ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1982, p. 513–515
0066-4804/82/030513-03$02.00/0 Vol. 21, No. 3

Lack of Efficacy of 2-Deoxy-D-Glucose in the Treatment of Experimental Herpes Genitalis in Guinea Pigs

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Received 26 October 1981/Accepted 9 December 1981

Topical treatment of herpes genitalis in female guinea pigs with 2-deoxy-D-glucose in either agarose gels or miconazole nitrate ointment failed to prevent the development of genital lesions or to reduce the mean titers of recoverable virus in vaginal swabs from infected animals. In contrast, phosphonoacetic acid was therapeutically effective.

The glucose analog 2-deoxy-D-glucose (dGlc) has been reported to exert selective antiviral effects against herpes simplex virus (HSV) and other herpesviruses in vitro (2, 4–7, 10–13). The compound inhibits those functions which have been attributed to virus-specific glycoproteins such as HSV-induced cell fusion (2, 7, 10–12), and this inhibitory activity has been correlated with an impairment or alteration in the glycosylation of virus-specific macromolecules (2, 4–6, 10, 11, 13). Ray et al. (14) have reported dGlc to be moderately effective in the topical treatment of herpetic keratitis in rabbits, but efficacy in these studies was dependent on the challenge virus inoculum employed. When higher input virus concentrations were used, dGlc treatment did not prevent lesion development but only reduced the severity of the lesions (2, 14). Gauri (8), in more recent studies, has reported dGlc to be essentially inactive in the local therapy of herpes simplex keratitis in rabbits, whereas phosphonoacetic acid (PAA) was found to exhibit marked efficacy in the inhibition of lesion development under the same experimental conditions. After the report by Blough and Giuntoli (1) that human herpes genitalis could be successfully treated by the topical application of dGlc, a number of laboratories have independently evaluated the therapeutic effectiveness of dGlc in the treatment of cutaneous and genital HSV infections in a variety of animal model systems. Kern et al. (E. R. Kern, L. A. Glasgow, R. J. Klein, and A. E. Friedman-Kien, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 514, 1980) have reported the failure of dGlc in the treatment of experimental HSV infections in vivo. In those studies, dGlc was found to be without therapeutic effect against HSV type 1 cutaneous infections in mice and HSV type 2 genital infections in both mice and guinea pigs. The purpose of this note is to communicate the results of an independent study, conducted in the Kettering-Meyer Laboratory, in which dGlc was examined for therapeutic efficacy against HSV type 2 genital infections in female guinea pigs, using methods similar to those employed by Kern et al. (9).

The MS strain of HSV type 2 was employed in these experiments. The virus, which was originally obtained from Earl R. Kern (University of Utah College of Medicine, Salt Lake City), was propagated and assayed in primary rabbit kidney cell cultures grown as monolayers in 75-cm^2 tissue culture flasks and Microtest II plates (Falcon Products Division of Becton, Dickinson & Co., Oxnard, Calif.). Eagle minimal essential medium containing 5.0% fetal bovine serum and antibiotics was utilized as the growth medium. Titrations of virus in rabbit kidney cells were performed using minimal essential medium supplemented with 2% fetal bovine serum and antibiotics as the maintenance medium. Young female albino guinea pigs, Hartley strain Crl: COBS (HA)BR, weighing 200 g, were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass. The animals were housed two to a cage and were given food and water ad libitum. All animals were quarantined for 1 week before use. Groups of guinea pigs were prepared for infection by preswabbing the vaginal mucosa of each animal with warm phospate-buffered saline (pH 7.4) about 2 h before virus inoculation. Guinea pigs were inoculated intravaginally with 10^5 50% cell culture infectious doses of HSV type 2 in a volume of 0.1 ml, using a disposable 1-ml tuberculin syringe equipped with a 0.5-in. (ca. 1.27-cm) plastic catheter. This concentration of virus usually resulted in a 95 to 100% genital infection rate, with only a 5 to 10% mortality rate. dGlc was obtained from Sigma Chemical Co. (St. Louis, Mo.) and was prepared for topical use at a final concentration of 1.0% (wt/vol) in either (i) 0.4% agarose (Seakem, Rockland, Maine) in phosphate-buffered saline (pH 7.4) or (ii) 2% miconazole nitrate ointment

