ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1986, p. 719–724
0066-4804/86/110719-06$02.00/0

Vol. 30, No. 5

Xylotubercidin against Herpes Simplex Virus Type 2 in Mice

ERIK DE CLERCQ[1]* AND MORRIS J. ROBINS[2]

Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Louvain, Belgium,[1] and Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2, Canada[2]

Received 17 June 1986/Accepted 13 August 1986

Of a series of newly synthesized derivatives of the pyrrolo[2,3-*d*]pyrimidine nucleosides tubercidin, toyocamycin, and sangivamycin, the xylosyl analog of tubercidin, xylotubercidin, exhibited the greatest potency and selectivity against herpes simplex virus type 2 (HSV-2) in vitro. At dosage regimens that were not toxic for the host, xylotubercidin proved efficacious in various HSV-2 animal model infections. When applied topically at 0.25, 0.5, or 1% in dimethyl sulfoxide or when administered systemically (intraperitoneally) at 12.5 or 25 mg/kg per day, xylotubercidin suppressed the development of herpetic skin lesions and the paralysis and mortality associated therewith in hairless mice inoculated intracutaneously with HSV-2. In this model, acyclovir was effective only if administered topically at 5 or 10% in dimethyl sulfoxide. When administered intraperitoneally over a dosage range of 10 to 50 mg/kg per day, xylotubercidin achieved a significant reduction in the mortality rate of mice infected intraperitoneally with HSV-2. Under the same conditions, acyclovir did not offer any protection even when administered at doses up to 250 mg/kg per day. Xylotubercidin thus appears to have considerable potential for both topical and systemic treatment of HSV-2 infections.

Acyclovir (Zovirax) is the only antiviral compound that has been licensed for the treatment of genital herpes. The compound has proven to be effective in the systemic and topical treatment of herpes simplex virus (HSV) type 2 (HSV-2) infections in both mice (11, 25, 31, 38) and guinea pigs (39, 43). Various clinical studies have demonstrated that acyclovir, when given systemically (either intravenously or perorally) or applied topically, is efficacious in the treatment of primary genital herpes (4, 7, 8, 29, 30) and, to a lesser extent, recurrent genital herpes (8, 30, 32, 33). Furthermore, oral administration of acyclovir has been shown to markedly reduce (but not completely abrogate) recurrences of genital HSV infection (17, 42), when administered daily for an extended time period (i.e., 4 months), and therefore, acyclovir could be advocated for long-term use in the prophylaxis of recurrent genital herpes.

Considering the possibility of breakthrough recurrences of genital herpes, as well as the risk for long-term side effects and, in particular, the emergence of drug-resistant virus strains that might be associated with a prolonged course of acyclovir treatment, it may be useful to have at hand alternative therapeutic modalities for the treatment of HSV-2 infections. These drugs should preferably act by a different mechanism than acyclovir and hence be effective against acyclovir-resistant HSV mutants which, as arising in the clinic, are mostly of the thymidine kinase-deficient (TK^-) type (5, 9, 40, 44).

Candidate antiviral drugs that have proven to be effective against HSV-2 infections in animal models and that therefore seem worth pursuing for their potential in the treatment of genital herpes and HSV-2 infections in general include cyclaradine (43) as well as its predecessor vidarabine (34); the 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)pyrimidine derivatives FMAU, FIAC, and FIAU (27, 28); 5-ethyl-2′-deoxyuridine (EDU) (39); phosphonoformate (1, 23, 24, 28); and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (21), particularly when combined with interferon (18–20). Cyclaradine, vidarabine, and phosphonoformate thereby offer the advantage that they should be effective against TK^- HSV strains as their antiviral activity is not dependent on phosphorylation by the viral thymidine kinase.

In this report we describe the potential of xylotubercidin, the xylosyl derivative of tubercidin (7-deazaadenosine), for the topical and systemic treatment of HSV-2 infections. In contrast with its parent compound, tubercidin, which was not selective in its antiviral activity, xylotubercidin inhibited the replication of HSV-2 in vitro at concentrations well below the cytotoxic concentrations; and these observations extended to the in vivo situation in which xylotubercidin was found to suppress the development of cutaneous HSV-2 lesions and mortality associated with HSV-2 infection when administered topically or systemically to mice at doses which were not toxic for the host.

