Ciclopiroxolamine: A Vaginal Product with Microbicide Potential

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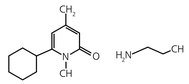
Introduction

To accelerate the development of microbicides in India, where HIV and other sexually transmitted infections (STIs) are a serious problem, more than 2,200 topical products marketed in that country were reviewed for potential anti-HIV and cytotoxic activity, and 60 pharmaceutical ingredients or formulations were tested more extensively. Those with significant HIV-inhibitory activity were evaluated for activity against gonococcus, chlamydia, lactobacillus, and sperm. The products with the best results were evaluated for safety in the rabbit vaginal irritation assay and condom compatibility test. Based on these tests, one of the most promising compounds is ciclopiroxolamine (CO). In addition to an extensive literature review, PATH performed a number of tests through its collaborators to extend the information regarding the activity and safety of CO.



Ciclopiroxolamine (6-cyclohexyl-1-hydroxy-4-methyl-2-[1H]-pyridone ethanolamine salt) (Mr: 268.35 Da; CAS no. 41621-49-2) is marketed worldwide, including in the United States and Europe, as a topical antifungal agent. It is a white to pale-yellow crystalline powder with a melting point of 144°C. It is slightly soluble in water but very soluble in alcohol and dichloromethane. The purchase cost is approximately $600/kg ($0.03/vaginal dose).

This active ingredient is found in at least 14 vaginal antifungal products marketed in India, Italy, Spain, Switzerland, Turkey, and other countries. The dosage forms include creams, ovules, foams, powders, solutions, and washes which usually contain 1% CO.

Efficacy

Table 1. Inhibition of Non-STI-Causing Bacteria and Fungi

Organisms	MIC, µ/mL
Yeasts, dermatophytes and fungi	0.9–31.3 [1, 2]
Candida albicans, Candida glabrata and other *Candida* species	0.9–3.9 [1, 2]
All gram-positive and gram-negative bacteria tested *(Staphylococcus, Streptococcus, Corynebacterium, Listeria, Erysiphelothrix, Bacillus, Sarcina, Pasteurella, Salmonella, Shigella, Escherichia, Aerobacter, Paracolobactrum, Klebsiella, Proteus, Pseudomonas, and Mycoplasma)*	0.2–125 [2-5]
Trichomonas vaginalis	50–100 [1, 4]
Chlamydia trachomatis and *C. psittaci*	10 [4, 6]

In addition, CO:

- Is an excellent anti-inflammatory agent. [3, 7-16]
- Is absorbed into the skin and vaginal wall, but only ~2%–6% reaches systemic circulation. [4, 5, 11-13]
- Has several mechanisms of antifungal action which show:
  - No effect on sterol synthesis. [3]
  - Transmembrane transport of substrates blocked for macromolecular synthesis. [14]
  - Metal-dependent enzymes inhibited. [3]
  - Binding to host cells prevented. [15]
- Does not cause *Candida albicans* to develop resistance even with long-term treatment. [16]

Table 2. Inhibition of HIV*

Assay	IC_{50} µ/mL
Binding inhibition assay (cells, virus + cpd mixed; no wash)	1.46 (IRF); 6.9 (BaL); 45.5(IIIB); Selectivity index: >100
Cell-effect assay (cells + cpd; wash; add virus)	0.96
Virucidal assay (virus + cpd; wash or high dilution; add cells)	No effect (lab 1); 2-log reduction at 0.09%; 2.7-log reduction at 0.3% (lab 2)
Cell-to-cell transmission assay	97.4

* Studies performed by Robin Shattock, Department of Infectious Diseases, St. George's Hospital Medical School, London, United Kingdom, and Gustavo F. Doncel, Contraceptive Research and Development Program (CONRAD), Norfolk, Virginia.

General comments on HIV inhibition:

- Several possible mechanisms of action:
  - Affect host cell to prevent viral fusion and/or entry.
  - Post-entry effect: anti-retroviral activity.
  - Potent inhibitor of deoxyhypusyl dehydroxylase; cellular hypusine needed for functional HIV-Rev protein, essential for HIV replication and HIV-directed protein synthesis. [17]
- Inhibits host cell enzyme and not a viral enzyme or nucleic acid so that HIV escape mutations should not occur.

