Estrogen metabolism to investigate carcinogenicity of *Schistosoma haematobium*

Mónica Botelho
UPSDNT
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1. Schistosoma haematobium: the neglected schistosome

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
Schistosomes: Life cycle

1. Eggs hatch releasing miracidia
2. Miracidia penetrate snail tissue
3. Measida penetrate snail tissue
4. Sporocyst in snail (successive generations)
5. Cercariae released by snail into water and free-swimming
6. Cercariae penetrate skin
7. Cercariae lose tails during penetration and become schistosomulae
8. Circulation
9. Migrate to portal blood in liver and mature into adults
10. Paired adult worms migrate to:
    a. Mesenteric vessels of bowel/rectum (laying eggs that circulate to the liver and shed in stools)
    b. Venous 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

PJ Hotez et al., *Lancet* 2010

Gryseels et al 2006

Orihel and Ash
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>Schistosoma japonicum</td>
<td>1 million</td>
<td>644</td>
<td>644</td>
<td>Steinmann et al. 2006 [1]</td>
</tr>
<tr>
<td>Schistosoma mansoni</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>Schistosoma haematobium</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

PJ Brindley and PJ Hotez, PLoS NTDs 2013

PJ Hotez et al., PLoS NTDs 2013
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)

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The *Schistosoma japonicum* genome reveals features of host–parasite interplay

The genome of the blood fluke *Schistosoma mansoni*

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Whole-genome sequence of *Schistosoma haematobium*

Neil D Young¹,¹¹, Aaron R Jex¹,¹¹, Bo Li²,²¹, Shiping Liu², Lifeng Yang², Zijun Xiong², Yingrui Li², Cinzia Cantacessi¹, Ross S Hall¹, Xun Xu², Fangyuan Chen², Xuan Wu², Adhemar Zerlotini³, Guilherme Oliveira³, Andreas Hoffmann¹,⁴, Guojie Zhang², Xiaodong Fang², Yi Kang², Bronwyn E Campbell¹, Alex Loukas⁵, Shoba Ranganathan⁶, David Rollinson⁸, Gabriel Rinaldi⁸,⁹, Paul J Brindley¹⁰, Huanming Yang², Jun Wang², Jian Wang² & Robin Gasser¹
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

Rinaldi et al., *PLoS NTDs* 2011
Animal models of urogenital Schistosomiasis

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

Mónica C. Botelho, M.Sc.\textsuperscript{a,b,*}, Paula A. Oliveira, Ph.D.\textsuperscript{c}, Carlos Lopes, Ph.D.\textsuperscript{d,e}, José M. Correia da Costa, Ph.D.\textsuperscript{a}, José C. Machado, Ph.D.\textsuperscript{b,f}

\textsuperscript{a} CIBP—Centre for Parasite Immunology and Biology, National Institute of Health, Porto, Portugal
\textsuperscript{b} IPATIMUP—Institute of Pathology and Molecular Immunology of Porto University, Porto, Portugal
\textsuperscript{c} CECAV—Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
\textsuperscript{d} ICBA—Institute of Biomedical Sciences Abel Salazar, Department of Cellular Biology and Immunology, Porto University, Porto, Portugal
\textsuperscript{e} IPO—Portuguese Institute of Oncology, Department of Pathology, Porto, Portugal
\textsuperscript{f} FMUP—Faculty of Medicine of Porto University, Porto, Portugal

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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\(^a,b,\ast\), 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\(^a,b\)

\(^a\) Center for the Study of Animal Science, ICETA, University of Porto, Portugal
\(^b\) INSA, National Institute of Health, Rue Alexandre Herculano, 321, 4000-055 Porto, Portugal
\(^c\) 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 Marta Mohamed-Illha de Luanda, Angola
\(^g\) Experimental Therapeutics and Pathology Research Group, Portuguese Institute of Oncology, Porto, Portugal

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\[\rightarrow\] **Normal urothelial cells (HCV 29)**

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

\[\rightarrow\] **Cell proliferation Apoptosis Oxidative stress Genotoxicity**
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
Pathways for Estrogen Carcinogenesis

Two different complementary pathways together probably contribute to the carcinogenicity of estrogen and to
- Initiation
- Promotion
- Progression


Unbalanced metabolism of endogenous estrogens in the etiology and prevention of human cancer

Ercole L. Cavalieri,¹,²,⁺, Eleanor G. Rogan,¹,²

¹ Epilect Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE 68198-6805, United States
² Department of Environmental, Agricultural and Occupational Health, College of Public Health, University of Nebraska Medical Center, Omaha, NE 68198-6805, United States

In relation to cancer initiation, estrogens should be considered as other chemicals, namely, their physico-chemical and biochemical properties lead them to follow the principles of chemical carcinogenesis elucidated by the Millers [6,7], rather than considering them as hormones. Substantial evidence supports a genotoxicity paradigm for the initiation of cancer by endogenous estrogens. Specific oxidative metabolites of estrogens can react
Major metabolic pathways in cancer initiation by estrogens

1. Estrone/Estradiol [E₁(E₂)]
   - E₁: R₂ = O
   - E₂: R₁ = -OH

2. CYP1B1
   - Converts to 4-OHE₁(E₂)

3. CYP150 or peroxidases
   - Converts to E₁(E₂)-3,4-Q

4. DNA
   - Depurinating Adducts
     - 4-OHE₁(E₂)-1-N7Gua
     - 4-OHE₁(E₂)-1-N3Ade

5. Bladder carcinoma with squamous differentiation

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

Adapted from Cavalieri, 2011
Zahid, 2013
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 region of Angola. (Potential for Biomarkers screening)