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Evaluation of (*E*)-5-(2-bromovinyl)- and 5-vinyl-1-β-D-arabinofuranosyluracil (BrVaraU, VaraU) in the treatment of experimental herpes simplex virus type 1 keratitis in rabbits: comparison with (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BrVUdR)

H. Töpke[1], M. Gräf[1], P. Wutzler[2], G. Herrmann[3] and J. Reefschläger[4]

[1]*Clinic of Eye Diseases, Medical Academy Erfurt, Erfurt, D.D.R.,* [2]*Institute of Medical Microbiology, Medical Academy Erfurt, Erfurt, D.D.R.,* [3]*Department of Cell Kinetics, Central Institute of Molecular Biology, G.D.R. Academy of Sciences, Berlin-Buch, D.D.R., and* [4]*Instititute of Medical Virology, Medical Department (Charité), Humboldt University of Berlin, Berlin, D.D.R.*

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Summary

The 5-substituted 1-β-D-arabinofuranosyl (araU) analogues, (*E*)-5-(2-bromovinyl)-araU (BrVaraU) and 5-vinyl-araU (VaraU), which can be considered as structural analogues of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BrVUdR), are potent and selective inhibitors of herpes simplex virus type 1 (HSV-1) replication in vitro. BrVaraU and VaraU have been compared with BrVUdR for their therapeutic effect on acute HSV-1 keratitis in rabbits. Both araU derivatives applied as 0.1% eyedrops suppressed the development of keratitis as monitored by the reduced number of herpes efflorescences. The healing effect of BrVaraU and VaraU was less pronounced than that of 0.1% BrVUdR eyedrops, the difference between BrVUdR and VaraU being statistically significant at the 10th day of treatment. As a further indication of the healing effect the number of corneae with opacities seen after cessation of drug treatment were 3.3, 7.4, 27.6 and 46.9% for the BrVUdR-, BrVaraU-, VaraU- and placebo-treated eyes, respectively.

Bromovinylarauracil (BrVaraU); Vinylarauracil (VaraU); Bromovinyldeoxyuridine (BrVUdR); Herpes simplex virus type 1 (HSV-1); Rabbit keratitis

Correspondence to: H. Töpke, Clinic of Eye Diseases, Medical Academy Erfurt, Nordhäuser Strasse 74, Erfurt 5010, D.D.R.

Introduction

The therapeutic value of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (bromovinyldeoxyuridine, BrVUdR) (De Clercq et al., 1979, 1980; Reefschläger et al., 1982a, 1987b; De Clercq and Walker, 1984; De Clercq, 1986) has been demonstrated in the treatment of different experimental eye infections in rabbits as well as in diseases of the human eye due to infection with herpes simplex virus type 1 (HSV-1) and varicella-zoster virus (VZV) (Maudgal et al., 1980, 1981, 1982a,b, 1984, 1985; Töpke et al., 1984). Most of the licensed antiherpes drugs currently used in ophthalmology are surpassed by BrVUdR with regard to both antiviral potency and selectivity (De Clercq et al., 1979, 1980; Reefschläger et al., 1982a, 1987b; Reefschläger, 1986). Eye infections which have become resistant to treatment with either idoxuridine, trifluridine, vidarabine or acyclovir can be successfully treated with BrVUdR eyedrops (Maudgal et al., 1985; Maudgal and De Clercq, 1988). It should be mentioned that BrVUdR has low activity against herpes simplex virus type 2 (HSV-2) (De Clercq et al., 1980; Reefschläger et al., 1982b), moderate solubility in water (6.5 mg/ml at 25°C), and is readily cleaved by pyrimidine nucleoside phosphorylases (Desgranges et al., 1983; Liermann and Herrmann, 1983; De Clercq, 1984).

1-β-D-arabinofuranosyl-(*E*)-5-(2-bromovinyl)uracil (BrVaraU) (Sakata et al., 1980; Busson et al., 1981; Reefschläger et al., 1983) and 1-β-D-arabinofuranosyl-5-vinyluracil (VaraU) (Machida et al., 1980; Reefschläger et al., 1983) were synthesized with the aim to obtain equally, if not more potent antiviral compounds than BrVUdR. Both araU derivatives proved to be potent inhibitors of HSV-1 replication (Busson et al., 1981; Machida et al., 1981; Reefschläger et al., 1983), and they showed a 10-fold higher selectivity index compared to BrVUdR (Machida, 1986; Reefschläger et al., 1986). Of all antiviral compounds described so far, BrVaraU is the most effective in inhibiting VZV replication in human embryonic lung fibroblast cell cultures (Machida et al., 1982). It is effective in the treatment of HSV-1 encephalitis in mice (Machida and Sakata, 1984; Reefschläger et al., 1986) and simian varicella virus infection in monkeys (Soike et al., 1984). However, BrVaraU is ineffective against HSV-2 in vitro (Machida et al., 1981; Reefschläger et al., 1983).

