Combination of Benznidazole and Nifurtimox plus Posaconazole enhances activity against *Trypanosoma cruzi* in experimental Chagas disease

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**Past** (1909)  
*Chagas disease discovery*  
(1909 – 2009)

**Present** (2009)  
- Improvements in Case Management – Heart Disease  
- Vector control/eradication programmes  
- Description of main clinical manifestations  
- *Trypanosoma cruzi* identification

**Future**  
- New Chemical Entities - Target Product Profile  
  - Old drugs with new Chemotherapy strategies  
  - Specific anti-*T. cruzi* treatment

Neglected populations
Evaluating combination treatment...

Combination with registered compounds (Benznidazole/Nifurtimox)

Aims: (i) improvement of efficacy
      (ii) improvement of safety and tolerability
      (iii) reduction of the dose and duration of the therapeutic regimen
      (iv) Potential impact on resistance development to each individual compounds from the combinations

Starting point:

Evaluation of combination therapy Nifurtimox/Benznidazole
  + Azole compounds
« Reduction in time, costs and risks »

Current available drugs

- Benznidazole and Nifurtimox: nitroheterocyclic drugs

- Parasiticidal activity: conversion of reactive intermediates within the parasite that generate superoxide, which causes oxidative damage to components of the parasite

- Compounds activated within the parasite, probably by a Type 1 Mitochondrial Nitroreductase enzyme

Wilkinson et al. PNAS 2008; 105:5022-7
Paulino et al. Mini Rev Med Chem. 2005
Trypanosoma cruzi
Ergosterol Biosynthesis Pathway

Azole class of compounds: Posaconazole, Ravuconazole

E.G. Hankins et al. / Molecular & Biochemical Parasitology 144 (2005) 68–75

Combination of nitroheterocyclic compounds Benznidazole and Nifurtimox plus Posaconazole

Swiss mice inoculated with 5.0 x 10^3 blood trypomastigotes of T. cruzi Y strain
Treatment initiated 4 days post-infection with confirmed parasitaemia
7 consecutive days with each single drug by gavage

Therapeutic dose

Half of the therapeutic dose

One forth of the therapeutic dose
Mice infected with Y strain of *Trypanosoma cruzi* treated with different doses of Benznidazole, Nifurtimox and Posaconazole

<table>
<thead>
<tr>
<th>Dose</th>
<th>Parasitemia suppression (dose±SD)</th>
<th>Parasitemia reactivation (day)</th>
<th>Parasitemia peak x 10^3 *</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Benznidazole</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mg</td>
<td>1.33±0.52</td>
<td>5</td>
<td>10.0 (17th)</td>
</tr>
<tr>
<td>50 mg</td>
<td>1.83±0.75</td>
<td>1</td>
<td>84.6 (16th)</td>
</tr>
<tr>
<td>25 mg</td>
<td>ND</td>
<td>-</td>
<td>246.6 (8th)</td>
</tr>
<tr>
<td><strong>Nifurtimox</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mg</td>
<td>1.0±0.0</td>
<td>5</td>
<td>8.3 (16th)</td>
</tr>
<tr>
<td>25 mg</td>
<td>ND</td>
<td>-</td>
<td>30.6 (8th)</td>
</tr>
<tr>
<td>12.5 mg</td>
<td>ND</td>
<td>-</td>
<td>396.6 (8th)</td>
</tr>
<tr>
<td><strong>Posaconazole</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ps 20 mg</td>
<td>1.33±0.51</td>
<td>12</td>
<td>11.3 (24th)</td>
</tr>
<tr>
<td>Ps 10 mg</td>
<td>1.5±0.54</td>
<td>11</td>
<td>49.3 (25th)</td>
</tr>
<tr>
<td>Ps 5.0 mg</td>
<td>1.17±0.41</td>
<td>11</td>
<td>318.6 (26th)</td>
</tr>
<tr>
<td><strong>No treated control group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>900.6 (8th)</td>
</tr>
</tbody>
</table>

Maximum parasitemia levels of the Y strain infected mice treated with different doses of Benznidazole, Nifurtimox and Posaconazole

Presented by DNDi at ASTMH 2009
Survival rates of the Y strain infected mice treated with different doses of Benznidazole, Nifurtimox and Posaconazole

