

Methods

Established protocols were used for developing *lacZ* expressing mycobacteriophage constructs (blue phage constructs) from temperature sensitive mutant phAE159 of mycobacteriophage TM4. Standard molecular biology techniques were used for developing the required cosmids and plasmids. Diagnostic efficiency of one of the constructs was further evaluated using known cultures and sputum deposits.

Results

Conditionally replicating mycobacteriophage constructs harboring *lacZ* gene capable of detecting and reporting the viable tubercle bacilli in pulmonary specimens were developed. Using cultures, the sensitivity of detection of the

phage construct was found to be 20 bacilli/ml. A preliminary evaluation of its diagnostic capability was done using 96 sputum samples processed by modified Petroff's method. The assay picked up 36 out of 45 conventional culture positives yielding a sensitivity of 80%. Among 51 culture negatives, 20 were assay negative yielding a specificity of 40% (table 13).

Table 13: Blue phage construct in comparison with conventional culture in diagnosis of TB

		LJ culture		Total
		Positive	Negative	
Blue phage	Positive	36	31	67
	Negative	9	20	29
		45	51	96

All 9 culture positives that were missed by the assay were found to be high grade culture positives with a short turn around time indicating heavy load of viable bacteria in the sample calling for suitable modification of the assay so as to establish an ideal ratio of cells to phage. Among 31 culture negative-assay positives, 4 were smear positive, 4 were from patients within 6 months of treatment, 6 within 1 year of treatment, 3 were follow up cases, 2 were established TB-HIV positive cases and 6 were single time samples from MDR suspects.

Conclusion

The construct shows promise based on the preliminary findings. The sensitivity and specificity of the assay can be greatly improved by optimizing the assay format and evaluating it against liquid based methods using larger number of samples. Such an attempt is fully warranted since the assay has the potential to completely eliminate the use of processing methods and expensive instruments.

[Contact person: Dr.Vanaja Kumar (E-Mail ID: vanajakumar@trchennai.in)]

Nevirapine and efavirenz concentrations in HIV-infected children treated with antiretroviral drugs in India

Background

Antiretroviral drugs for pediatric use were made available by the National AIDS Control Organization (NACO) at the Government ART centres from November, 2006. The most common generic formulation consists of NVP with 3TC and d4T in three different ratios, which are administered to children based on their body weight. Underdosing is a major threat to the long term success of ART, and is of particular concern for children facing a life-time requirement for ART. It is therefore important to ensure that children receive adequate doses of the drugs, and plasma concentrations of ARV drugs are maintained within the therapeutic range.

Aim

- To examine the influence of age, drug dose, type of formulation and nutritional status on blood levels of NVP in HIV-infected children receiving treatment with generic adult or pediatric formulations

Methods

This was a multi-centric study conducted at four sites in three cities. HIV-infected children receiving treatment from the Government ART centres at the Government Rajaji Hospital, Madurai, B.J.Wadia Hospital, Mumbai, Government Hospital of Thoracic Medicine, Tambaram, Chennai and Kilpauk Medical College and Hospital, Chennai, and meeting the study criteria were recruited to the study. On the day of the study, blood samples (2 ml) were collected in heparinised vacutainers prior to drug intake (trough concentration) and at 2 hrs after administration of NVP and other ARV drugs under supervision.

Assessment of nutritional status

Nutritional status was graded and each child was assessed for underweight, wasting and stunting according to the WHO classification of malnutrition using Epi Info version 6.04d. Z scores of height-for-age, weight-for-age and weight-for-height were used to measure stunting, underweight and wasting respectively.

Drug estimations

Plasma concentrations of NVP were determined by validated HPLC assays with UV detection. A total of 80 HIV-infected children have been recruited to the study. The study data is being analysed.

[Contact person: Dr.Geetha Ramachandran (E-Mail ID: geethar@trcchennai.in)]

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

Background

Although it is known that RMP significantly reduces the bioavailability of EFV and NVP, the clinical significance of this reduction is unclear. In an ongoing controlled clinical trial at the Centre, two different ARV regimens along with RMP containing ATT are being evaluated in patients with HIV-1 and TB.

Aim

- To study the trough levels of EFV and NVP while receiving ART (with and without ATT) and correlate with treatment outcome (viral load and CD4 cell count measurements) and NVP/EFV resistance pattern

Methods

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

So far, 64 and 94 patients receiving NVP and EFV respectively have been recruited into this study.

The study is in progress.

[Contact person: Dr.Geetha Ramachandran (E-Mail ID: geethar@trcchennai.in)]