MATERIALS AND METHODS

Test compounds. Tubercidin was purchased from The Upjohn Co. (Kalamazoo, Mich.). Toyocamycin, sangivamycin, and xylotubercidin (6, 35), and the arabinosyl (22), 2′-deoxyribosyl (37), 3′-deoxyribosyl (36), and 2′-deoxyxylosyl derivatives of tubercidin, toyocamycin, and sangivamycin were obtained from M. J. Robins. The synthesis and in vitro biological properties of the sugar-modified analog of tubercidin, toyocamycin, and sangivamycin, which all are pyrrolo[2,3-*d*]pyrimidine nucleosides, will be published elsewhere. Acyclovir (acycloguanosine; Zovirax) was purchased from Wellcome (Aalst, Belgium). The formulas of tubercidin, toyocamycin, sangivamycin, and xylotubercidin are presented in Fig. 1.

Viruses. The origin of the viruses was as follows: HSV type 1 (HSV-1) (KOS), HSV-1 (F), HSV-1 (McIntyre), TK^- HSV-1 (B2006), HSV-2 (G), HSV-2 (196), HSV-2 (Lyons), vaccinia virus, and vesicular stomatitis virus (14); coxsackievirus type B4, poliovirus type 1, Sindbis virus, and measles virus, (15); reovirus type 1 (ATCC VR-230) and parainfluenza virus type 3 (ATCC VR-93) were obtained from the American Type Culture Collection (Rockville, Md.). The TK^- HSV-1 [C1 (101)] strain was obtained from H. J. Field (Cambridge, England). The TK^- HSV-1 (VMW-1837) strain was isolated from an immunosuppressed patient who had

* Corresponding author.

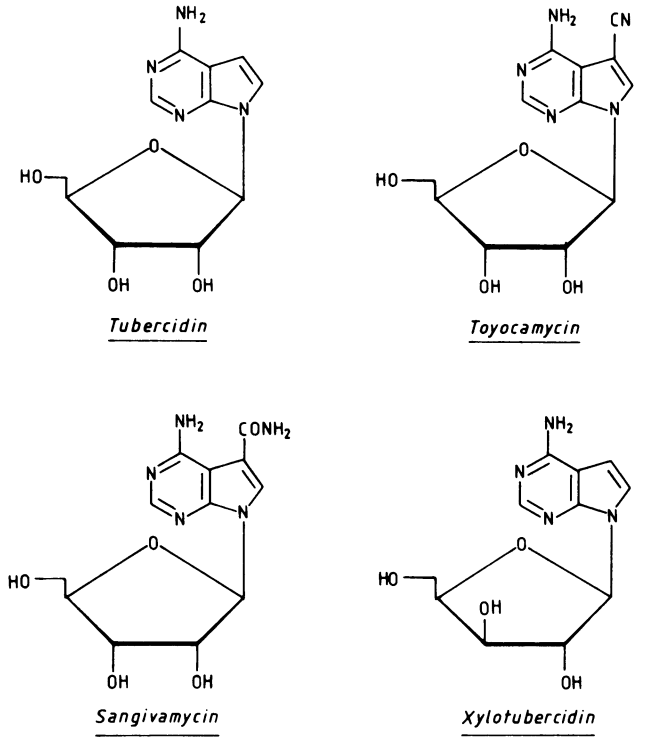

FIG. 1. Formulas of tubercidin, toyocamycin, sangivamycin, and xylotubercidin.

been treated with acyclovir for an oral HSV-1 infection and who had become resistant to acyclovir. The HSV-1 strain isolated from this patient was resistant to acyclovir and coresistant to several antiviral drugs including EDU. The virus stocks were grown in primary rabbit kidney (PRK) cells (HSV-1, HSV-2, and vesicular stomatitis virus), HeLa cells (poliovirus), African green monkey kidney (Vero) cells (coxsackievirus B4, measles virus, reovirus, and parainfluenza virus), chick embryo cells (Sindbis virus), or chorioallantoic membrane cells (vaccinia virus).

