New insights into the carcinogenesis induced by Schistosomes

Mónica Botelho
29/08/2015
**Schistosomes: Life cycle**

- Eggs hatch releasing miracidia in the water
- Miracidia penetrate the snail tissue
- Micronized tissue (successive generations)
- Cercariae released by snail into water and free-swimming
- Cercariae penetrate the skin
- Circulation
- Migrate to portal blood in liver and mature into schistosomulae
- In feces
- In urine
- S. mansoni
- S. japonicum
- S. haematobium
- Paired adult worms migrate to:
  - Intravascular veins of bowel/rectum (laying eggs that circulate to the liver and shed in stools)
  - Vascular plexus of bladder

**Urogenital Schistosomiasis**

- Eggs provoke granulomatous inflammation that leads to small fibrotic nodules known as “sandy patches”, ulceration, and pseudopolyposis of the vesical and ureteral walls. Urinary granulomas.
- Dysuria, pollakisuria, proteinuria and HEMATURIA
- Bacterial superinfection
- Obstructive uropathy. Hydronephrosis

Sources:
- CDC [http://www.dpd.cdc.gov/DPDX](http://www.dpd.cdc.gov/DPDX)
- PJ Hotez et al., Lancet 2010
- Gryseels et al 2006
- Orihel and Ash
Top 10 World’s deadliest animals

If you’re thinking about sharks, snakes and lions...think again!

200,000 people killed per year
Mosquito Week

The Deadliest Animal in the World
By Bill Gates
| April 25, 2014
New Diseases
To improve prospects for curbing six newly targeted diseases—ascaris, trichuris, hookworm, schistosomiasis, Buruli ulcer, and Chagas disease—we are investing in research to better understand their transmission patterns and what tools or interventions are needed to fight them.
## The most neglected schistosome among schistosomes

### Table 1. Number of citations in PubMed over the last five years, 2008–2012.

<table>
<thead>
<tr>
<th>Parasite Species</th>
<th>Approximate Number of Human Cases</th>
<th>Number of PubMed Citations over the Last Five Years&lt;sup&gt;b&lt;/sup&gt;</th>
<th>PubMed Citations per Millions of Human Cases</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Schistosoma japonicum</em></td>
<td>1 million</td>
<td>644</td>
<td>644</td>
<td>Steinmann et al. 2006 [1]</td>
</tr>
<tr>
<td><em>Schistosoma mansoni</em></td>
<td>54 million&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,371</td>
<td>25</td>
<td>Van der Werf et al. 2003 [3]</td>
</tr>
<tr>
<td><em>Schistosoma haematobium</em></td>
<td>112 million&lt;sup&gt;a&lt;/sup&gt;</td>
<td>342</td>
<td>3</td>
<td>Van der Werf et al. 2003 [3]</td>
</tr>
</tbody>
</table>

<sup>a</sup> Sub-Saharan Africa only  
<sup>b</sup> Search conducted on July 14, 2012

1. Group 1 carcinogen responsible for a unique squamous cell carcinoma of the bladder

2. Female Genital Schistosomiasis (FGS) – Infertility?

3. FGS: 3 – 4 times increased risk in acquiring HIV infection
The neglected schistosome

- Absence of available animal models of urogenital schistosomiasis

- Absence of (1) in vitro culture methodologies for developmental stages and (2) Functional Genomic toolkit to address basic biological questions

- In 2012 Schistosoma haematobium got into the postgenomic era with S. mansoni and S. japonicum (in 2009)
In vitro culture of *Schistosoma haematobium* developmental stages

Eggs isolated from liver of infected hamsters

Eggs isolated from intestine of infected hamsters

Rinaldi et al., *PLoS NTDs* 2011
In vitro culture of *Schistosoma haematobium* developmental stages

Adults obtained by portal perfusion from infected hamsters

Cercariae obtained by shedding infected *Bulinus truncatus* snails

Schistosomules obtained by mechanical transformation of cercariae

Michael Hsieh, MD, PhD
Stirewalt Endowed Director
Principal Investigator of Schistosomiasis Resource Center at BRI
http://www.afbr-bri.com/schistosomiasis/
mhsieh@afbr-bri.com
Animal models of Schistosomiasis associated bladder cancer

Urothelial dysplasia and inflammation induced by *Schistosoma haematobium* total antigen instillation in mice normal urothelium

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ª ICBAS–Institute of Biomedical Sciences Abel Salazar, Department of Cellular Biology and Immunology, Porto University, Porto, Portugal
ª IPO–Portuguese Institute of Oncology, Department of Pathology, Porto, Portugal
ª FMUP–Faculty of Medicine of Porto University, Porto, Portugal

Received 19 August 2009; received in revised form 25 September 2009; accepted 29 September 2009

*Urologic Oncology* 29 (2011) 809 – 814
Carcinogenic potential of S. haematobium eggs

Tumour-like phenotypes in urothelial cells after exposure to antigens from eggs of Schistosoma haematobium: An oestrogen–DNA adducts mediated pathway?