In contrast, VaraU not only inhibits HSV-1 and VZV in vitro (Machida et al., 1980, 1982; Reefschläger et al., 1983, 1987a) and is highly effective against HSV-1 encephalitis in mice (Reefschläger et al., 1986), but it also demonstrates a marked activity against different clinical HSV-2 isolates in cell culture, similar to that of acyclovir (Reefschläger et al., 1987a).

In the present study we compared the efficacy of BrVaraU and VaraU with that of BrVUdR in the topical treatment of experimental HSV-1 keratitis in rabbits.

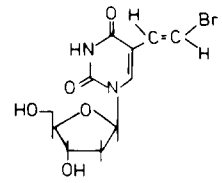
Materials and Methods

Drugs

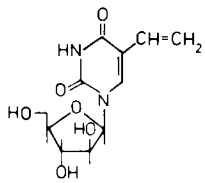
BrVUdR, BrVaraU and VaraU (Fig. 1) were synthesized as described previously (Reefschläger et al., 1982a, 1983) and formulated as 0.1% eyedrops. Per 10 eyedrops, pH 4.25, there was 0.01 g test compound, 0.07 g NaCl, 0.2 g hydroxyethylcellulose and 0.002 g thiomersal; 0.9% NaCl was used as placebo.

Animal model

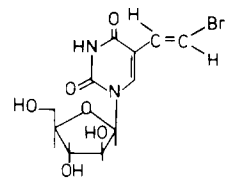
The rabbits weighed between 2 and 3 kg and were housed in single boxes under conventional conditions. Corneas were scarified by a modified Likar technique. In each quadrant a ring-shaped epithelial defect with a diameter of 1 mm was caused by turning the end of glass capillary touching the eye. The 20 µl suspension of HSV-1 (strain Kupka) was inoculated into the conjunctival sac. The inoculum contained about 5×10^6 $TCID_{50}$. In each experimental group (18 animals) both eyes of the rabbits were infected. Treatment was initiated 3 days post infection. At this time 2/3 (50–80%) of the eyes exhibited herpes simplex keratitis. Each eye was treated with one drop of the 0.1% drug solution 5 times daily at 4–hourly intervals from 7 a.m. to 11 p.m. The duration of treatment was 18 days. During the first week the state of the cornea was assessed daily, during the second week every other day, and during the third week every 3rd day. The examination by a slitlamp (biomicroscope) after application of 1% fluoresceine-eyedrops was always performed by the same person. For each cornea the number and type of epithelial lesions (punctate lesions, dendrites, ulcers) were documented, and after healing the number of corneal opacities as a parameter of keratitis severity was assessed.



(E)-5-(2-Bromovinyl)-2'-deoxyuridine
Bromovinyldeoxyuridine, BVDU, Br VUdR



1-ß-D-Arabinofuranosyl-5-vinyluracil
Vinyl-ara U, Vara U



1-ß-D-Arabinofuranosyl-(E)-5-(2-bromovinyl)uracil
Bromovinyl-ara U, Br Vara U

Fig. 1. Structural formulae of nucleoside analogues.

Results

At the beginning of the treatment, that is three days after virus inoculation, we observed about the same number of epithelial herpetic lesions in all test groups. During the next three days the number of punctate lesions and dendrites continued to increase, in the placebo group 4-fold, in the VaraU and BrVaraU groups 2–3-fold and in the BrVUdR group 1.4-fold (Fig. 2). BrVUdR suppressed the development of HSV-1 keratitis most strongly, the difference with the placebo group being statistically significant for the 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th day of treatment ($P < 0.05 - P < 0.001$). BrVaraU and VaraU 0.1% eyedrops achieved a reduction in the severity of herpetic eye lesions; as compared to the placebo group the healing effect was significant for VaraU on days 3 and 4 and for BrVaraU it was significant on days 3 and 5 ($P < 0.05$). Throughout the whole observation period BrVUdR was more effective than VaraU and BrVaraU; a statistically significant difference was seen between BrVUdR and VaraU on the 10th treatment day. In all groups, except for the VaraU group, the number of corneal lesions regressed from the 5th treatment day onwards. On day 18 almost all corneal lesions had healed, and the number of eyes with corneal opacities was assessed (Table 1). The number of cornea with opacities was 3.3., 27.6, 7.4 and 46.9% for the BrVUdR, VaraU, BrVaraU and placebo groups, respectively.

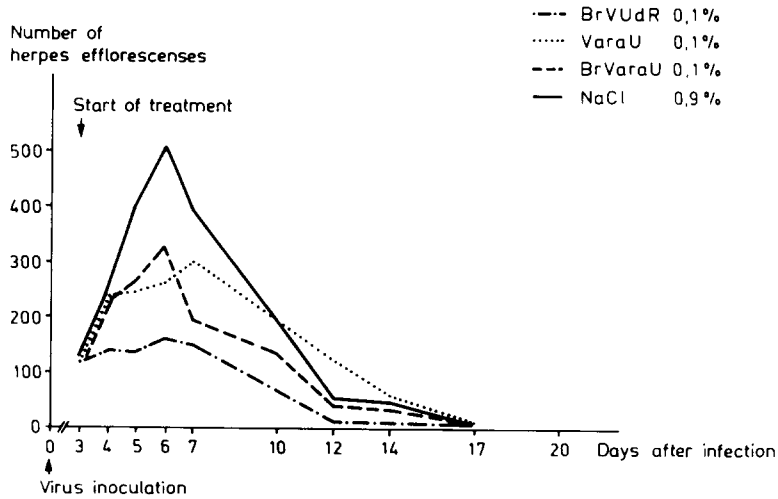


Fig. 2. Therapeutic effect of nucleoside analogues on experimental HSV-1 keratitis. Treatment was started 72 h after virus infection.

