Potentiating Effect of Organomercurials on Bisphenols

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Abstract □ The activity of organomercurial–bisphenol combinations was checked by *in vitro* methods against *C. albicans*, *S. aureus*, *E. coli*, *B. subtilis*, and *S. faecalis*. The *in vivo* activity of these combinations was determined against *M. canis* and *T. mentagrophytes* in guinea pig infections. The methods for both *in vitro* and *in vivo* testings are given. A potentiating effect was observed both *in vitro* and *in vivo* when phenylmercuric acetate is added to hexachlorophene or dichlorophene.

Keyphrases □ Hexachlorophene, dichlorophene activity, potentiation—organomercurials □ Antimicrobial activity—bisphenol–organomercurial combinations □ Optical density—microbial culture concentration

Recent Food and Drug Administration regulations, which prohibit the use of most antibiotics in topical ointments, and the failure of most systemic medication in the treatment of topical fungal infections have brought about a renewed interest in some of the older antimicrobial agents. These agents, while active against specific organisms, did not inhibit a broad spectrum of infective microorganisms.

Earlier experiments in this laboratory indicated that by the combination of two of these agents the desired results could be obtained *in vitro* (1). In an earlier report the activity of phenylmercuric acetate and a bisphenol was reported. While Barker (2) reported that some cationic and some anionic agents such as surfactants tend to inactivate phenylmercuric compounds, later findings (3) show that the mercury is not inactivated by all such compounds and, in particular, the nonionic surfactants actually may potentiate the activity of the mercurials. It has been established that the surfactants potentiate the activity of the bisphenols.

This study was a continuation of earlier work at the laboratory (4) which reported on the activity of the potentiating effect of organomercurials upon the bisphenols *in vitro* and *in vivo*. In this study the active ingredients were combined in a cream base and checked for activity *in vitro* and *in vivo* in animals that were infected with *Microsporum canis* and *Trichophyton mentagrophytes*.

METHODS AND MATERIALS

In Vitro—The test organisms used in this experiment were *Staphylococcus aureus* (ATCC 9144), *Candida albicans* (ATCC 10231) *Escherichia coli* (ATCC 8330), *Bacillus subtilis* (ATCC 6633), and *Streptococcus faecalis* (ATCC 10541). The organisms were incubated in Trypticase soy broth[1] for 18 hr. at 37°. The cultures were then centrifuged and washed twice with sterile 0.85% saline and finally resuspended in sterile saline. The cultures then were diluted with sterile saline to give 75% light transmission at the 550 mμ wavelength on the Spectronic 20 colorimeter.[2] In all cases exactly 0.1 ml. of this standardized culture was used for the inoculum, and fresh suspensions were prepared daily.

The compounds tested were phenylmercuric acetate, nitromersol, dichlorophene, hexachlorophene, and combinations of the mercury salts with the bisphenols. The solutions were dissolved in ethanol and sterilized by filtration. Following the sterilization they were diluted in sterile distilled water. The agents were then serially diluted through sterile Trypticase soy broth, in 18- × 150-mm. metal cap tubes, and inoculated with 0.1 ml. of the above suspension of organisms. The tubes were incubated at 35–37° for 24 hr. and observed for growth. Control tubes using only the solvent were also tested and found to have no inhibitory properties at the levels used in these tests.

In Vivo—The test organisms in this study were *Microsporum canis* and *Trichophyton mentagrophytes*. The cultures were prepared by inoculating Sabouraud dextrose agar (Difco) with the test organism and incubating at 28° for 7 days. The mycelial mat was removed and placed in 10 ml. of sterile physiological saline in a ground-glass homogenizer and macerated until there was an even suspension of the culture. The inoculum used for each guinea pig was 0.5 ml. of this suspension.

Seventy male guinea pigs weighing 225–275 g. were infected with *Microsporum canis* and 70 were infected with *Trichophyton mentagrophytes*. The methd used to infect the animals was to remove the hair from the right side of each animal by use of electric clippers and a safety razor. The culture was then placed on the cleared area and ground into the tissue by use of No. 1 sandpaper. The animals were unmedicated for 5 days to allow the infection to become established. Following this period, cultures were made by skin scrapings onto Mycobiotic agar (Difco) and incubated at 28° for 7 days. After cultures were made, each of the 10 animals in each group were treated twice daily, with a vanishing cream[3] containing the test substance, for a period of 7 days with one of the five test substances. Ten animals were unmedicated and served as controls. The vanishing cream base was selected after it was determined that there was no antagonism between the base and the active ingredients.

