A natural microbicide (BP36) against HIV-1

4th Biennial Conference

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Microbicide as a potential solution to HIV transmission

- Sub-Saharan Africa burdened by HIV infection, Sexually active women contribute to > 50% infections

- A “microbicide” is a product that will prevent sexual transmission of HIV and potentially other STIs, and is likely to be applied topically to the vagina as a gel, cream, film, suppository, or vaginal ring

  - Alternative means for women to protect and control sexual transmission of HIV
  - Protects at main portal of transmission
<table>
<thead>
<tr>
<th>Candidate name</th>
<th>Mode of action</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonoxynol-9 (N9)</td>
<td>Surfactant</td>
<td>Risk of acquiring HIV was increased with frequent use of product. Product shown to be harmful.</td>
</tr>
<tr>
<td>SAVVY®</td>
<td>Surfactant</td>
<td>Product did not produce a meaningful result owing to lower than expected HIV incidence in the study population.</td>
</tr>
<tr>
<td>Carraguard®</td>
<td>Non specific blockers (Electrostatic interference with virus)</td>
<td>Unsuccessful in demonstrating efficacy. Risk of acquiring HIV not reduced.</td>
</tr>
<tr>
<td>cellulose sulphate</td>
<td>Non specific blockers (Electrostatic interference with virus)</td>
<td>Unsuccessful in demonstrating efficacy and product may be harmful.</td>
</tr>
<tr>
<td>BufferGel™</td>
<td>Acidifying agent (Maintaining natural flora of vagina)</td>
<td>Unsuccessful in demonstrating efficacy.</td>
</tr>
<tr>
<td>0.5% PRO 2000</td>
<td>Non specific blockers (Electrostatic interference with virus)</td>
<td>Demonstrated efficacy but short of statistical significant levels.</td>
</tr>
<tr>
<td>Tenofovir</td>
<td>RT inhibitor</td>
<td><strong>Caprisa 004 study: reduced women’s risk of infection by 39%</strong>&lt;br&gt;<strong>Voice MTN 003: Gel was ineffective in reducing risk of infection</strong></td>
</tr>
</tbody>
</table>
An ideal microbicide

- Physical barrier, lubrication
- Maintenance of normal microflora
- Effective against Other STIs

- Non inflammatory
- Affordable
- Ease of use, acceptability-compliance

Multiple mode of action e.g. entry, fusion, RT inhibitors

- Inhibition of HIV uptake by dendritic cells (e.g. anti-DC-SIGN)
- Inhibition of reverse transcriptase
- Fusion/absorption inhibition (e.g. polyanions, co-receptor antagonists)
Traditional Medicines and relevance to HIV

- South Africa has a long tradition of medicinal use of indigenous plants
- >200 000 Traditional Health Practitioners (THP) active throughout country, ~ 20 000 university trained MD
- 70% of population visits a THP
- 350 commonly used medical plants
- Recently significant increase in HIV infected patients visiting THPs
- Scientific research based on TMs has significant potential to lead to new treatments for HIV
  - 25% of prescription medicines are plant derived

70% of South Africans consult a THP

52% of all drugs has some relation to natural compounds
Background: Traditional use to BP36

- Information on traditional use of Eastern Cape indigenous plant species received during 2003

- Benefit Sharing Agreement signed in 2006 between knowledge holder and CSIR.

- Plant originally used to treat “skin diseases or ailments, womb problems and blood related diseases, arthritis, diabetes, high blood pressure, TB, cancers, eye and ear infection”.

- More recently used to treat HIV infected patients

- Method of Preparation
  - Leaves, stems dried and ground
  - Boiling water added to powder
  - Drink as strong tea
Production of active ingredient

- Production in Clinical and Botanical Supplies Unit

1. Ground plants
2. Plants and water
3. Boil in water
4. Filter and spray dry
5. Decolouration
6. Precipitation
Chemical characterization

- Hydrolysis, derivatization process followed by GC analysis

<table>
<thead>
<tr>
<th>Monosaccharide</th>
<th>% of total carbohydrate present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinose</td>
<td>11.5</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>5.5</td>
</tr>
<tr>
<td>Xylose</td>
<td>1.9</td>
</tr>
<tr>
<td>Mannose</td>
<td>4</td>
</tr>
<tr>
<td>Galactose</td>
<td>10.5</td>
</tr>
<tr>
<td>Glucose</td>
<td>26</td>
</tr>
<tr>
<td>Glucuronic acid</td>
<td>4.3</td>
</tr>
<tr>
<td>Galacturonic acid</td>
<td>36.3</td>
</tr>
</tbody>
</table>

Pectin like molecule, high molecular weight
Anti HIV efficacy

- Testing of Active: HIV-1 pseudovirus inhibition assay
- Completed assaying against 11 subtype C, 3 subtype A and 3 subtype B strains

**IC$_{50}$ range against HIV-1 subtype C pseudoviruses**

- **BP36 active**: 0.1 – 7.9µg/ml
- **T20**: 0.1 – 7.5µg/ml
- **Tenofovir**: 0.2 – 1.2µg/ml
Cytotoxicity towards non infected cells

- Compound CC<sub>50</sub>: 1477 µg/ml
- Etoposide CC<sub>50</sub>: 54.37 µg/ml
Mode of action – stage of viral cycle compound inhibits

Time of Addition against ZM53

% Inhibition

Time of drug addition (hours post infection)

- Compound
- T20
- Maraviroc
- Tenofovir
- Nevirapine
- Raltegravir
- ZM53 VC
Active ingredient loses its ability to inhibit infection after viral attachment suggesting that it acts as an attachment inhibitor.
Formulation studies – gels

- Work done by CSIR materials scientists
- Formulation of active into hydroxyethyl cellulose (HEC) gel and maintaining rheological properties
- Investigated rheometry of 3 different concentrations of active, 5, 30, 60mg/mL gel
- 60mg/mL active combined with 0.1% or less HEC - gel much higher viscosity profile
- HEC concentration cannot be dropped further as gel-like properties will not be retained in the vagina without leakage occurring.
**Formulation studies – Caplet**

- Work done by Wits University, Drug Delivery Platform
- Intra vaginal composite polymeric drug delivery system
- Target – slow release of actives over 30-60 days through insertion of a caplet in posterior fornix of vagina
- BP36 and AZT microsphere encapsulation prepared with extended release
Production of BP36: Cultivation trials

- Work done by CSIR Enterprise Creation for Development
- Obtained regulatory approval for the collection of seeds of the indigenous plant species (BP36)
- Developed methods for germination of the seed of BP36
- Seedlings have been transplanted to establish a mother plantation
  - Ensures commercial scale cultivation can be undertaken without disturbance of wild populations
Way ahead

• Preclinical Screening
  • Efficacy against other viral infections e.g. herpes simplex virus type 2 (HSV-2), human papillomavirus (HPV)
  
  • Additional Efficacy in PBMC assay

• Toxicity profiles
  • in vitro
  • Lactobacilli
  • Inflammatory

• Drug release profiles and irritancy
  • pH stability
  • In vivo (pig vagina study)
Thank you

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