TABLE 1

Effect of BrVUdR, VaraU and BrVaraU topical eyedrops (0.1%) on the development on healing of experimental HSV-1 keratitis in rabbits.

Compounds	BrVUdR 0.1%	VaraU 0.1%	BrVaraU 0.1%	NaCl 0.9%
Treated eyes	36	36	36	36
Epithelial lesions, when treatment was initiated (day 3)	114	112	115	118
Eyes with lesions, when treatment was initiated (day 3)	30	29	27	32
Corneae with opacities after treatment (day 18)	1	8	2	15
In %	3.3	27.6	7.4	46.9

Discussion

Members of the 'second generation' of antiherpes drugs, although highly selective in their antiviral action, have some properties restricting their efficiency and clinical use, i.e. narrow activity spectrum, enzymatic inactivation, poor oral bioavailability and/or low solubility in water (De Clercq, 1984, 1985). (9-[2-Hydroxyethoxymethyl]guanine (acyclovir) is the only representative of the 'second generation' of antiherpetic drugs which has been approved as a 3% ointment for the topical treatment of herpetic eye infections in man. Because of its low solubility in water (0.2% at 25°C) the application of acyclovir at sufficiently high concentrations in eyedrops is not feasible.

In the D.D.R. 0.1% BrVUdR eyedrops are currently undergoing multicentered clinical trials, their therapeutic efficacy and safety in the treatment of experimental HSV-1 keratitis having been clearly shown (Töpke et al., 1984). In the future, each new drug developed for the treatment of herpetic eye infections may be compared with acyclovir or BrVUdR. In both experimental herpesvirus infections and clinical studies, either drug may thus serve as a reference compound. We have compared the efficacy of 0.1% BrVaraU and VaraU eyedrops with the efficacy of 0.1% BrVUdR eyedrops and placebo (0.9% NaCl) eyedrops in the treatment of experimental HSV-1 keratitis in rabbits. The statistically most significant effect was displayed by BrVUdR eyedrops. BrVaraU and VaraU also achieved a statistically significant effect but it was less pronounced than that of BrVUdR.

Maudgal and De Clercq (1985) found that, when formulated as either 0.1% or 0.5% eyedrops, BrVaraU had a healing effect on HSV-1 keratitis in rabbits, but the healing effect obtained with 0.1% BrVUdR eyedrops was significantly better. Rajčani and Reefschläger (1987) recently compared the efficacy of BrVUdR, BrVaraU, VaraU and acyclovir, each as 0.1% eyedrops in the topical therapy of acute HSV-1 keratitis in rabbits. As based upon the reduction of virus titer in the conjunctival swabs of the rabbits, the relative order of decreasing activity was as follows: BrVUdR > acyclovir > BrVaraU > VaraU. Whereas BrVUdR and acyclovir completely prevented the establishment of ganglionic latency, BrVaraU and

VaraU treatment reduced the number of explanted HSV-1-positive ganglio ments to 6% or 1.3%, compared with 43% from placebo-treated rabbits, r tively (Rajčani and Reefschläger, 1987).

In the present study, the BrVUdR group was the first to proceed to co healing, followed by the BrVaraU group, whereas re-epithelisation in the group occurred more slowly, even at a slower pace, than in the placebo However, three animals in the control group died from encephalitis. At t of the treatment period (18th day), residual lesions were scored in all anima ble 1). Following BrVUdR and BrVaraU treatment, the number of cornea ities was significantly lower than following VaraU or placebo treatment. half of the corneae of the placebo group remained affected. This is a prob practical importance since restoration of visual acuity does not only depend rapid elimination of infection but also on the disappearance of corneal op From this view-point BrVUdR and BrVaraU seem more suitable for clini than VaraU. To what extent higher VaraU concentrations (0.5%–2% eye may lead to an improved healing effect remains to be established. The hi ubility of VaraU and BrVaraU as well as their high selectivity index against 1 in cell culture (Machida, 1986; Reefschläger et al., 1986) make it possibl crease their concentration.

Taking into consideration that, on the one hand, VaraU inhibits the repl of different HSV-2 isolates at similar concentrations as acyclovir, and that, other hand, VaraU is effective in the treatment of HSV-2 encephalitis i (Reefschläger et al., 1987a), it seems worth further exploring VaraU for tential in the treatment of herpesvirus infections.

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