

(E)-5-(2-Bromovinyl)-2'-Deoxyuridine in the Treatment of Experimental Herpes Simplex Keratitis

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IDU (5-iodo-2'-deoxyuridine), BVDU [(E)-5-(2-bromovinyl)-2'-deoxyuridine], and placebo ointments were studied for their effects on experimental herpes simplex (type 1) keratoconjunctivitis in rabbits. When treatment was begun 65 h after virus inoculation, both BVDU and IDU prevented development of keratitis. Both BVDU and IDU were also effective in suppressing the severity of conjunctivitis, and in this respect, BVDU proved significantly better than IDU. When treatment was started 110 h after virus inoculation, BVDU proved significantly better than IDU in promoting healing of established keratitis.

Since its introduction in ophthalmological practice in 1962 (5), IDU (5-iodo-2'-deoxyuridine) has remained the drug of choice in the treatment of herpes simplex keratitis (4). However, its toxicity, the emergence of IDU-resistant virus strains, and the inability of IDU to prevent recurrences have led to the search for more effective and less toxic antiherpes agents. Of many antiherpes compounds developed so far, only a few drugs, i.e., ara-A (9- β -D-arabinofuranosyladenine) (9, 11), phosphonoacetic acid (8), TFT (trifluorothymidine or 5-trifluoromethyl-2'-deoxyuridine) (6), and acyclovir [acycloguanosine or 9-(2-hydroxyethoxymethyl)guanine] (4b, 7, 10) have shown usefulness in the treatment of herpetic keratitis of animals (rabbits) or humans or both.

BVDU [(E)-5-(2-bromovinyl)-2'-deoxyuridine], a newly synthesized thymidine analog (12), has been evaluated by De Clercq et al. (1, 2) for its potency and selectivity as an antiherpes agent in primary rabbit kidney and human skin fibroblast cell cultures. In these cell cultures BVDU was about 20 times more active against the replication of herpes simplex virus (HSV) type 1 (strain KOS) and 60 times less toxic than IDU as judged by inhibition of deoxyuridine incorporation into host cell deoxyribonucleic acid. BVDU surpassed various other established antiherpes compounds, including phosphonoacetic acid, TFT, ara-A, ara-T (1- β -D-arabinofuranosylthymine), and acycloguanosine, in activity against HSV type 1 in PRK cells.

When applied as a 1% topical ointment, BVDU was about as effective as acycloguanosine, phosphonoacetic acid, and trisodium phosphonoformate in preventing development of skin lesions in athymic nude mice inoculated intra-

cutaneously with HSV type 1 (strain KOS) (3). Under the same conditions, IDU effected but a slight inhibition of vesicle formation. When applied as a 0.1% ointment, BVDU and phosphonoacetic acid, but not acycloguanosine, inhibited development of skin lesions. Once vesicles began to appear (4 days after virus inoculation), neither phosphonoacetic acid nor BVDU, applied topically, suppressed evolution of the lesions. BVDU, however, effected a reduction in the severity of the disease when administered intraperitoneally (at a dose of 60 mg/kg) (2).

In this study we have compared the effectiveness of BVDU and IDU in preventing the evolution of experimental herpes simplex keratoconjunctivitis in rabbits and in treating the established disease.

MATERIALS AND METHODS

IDU was obtained from Ludeco, Brussels. BVDU was synthesized by procedures that have been published (4a, 13) or will be published shortly (R. Busson and H. Vanderhaeghe, manuscript in preparation).

The rabbits used in our experiments originated from a cross breed of Californian albino and Dendermonde white rabbits. They were 12 weeks old, of both sexes, and weighed 1.5 to 2 kg.

