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Novobiocin and Coumermycin A_1 Inhibit Viral Replication and the Reactivation of Herpes Simplex Virus Type 1 from the Trigeminal Ganglia of Latently Infected Mice

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Herpes simplex virus type 1 was reactivated from the trigeminal ganglia of latently infected mice in a quantitative and time-dependent manner. Novobiocin and coumermycin A_1 reversibly inhibited the reactivation of herpes simplex virus type 1. They did not inhibit viral replication in permissive cells (CV-1) but did inhibit replication in cells of neuronal origin (C1300) and acutely infected trigeminal ganglia.

Reactivation of latent herpes simplex virus to produce recrudescent disease is frequent and can be life threatening in immune system-suppressed individuals (23). The study of reactivation would be aided if inhibitors were available, and these inhibitors might have clinical importance. Since the structure of latent herpes simplex virus type 1 (HSV-1) DNA differs from that of linear virion DNA (7, 26, 27) and since topoisomerases can mediate the integration (13, 14), recombination (29), circularization, catenation, and decatenation of DNA (for reviews, see references 5, 10, 30, and 31), it was hypothesized that topoisomerase inhibitors affect the reactivation process. In this paper, a reactivation model based on the work of Klein (15) that was efficient, quantitative, and time dependent was used to evaluate the effects of topoisomerase inhibitors.

Mice were infected, after corneal scarification, with 10^6 to 10^7 PFU of HSV-1(F) per eye (26, 27). At 4 weeks postinoculation, infectious virus was no longer detectable in the central nervous system and peripheral nervous system but could be reactivated after explant cultivation of the trigeminal ganglia (Fig. 1 and Table 1). Infectious virus was first detectable at 2 days postexplant, and by 4 days postexplant virus could be recovered from all of the ganglia (Fig. 1). The effect on reactivation was assayed by adding the topoisomerase inhibitors to ganglia at explant. The appearance of reactivated HSV-1 was completely inhibited by novobiocin (160 μM) and coumermycin A_1 (9 μM) at 4 days (Table 1) or 8 days (data not shown) postexplant. The effects of novobiocin and coumermycin A_1 on HSV-1 reactivation were reversible. When novobiocin (160 μM) or coumermycin A_1 (9 μM) was added at explant and removed at day 4, reactivation occurred in all of the ganglia by day 8. When two other topoisomerase inhibitors, nalidixic acid and oxolinic acid, were used, reactivation occurred in 100% of the ganglia (Table 1).

Concentrations of novobiocin and coumermycin A_1 that inhibited reactivation were weak inhibitors of HSV-1 replication in permissive cells (CV-1) infected at a multiplicity of infection of 1 PFU per cell (Table 2) or 0.01 PFU per cell (data not shown). Nalidixic acid and oxolinic acid were also weak inhibitors of HSV-1 replication in CV-1 cells (Table 2), consistent with results obtained by using BHK cells (9). Since a compound with antiviral activity might indirectly affect reactivation, phosphonoacetic acid, an inhibitor of HSV-1 replication (24), and its encoded DNA polymerase (18) were studied. At concentrations that exhibited the maximum antiviral activity in CV-1 cells (Table 2), 1.4 to 2.9 mM (200 to 400 μg/ml), phosphonoacetic acid affected HSV-1 reactivation (Table 1). Thus, any effect of phosphonoacetic acid on reactivation could not be distinguished from its antiviral activity.

Although novobiocin and coumermycin A_1 did not exhibit potent antiviral activity in CV-1 cells, their effects in C1300 cells (Table 2), a neuroblastoma cell line (20), and in acutely infected ganglia (Table 3) were significant. Nalidixic acid and oxolinic acid were not more potent in C1300 cells than in CV-1 cells (Table 2). The difference in antiviral activity of novobiocin in CV-1 and C1300 cells was not due to a difference in cytotoxicity, since novobiocin (160 μM) increased the doubling times of both cell lines from about 24 h to 48 h (data not shown). Because novobiocin and coumermycin A_1 inhibited HSV-1 replication in cells of neuronal origin and in acutely infected ganglia to a greater extent than they did in CV-1 or BHK (9) cells, the antiviral activity may be the result of an effect on the host cell or on a viral function required only in some cell types. This antiviral activity may account for the effects of novobiocin and coumermycin A_1 on HSV-1 reactivation.

To determine whether the inhibitors had an effect on the structure of the latent genome and the synthesis of viral DNA, DNA was extracted from ganglia (27) that had been incubated for 4 days in explant culture. Latent HSV-1 DNA lacks free termini and can be distinguished from linear virion DNA by *Bam*HI digestion and Southern blot analysis (7, 26, 27). In the presence of novobiocin or coumermycin A_1 the terminal *Bam*HI fragments, P and S, were not detectable and the amount of HSV-1 DNA did not increase greatly relative to the latent sample (Fig. 2, lanes 1 to 3). In the reactivated sample the amount of HSV-1 DNA increased 1,000-fold, and *Bam*HI fragments P and S were present (Fig. 2, lane 4).

