THIORIDAZINE: EVIDENCE FROM IN VIVO, EX VIVO, IN VIVO (MOUSE AND HUMAN) SUPPORTING ITS USE FOR THERAPY OF MDR/XDR TB INFECTIONS.

TARGETTING THE HUMAN MACROPHAGE FOR ENHANCED KILLING OF INTRACELLULAR MDR/XDR Mtb: A NEW CONCEPT FOR THERAPY OF MDR/XDR TB.

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**Mycobacterium tuberculosis**

- Causative agent of Tuberculosis (TB)

- Emergence of **Multi-Drug Resistant strains (MDR-TB)**
  - Resistance to at least isoniazid (INH) + rifampicin (RIF)

- Emergence of **Extensively-Drug Resistance strains (XDR-TB)**

XDR = Multidrug-resistant TB (MDR-TB) plus resistance to (i) any fluoroquinolone, and (ii) at least 1 of 3 injectable second-line drugs: capreomycin, kanamycin, amikacin

WHO; Weekly Epidemiol Record; 2008
The STOP TB Strategy

Components of the STOP TB Strategy

1. Pursue high-quality DOTS expansion and enhancement
   a. Political commitment with increased and sustained financing
   b. Case detection through quality-assured bacteriology
   c. Standardized treatment with supervision and patient support
   d. An effective drug supply and management system
   e. Monitoring and evaluation system, and impact measurement

2. Address TB/HIV, MDR-TB and other challenges
   - Implement collaborative TB/HIV activities
   - Prevent and control multidrug-resistant TB
   - Address prisoners, refugees and other high-risk groups and special situations

3. Contribute to health system strengthening
   - Actively participate in efforts to improve system-wide policy, human resources, financing, management, service delivery, and information systems
   - Share innovations that strengthen systems, including the Practical Approach to Lung Health (PAL)
   - Adapt innovations from other fields

4. Engage all care providers
   - Public-Public, and Public-Private Mix (PPM) approaches
   - International Standards for TB Care (ISTC)

5. Empower people with TB, and communities
   - Advocacy, communication and social mobilization
   - Community participation in TB care
   - Patients’ Charter for Tuberculosis Care

6. Enable and promote research
   - Programme-based operational research
   - Research to develop new diagnostics, drugs and vaccines

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Multi-Drug Resistant *M. tuberculosis*

New approaches - Phenothiazines

Purpose of the study

*In vitro, ex vivo and in vivo* studies

Macrophage model

2 TO 3 BILLION INFECTED WITH Mtb.

10.5 MILLION NEW CASES.

OVER 2 MILLION DEATHS.

511,000 NEW CASES OF MDR TB.

XDR TB ??????
Develop new anti-TB drugs effective against intracellular MDR/XDR-TB:
Phenothiazines

Amaral et al; others
- Mellaril®
- less toxic than CPZ
- Drowsiness (most common)

Schnetzler & Carrel

Charpentier

Guttman & Erhlich

- Serious side-effects (toxicity)
- Gradually replaced by TZ

Wide gamut of *in vitro* antimicrobial activity.

1891 1953 1964 2004-2008
PHENOTHIAZINES

Thioridazine (TZ)

Chlorpromazine (CPZ)

L Amaral, Nov 2010
The antimycobacterial activity of thioridazine derivatives against drug resistant *Mycobacterium tuberculosis*:

*in vitro, ex vivo and in vivo* studies
<table>
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<th>Resistance to:</th>
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</table>

INH, Isoniazid; PZA, pyrazinamid; RIF, rifampicin; SM, streptomycin; EMB, ethambutol.

*Lowest concentration (mg/L) of chlorpromazine or thioridazine that totally inhibited generation of 14CO2.


L Amaral, Nov 2010
Figure 2. The mean effects of chlorpromazine (■) and thioridazine (□) on the generation of $^{14}$CO$_2$ by 20 and 13 strains of *M. tuberculosis*, respectively. Standard deviations are depicted as bars.
HOWEVER, *IN VITRO* CONCENTRATIONS ARE CLINICALLY IRRELEVANT SINCE THE MAXIMUM CONCENTRATION OF THESE COMPOUNDS THAT CAN BE ACHIEVED IN THE PATIENT IS 0.5 mg/L OF PLASMA!
Infection studies in human macrophages

- **Low killing activity** – important for the study of compounds that present intracellular activity against phagocytosed bacteria

**Peripheral blood – human voluntary healthy donors**

**Mononuclear cells**

(monocytes + lymphocytes)

**Blood collection**

**Isolation**

(histopaque)

(1400 rpm x 30 min)

**Adjustment**

(1x10^6 cells/mL)

**Incubation**

(37ºC; 5.5% CO₂)

**Viability assays**

(trypan blue)

**Cell culture**
KILLING ACTIVITY OF HUMAN MACROPHAGES


L Amaral, Nov 2010
ENHANCED KILLING BY CPZ AND TZ

Experimental outline

Screening of TZ derivatives (22)

Toxicity in lymphocytes (Trypan blue exclusion method)

Mutagenicity Assay (Ames test)

Non-toxic and non-mutagenic derivatives

S. aureus / MRSA (model)
MIC and MBC – microdilution method

M. tuberculosis / MDR-TB
MIC and MBC – BACTEC 460™

Infection studies in macrophages (phagocytosis assay)

Toxicity assays (Balb/C) – TZ and derivatives

Infection studies – TZ and derivatives

Studies

In vitro

Ex vivo

In vivo

Minimum Inhibitory Concentration (MIC)
Minimum Bactericidal Concentration (MBC)
Synthesis: Prof. György Hajós (Chemistry Institute of Budapest, Hungary); Chemical manipulation of the parent compound (TZ)

TZ derivatives

- TZ derivatives

TZ
**In Vitro** Growth inhibition of *Mycobacterium tuberculosis* by Thioridazine derivatives.

