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Topical butylated hydroxytoluene treatment of genital herpes simplex virus infections of guinea pigs*

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Summary

The effect of topical treatment with butylated hydroxytoluene (BHT) was evaluated in primary and recurrent genital herpes simplex virus type 2 (HSV-2) infection of guinea pigs. In the first experiment, treatment with placebo, 5%, 10%, or 15% BHT was initiated 48 h after viral inoculation and continued 4 times daily for 15 days. During primary infection no differences in maximum lesion severity or titers of virus in lesions were observed, however, lesion duration was reduced in BHT-treated animals resulting in a significantly smaller lesion score-day area under the curve. In a second experiment using U.S.P. mineral oil as an additional placebo, BHT placebo and 15% BHT in a double blind trial, similar results were obtained. Treatment of the recurrent infection in either experiment failed to alter the number of recurrent episodes or days with lesions.

genital herpes; butylated hydroxytoluene; animal model; herpes simplex virus; recurrent disease

Introduction

Primary and recurrent infections of the human genital tract with herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) have reached epidemic proportions [14].

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Primary infection is generally more severe resulting in multiple lesions on the genitalia often accompanied by urinary tract involvement and neurologic complications. Recurrent episodes are generally less severe and of shorter duration, but cause significant physical and psychological discomfort [1,3].

We have reported previously that intravaginal inoculation of guinea pigs with HSV-2 results in a primary infection which simulates human disease and that after recovery from the primary infection, recurrent vesicular lesions appear spontaneously on the external genitalia [10,12,17]. This infection provides an experimental model of genital HSV infection in which a potential antiviral compound can be evaluated for its effect both on primary and recurrent disease.

It has been reported that a group of hydrophobic molecules have activity against numerous viruses in vitro [6,13,16] and in vivo [2,9]. One of these compounds, butylated hydroxytoluene (BHT), applied topically, has been shown to reduce the severity of HSV-1 induced skin lesions in hairless mice [9]. The purpose of our studies was to determine the effect of topical BHT therapy on: (1) the development and severity of primary lesions, (2) virus replication in the vaginal tract and external skin lesions, and (3) the frequency and severity of recurrent lesions in a genital HSV-2 infection of guinea pigs.

Materials and Methods

Animals and viral inoculation

Female Hartley strain guinea pigs (Charles River Breeding Laboratories, Wilmington, Mass.) weighing 180–200 g were used in these studies. Groups of 10 animals each were inoculated intravaginally with 10^5 plaque forming units (pfu) of the MS strain of HSV-2. 2 h prior to viral inoculation, vaginal secretions were removed with a PBS-moistened dacron-tipped applicator. Animals used as toxicity controls were sham infected with media. This procedure has been described in detail previously [12].

Antiviral drug and treatment

BHT and corresponding placebo were provided by Key Pharmaceuticals, Inc., Miami, Florida through the Antiviral Substances Program, NIAID, NIH, Bethesda, Maryland. In the first study, placebo, 5, 10, and 15% BHT solutions were utilized. The second study was a double blind two placebo trial. After completion of the study, the code was broken and the following compounds were identified: 15% BHT; U.S.P. mineral oil; and BHT placebo (mineral oil). In the primary infection, treatment consisting of 2–4 drops (0.2 ml) per animal applied to the external genital skin was initiated 48 h after viral inoculation and continued every 6 h for 15 days. Sham infected animals were treated in the same manner and observed for adverse drug effects. In the first experiment, treatment of recurrent disease was initiated 30 days after viral inoculation and continued every 6 h for 16 days. In the second experiment treatment was begun 41 days after infection and continued every 6 h for 21 days.

Sample collection and lesion scoring

Samples for recovery of HSV-2 in primary lesions were obtained on days 3–10 after inoculation utilizing a media moistened applicator. In the second experiment, samples of vaginal secretions were also obtained on days 1, 3, 5, 7, and 10. The applicators were placed in tubes with 1.0 ml of media, vortexed and frozen at −70°C until titrated on rabbit kidney (RK) cells for quantitation of HSV [12].

