Haloprogin: a Topical Antifungal Agent

E. F. HARRISON, P. ZWADYK, JR.,1 R. J. BEQUETTE, E. E. HAMLLOW, P. A. TAVORMINA,
AND W. A. ZYGMUNT

Mead Johnson Research Center, Evansville, Indiana 47721

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Haloprogin was shown to be a highly effective agent for the treatment of experimentally induced topical mycotic infections in guinea pigs. Its in vitro spectrum of activity also includes yeasts, yeastlike fungi (Candida species), and certain gram-positive bacteria. The in vitro and in vivo antifungal activity of haloprogin against dermatophytes was equal to that observed with tolnaftate. The striking differences between the two agents were the marked antimonilial and selective antibacterial activities shown by haloprogin, contrasted with the negligible activities found with tolnaftate. Addition of serum decreased the in vitro antifungal activity of haloprogin to a greater extent than that of tolnaftate; however, diminished antifungal activity was not observed when haloprogin was applied topically to experimental dermatophytic infections. Based on its broad spectrum of antimicrobial activity, haloprogin may prove to be a superior topical agent in the treatment of dermatophytic and monilial infections in man.

Haloprogin (2,4,5-trichlorophenyl-γ-i odopropargyl ether; Fig. 1) was synthesized by Seki et al. (7) as part of a series of new acetylenic compounds closely related to capillolin and lenamycin. These investigators found certain γ-iodopropargyl aryl ethers to possess strong in vitro antimicrobial activity. Preliminary data on the antifungal properties of haloprogin, originally identified as M-1028, were reported by Seki and associates in 1964 (8).

This communication confirms the antimicrobial properties of haloprogin previously reported by Seki et al. (8) and extends the evaluation to include a wider spectrum of dermatophytes and other microorganisms. In addition, haloprogin (Halotex, Mead Johnson Laboratories) was compared with tolnaftate both in vitro and in animals treated with steroid to prevent spontaneous remission of the trichophyton infections (2).

MATERIALS AND METHODS

Drugs. Crystalline haloprogin was supplied by Meiji Seika Kaisha, Ltd., Tokyo, Japan. Crystalline tolnaftate was kindly supplied by Schering Corp., Bloomfield, N.J. Undecylenic acid was purchased from Mann Research Laboratories, New York City. For in vivo study, the following haloprogin formulations were prepared by our pharmaceutical product development group. Formulation A contained 1% haloprogin in a water-dispersible semisolid base. The contents per gram were 10 mg of haloprogin, 200 mg of diethyl sebacate, 530 mg of polyethylene glycol 400, 250 mg of polyethylene glycol 4000, and 10 mg of polyvinyl-pyrrolidone-K 90. Formulation B contained 1% haloprogin and 5% diethyl sebacate, in Plastibase. Formulation C contained 1% haloprogin, and 5% polyethylene glycol 400 dilurate in Plastibase. Formulation D contained 1% haloprogin, and 5% isopropyl myristate in Plastibase. Formulation E contained 1% haloprogin in polyethylene glycol 400. Formulation F contained 1% haloprogin solution. Each milliliter contained 10 mg of haloprogin, 200 mg of diethyl sebacate, and ethyl alcohol to volume. Tinactin (1% tolnaftate solution, Schering Corp.), used as a reference agent, was obtained commercially.

In vitro antifungal tests. The in vitro fungistatic concentrations were determined by a serial dilution method using Sabouraud’s liquid medium (BBL) (1, 3). The concentration of drugs tested ranged from 0.19 to 100 μg/ml. All tubes were inoculated with approximately 106 viable macrospores obtained by washing the surface of a 14-day slant culture of the test dermatophyte with Sabouraud’s liquid medium and diluting to the desired concentration. The inoculated assay tubes were incubated at room temperature (28 C) for 7 days and examined for visible growth. The minimal inhibitory concentration (MIC) was the lowest level of drug that completely prevented growth of the dermatophyte.