Mice. Either hairless (*hr/hr*) mice (age, 25 to 30 days; weight, 18 to 20 g) or Naval Medical Research Institute (NMRI) mice (age, 15 days; weight, 10–12 g) were used for the in vivo experiments. The NMRI mice were randomly bred; the hairless mice were bred by backcross and intercross of the homozygous parents. All the mice were obtained from the Animal Production Center (Proefdierencentrum) of the Katholieke Universiteit Leuven. They were housed under conventional conditions in groups of five with unrestricted access to food and drinking water. Female and male mice were used at random in all experiments.

In vitro tests. The antiviral assays were based on inhibition of virus-induced cytopathogenicity in PRK, HeLa, or Vero cell cultures, following previously established procedures (14, 15). The compounds were added immediately after the 1-h virus adsorption period. In tests that were run in parallel with the antiviral assays, the compounds were examined for their effects on the morphology of normal, uninfected cell cultures, which had been in contact with the compounds for the same time period as the virus-infected cells. A microscopically detectable disruption of the normal cell morphology was taken as the endpoint of cytotoxicity. Cytotoxicity was also assessed by inhibition of cell proliferation; these tests were carried out with murine leukemia L-1210 cells in their exponential growth phase (13). Inhibition of DNA, RNA, and protein synthesis was evaluated, as described previously (13), by monitoring the incorporation of [*methyl*-^{3}H]thymidine, [5-^{3}H]uridine, and [4,5-^{3}H]leucine, respectively.

TABLE 1. Comparative cytotoxicity and antiviral activity of different pyrrolopyrimidine nucleoside analogs in primary rabbit kidney cell cultures

Compound	$MIC_{1/2}$ (μg/ml)[a]		MTC (μg/ml)[b]
	HSV-1 (KOS)	HSV-2 (G)	
Tubercidin	0.1	0.1	0.4
Toyocamycin	≥0.04	≥0.04	0.04
Sangivamycin	0.07	0.07	1
Aratubercidin	125	40	>400
Aratoyocamycin	7	2	10
Arasangivamycin	4	1	40
Xylotubercidin	0.2	0.07	10
2′-Deoxytubercidin	50	40	>400
2′-Deoxytoyocamycin	≥1	0.3	4
2′-Deoxysangivamycin	2	1	10
3′-Deoxytubercidin	≥10	≥10	10
3′-Deoxytoyocamycin	≥0.4	≥0.4	1
3′-Deoxysangivamycin	≥10	≥10	10
2′-Deoxyxylotubercidin	70	70	>400
2′-Deoxyxylosangivamycin	70	70	>400

[a] MIC required to reduce virus-induced cytopathogenicity by 50%. Values are means for at least three independent experiments.
[b] Minimum toxic concentration (MTC) required to cause a microscopically detectable change in morphology of normal uninfected cells treated with the compounds and run in parallel with the infected treated cells.

In vivo tests. In vivo antiviral activity was explored in hairless mice inoculated intracutaneously in the lumbosacral area with HSV-2 (196) at $10^{3.7}$ PFU/0.05 ml per mouse. For topical use, the test compounds were formulated in dimethyl sulfoxide (DMSO) at different concentrations (ranging from

TABLE 2. Antiviral activity spectrum of xylotubercidin

Virus	Cell system	$MIC_{1/2}$ (μg/ml)[a]
HSV-1 (KOS)	PRK	0.2
HSV-1 (F)	PRK	0.2
HSV-1 (McIntyre)	PRK	0.2
TK$^-$ HSV-1 (B2006)	PRK	0.7
TK$^-$ HSV-1 [C1(101)]	PRK	0.4
TK$^-$ HSV-1 [VMW-1837]	PRK	0.7
HSV-2 (G)	PRK	0.07
HSV-2 (196)	PRK	0.1
HSV-2 (Lyons)	PRK	0.07
Vaccinia	PRK	0.3
Vesicular stomatitis	PRK	≥10
(Cytotoxicity	PRK	10[b])
Vesicular stomatitis	HeLa	≥4
Coxsackie B4	HeLa	≥4
Polio type 1	HeLa	≥4
(Cytotoxicity	HeLa	4[b])
Reo type 1	Vero	≥40
Parainfluenza type 3	Vero	≥40
Sindbis	Vero	≥40
Coxsackie B-4	Vero	≥40
Measles	Vero	≥40
(Cytotoxicity	Vero	40[b])