During the primary infection (days 0–21), lesion severity was scored on a 0 to 4+ scale in 0.5 increments. For the recurrent disease, lesions were recorded by day, site, lesion stage (erythema or vesicle) and number of lesions. A recurrent episode was determined to be a day or days with lesions present with 1 or more lesion free days preceding and following. A detailed description of sample collection and lesion scoring has been published previously [12,17].

Virus assays

The amount of HSV-2 present in lesion and vaginal swab samples was determined by plaque assay on RK cell monolayers in 35 mm, 6-well Linbro tissue culture plates as has been described previously [12].

Statistical evaluation

Virus titer-day and lesion score-day areas under the curve were generated by a computer program. These areas, mean peak virus titer, mean peak lesion score, time to healing, mean number of recurrent episodes and number of recurrent lesion days were compared by the Mann–Whitney U rank sum test. A *P*-value of 0.05 or less was considered significant.

Results

Toxicity

In the initial experiment, a local irritation was observed in BHT and placebo-treated animals. This appeared as a slight redness on the external genital skin beginning 2 days after the initiation of treatment. The intensity of the redness did not increase, however, a sloughing or peeling of the keratinized epithelium occurred during day 3 and 4 of therapy. The redness persisted throughout the course of therapy and totally resolved by the second day after treatment was terminated. This local irritation seemed minor, so treatment regimens were not altered. Although there was no mortality in the uninfected drug control animals, all of the placebo-treated HSV infected animals died during the primary infection (Table 1).

Due to the unusually high mortality in the placebo-treated group in the first experiment, a second double blinded experiment utilizing USP mineral oil, BHT-placebo (mineral oil) and 15% BHT was performed. The local irritation observed in the first experiment was again observed with both of the mineral oil placebos as well as the BHT solution. The BHT-placebo group again had a higher mortality, however, this was not significantly different from the untreated control group. The increased mortality did not appear to be due to toxicity of the placebo preparations as there were no

TABLE 1

Effect of topical BHT treatment on lesion scores, duration, and mortality in an HSV-2 genital infection of guinea pigs

Group	Mean peak lesion score	Mean lesion duration (days)	Mean area under the lesion score-day curve	Mortality
Experiment 1				
Control	3.0	11.4	25.1	1/10
BHT placebo	3.4		24.0	10/10[c]
5% BHT	2.7[a]	7.8[c]	16.0[a,d]	2/10
10% BHT	2.9[b]	7.0[c]	15.8[a,d]	1/10
15% BHT	2.6[a]	7.2[c]	14.1[a,d]	1/10
Experiment 2				
Control	3.5	11.2	24.8	4/10
U.S.P. mineral oil	3.5	11.7	22.1	3/10
BHT placebo	3.2	10.8	21.3	5/10
15% BHT	3.0	8.4[c,e]	14.5[d,e]	2/10

[a] $P<0.01$ compared to BHT placebo-treated animals.
[b] $P<0.05$ compared to BHT placebo-treated animals.
[c] $P<0.01$ compared to untreated control animals.
[d] $P<0.05$ compared to untreated control animals.
[e] $P<0.05$ compared to mineral oil-treated animals.

deaths in the drug control animals or in animals treated for 3 weeks during recurrent disease.

Effect of BHT therapy on lesion severity in the primary infection

During the primary infection, lesions on the external genitalia began to appear on days 3–4 after HSV inoculation. These lesions progress to an ulcerative (maximum score) stage by days 6–7, crust and gradually heal by days 15–21. The effect of treatment with 5%, 10%, or 15% BHT solution on lesion severity is summarized in Fig. 1 and Table 1. There were no significant differences in the mean peak lesion score between untreated or BHT-treated animals. In contrast, the placebo-treated group had more severe lesions than either the untreated or BHT-treated groups, and all animals in this group died. The BHT-treated animals had an average lesion duration of 7–8 days compared to 11.4 days in untreated control animals. Time to healing in the placebo-treated group could not be calculated as all the animals died prior to the time that the infection had cleared. The time to healing was significantly reduced in the BHT-treated groups when compared to the untreated control group. The areas under the lesion score-day curve were significantly smaller when compared to either untreated or placebo-treated animals. This difference appeared to be the result of the more rapid healing of lesions rather than a reduction in development and spread of the lesions.