In the first experiment 30 rabbits were infected at a single setting in both eyes with HSV type 1 (strain McIntyre). The eyelids were rubbed gently over the cornea for 30 s after instillation of one drop of the virus inoculum containing either $10^{5.2}$ plaque-forming units (PFU) or $10^{4.2}$ per ml. The animals were divided into three groups of 10 rabbits, each group containing 5 rabbits infected with the lower and 5 with the higher virus dilution. Virus PFU were determined in Vero cell cultures. Treatment was begun 65 h after virus inoculation; ten animals were treated with 0.5% IDU

ointment, ten animals were treated with 0.5% BVDU ointment, and the other ten animals were treated with placebo ointment [sterile eye ointment containing liquid paraffin, yellow soft paraffin, and wool fat at a 10:80:10 (wt/wt/wt) ratio, according to *Pharmacopoeia (British)* (12)]. The ointments were applied five times a day at 2-h intervals during 5 consecutive days. At each application, about 1 cm of ointment was pressed out of an ophthalmic dispensing tube into the lower cul-de-sac of the eye. Rabbits were examined daily on a blind basis by the same individual.

In the second experiment, another 30 rabbits were infected at a single setting in both eyes with HSV type 1 (strain McIntyre). Only one virus dilution containing $10^{5.2}$ PFU/ml was employed in this study. Treatment was started 110 h after virus inoculation. The ointments and schedule and duration of therapy were the same as in the first experiment. Clinical evaluations were done by the same examiner but not on a blind basis.

Keratitis was graded after staining with a 2% fluorescein solution by using a 2× magnifier. The severity of conjunctivitis was scored from grade 0 to 4. Grade 0 meant no conjunctivitis, and grade 4 meant marked conjunctivitis with follicle formation and discharge. Keratitis scores were as follows: grade 0, no keratitis; grade 1, one to five punctate lesions involving less than 1/3 of the surface of the cornea; grade 2, one to five punctate lesions or small ulcers involving up to 1/3 of the surface of the cornea; grade 3, six or more dendritic lesions or ulcers involving more than 1/3 but less than 2/3 of the surface of the cornea; grade 4, dendritic lesions or small ulcers involving more than 2/3 of the surface of the cornea but not the total cornea; grade 5, total corneal ulcers.

Statistical analysis of the conjunctivitis and keratitis scores between the IDU and BVDU groups was

done by Student's *t* test. *P* values <0.05 were considered significant.

RESULTS AND DISCUSSION

In our experiments both IDU and BVDU, when applied as 0.5% ointments, suppressed development of herpes simplex keratitis and promoted healing of established keratitis and conjunctivitis.

Among rabbits which had been infected with $10^{4.2}$ or $10^{5.2}$ PFU of HSV per ml (experiment 1), there was no marked difference in the severity of keratitis, neither at the time treatment was started nor later on during the further evolution of the disease. Hence, for all groups (whether placebo, IDU, or BVDU treated), the results were presented for the whole group, irrespective of the virus inoculum dose ($10^{4.2}$ or $10^{5.2}$ PFU/ml). When treatment was started (65 h after infection) most rabbits showed an early stage of herpetic keratitis. The severity of keratitis steadily increased in the placebo group, whereas IDU and BVDU ointments suppressed the development of keratitis (Table 1, Fig. 1). Six rabbits of the BVDU group had grade 1 keratitis on day 1 of treatment. The eyes gradually healed, so that after 5 days of treatment only one eye showed mild grade 1 keratitis. No new lesions appeared during the treatment period. Of the IDU group, only one eye had grade 1 keratitis on day 1 of treatment. This eye gradually healed, but grade 1 keratitis appeared in eight other eyes throughout the treatment period. Three of these eyes