[a] See footnote *a* to Table 1.
[b] See footnote *b* to Table 1.

TABLE 3. Effects of tubercidin, toyocamycin, sangivamycin, xylotubercidin, and acyclovir on the development of herpetic skin lesions, paralysis of the hind legs, and mortality of hairless mice inoculated intracutaneously with HSV-2 (196)[a]

Compound	Concn (%)	No. of mice	No. of survivors on the following day after virus inoculation:						% survivors
			4	6	8	10	15	20	
Tubercidin	0.25[b]	10	10 (0/0)[c]	9 (0/0)	9 (0/1)	8 (0/0)	8 (0/0)	8 (0/0)	80
	0.1[b]	10	10 (0/0)	10 (2/0)	7 (1/1)	5 (0/0)	5 (0/0)	5 (0/0)	50
	0.05	10	10 (2/0)	10 (2/4)	4 (1/1)	3 (0/1)	2 (0/0)	2 (0/0)	20
	0.025	10	10 (3/0)	10 (6/2)	4 (0/2)	2 (0/0)	1 (0/0)	1 (0/0)	10
	0	10	10 (3/0)	9 (4/3)	2 (0/2)	0	0	0	0
Toyocamycin	0.25[b]	10	5 (0/0)	5 (0/0)	4 (0/0)	2 (0/0)	0	0	0
	0.1[b]	10	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	8 (0/0)	8 (0/0)	80
	0.05[b]	10	10 (0/0)	10 (0/0)	10 (0/0)	9 (0/0)	9 (0/0)	9 (0/0)	90
	0.025[b]	10	10 (0/0)	9 (2/1)	6 (1/2)	3 (0/0)	3 (0/0)	3 (0/0)	30
	0	10	10 (0/0)	7 (3/3)	1 (0/1)	0	0	0	0
Sangivamycin	0.25[b]	10	8 (0/0)	2 (0/0)	0	0	0	0	0
	0.1[b]	10	10 (0/0)	10 (0/0)	9 (0/0)	9 (0/0)	9 (0/0)	9 (0/0)	90
	0.05[b]	10	10 (0/0)	10 (1/0)	8 (1/0)	7 (0/0)	5 (0/0)	5 (0/0)	50
	0.025[b]	10	10 (1/0)	10 (2/3)	6 (1/0)	3 (1/0)	2 (0/0)	2 (0/0)	20
	0	10	10 (3/0)	8 (7/1)	0	0	0	0	0
Xylotubercidin	1	10	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	100
	0.5	10	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	8 (0/0)	8 (0/0)	80
	0.25	10	10 (0/0)	10 (0/0)	10 (1/0)	9 (0/0)	8 (0/0)	8 (0/0)	80
	0.1	10	10 (0/0)	10 (3/0)	8 (3/3)	4 (1/1)	1 (0/0)	1 (0/0)	10
	0	10	10 (0/0)	6 (4/2)	1 (0/1)	0	0	0	0
Acyclovir	10	10	10 (0/0)	10 (0/0)	10 (0/0)	10 (1/0)	9 (0/0)	9 (0/0)	90
	5	10	10 (0/0)	10 (0/0)	10 (0/2)	8 (1/0)	7 (0/1)	6 (0/0)	60
	2.5	10	10 (0/0)	10 (4/0)	8 (3/3)	4 (1/2)	1 (0/0)	1 (0/0)	10
	1	10	10 (0/0)	10 (3/2)	6 (3/2)	5 (0/4)	0	0	0
	0	10	10 (2/0)	9 (3/6)	1 (0/1)	0	0	0	0

[a] Drugs were at concentrations ranging from 0.025 to 10% in DMSO.
[b] Local toxicity (erythema, crust formation) was noted at this concentration; toyocamycin and sangivamycin also showed systemic toxicity (as reflected by the 0% survival rate) at a concentration of 0.25%.
[c] Numbers in parentheses are number of mice with herpetic skin lesions only/number of mice with herpetic skin lesions plus paralysis.

