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Effects of Adenosine Monophosphate on the Reactivation of Latent Herpes Simplex Virus Type 1 Infections of Mice

WILLIAM T. BLUE,* ROGER D. WINLAND, DARLENE G. STOBBS, DONNY F. KIRKSEY, AND RUSSELL E. SAVAGE

Department of Zoology and Microbiology, and the Biomedical Sciences Division, College of Osteopathic Medicine, Ohio University, Athens, Ohio 45701

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Adenosine monophosphate pretreatment of mice with latent herpes simplex virus type 1 infections significantly reduced the rate of reactivation of latent virus. Adenosine monophosphate pretreatment did not, however, eradicate latent virus.

Recently, Sklar and Buimovici-Klein (8) presented evidence suggesting that adenosine monophosphate (AMP), a naturally occurring cellular metabolite, is an effective treatment for recurrent herpes simplex virus type 1 (HSV-1) infections in humans. They also suggested that AMP could prevent the establishment of a latent infection and that it may eradicate latent HSV-1 from the central nervous system. We therefore tested AMP for these effects in a mouse model.

The mouse ear model described by Hill et al. (4, 5) was initially utilized in our studies. The right ear pinnae of 4-week-old Swiss white mice were inoculated subdermally with 50 μl of HSV-1 (strain LP, obtained from Bernard Roizman) containing 8×10^4 plaque-forming units. Lesions of ear pinnae, characterized by inflammation or vesicle formation, were allowed to heal completely. After 2 weeks, the areas of these former lesions were stripped six times with cellophane tape. Because the inflammation associated with tape stripping often persisted for 2 to 5 days and overlapped with that due to true reactivation, an alternative reactivation method which did not produce physical trauma was developed and applied. This procedure involved intraperitoneal administration of amphetamine sulfate. As the results of a comparative study (Table 1, groups II and III) show, the primary reactivation rates after injection of 5 mg of amphetamine sulfate per kg of body weight and stripping were essentially identical. In different experiments, reactivation rates by either procedure ranged from 40 to 50%. The data also show that over 80% of the animals already shown to harbor latent virus (via amphetamine reactivation) could be reactivated once again with amphetamine (Table 1, group IV).

AMP (Chemical Dynamics Corp., Plainfield, N.J.) was next tested for its ability to prevent amphetamine-induced reactivation of latent HSV-1. At 1 h before amphetamine administration, animals with healed primary HSV-1-induced lesions received 2 mg of AMP per kg of body weight intraperitoneally and were observed for 7 days for reactivation of lesions. The results shown for group V indicate that AMP pretreatment significantly reduced the reactivation rate. When mice from the same group were allowed to rest for 2 weeks and were then retreated with amphetamine, but without AMP pretreatment, approximately 43% exhibited reactivation of the lesions (Table 1, group VI). These results show that although AMP can inhibit reactivation of lesions by latent HSV-1, it does not eradicate latent virus. AMP was also tested for the ability to inhibit secondary rounds of amphetamine reactivation; i.e., a population of mice was used which contained only animals previously demonstrated to harbor latent virus (reactivated animals from group III). As the data show, AMP pretreatment again significantly reduced the reactivation rate to 11% from an expected 80% (Table 1, group VII).

Mice which were pretreated with AMP demonstrated virtually identical physical reactions to amphetamine administration as did untreated animals. Nevertheless, to rule out the possibility that AMP may somehow block the reactivating ability of amphetamine by some nonspecific interaction with the drug, rather than a specific effect on the cells harboring latent virus, we repeated the AMP inhibition studies, utilizing stripping as the reactivant instead of amphetamine. As the data show, AMP pretreatment significantly reduced the stripping reactivation rate (Table 1, group VIII).

Finally, AMP was tested for the ability to prevent the symptoms of a primary infection or the establishment of a latent infection. Mice were split into two groups of 20 which were either infected with HSV-1 alone or were pretreated with AMP (2 mg per kg of body weight) 1 h before HSV-1 infection. Only 1 of 20 animals

TABLE 1. *Inhibition of reactivation of latent HSV-1 by AMP*

Group[a] (no. of expt)	Reactivation treatment	Total no. of animals tested	No. of animals reactivated (%)
I (2)	None	100	0 (0)
II (3)	Stripping	123	53 (43.1)
III (6)	Amphetamine	379	161 (42.5)
IV (2)	Amphetamine[b]	59	49 (83)
V (2)	AMP + amphetamine	92	4 (4.3)[c]
VI (1)	amphetamine[d]	23	10 (43.4)
VII (1)	AMP + amphetamine[e]	18	2 (11.1)[f]
VIII (1)	AMP + stripping	20	2 (10)[g]

[a] All animals used for subsequent reactivation treatments had developed an initial, observable ear infection after inoculation of 8×10^4 plaque-forming units of HSV-1 into their right ear pinnae.

[b] These were 59 animals from group III which had been allowed to heal after an initial reactivation with amphetamine.

[c] Compared with group III, $t_x = 8.62$ and $P < 0.001$.

[d] These were 23 animals from group V which did not develop a recurrent ear infection after initial treatment with AMP plus amphetamine.

[e] These were 18 animals from group III which had been allowed to heal after an initial reactivation with amphetamine.

[f] Compared with group IV, $t_{60} = 5.99$ and $P < 0.001$.

[g] Compared with group II, $t_{120} = 3.27$ and $P < 0.001$.

pretreated with AMP developed observable signs of infection, as compared with 17 in the control (untreated) group. Two weeks after healing, all of the animals were treated with amphetamine for reactivation. None of the AMP-pretreated animals showed the symptoms of reactivation, whereas eight of the control animals did (47% of the animals which showed a primary lesion). These results indicate that AMP pretreatment could prevent the establishment of both primary and latent HSV-1 infections in some mice.

Our results indicate that AMP has prophylactic efficacy against both primary and recurrent HSV-1 infections in mice. Therapeutic trials, i.e., use of the agent after the establishment of a primary or recurrent HSV-1 infection, are currently under way. These results would, however, support the observations of Sklar and Buimovici-Klein (8) on the possible efficacy of AMP treatment of human patients, although they do not support eradication of latent virus as a possible mechanism of AMP action. The actual mechanism of action of AMP is unknown. An attractive hypothesis is that it acts via adenosine, which recently has been widely investigated for its effects on cells of the nervous system (1, 3, 9). Of several effects described, adenosine binding to specific cell membrane receptors decreased intracellular cyclic AMP levels, thus depressing cellular metabolism (1, 10). A depressed cellular metabolic rate may render cells harboring latent virus incapable of supporting virus replication. Recently, Schnitzlein and Reichmann (7) have shown that adenosine inhibits replication of vesicular stomatitis virus ribonucleic acid by a mechanism as yet unknown. AMP, in contrast, does not appear to directly inhibit replication of HSV-1 in tissue culture cells (T. North, personal communication). It would thus appear doubtful that AMP has any direct antiviral activity against HSV-specific enzymes, as do arabinosyl adenine (6) and acyclovir (2). However, if the efficacy of AMP is confirmed, especially in humans, it offers the advantage over other currently available or experimental antiherpetic agents of being relatively nontoxic, and it would be doubtful that resistant mutants should arise. One might also expect that AMP would be effective against HSV-2 and varicella-zoster virus, both of which remain latent in nervous tissues. Verification awaits testing in suitable animal models or in humans.

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