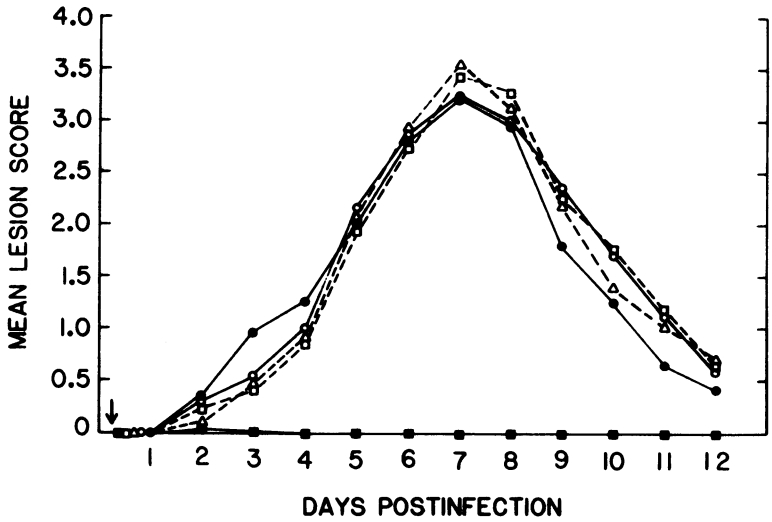

FIG. 1. Failure of dGlc to prevent HSV type 2 genital lesion development in female guinea pigs. Groups of 10 animals were treated topically twice a day for 7 days, starting 6 h after intravaginal inoculation with virus at a concentration of 10^5 50% cell culture infectious doses per 0.1 ml. The data represent mean lesion scores for untreated virus-infected control animals (●), for placebo-treated virus-infected control animals (○), for virus-infected animals treated with 1.0% dGlc in 0.4% agarose (□), for virus-infected animals treated with 1.0% dGlc in 2% miconazole nitrate ointment (△), and for virus-infected animals treated with 5.0% PAA in 0.4% agarose gels (■).

(Monistat-7 Vaginal Cream; Ortho Pharmaceutical Corp., Raritan, N.J.). The disodium salt of PAA was provided by Abbott Laboratories (North Chicago, Ill.) and was prepared for topical use at a final concentration of 5.0% (wt/vol) in phosphate-buffered saline containing 0.4% agarose.

For topical treatment studies, 30 animals served as virus-infected controls (10 untreated and 20 agarose or miconazole placebo treated), while groups of 10 virus-infected guinea pigs were treated intravaginally with 1.0% dGlc (prepared in either agarose gels or miconazole ointment) or with 5.0% PAA (positive control drug) prepared in agarose gels, administered in a volume of 0.1 ml twice a day for 7 days starting at 6 h after virus inoculation. Groups of five uninfected animals were treated with each of the drug formulations as toxicity controls, and five uninfected animals were held as normal, untreated controls. Vaginal swabs for isolation of HSV were obtained on days 1, 3, 5, 7, and 10 postinoculation, placed in 1.0 ml of cell culture medium containing gentamicin, and stored frozen at −80°C until assayed for infectious virus on rabbit kidney cell monolayers. The geometric mean virus titers for each group of animals on each of the virus assay days were subsequently determined. To rule out carryover of drug from the genital tract to the virus assay system, all swabs were collected at least 12 h after previous treatment. Samples from drug-treated control animals were also tested in vitro for residual antiviral activity. (No evidence of drug in sufficient concentration to inhibit HSV replication was detected in the vaginal swabs thus tested.) Virus-induced lesions were scored daily (days 1 through 12) according to the following scale: 0, no apparent evidence of virus infection; 0.5, redness and swelling; 1.0, distinct area where vesicle might form; 1.5, single discrete vesicle; 2.0, several discrete vesicles; 2.5, many small discrete vesicles; 3.0, many large discrete vesicles; 3.5, many large vesicles coalescing; and 4.0, large ulcers and maceration. A declining score was used during the healing stage, and a mean lesion score for all of the animals in each group was calculated for each day of observation.

The lack of efficacy of dGlc in the intravaginal treatment of HSV type 2 genital infections in guinea pigs is obvious from the data presented in Fig. 1. In this typical experiment, external lesions first appeared on day 3, and peak mean lesion scores were observed on day 7. No significant differences were observed between the mean lesion scores of the untreated or placebo-treated HSV-infected controls and those of the dGlc-treated animals. Treatment with 5.0% PAA, starting at 6 h after virus inoculation, was markedly effective, however, in completely preventing external lesion development (Fig. 1). As in the earlier studies reported by Kern et al. (9), the untreated and placebo-treated HSV-infected

control animals had peak mean vaginal virus titers on days 1 and 3 post-inoculation, and these gradually declined through day 10. Treatment with dGlc failed to reduce the mean virus titers when compared with controls (data not shown). In contrast, treatment with 5.0% PAA resulted in an almost complete inhibition of virus replication in the genital tract, with no detectable virus present in the vaginal swab samples obtained from most PAA-treated animals.

These data clearly indicate that 1.0% dGlc is not effective in the early topical treatment of herpes genitalis in female guinea pigs, whereas 5.0% PAA treatment under similar conditions produces a significant therapeutic benefit (15). Our results closely correspond with the independent findings of Kern et al. (E. R. Kern, L. A. Glasgow, R. J. Klein, and A. E. Friedman-Kien, J. Infect. Dis., in press), and the uniformly negative data from these combined animal model studies, along with the questions raised after the clinical trials (3), should serve to emphasize the fact that a careful evaluation of the therapeutic potential of dGlc in additional double-blind, controlled clinical trials will be required before the claim of antiviral efficacy in human patients with genital herpesvirus infections can be either substantiated or refuted in an objective manner.

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