**Drug Combination experiments**

**Drug combinations:**

**Benznidazole (Bz) plus Posaconazole (Ps)**
- 25 and 50 mg/kg.day of Bz
- 5 and 10 mg mg/kg/day of Ps

**Nifurtimox (Nfx) plus Posaconazole (Ps)**
- 12.5 and 25 mg/kg.day of Nfx
- 5 and 10 mg mg/kg.day of Ps
## Benznidazole plus Posaconazole treatment

<table>
<thead>
<tr>
<th>Treatment scheme (n=6)</th>
<th>Parasitemia suppression (dose±SD)</th>
<th>Parasitemia reactivation (day)</th>
<th>Parasitemia peak</th>
<th>Survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bz/Ps (50/10mg)</td>
<td>1.4±0.55</td>
<td>10</td>
<td>21.600 (22nd)</td>
<td>100%</td>
</tr>
<tr>
<td>Bz/Ps (50/5mg)</td>
<td>1.33±0.52</td>
<td>10</td>
<td>60.000 (22nd)</td>
<td>100%</td>
</tr>
<tr>
<td>Bz/Ps (25/10mg)</td>
<td>1.67±0.52</td>
<td>10</td>
<td>40.166 (26th)</td>
<td>100%</td>
</tr>
<tr>
<td>Bz/Ps (25/5mg)</td>
<td>1.16±0.41</td>
<td>7</td>
<td>11.133 (27th)</td>
<td>100%</td>
</tr>
<tr>
<td>Bz 100mg</td>
<td>1.33±0.52</td>
<td>5</td>
<td>10.000 (17th)</td>
<td>100%</td>
</tr>
<tr>
<td>Ps 20 mg</td>
<td>1.33±0.51</td>
<td>12</td>
<td>11.333 (24th)</td>
<td>100%</td>
</tr>
</tbody>
</table>

### Parasiteaemia

**Maximum parasitemia – log parasite/0.1 mL of blood**
Nifurtimox plus Posaconazole treatment

<table>
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<tr>
<th>Treatment scheme (n=6)</th>
<th>Parasitemia suppression (dose±SD)</th>
<th>Parasitemia reactivation (day)</th>
<th>Parasitemia peak</th>
<th>Survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nfx /Ps (25/10mg)</td>
<td>1.5±0.55</td>
<td>11</td>
<td>63.333 (27th)</td>
<td>100%</td>
</tr>
<tr>
<td>Nfx /Ps (25/5mg)</td>
<td>1.33±0.52</td>
<td>11</td>
<td>27.333 (27th)</td>
<td>100%</td>
</tr>
<tr>
<td>Nfx /Ps (12.5/10mg)</td>
<td>1.0±0.0</td>
<td>10</td>
<td>68.666 (27th)</td>
<td>84%</td>
</tr>
<tr>
<td>Nfx /Ps (12.5/5mg)</td>
<td>1.16±0.41</td>
<td>9</td>
<td>68.666 (27th)</td>
<td>100%</td>
</tr>
<tr>
<td>Ps 20 mg</td>
<td>1.33±0.51</td>
<td>12</td>
<td>11.333 (24th)</td>
<td>100%</td>
</tr>
<tr>
<td>Nfx 50 mg</td>
<td>1.0±0.0</td>
<td>5</td>
<td>8.333 (16th)</td>
<td>100%</td>
</tr>
</tbody>
</table>

Parasitemia peak
Treatment scheme
Parasitemia suppression (dose±SD)
Parasitemia reactivation (day)
Survival rate (%)

Nifurtimox plus Posaconazole treatment

Parasitaemia

Maximum parasitemia – log parasite/0.1 mL of blood
Conclusions and future directions

✓ The combination of Ps and Bz or Nfx was significantly more efficacious against T. cruzi than the same dose of each drugs alone.

✓ Need for confirmation of findings in experimental models with prolonged exposure and immunosuppression for assessment of cure

✓ Alternative combination regimens for Chagas disease should be further investigated

“A one-shot inexpensive, nontoxic drug to be used in individual cases as well as for preventing Chagas disease transmission is still a vague dream.”

Brener Z: Chemotherapy of Trypanosoma cruzi infection”

Collaborators:

Post-graduated students: Isabel Mayer, Livia Figueiredo, Ivo Caldas, Sergio Caldas

Graduated students: Andre Gravel, Alvaro Neto, Tassiane Martins

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DNDi: F. Alves, B. Bourdin, R. Don, E. Torreele

Presented by DNDi at ASTMH 2009