Following the last day of treatment, any remaining drug was removed by washing the treated area with warm tap water. Cultures of skin scrapings were made on Mycobiotic agar immediately following the washing and again at 7 and 14 days posttreatment. At the time each culture was made, scales and newly grown hairs from the infected site of each animal were examined microscopically.

RESULTS

The *in vitro* results are recorded in Table I. It is evident from the data in this table that phenylmercuric acetate exerts an effect in combination with the bisphenols which one would not expect from an additive effect alone. Against *Candida albicans* and *Escherichia coli*, the phenylmercuric acetate–dichlorophene combination was the most active, while the most active combination against the Gram-positive organisms was phenylmercuric acetate–hexachlorophene. The combination of nitromersol with the bisphenols displays a potentiating effect; it is less than that observed with the other combinations.

The *in vivo* results are recorded in Table II. The data indicates that the combinations of phenylmercuric acetate–bisphenol is more active against *Microsporum canis* than it is against *Trichophyton mentagrophytes*. The combinations are more effective than either agent alone.

DISCUSSION

The data obtained in this study indicate that a product containing phenylmercuric acetate and a bisphenol such as dichlorophene would be of use in the treatment of superficial fungus infections such

[1] Baltimore Biological Laboratories.
[2] Bausch & Lomb, Inc., Rochester, N. Y.
[3] Contains boric acid, glycerin, cetyl alcohol, stearyl alcohol, Span 20, Tween 20, sodium lauryl sulfate, and water.

Table I—Minimum Inhibitory Concentrations, in p.p.m.

Test Organism	Hexachlorophene	Dichlorophene	Phenylmercuric Acetate	Nitromersol	Hexachlorophene + 0.1 Part Phenylmercuric Acetate[a]	Dichlorophene + 0.1 Part Phenylmercuric Acetate[b]	Hexachlorophene + 0.1 Part Nitromersol[a]
C. albicans	31.2	50.0	0.8	25.0	2.0	1.6	12.5
S. aureus	10.0	10.0	1.7	6.2	1.0	1.6	1.6
E. coli	250.0	200.0	8.2	20.0	20.0	12.6	20.0
B. subtilis	10.0	15.0	0.8	8.0	1.0	1.6	1.8
S. faecalis	15.0	20.0	1.7	4.0	1.2	1.7	2.2

[a] Expressed as p.p.m. hexachlorophene. [b] Expressed as p.p.m. dichlorophene. The results of the tests reported in this table are for 10 tests on each organism per compound or combination.

Table II—Efficacy of Products Tested; Number Animals Infected/Number Treated

Compound Tested	*Microsporum canis* Day 1 Post-Treatment	*Microsporum canis* Day 7 Post-Treatment	*Microsporum canis* Day 14 Post-Treatment	Efficacy, %	*Trichophyton mentagrophytes* Day 1 Post-Treatment	*Trichophyton mentagrophytes* Day 7 Post-Treatment	*Trichophyton mentagrophytes* Day 14 Post-Treatment	Efficacy, %
Phenylmercuric acetate (0.05%)	7/10	7/10	7/10	30	7/10	8/10	8/10	20
Hexachlorophene (0.5%)	9/10	9/10	8/10	20	10/10	10/10	9/10	10
Dichlorophene (0.5%)	8/10	8/10	8/10	20	8/10	8/10	8/10	20
Dichlorophene (0.5%) Phenylmercuric acetate (0.05%)	3/10	2/10	2/10	80	4/10	4/10	4/10	60
Hexachlorophene (0.5%) Phenylmercuric acetate (0.05%)	2/10	2/10	2/10	80	7/10	6/10	6/10	40
Vanishing cream base	10/10	10/10	9/10	10	10/10	10/10	10/10	0
Control	10/10	10/10	10/10	0	10/10	10/10	10/10	0

as ringworm and athlete's foot. This combination displayed good activity when tested *in vitro* and the potentiated activity remained when the product, in ointment form, was checked *in vivo*.

While this study indicates that excellent results were obtained with twice-daily treatment for a period of 7 days, it must be remembered that these were fresh infections and treatment of longstanding infections of *Microsporum canis* might require treatment of 14 days or even longer in some cases. The results obtained also indicate that a treatment period longer than 7 days would have increased the percentage efficacy of the tested products; however, it has been found that at 21–28 days some of the control animals do not give positive cultures from one test period to another and for this reason the test must be shortened.

The *in vitro* data obtained along with the fact that these combinations are not inactivated *in vivo* would indicate that a product for the treatment of *Candida albicans* both topically and intravaginally can be developed using the tested agents.

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