Mónica C. Botelho a,b,*, Nuno Vale c, Maria João Gouveia c, Gabriel Rinaldi d,e, Julio Santos f, Lucio L. Santos g, Paula Gomes c, Paul J. Brindley d, José Manuel Correia da Costa a,b

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c CIQUP, Chemistry and Biochemistry Department, Faculty of Sciences, University of Porto, Porto, Portugal
d Department of Microbiology, Immunology and Tropical Medicine, Research Center for Neglected Diseases of Poverty, School of Medicine & Health Sciences, George Washington University Washington, DC 20037, USA
e Departamento de Genética, Facultad de Medicina, Universidad de la República, (UDELAR), Montevideo 11800, Uruguay
f Clínica da Sagrada Esperança, Avenida Martala Mohamed-ilha de Luanda, Angola
g Experimental Therapeutics and Pathology Research Group, Portuguese Institute of Oncology, Porto, Portugal

International Journal for Parasitology 43 (2013) 17–26

→ Normal urothelial cells (HCV 29)

→ Liquid Chromatography Diode Array Detection Electron Spray Ionisation Mass Spectrometry (LC/UV-DAD/ESI-MS) – investigation of oxysterols (oxidized derivatives of cholesterol)

Cell proliferation
Apoptosis
Oxidative stress
Genotoxicity
The Hallmarks of Cancer

• Proliferation
• Apoptosis
• Migration
• Invasion
• Metastasis
• Angiogenesis

• Metabolism
• Immunity
• Genome instability
• Inflammation
Soluble eggs antigens induced tumor-like phenotype in urothelial cells

Cell proliferation

Genotoxicity

Apoptosis

Oxidative stress

Transferase-mediated deoxyuridine triphosphate nick end-labelling (TUNEL)
MS identified molecules extracted from *S. haematobium* CATECHOL-OESTROGENS (oxidative metabolites derived from estrogens)

- m/z 716
- m/z 803
- m/z 813
- m/z 817

Botelho et al., IJP, 2013
Pathway for Schistosomiasis Bladder Cancer

Catechol

Quinone

CYP1B1

4-OHE1(E2)

E1(E2)-3,4-Q

DNA

Depurinating Adducts

4-OHE1(E2)-1-N7Gua

4-OHE1(E2)-1-N3Ade

E1: R, =O
E2: R, -OH

Bladder carcinoma with squamous differentiation

Cancer ← Mutations ← Error-prone base excision repair ← DNA with apurinic sites

Botelho et al, 2015
Thank you for your attention
Infertility-associated schistosomiasis

The role of estrogens and estrogen receptor signaling pathways in cancer and infertility: the case of schistosomes

Mónica C. Botelho¹,², Helena Alves¹, Alberto Barros³,⁴, Gabriel Rinaldi⁵, Paul J. Brindley⁶, and Mário Sousa⁶

• Homonal imbalance caused by estrogen-like molecules produced by schistosomes
Schistosomes estrogen-like molecules and down-regulation of estrogen receptor

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>E2 Range</th>
<th>Testosterone</th>
<th>LH Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>4</td>
<td>62,8</td>
<td>0-22</td>
<td>&lt;15,0</td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>30,8</td>
<td>0-25</td>
<td>77,5</td>
</tr>
<tr>
<td>Male</td>
<td>14</td>
<td>79,8</td>
<td>0-25</td>
<td>363</td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>45,7</td>
<td>0-25</td>
<td>724</td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>31,9</td>
<td>0-25</td>
<td>535</td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
<td>68,3</td>
<td>&lt;56,0</td>
<td>982</td>
</tr>
</tbody>
</table>

Gene Expression

![Gene Expression Chart]

Botelho et al. Exp Parasitol 2010
Infertility-associated Schistosomiasis haematobia in women

Urinary Estrogen Metabolites and Self-Reported Infertility in Women Infected with *Schistosoma haematobium*

Júlio Santos¹, Maria João Gouveia², Nuno Vale², Maria de Lurdes Delgado³, Ana Gonçalves⁴, José M. Teixeira da Silva⁴, Cristiano Oliveira⁴, Pedro Xavier⁴, Paula Gomes², Lúcio L. Santos¹,⁵, Carlos Lopes¹,⁶, Alberto Barros⁴,⁷, Gabriel Rinaldi⁸,⁹, Paul J. Brindley⁸, José M. Correia da Costa³,¹⁰, Mário Sousa¹¹, Mónica C. Botelho³,¹⁰*