Beginning with the lowest concentration of drug that inhibited growth in the MIC test, streak plates were made on Sabouraud’s dextrose agar from the tubes containing the next five higher drug concentrations. The plates were incubated at 28 C for 7 days and examined for the presence of fungal growth. The lowest concentration of drug that prevented growth upon subculture was considered to be the minimal fungicidal concentration (MFC).
Infected guinea pigs were housed in individual wire-bottom cages and were provided food and water ad libitum. On the 3rd day after infection, the animals were divided at random into groups of 5 animals each, and treatment was initiated. One group of animals did not receive treatment and was used as an infection control. Treatment consisted of topical application to the infection sites of measured amounts of test formulation for 7 consecutive days. When treatment was terminated, the animals were sacrificed, and the infected sites were washed with a soap solution and thoroughly rinsed. Skin scrapings and hairs from each test site were cultured on Littman oxgall medium (Difco). The inoculated plates were incubated at 28 C for 7 days and examined for evidence of visible growth.

**In vivo antifungal evaluation with steroid-treated animals.** Steroid treatment has been used to prolong experimentally induced dermatophyte infections in guinea pigs (2). The scarified skin lesions were infected with T. gypseum var. asteroide as previously described. On the third day after infection, the animals were divided at random into groups of 15 animals, and the antifungal formulations were applied topically twice daily at 10- to 14-hr intervals for 12 consecutive

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**Table 1. In vitro antifungal spectra for haloprogin and tolnaftate**

| Organism                              | Antifungal activity (μg/ml)* |
|---------------------------------------|------------------------------|
|                                       | MIC  | MFC  | MIC  | MFC  |
| Haloprogin                            |      |      | Haloprogin |      |      |
| Tolnaftate                             |      |      | Tolnaftate |      |      |
| **Allescheria boydii SUI 807**         | 0.095| >25  | 0.39 | >25  |
| **Alternaria species**                 | 0.78 | >100 | ND   | >100 |
| **Aspergillus niger**                  | 0.78 | 0.19 | ND   | ND   |
| **Fusarium species SUI 1900**          | 0.19 | >25  | 0.19 | >25  |
| **Geotrichum species SUI 1400**        | 1.56 | >25  | >25  | >25  |
| **Hormodendrum compactum UT 2**        | >25  | >25  | >25  | >25  |
| **Keratinomyces ajelloi SUI 1123**     | 0.012| 0.0007| 0.012| 0.0007|
| **Microsporum canis UT 4**             | <0.047| <0.047| <0.047| <0.047|
| **M. cookei SUI 1128**                 | 0.19 | 0.095| 0.19 | 0.095|
| **M. distortum SUI 1118**              | 0.003| 0.0015| 0.003| 0.0015|
| **M. gypseum UT 5**                    | 0.19 | <0.047| 0.19 | <0.047|
| **Monosporium apiospermum SUI 804**    | 0.19 | >25  | 0.19 | >25  |
| **Nigrospora species SUI 4200**        | <0.047| <0.047| <0.047| <0.047|
| **Penicillium chrysogenum**            | 0.19 | >100 | ND   | >100 |
| **Philohora jeanselmei UT 7**          | >25  | >25  | >25  | >25  |
| **P. verrucosa UT 8**                  | 25   | 25   | 25   | 25   |
| **Rhizopus species SUI 2100**          | >25  | >25  | >25  | >25  |
| **Trichophyton concentricum SUI 1221** | <0.047| >25  | <0.047| >25  |
| **T. ferrugineum SUI 1229**            | 0.0015| 0.19 | 0.0015| 0.19 |
| **T. galliae SUI 1210**                | 0.047| 0.39 | 0.047| 0.39 |
| **T. megundi SUI 1209**                | 0.095| <0.047| 0.095| <0.047|
| **T. rubrum SUI 1248**                 | 0.012| <0.047| 0.012| <0.047|
| **T. rubrum SUI 1200**                 | 0.012| <0.047| 0.012| <0.047|
| **T. tonsurans SUI 1211**              | 0.047| 0.78 | 0.095| 0.78 |
| **T. tonsurans UT 11**                 | <0.047| <0.047| <0.047| <0.047|
| **T. verrucosum SUI 1240**             | 0.0015| <0.047| 0.0015| ND   |
| **T. violaceum UT 12**                 | <0.047| <0.047| <0.047| <0.047|