In explanted ganglia exposed to novobiocin (160 μM) for 3 days and labeled overnight, the incorporation of thymidine into acid-precipitable macromolecules was inhibited by about 95 ± 4% (data not shown), consistent with an effect on DNA synthesis. Novobiocin had a less inhibitory effect on the incorporation of uridine and amino acids (30 and 40%,

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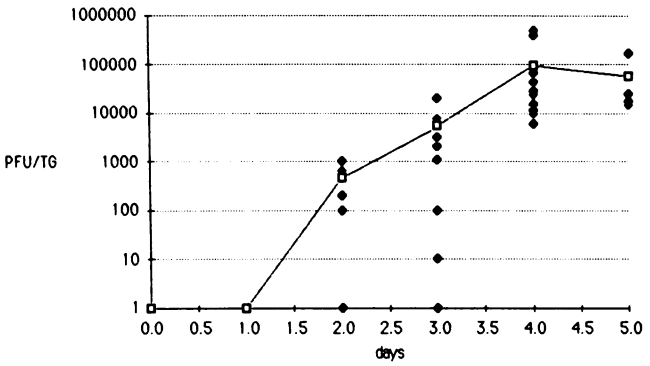

FIG. 1. Explant reactivation of HSV-1 from the trigeminal ganglia of latently infected mice. Ganglia were explanted from latently infected mice (≥4 weeks postinfection) and incubated in Eagle minimum essential medium–5% fetal calf serum at 37°C, with 5% CO_2. At each time point after explant the ganglia were Dounce homogenized and the amount of infectious HSV-1 was determined by plaque assay with CV-1 cells (28). Symbols: ◆, individual ganglion; □, ganglia averages. PFU/TG, PFU per trigeminal ganglion. Day 0 was the time of explant. Points on the abscissa indicate ganglia in which no HSV-1 was detected.

TABLE 1. Effects of topoisomerase inhibitors and PAA on HSV-1 reactivation[a] from the trigeminal ganglia of latently infected mice

Compound or titer/concn	No. of mice with reactivated HSV-1/total no.
Novobiocin	
80 μM	(14/39)
160 μM	(0/15)[b]
Coumermycin A_1	
4.5 μM	(6/7)
9 μM	(0/15)
18 μM	(0/4)
Nalidixic acid	
862 μM	(7/7)
2,600 μM	(4/4)
Oxolinic acid	
766 μM	(7/7)
2,600 μM[c]	(4/4)
PAA	
0.18 mM	(8/8)
0.36 mM	(7/8)
0.71 mM	(5/9)
1.4 mM	(0/12)
Controls (no drug)	
0	(44/44)[d]
Titer at explant	
0	(0/16)

[a] Trigeminal ganglia of latently infected mice were incubated in explant culture for 4 days and then homogenized, and titers were determined (28). PAA, Phosphonoacetic acid.

[b] No plaques were detected, even when the undiluted homogenates were plated onto CV-1 cell dilutions. Reactivation was considered positive if even a single plaque was detected.

[c] This concentration of oxolinic acid was above the solubility limit.

[d] The virus yield in untreated ganglia was 10^4 to 10^6 PFU per trigeminal ganglion.

TABLE 2. Effects of topoisomerase inhibitors and PAA on HSV-1 replication[a] in CV-1 and C1300 cells

Compound/concn	HSV-1 yield (log_{10} vs control)	
	CV-1[b]	C1300[c]
Novobiocin		
80 μM	−0.1	−1.3
160 μM	M−0.5	−2.9
Coumermycin A_1		
4.5 μM	−0.2	NT[d]
9 μmM	−0.5	−2.1
18 μM	−0.9	NT
Nalidixic acid		
862 μM	−1.1	−0.2
Oxolinic acid		
766 μM	−0.5	−0.7
PAA		
0.18 mM	−0.4	NT
0.36 mM	−1.0	NT
0.71 mM	−2.4	NT
1.4 mM	−3.7	NT
2.9 mM	−4.1	NT

[a] Compounds were present continuously from 1 h prior to infection until the cultures were freeze-thawed at 20 to 24 h postinfection and until titers were determined (28). PAA, Phosphonoacetic acid.

[b] The average yield in control cultures was 200 PFU per cell.

[c] The average yield in control cultures was 30 PFU per cell.

[d] NT, Not tested.

respectively). Phosphonoacetic acid (1.4 mM) did not inhibit the incorporation of any precursor significantly (data not shown), as previously reported (24). UV light can stimulate the reactivation of latent HSV-1 (3, 6, 11), and repair DNA synthesis stimulated by UV-induced DNA damage is sensitive to novobiocin (4, 19). These results and those described above (Fig. 2) suggest that the inhibition of reactivation was due to an effect on one or more events involved in, or prior to, HSV-1 DNA synthesis.