The effect of BHT treatment on lesion severity in the second experiment is shown in

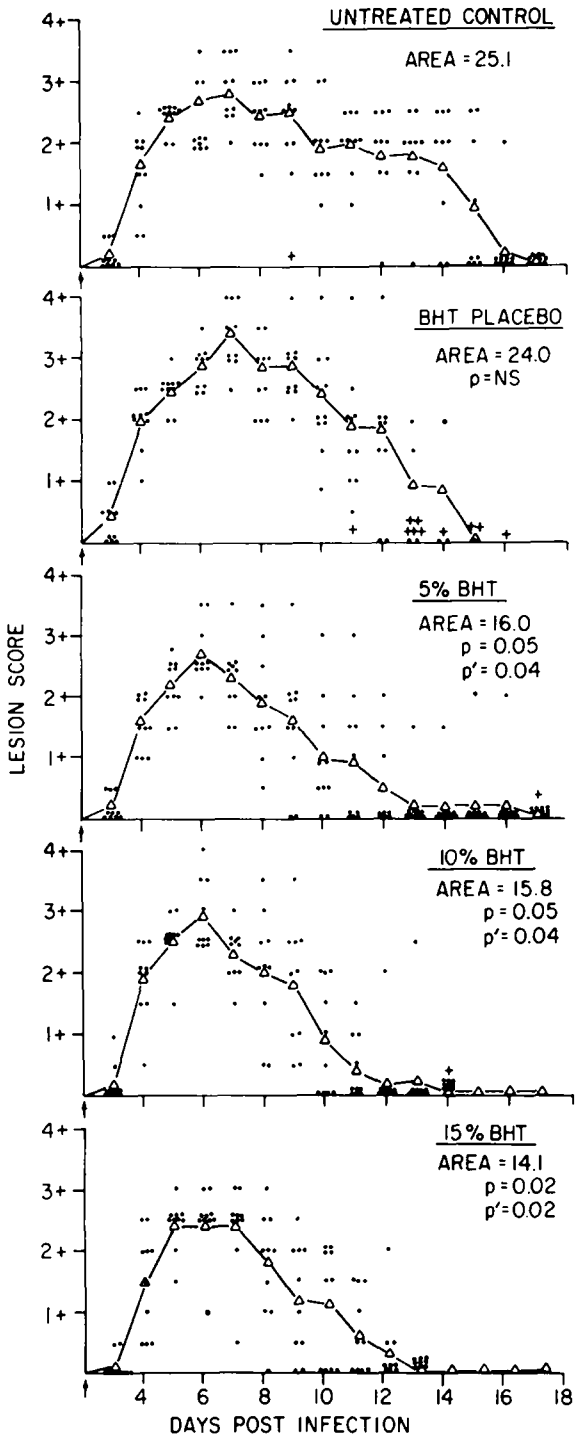

Fig. 1. Effect of topical BHT treatment on the severity of primary lesions in a genital HSV-2 infection of guinea pigs. Values of significance are: p = comparison with placebo-treated animals, and p' = comparison with untreated control animals.

Fig. 2 and summarized in Table 1. There was no difference in the average peak lesion score in untreated, USP mineral oil-treated, placebo-treated or BHT-treated animals. The lesion duration between BHT-treated and USP mineral oil-treated or control animals was again significantly reduced. However, there were no differences when compared to the BHT placebo. The difference in lesion duration was again reflected in the area under the lesion score-day curve when compared to control or mineral oil-treated animals. There was no difference when compared to the BHT placebo-treated animals, however, as there were numerous deaths in the placebo-treated group prior to healing (Table 1), the lesion duration and area under the curve may be skewed toward less severely infected animals.

Effect of BHT treatment on vaginal and lesion virus titers

Samples collected for quantitation of HSV-2 were assayed on RK cells and viral titers determined. The results of these assays are illustrated in Fig. 3 and summarized in Table 2. There were no differences in the mean peak lesion titers or area under the lesion virus titer-day curves in either experiment. There were also no differences in mean time to viral clearance. In the second experiment, vaginal virus titers were also unaltered with BHT therapy.