*Abbreviations: MIC, minimal inhibitory concentration; MFC, minimal fungicidal concentration; and ND, not determined.*
Epidermophyton floccosum UT 1...........<0.047 <0.047 <0.047 <0.047 3.12 3.12 <0.19 <0.19
Microsporum audouini UT 3.........<0.047 <0.047 <0.047 <0.047 3.12 6.25 <0.19 3.12
M. audouini ATCC 9079..................0.39 0.78 0.095 0.095 25 25 3.12 3.12
M. canis ATCC 10241.................0.39 0.39 0.095 0.095 50 50 3.12 3.12
M. cookei SUI 1127......................0.095 0.19 <0.047 0.003 3.12 3.12 0.34 0.39
M. gypseum SUI 1101....................0.095 0.19 0.095 0.095 6.25 6.25 0.39 0.78
Trichophyton mentagrophytes (asteroides) ATCC 8757.................0.095 0.095 0.024 0.024 12.5 12.5 0.39 0.39
T. mentagrophytes UT 9...............0.39 0.39 <0.047 <0.047 25 25 0.78 0.78
T. mentagrophytes SUI 1204..............0.095 0.095 3.12 3.12 6.25 6.25 3.12 3.12
T. mentagrophytes (gypseum) ATCC 9129...................0.39 0.78 0.047 0.047 25 25 3.12 3.12
T. mentagrophytes (interdigitale) ATCC 9972.......................0.78 0.78 3.12 3.12 25 25 1.56 1.56
T. rubrum UT 10.........................<0.047 <0.047 <0.047 <0.047 1.56 1.56 <0.19 <0.19
T. schoenleinii SUI 1205.................0.0015 0.0015 <0.047 <0.047 0.0007 0.78 0.78 <0.19 <0.19

a Abbreviations: MIC, minimal inhibitory concentration; MFC, minimal fungicidal concentration.

Table 3. Comparison of in vivo antifungal activity of haloprogin and tolnaftate against Trichophyton gypseum var. asteroides

| Formulation          | No. of observations | Antifungal cure rate |
|----------------------|---------------------|---------------------|
|                      | Animals | Infected sites |                   |
| Haloprogin A         | 8       | 16              | 14/16 (88%)        |
| Haloprogin B         | 8       | 16              | 14/16 (88%)        |
| Haloprogin C         | 6       | 12              | 8/12 (67%)         |
| Haloprogin D         | 7       | 14              | 9/14 (64%)         |
| Haloprogin E         | 8       | 16              | 9/16 (56%)         |
| Tolnaftate, 1% solution | 8   | 16              | 15/16 (94%)        |
| Untreated controls   | 8       | 16              | 6/16 (38%)         |

a Topical drug treatment was administered twice daily for 7 consecutive days.

b Ratio expresses number of sites free of fungi on subculture per number of sites cultured. Infected sites were cultured the day after treatment was terminated. Numbers in parentheses are percent values.

days. Beginning with the first day of therapy, each animal received a daily subcutaneous dose of 20 mg of triamcinolone acetonide per kg (Kenalog-IM; E. R. Squibb & Sons, New York, N.Y.) for 12 consecutive days. The untreated infection controls received steroid treatment only.

Hair was plucked from each infected site on all animals immediately before treatment and on the 4th, 8th, and 12th day of treatment. The hair was cultured on Littman oxgall agar with additions of streptomycin (50 μg/ml) and cycloheximide (500 μg/ml). Cultures were considered negative when no fungal colonies developed after 7 days of incubation at 28 C.

In vitro anti-yeast activity. Yeast nitrogen base medium (Difco) supplemented with 1.5% dextrose and adjusted to pH 4.5 was used to determine the MIC. Total assay volumes were 10 ml in 50-ml Erlemeyer flasks. Cells for the inoculum consisted of suspending the growth from an 18- to 24-hr slant culture in sterile saline solution, centrifuging, washing, and resuspending the cells to a standardized optical density reading (0.085). Two drops of this suspension were used to inoculate each flask. All flasks were incubated at 28 C with rotary agitation.