Novobiocin and coumermycin A_1 may affect the ability of C1300 cells or cells in ganglia to express cellular functions required for HSV-1 permissiveness or may affect viral functions necessary for latent HSV-1 to reenter the replication cycle. The induction of early viral antigens in cells latently infected with Epstein-Barr virus, after a reactivation stimulus, can be blocked reversibly by novobiocin and coumermycin A_1 (12). Other possible explanations for the inhibition by novobiocin and coumermycin A_1 of HSV-1 replication in ganglia and C1300 cells but not in CV-1 cells include differences in the cellular topoisomerases present, drug uptake, or metabolism.

Topoisomerase activities have been found in many cell types (5, 10, 30, 31) and are induced in vaccinia virus (1, 8)- and HSV-1-infected cells (2, 16, 21). The sensitivity of the HSV-1-induced topoisomerase activity to inhibitors is not known, but a vaccinia virus-encoded enzyme is inhibited 50% by 180 μM novobiocin (8). *Drosophila* topoisomerase type II is inhibited by novobiocin and coumermycin A_1, with K_is of 210 and 10 μM, respectively (22). These levels are similar to those that inhibited HSV-1 reactivation (Table 1). However, it would be speculative to propose that the HSV-1-induced topoisomerase activity plays a role in HSV-1 replication, latency, or reactivation.

The data do not allow us to distinguish between the effects

TABLE 3. Effect of novobiocin and coumermycin A_1 on the replication of HSV-1 in acutely infected trigeminal ganglia

Treatment	No. of infectious HSV-1 ± SD (PFU/ganglion)		Log_{10} vs control
	At explant[a]	4 days postexplant	
Control	$1.3 \times 10^4 \pm 0.8 \times 10^4$ (4)	$5.4 \times 10^5 \pm 2.4 \times 10^5$ (5)	
Novobiocin (160 μM)		$8.0 \times 10^2 \pm 8.3 \times 10^2$ (5)	−2.8
Coumermycin A_1 (9 μM)		$4.5 \times 10^3 \pm 2.8 \times 10^3$ (5)	−2.1

[a] Ganglia were explanted 3 days after the mice were infected, which was the earliest time that HSV-1 could be consistently detected. Compounds were added to the media at the time of explant and were present at 4 days, at which time the ganglia were assayed for infectious HSV-1 (28; see the legend to Fig. 1).

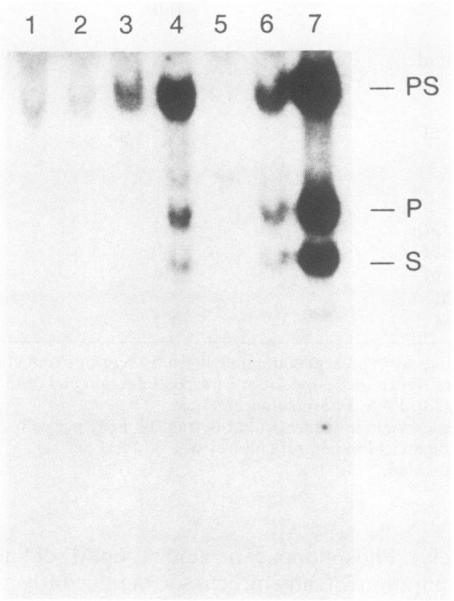

FIG. 2. Structure of HSV-1 DNA during latency and reactivation and the effect of inhibitors. DNA was extracted from latent trigeminal ganglia (27) at explant (lane 1) and after explant reactivation for 4 days in the presence of 160 μM novobiocin (lane 2) or 9 μM coumermycin A_1 (lane 3) or without added compounds (diluted 1:100 with mouse DNA; lane 4). Uninfected mouse brain control (lane 5) and 10^{-5} and 10^{-4} dilutions of HSV-1 DNA in control mouse DNA (lanes 6 and 7) are included for comparison. The DNA samples were digested with *Bam*HI, electrophoresed through 0.7% agarose, Southern blotted to nitrocellulose, and hybridized to a nick-translated (17) *Bam*HI PS fragment probe (25), as previously described (26, 27). Kodak XAR film was exposed for 3 days. The positions of the *Bam*HI restriction fragments PS, P, and S are labeled.

of novobiocin and coumermycin A_1 on the virus or on the host cell. Indeed, it should be emphasized that the possibility that they inhibit reactivation and replication in trigeminal ganglia through a mechanism other than an effect on a DNA topoisomerase of cellular or viral origin cannot be ruled out. However, the reactivation system presented in this manuscript may be of value to identify the critical events during the reactivation of latent HSV-1, to study the effect of inhibitors, and to analyze HSV-1 mutants.

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