Plos One 9 (2014) e96774

<table>
<thead>
<tr>
<th></th>
<th>E + (n = 25)</th>
<th>E - (n = 21)</th>
<th>OR</th>
<th>95% CI</th>
<th><em>P</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertile women (ages)</td>
<td>2 (29, 63)</td>
<td>6 (28–94)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2 (ages)</td>
<td>15 (19–41)</td>
<td>2 (21–34)</td>
<td>4.33</td>
<td>1.13–16.70</td>
<td>0.03</td>
</tr>
<tr>
<td>Group 3 (ages)</td>
<td>9 (18–20)</td>
<td>1 (21)</td>
<td>2.67</td>
<td>0.60–11.80</td>
<td>n.a.</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>≤12 years</th>
<th>8</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>25</td>
<td>21</td>
</tr>
</tbody>
</table>

Women unable to become pregnant after one year of trial (Self-reported primary infertility - Group 2) and those who had borne fewer children than desired (Self-reported secondary infertility - Group 3).

OR, odds ratio; CI, confidence interval.
Schistosoma mansoni infection impairs reproduction in mice

Reding C¹, Reding A¹, Lopes G², Sousa M³, Gartner F⁴, Alves H⁵, Richter J⁶, Oliveira PA⁷, Botelho MC⁵

Unpublished results

• Mating
• Gestational period
• Synchronization
• Number pups
Infertility associated Schistosomiasis mansoni

<table>
<thead>
<tr>
<th>Animals</th>
<th>Gestational length (days)</th>
<th>Synchronicity (days)</th>
<th>Number of pups</th>
</tr>
</thead>
<tbody>
<tr>
<td>2FCx1MC</td>
<td>25</td>
<td>0-1</td>
<td>15.1</td>
</tr>
<tr>
<td>2FCX1MI</td>
<td>25.6</td>
<td>0-2</td>
<td>14.5</td>
</tr>
<tr>
<td>2FIX1MC</td>
<td>22.8</td>
<td>1-6</td>
<td>13.8</td>
</tr>
<tr>
<td>2FIX1MI</td>
<td>21.8</td>
<td>3-8</td>
<td>11.9</td>
</tr>
</tbody>
</table>

**Control**

- **Ovary**
- **Tube**
- **Testes**

**S. mansoni**

- **Ovary**
- **Tube**
- **Testes**
GnRH → LH, FSH → Follicle maturation → LH surge → Ovulation → Estrogen Receptor (pituitary) → E2 → Ovocyte with follicular cells

A – Polar body
B – Zona pellucida
Mechanism for Schistosomiasis Female Infertility

GnRH

LH
FSH

Follicle maturation

E2

Ovulation

LH surge

Estrogen Receptor (pituitary)

Ovocite with follicular cells

A – Polar body
B – Zona pellucida
Conclusions

1. It is feasible to culture *in vitro* developmental stages of *S. haematobium*

2. Soluble extracts from *S. haematobium* eggs induced carcinogenesis of the bladder in animal models

3. Soluble extracts from *S. haematobium* eggs induced tumor-like phenotype in urothelial cells

4. Novel catechol-oestrogen molecules derived from the eggs could be involved in the carcinogenesis process of the bladder
Future Perspectives

1. Functional genomics, such as RNAi to address biological relevant questions related to *S. haematobium* and its carcinogenic potential (e.g. The draft genome of *S. haematobium* encodes a homolog of estradiol 17β dehydrogenase, also known as 17β hydroxysteroid dehydrogenase or 17β HSD, which has a known role in the synthesis of estradiol and testosterone.)

2. Synthesize and/or purify and/or isolate reactive catechol-estrogens.

3. Evaluate impact of catechol estrogens on urothelial cells *in vitro*, at the phenotypic and gene expression levels.

4. Evaluate impact of catechol estrogens in an informative mouse model.

5. Investigate schistosome catechol estrogen–DNA adducts in informative human cases from a schistosomiasis haematobia endemic regions. (Potential for Biomarkers screening)
1. *Schistosoma haematobium*: the neglected schistosome
   • Facts of figures

2. Development of functional tools for schistosomes
   • Culture of developmental stages of *Schistosoma haematobium*
   • Animal models of *S. haematobium* induced bladder cancer

3. Carcinogenic potential of *S. haematobium* eggs
   • Effect of egg extract on urothelial cells
   • Catechol-estrogens isolated from *Schistosoma haematobium*
The Hallmarks of Cancer

• Proliferation
• Apoptosis
• Migration
• Invasion
• Metastasis
• Angiogenesis
Two different complementary pathways probably contribute to estrogen imbalance leading to:

• Initiation and promotion of cancer progression