Table 4. In vivo activity of haloprogin and tolnaftate in steroid-treated guinea pigs experimentally infected with Trichophyton gypseum var. asteroides

| Formulation          | Ratio of lesions with negative cultures to lesions examined on days |
|----------------------|---------------------------------------------------------------|
|                      | 0 | 4 | 8 | 12 |
| Haloprogin, 1% solution (formulation F) | 0/30 | 29/30 | 27/30 | 26/26 |
| Haloprogin, 1% semisolid (formulation A) | 0/30 | 19/30 | 21/28 | 24/24 |
| Tolnaftate solution | 0/30 | 12/30 | 30/25 | 26/25 |
| Untreated controls | 0/30 | 0/30 | 0/30 | 0/28 |

a Topical drug treatment was administered twice daily for 12 consecutive days.
for 20 hr, after which the MIC was determined by visual observation.

In vitro antibacterial activity. Conventional twofold tube dilution methods were used to determine the antibacterial activity. Various drug concentrations were added to Trypticase Soy Broth (BBL) and inoculated with approximately $10^8$ bacteria per ml. After incubating for 18 hr at 37 C, the MIC was determined by visual observation.

**RESULTS AND DISCUSSION**

The MIC values obtained with haloprogin against several clinically important strains of *Microsporum* and *Trichophyton* species ranged from 0.0015 to 0.39 µg/ml (Tables 1 and 2). Tolnaftate produced a similar level of activity against these dermatophytes. Haloprogin, however, was effective against a wider spectrum of fungi than tolnaftate. Low MIC values were obtained with *Allescheria*, *Alternaria*, *Aspergillus*, *Geotrichum*, *Keratinomyces*, *Monosporium*, *Nigrospora*, and *Penicillium* species by using haloprogin, whereas tolnaftate was essentially inactive against these cultures. Neither haloprogin nor tolnaftate significantly inhibited cultures of *Hormodendrum*, *Phialophora*, and *Rhizopus* species. The MIC values of haloprogin are in general agreement with those reported by Seki et al. (8). Likewise, the degree of antifungal activity ob-

### Table 5. Comparative in vitro activity of various antifungal agents against yeast and yeast-like fungi

| Organism                                | Minimal inhibitory concentration (µg/ml) |
|-----------------------------------------|------------------------------------------|
|                                         | Haloprogin | Tolnaftate | Undecylenic Acid |
| *Candida albicans* ATCC 10231           | 0.2        | >25         | >25              |
| *C. albicans* NRRL Y477                 | 0.05       | >25         | >25              |
| *C. tropicalis* NRRL Y410               | 0.4        | >100        | 25               |
| *C. utilis* NRRL Y900                   | 0.8        | >100        | 25               |
| *Debaryomyces subgloboius* NRRL Y6666   | 0.05       | >100        | 25               |
| *Geotrichum candidum* NRRL Y552         | 0.4        | >25         | >25              |
| *Hansenula anomala* NRRL Y1737          | 0.2        | >100        | 25               |
| *H. saturnus* NRRL Y1304                | 0.4        | >100        | 25               |
| *Kloeckera brevis* NRRL Y6671           | 0.4        | >100        | 25               |
| *Pichia saitoi* NRRL Y1595              | 0.4        | >100        | 25               |
| *Rhodotorula sanniei* NRRL Y1595        | 0.4        | >100        | 10               |
| *Saccharomyces cerevisiae* NRRL Y9763   | 0.05       | >25         | >25              |