Effect of BHT treatment of recurrent lesions

After recovery from the primary infection, spontaneous recurrent vesicles appear on the external genitalia. These vesicles are generally few in number, last only 1–3 days and do not progress to the ulcerative state. The effect of BHT treatment on these

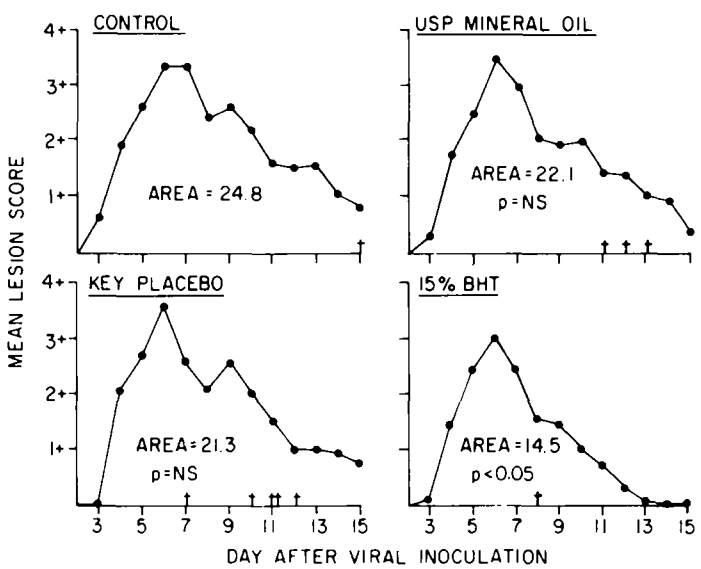

Fig. 2. Effect of topical treatment with USP mineral oil, BHT placebo or 15% BHT on the severity of primary lesions in a genital HSV-2 infection of guinea pigs.

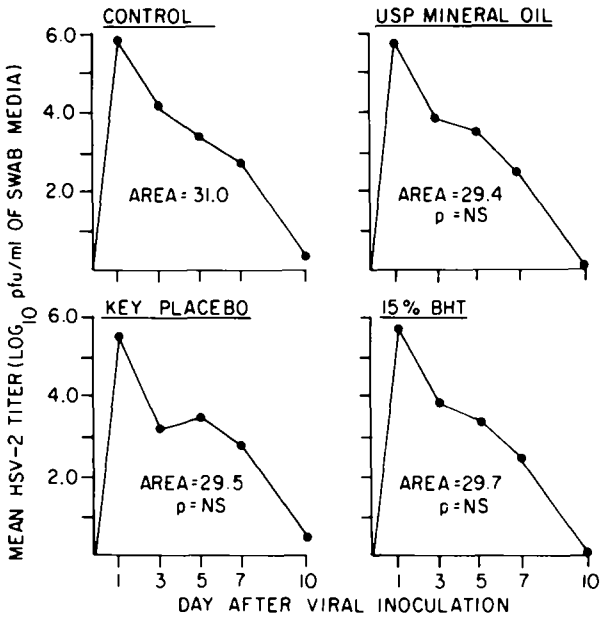

Fig. 3. Effect of topical treatment with USP mineral oil, BHT placebo or 15% BHT on vaginal virus titers of guinea pigs inoculated intravaginally with HSV-2.

TABLE 2

Effect of BHT treatment on virus titers in external genital HSV lesions

Group	Average peak lesion virus titer	Mean time to viral clearance (days)	Mean area under the virus titer-day curve
Experiment 1			
Control	3.2	7.8	8.3
0% placebo	3.9	8.2	12.6
5% BHT	3.6	7.9	12.8
10% BHT	3.4	7.8	10.6
15% BHT	3.1	8.3	9.9
Experiment 2			
Control	3.3	8.7	10.3
U.S.P. mineral oil	3.2	7.6	7.6
BHT placebo	3.2	8.7	8.0
15% BHT	3.2	7.6	7.2

recurrent lesions is summarized in Table 3. In the first experiment, BHT therapy was resumed 30 days after HSV inoculation and continued 4 times daily for 16 days. In the second experiment, a 3-week observation period (days 20–40) was utilized to establish baseline recurrence rates prior to the initiation of treatment on day 41. In both experiments animals were also observed for an additional 3 weeks following the treatment period. No differences in recurrence rates or number of days with lesions in untreated, mineral oil- or placebo-treated and BHT-treated animals were observed prior to treatment, during treatment or after treatment was terminated.