TABLE 1. Keratitis scores of different eyes in experiment 1

Treatment group	Grade lesions	No. of eyes exhibiting grade lesions on (day after start of treatment):					
		0	1	2	3	4	5
BVDU	None	14	14	14	14	17	19
	1	6	6	6	6	3	1
	2	— ^a	—	—	—	—	—
	3	—	—	—	—	—	—
	4	—	—	—	—	—	—
	5	—	—	—	—	—	—
IDU	None	19	19	15	16	16	15
	1	1	1	5	3	2	3
	2	—	—	—	1	—	—
	3	—	—	—	—	1	—
	4	—	—	—	—	1	—
	5	—	—	—	—	—	2
Placebo	None	13	13	1	—	—	—
	1	6	6	5	1	1	—
	2	1	1	6	3	—	—
	3	—	—	7	6	4	—
	4	—	—	1	8	11	5
	5	—	—	—	2	4	15

^a —, None.

healed, and two deteriorated to total corneal ulcers (grade 5 keratitis) in spite of the treatment. Three eyes showed grade 1 keratitis at the end of the experiment. Although BVDU appeared clinically more effective than IDU in suppressing the development of keratitis in this experiment (Table 1, Fig. 1), the difference was not statistically significant (Fig. 1).

Both IDU and BVDU caused a rapid lessening of the conjunctivitis as compared to the placebo-treated group (Table 2, Fig. 2). The improvement was evident within 48 h and continued throughout the treatment period. BVDU was significantly better than IDU ($P = 0.005$) in suppressing conjunctivitis on days 4 and 5 of treatment (Fig. 2). The latter observation fur-

ther suggests that BVDU by itself is nonirritating to the eye.

In the second experiment the delayed applications of BVDU and IDU promoted healing of the keratitis. Most animals had developed a rather severe keratitis when treatment was started (110 h after infection) (Table 3, Fig. 3). In the placebo group the severity of keratitis increased progressively (conjunctivitis was not scored in this experiment). In the IDU group the existing lesions progressed before they began to regress and new lesions appeared during treatment. The healing effect of BVDU was significantly better than that of IDU ($P = 0.003$ on day 2 of treatment) (Fig. 3). Throughout BVDU

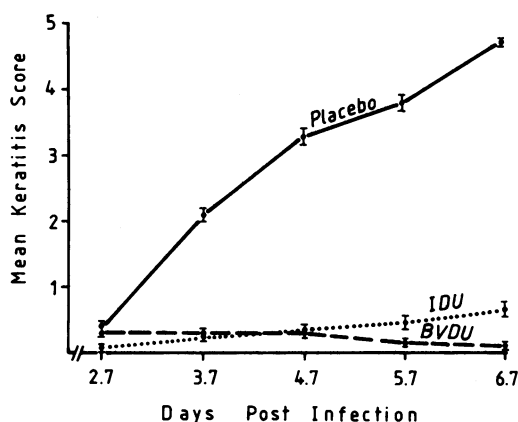


FIG. 1. Mean (\pm standard error of the mean) keratitis response curves by treatment group. Treatment was started 65 h after virus inoculation (corresponds to day 2.7 on the graph).

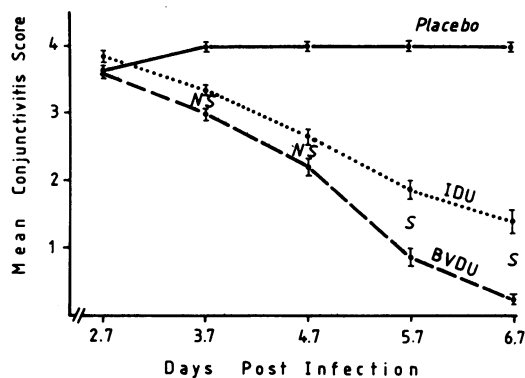


FIG. 2. Mean (\pm standard error of the mean) conjunctivitis response curves by treatment group. Treatment was started 65 h after virus inoculation (corresponds to day 2.7 on the graph). S, Significant; NS, not significant. Significance or lack of significance refers to the differences between the IDU and BVDU groups.