Table 3. Inhibition of other STI-Causing Microbes

Organism	Test Conditions and Results	Researchers
Herpes simplex virus type 2 (HSV 2)	IC_{50} = 152 µg/mL (cells + CO for 1 hour; add virus for 1 hour; wash; plaque assay); 40% inhibition at 400 µg/mL CO (virucidal assay: virus + CO for 1 hour, dilute 1000X; add cells for 1 hour; wash; plaque assay)	Gong**
Gonococci	IC_{50} = 1 µg/mL	Cooper†
Chlamydia	IC_{50} = 1.6 mg/mL	Cooper

** Yunhao Gong, Department of Pharmacology and Therapeutics, The University of British Columbia, Vancouver, Canada.
† Morris Cooper, Microbicide Research Laboratory, Department of Medical Microbiology and Immunology, Southern Illinois University, Springfield, Illinois.

Table 4. Inhibition of Non-STI-Causing Bacteria and Fungi

Organisms	Test Conditions and Results	Researcher
BV-causing anaerobes (all)	MIC = 40–80 µg/mL (*G. vaginalis*, *Mobiluncus*, *Bacteroides*, etc)	Citron‡
Aerobes (all)	MIC = 40-160 µg/mL (*E. coli*, *Strep*, *Staph*, *Enterococcus*, *Listeria*, *Pseudomonas*, etc)	Citron
Candida albicans and glabrata	MIC = 20 µg/mL	Citron

‡ Diane M. Citron, B.S., M-ASCP, Associate Director, R.M. Alden Research Laboratory, Santa Monica, California.

Safety

- Approved for vaginal use: 2–3 times daily for up to 3 weeks. Also approved for use on skin (up to 4 weeks). [18, 19]
- Extensive animal safety testing performed, including long-term vaginal irritation, dermal safety, reproductive toxicology, mutagenicity, carcinogenicity, and others. [3, 5, 13, 20-24]
- More than 10 clinical studies with vaginal dosage forms showed minimal or no side effects. [25-37]

Table 5. Inhibition of Lactobacilli

Assay	Results	Researcher
Microdilution assay (4 species)	MIC = 20–100 µg/mL	Citron
Steers time kill assay (4 species)	1,000 µg/mL: no death in 24 hours; 10,000 µg/mL: 1–24 (*L. crispatus* >24 hours); *L. salivarius* >8<24 hrs; *L. gasseri* and *jensii*: 1–3 hours)	Citron
Agar dilution assay	MIC = 60–1,000 µg/mL	Citron

Table 6. *In vitro* cytotoxicity

Test Conditions, Results	Researchers
CC_{50} = 25–900 µg/mL (cells: PM-1, HeLa, P4-R5, VK-2, Vero)	Cooper, Doncel, Gong, Shattock

Table 7. Other Tests

Test Performed	Test conditions/Type	Results	Researcher
Spermicidal activity	*In vitro* assay	MEC = 1,200–8,800 µg/mL	Doncel
Rabbit vaginal irritation	10-day application of 1% cream (Olamin)	Score: 3.93 (mild)	Waller
Condom stability	1-hour application of 1% cream (Olamin)	No effect	Carter§

§ Eli Carter, Family Health International (FHI), Durham, North Carolina.

General Summary

As a potential microbicide, CO:

- Inhibits HIV and other STI-causing pathogens as well as BV-causing bacteria and fungi.
- Has minimal potential for development of HIV mutations or fungal resistance.
- Has anti-inflammatory properties that help maintain vaginal health.
- Will enter vaginal/cervical tissue to contact target cells.
- Has limited cytotoxicity towards host cells and is safe in animal vaginal irritation assays.
- May affect lactobacillus growth but causes no death.
- Is safe: (1) marketed worldwide for more than 20 years; (2) extensive animal safety testing conducted; and (3) many clinical studies performed to evaluate the efficacy and tolerance of CO as topical for skin, nails, and vagina.
- Has no effect on latex condom stability.

Conclusion

Ciclopiroxolamine is an exciting microbicidal candidate with a long history of proven safety in clinical use as a vaginal and skin product with anti-infective properties. It is already formulated, manufactured, and marketed worldwide in vaginal products. The next step is to obtain additional data on anti-HIV activity.

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