TABLE 3

Effect of BHT treatment on recurrent HSV-2 infection in guinea pigs

Parameter	Treatment			
	None	BHT placebo	10% BHT	15% BHT
Experiment 1[a]				
Mean No. of episodes				
day 30–46	3.0	2.0	2.3	3.1
day 47–63	1.8	1.9	1.6	2.0
Mean No. of lesion days				
day 30–46	4.6	3.1	3.5	5.5
day 47–63	2.6	2.3	2.8	4.4
	None	U.S.P. mineral oil	BHT placebo	15% BHT
Experiment 2[b]				
Mean No. of episodes				
day 20–40[c]	3.5	2.7	4.2	2.4
day 41–61[d]	3.3	1.9	4.2	3.2
day 62–82[e]	2.3	1.6	2.6	3.2
Mean No. of lesion days				
day 20–40[c]	4.2	4.3	6.0	4.0
day 41–61[d]	6.5	2.4	6.8	4.5
day 62–82[e]	4.5	2.1	4.4	5.2

[a] Treatment was initiated on day 30 and continued 4 times daily for 16 days.
[b] Treatment was begun on day 41 and continued 4 times daily for 21 days.
[c] Before treatment (days 20–40).
[d] During treatment (days 41–61).
[e] After treatment (days 62–82).

Discussion

The guinea pig model of genital HSV infection exhibits many similarities to the disease in humans [11,17]. Viral replication in the vaginal tract and external lesions may be monitored as well as the severity of lesions during the primary infection. Additionally, spontaneous episodes of recurrent disease can also be followed. We have reported previously that topical therapy with acyclovir (ACV) was highly effective in reducing viral replication in the vaginal tract and external genital lesions and significantly reduced the severity of these lesions during primary infection [10]. Topical treatment with 5% ACV, however, failed to alter subsequent recurrence rates, and prophylactic administration of ACV during recurrent disease did not reduce the frequency of recurrent episodes (unpublished results). Similar observations have been reported with topical ACV treatment for primary [4] and recurrent [15] genital HSV infections in humans. The predictability of the guinea pig model has been further documented with the use of oral ACV for treatment of primary and recurrent genital HSV-2 infections [11].

BHT has been shown to inactivate numerous enveloped viruses in vitro [2,13,16] and one possible mechanism of action is alteration of the lipid membrane of the virus thereby hindering adsorption to susceptible cells [6,9]. However, in our studies, the virologic course of primary HSV-2 infection in guinea pigs was not altered with BHT treatment. Virus titers in lesions and vaginal secretions were not different between untreated, placebo-treated and BHT-treated animals. In other studies diethyl ether, which destroys the lipid membrane, also failed to alter virus titers when applied topically to genital HSV-2 infections of mice (unpublished data) and to oral [8] or genital [5] lesions in humans. It would appear that BHT might inactivate free virions with which it comes in contact, but its effects on intracellular viral replication and cell to cell spread are not known.

In the current studies, topical BHT treatment failed to alter viral replication in the genital tract or the development of external genital lesions during primary infection, but did reduce time to healing of the external lesions in guinea pigs. This effect on healing time was similar to that observed by Keith et al. [9] on cutaneous HSV-1 lesions in hairless mice. Additionally, the local toxicity observed on the external genital skin was similar to that described on the hairless mouse [9].

In our studies, BHT treatment failed to reduce the mean maximum lesion score or the number of new contiguous lesions. The inability of therapy to alter viral replication may have precluded its having an effect on lesion progression or contiguous lesion development or in preventing recurrent lesions when administered prophylactically to guinea pigs infected with HSV-2.

These studies suggest that topical BHT therapy should not alter the virologic course of primary or recurrent genital herpes in humans. The promotion of healing could be beneficial, however, in reducing the duration of lesions. In a clinical trial in patients with recurrent herpes labialis, topical treatment with BHT reduced the time to crust formation, but did not alter the virologic course or other clinical measures of efficacy [7]. Additional clinical studies will have to be performed to determine whether BHT has a role in the management of HSV infections in humans.

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