Compounds MIC (mg/L).

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ENHANCED KILLING OF MYCOBACTERIUM tuberculosis by 0.1 mg/L of DERIVATIVES OF THIORIDAZINE.


L Amaral, Nov 2010
INTRACELLULAR ACTIVITY OF OTHER NON-PHENOTHIAZINES

L Amaral, Nov 2010
Animal model: Balb/C

- Daily inoculations
- Daily weighing

In all the concentrations tested no toxicity was obtained

Concentrations selected for treatment: 100, 400 and 1200 mg TZ/Kg/day
Infection studies with *M. tuberculosis*

Day 0
- I.P. injection

Infection with TB
(1×10⁶ CFU/mL)

Day 30
- Treatment (TZ)

Except the Control group

- NO TZ
- mg TZ/kg/day

Control
- 100
- 400
- 1200

Organs removal (lungs, liver and spleen) – plating – CFU
Mice treated with TZ (equivalent in the human to 1200 mg/Kg/day)

colony forming units (CFU) reduction – lungs

In Vivo Study 2010.
Confirmation of effect of Thioridazine

Treatment after 60 days post infection 32 and 70 mg/Kg/day;
Black bars @ end of 30 days; White bars @ end of 60 days.

Thioridazine in combination with INH, Rifampin and PZA.

**Figure 3.** Effect of combined treatment with standard anti-tuberculosis treatment and thioridazine on lung bacillary load in mice infected with *M. tuberculosis* H37Rv. Animals were treated from day 60 with conventional chemotherapy alone (isoniazid [H], rifampcin [R] and pyrazinamide [Z], gray bars), or in combination with thioridazine 32 mg daily (black bars). In comparison with untreated control mice (white bars), both treatments produced significant reduction of bacilli loads after 30 and 60 days of treatment, being higher and faster in the combined treatment group. Data are expressed as means ± SD, 8 mice per time point, asterisks represent statistical significance (p<0.05). T32 HRZ, first line anti-tuberculosis treatment with adjunctive thioridazine 32 mg/kg; HRZ, first line treatment only; C, controls.

doi:10.1371/journal.pone.0012640.g003
H37Rv Control Infection
(A) Extensive lung consolidation (arrows) is visible in control animals after 120 days of infection by drug-sensitive control Strain H37Rv.

MDR Control Infection
(C) Control mice after 120 days of infection with MDR strain show extensive pneumatic areas (arrow)

H37Rv Infection Plus Thioridazine
(B) In contrast, less pneumonia (arrow) is seen in the lung of Mice treated with Thioridazine 32 mg/kg daily by intragastric cannula.

MDR Infection Plus Thioridazine
(D) In comparison, less lung consolidation (arrow) is seen in the lung of mice infected by the MDR-TB strain and treated daily during two months with 70 mg/kg of thioridazine.
Thioridazine cures XDR TB

Abbate E et al. 2007; Tuberculosis extensamente resistente (XDR TB) en Argentina: aspectos descatables, epidemiologicos, bacteriologicos, terapeuticos y evolutivos. Revista Argentina de Medicina Respiratoria.
ARTICULO ORIGINAL

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Tuberculosis extensamente resistente (XDR-TB) en Argentina: aspectos destacables epidemiológicos, bacteriológicos, terapéuticos y evolutivos

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²Servicio de Micobacterias, Instituto Nacional de Enfermedades Infecciosas Carlos G. Malbrán.
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MECHANISM OF ACTION
Results – *Ex vivo* studies with the other Ca\(^{2+}/K^+\) pump inhibitors

**MRSA**

- Enhancement of the macrophage killing activity
- Reduction of CFU with all the inhibitors tested

In phagocytosed MRSA:

- **Enhancement of the macrophage killing activity**
- **Verapamil**: more active than TZ (higher concentrations)
- **MACROPHAGE MODEL**

**M. tuberculosis**

Enhancement of the macrophage killing activity

- Reduction of CFU

OTHER MECHANISMS OF ACTION

Thioridazine kills dormant M.tb!

and

Inhibits the expression of essential genes!
CONCLUSIONS

• Thioridazine cures MDR/XDR TB!
• However, patients must be monitored for cardiac functions prior to and periodically during therapy. Thioridazine in some patients prolongs the QT interval.
• Nevertheless, it may be used on the basis of compassionate grounds (prognosis is poor).
• Global clinical trials planned for 2011.
• INTERESTED?