### Table 6. Comparison of the in vitro antibacterial activity of various antifungal agents

| Organism                                | Minimal inhibitory concentration (µg/ml) |
|-----------------------------------------|------------------------------------------|
|                                         | Haloprogin | Tolnaftate | Undecylenic Acid |
| *Gram-positive*                         |            |            |                  |
| *Bacillus subtilis* (Meiji)              | 25         | >100       | >100             |
| *B. cereus* (mycoides)                  | 50         | >100       | >100             |
| *Diplococcus pneumoniae* (type III)     | 50         | >100       | 50               |
| *Staphylococcus aureus* (Smith)         | 1.56       | >100       | >100             |
| *S. aureus* (Smith K6)                  | 3.12       | >100       | >100             |
| *S. aureus* (Giorgio)                   | 3.12       | >100       | >100             |
| *S. aureus* (209 P)                     | 3.12       | >100       | >100             |
| *S. aureus* (I-4768)                    | 3.12       | >100       | >100             |
| *Streptococcus faecalis*                | 100        | >100       | >100             |
| *S. pyogenes*                           | 0.78       | >100       | 100              |
| *S. pyogenes* (C203)                    | 0.78       | >100       | 100              |
| *S. pyogenes* (hemolyticus)             | 50         | >100       | >100             |
| *S. salivarius*                         | >100       | >100       | >100             |
| *Gram-negative*                         |            |            |                  |
| *Escherichia coli* 0111: Bb              | >100       | >100       | >100             |
| *Pseudomonas aeruginosa* ATCC 9027      | >100       | >100       | >100             |
| *Salmonella typhimurium*                | >100       | >100       | >100             |
served with tolnaftate closely approximates that found by Weinstein, Oden, and Moss (11).

The fungicidal activities closely parallel the fungistatic levels and usually differ by only one tube dilution. This holds true for both haloprogin and tolnaftate. The importance of this feature is obvious when one considers clinical application.

Addition of 5% human plasma to the in vitro test system resulted in elevated MIC and MFC values for both antifungal agents (Table 2). The in vitro antifungal activity of haloprogin appeared to be affected to a greater degree by the added protein than was tolnaftate. The relevance of this observation was not borne out in vivo, however, since both drugs demonstrated similar magnitudes of activity (Tables 3 and 4).

Haloprogin in several different vehicles and tolnaftate in its marketed base appeared to be equally effective in eradicating T. gypseum var. asteroides infections in guinea pigs. It should be pointed out that this experimental infection is somewhat self-limiting and that, in our study, the spontaneous cure rate was about 35% (Table 3).

By dosing the infected animals with triamcinolone acetonide, a prolonged dermatophyte infection was maintained. Significant antifungal activity was detected with haloprogin formulations A and F as early as the 4th day of treatment (Table 4). By the 12th day of treatment, virtually all of the infected sites had negative cultures. In contrast, the untreated infection control animals maintained a high percentage of positive cultures throughout the test period. By day 8, the antifungal activity of tolnaftate approximated that of haloprogin.

The maintenance of the infection by chronic administration of steroid is not without certain undesirable effects. By the 12th day of steroid treatment, increased mortality was noted not only in the groups receiving antifungal treatment, but also in the infection control group (Table 4). Tonelli reported a progressive increase in mortality after a single subcutaneous injection of triamcinolone acetonide to rats and mice (9, 10).

Haloprogin demonstrates good in vitro inhibitory activity against different species of yeast and yeastlike fungi. All cultures are very sensitive to haloprogin (MIC values < 1 μg/ml). Considerably higher concentrations of undecylenic acid (10 to 25 μg/ml) are required for inhibition of these cultures. Tolnaftate is essentially inactive since no significant inhibition of growth is observed at concentrations from 25 to 100 μg/ml (Table 5).

In addition, haloprogin shows good in vitro inhibitory activity against gram-positive cocci (Staphylococcus and Streptococcus species). Undecylenic acid and tolnaftate are essentially inactive against all of the bacterial cultures tested (Table 6).

In summary, haloprogin possesses potent antifungal activity both in vitro and in vivo. Its spectrum of activity also includes yeast, yeastlike fungi, and gram-positive bacteria. Based on its broader spectrum of antimicrobial activity, haloprogin may prove to be a superior topical agent in the treatment of dermatophytic and monilial infections in man.

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