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

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Past (1909)

New Chemical Entities - Target Product Profile

Old drugs with new Chemotherapy strategies

Specific anti- T. cruzi treatment

Improvements in Case Management – Heart Disease

Vector control/eradication programmes

Description of main clinical manifestations

Trypanosoma cruzi identification

Present (2009)

Future

Neglected populations

Chagas disease discovery

(1909 – 2009)
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 »

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**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 10^1 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

(n=6) Y strain

Therapeutic dose

(n=6) Y strain

Half of the therapeutic dose

(n=6) Y strain

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>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>
</tbody>
</table>

**Benznidazole**

**Nifurtimox**

<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>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>
</tbody>
</table>

**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>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>
</tbody>
</table>

**No treated control group**

<table>
<thead>
<tr>
<th>Control</th>
<th>Parasitemia suppression (dose±SD)</th>
<th>Parasitemia reactivation (day)</th>
<th>Parasitemia peak x 10^3 *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ND</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

**Benznidazole**

**Nifurtimox**

**Posaconazole**
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 kg/day of Ps

**Nifurtimox (Nfx) plus Posaconazole (Ps)**
12.5 and 25 mg/kg.day of Nfx 
+ 5 and 10 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 (22&lt;sup&gt;rd&lt;/sup&gt;)</td>
<td>100%</td>
</tr>
<tr>
<td>Bz/Ps (50/5mg)</td>
<td>1.33±0.52</td>
<td>10</td>
<td>60.000 (22&lt;sup&gt;nd&lt;/sup&gt;)</td>
<td>100%</td>
</tr>
<tr>
<td>Bz/Ps (25/10mg)</td>
<td>1.67±0.52</td>
<td>10</td>
<td>40.166 (26&lt;sup&gt;th&lt;/sup&gt;)</td>
<td>100%</td>
</tr>
<tr>
<td>Bz/Ps (25/5mg)</td>
<td>1.16±0.41</td>
<td>7</td>
<td>11.133 (27&lt;sup&gt;th&lt;/sup&gt;)</td>
<td>100%</td>
</tr>
<tr>
<td>Bz 100mg</td>
<td>1.33±0.52</td>
<td>5</td>
<td>10.000 (17&lt;sup&gt;th&lt;/sup&gt;)</td>
<td>100%</td>
</tr>
<tr>
<td>Ps 20 mg</td>
<td>1.33±0.51</td>
<td>12</td>
<td>11.333 (24&lt;sup&gt;th&lt;/sup&gt;)</td>
<td>100%</td>
</tr>
</tbody>
</table>

### Parasitaemia

Maximum parasitemia = log parasite/0.1 mL of blood

**Presented by DNDi at ASTMH 2009**
Parasitemia peak
Treatment scheme (n=6)  Parasitemia suppression (dose±SD)  Parasitemia reactivation (day)  Parasitemia peak  Survival rate (%)
Nfx /Ps (25/10mg)  1.5±0.55  11  63.333 (27th)  100%
Nfx /Ps (25/5mg)  1.33±0.52  11  27.333 (27th)  100%
Nfx /Ps (12.5/10mg)  1.0±0.0  10  68.666 (27th)  84%
Nfx /Ps (12.5/5mg)  1.16±0.41  9  68.666 (27th)  100%
Ps 20 mg  1.33±0.51  12  11.333 (24th)  100%
Nfx 50 mg  1.0±0.0  5  8.333 (16th)  100%

Parasitemia
Nifurtimox plus Posaconazole treatment

Maximum parasitemia – log parasite/0.1 mL of blood
Drug combination experiments

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 drug 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”

Advances in Pharmacology and Chemotherapy,

Prof. Z Brener (1928-2002)