TABLE 2. Conjunctivitis scores of different eyes in experiment 1

Treatment group	Grade conjunctivitis	No. of eyes exhibiting grade conjunctivitis on (day after start of treatment):					
		0	1	2	3	4	5
BVDU	None	— ^a	—	—	—	7	14
	1	—	—	1	4	10	6
	2	—	—	4	9	2	—
	3	8	8	9	6	1	—
	4	12	12	6	1	—	—
IDU	None	—	—	—	—	1	3
	1	—	—	—	1	7	12
	2	—	—	2	7	8	3
	3	3	3	9	10	2	—
	4	17	17	9	2	2	2
Placebo	None	—	—	—	—	—	—
	1	—	—	—	—	—	—
	2	—	—	—	—	—	—
	3	7	7	—	—	—	—
	4	13	13	20	20	20	20

^a —, None.

TABLE 3. Keratitis scores of different eyes in experiment 2

Treatment group	Grade lesions	No. of eyes exhibiting grade lesions on (day after start of treatment):					
		0	1	2	3	4	5
BVDU	None	—	— ^a	—	7	15	19
	1	10	10	20	13	5	1
	2	2	2	—	—	—	—
	3	4	4	—	—	—	—
	4	4	4	—	—	—	—
	5	—	—	—	—	—	—
IDU	None	5	5	1	5	8	14
	1	1	1	6	9	9	3
	2	3	3	5	2	2	3
	3	7	7	8	4	1	—
	4	4	4	—	—	—	—
	5	—	—	—	—	—	—
Placebo	None	6	6	3	1	—	—
	1	4	4	4	4	2	—
	2	6	6	4	6	4	6
	3	3	3	5	2	3	—
	4	1	1	4	3	4	3
	5	—	—	—	4	7	11

^a —, None.

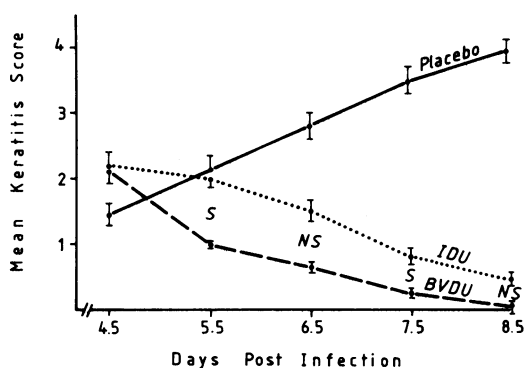


FIG. 3. Mean (\pm standard error of the mean) keratitis response curves by treatment group. Treatment was started 110 h after virus inoculation (corresponds to day 4.5 on the graph). S, Significant; NS, not significant. Significance or lack of significance refers to the differences between the IDU and BVDU groups.

treatment the existing lesions gradually regressed and no new lesions appeared. Although BVDU appeared better than IDU throughout the whole treatment period, the difference in their effect became progressively smaller and was no longer statistically significant after 5 days of therapy ($P = 0.05$ on day 4 and $P = 0.08$ on day 5 of treatment) (Fig. 3). The difference on day 3 of treatment was not significant because of a large standard error in scoring.

We did not observe any local or general untoward side effects with the 0.5% BVDU eye ointment. Even when applied as a 2.5% ointment, BVDU did not produce any toxicity to the eye, whereas both 2.5 and 0.1% BVDU ointments were found to be equally effective as a 0.5% BVDU ointment in treating herpetic keratitis (data not shown). BVDU does not affect the growth or metabolism of cultured cells, unless concentrations are employed that are 5,000- to 10,000-fold greater than those required to inhibit virus multiplication (1, 2). This implies that BVDU is very selective in its antiherpes action. Apparent lack of toxicity of BVDU has also been demonstrated in athymic nude mice after intraperitoneal administration of the drug at 600 mg/kg (2).

The exact mode of action of BVDU and the basis for its selective antiherpes activity are not yet known. However, its high antiherpes activity, efficacy, and apparent freedom from toxicity in vitro and animal model systems, makes it a promising compound for the exploration of antiherpes potentials in humans.

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