0.025 to 10%; wt/vol) and applied topically four times a day (at 9 a.m., 11 a.m., 2 p.m., and 4 p.m.) for 5 days, starting immediately after virus infection. For systemic use, the test compound was injected intraperitoneally twice a day at a daily dose of 5, 12.5, 25, or 50 mg/kg for 5 days, starting immediately after virus infection. For the hairless mice inoculated intracutaneously with HSV-2, several parameters of infection were followed: (i) herpetic skin lesions, presenting as epidermal ulcerations and considered as positive if they were at least 0.5 cm long, (ii) paralysis of the hind legs (invariably accompanied by herpetic skin lesions), and (iii) the number of survivors (survival rate) at 1 through 20 days postinfection.

In vivo antiviral activity was also determined in NMRI mice inoculated intraperitoneally with HSV-2 (196) at 100 times the 50% cell culture infective dose per 0.2 ml per mouse and treated subsequently by the test compounds given via the intraperitoneal route twice a day (at a daily dose of 10, 25, 50, 100, or 250 mg/kg) for 5 days, starting immediately after virus infection. The survival rate of the mice was followed until day 20 postinfection.

Statistical significance of the differences in the final survival rates (20 days postinfection) was assessed by the χ^2 test with the Yates correction for small numbers.

RESULTS

Of the various pyrrolopyrimidine nucleoside analogs that were evaluated for their anti-HSV activity in vitro (Table 1), xylotubercidin emerged as the most selective inhibitor of both HSV-1 and HSV-2. It inhibited the cytopathic effect of HSV-2 (G) at a concentration of 0.07 μg/ml; that is, about 150-fold lower than the concentration at which the compound itself proved cytopathic, as based on either alteration of cell morphology (Table 1) or inhibition of DNA, RNA, or protein synthesis (E. De Clercq, J. Balzarini, D. Madej, F. Hansske, and M. J. Robins, J. Med. Chem., in press). Other pyrrolopyrimidine nucleosides, i.e., tubercidin, toyocamycin, and sangivamycin, were equally or even more potent than xylotubercidin as inhibitors of HSV-1 or HSV-2; but in contrast with xylotubercidin, these other compounds showed little, if any, selectivity as antiviral agents. Xylotubercidin was also active against TK^- HSV-1 strains (Table 2). However, RNA viruses, i.e., vesicular stomatitis, coxsackie, polio, reo, parainfluenza, Sindbis, and measles, were not inhibited, unless xylotubercidin was used at concentrations that were toxic for the host cells.

When evaluated for its efficacy in the topical treatment of an intracutaneous HSV-2 infection in hairless mice, xylotubercidin suppressed the development of herpetic skin lesions, paralysis of the legs, and mortality, when administered at a concentration of 0.25, 0.5, or 1% in DMSO (Table 3). With these treatment regimens the final survival rate was increased from 0 to 80, 80, and 100%, respectively. Evidence of either local or systemic toxicity for the host was not observed with xylotubercidin applied topically at 0.25, 0.5, or 1%. Local toxicity (erythema, crust formation) and systemic toxicity (about 40% mortality) became apparent, however, when xylotubercidin was used topically at 2.5% (data not shown).

Tubercidin, toyocamycin, and sangivamycin also conferred a protective effect against the different parameters of intracutaneous HSV-2 infection in hairless mice (Table 3), when applied at the appropriate concentrations (0.1 and 0.25% for tubercidin, 0.05 and 0.1% for toyocamycin and sangivamycin); but at these concentrations, and for

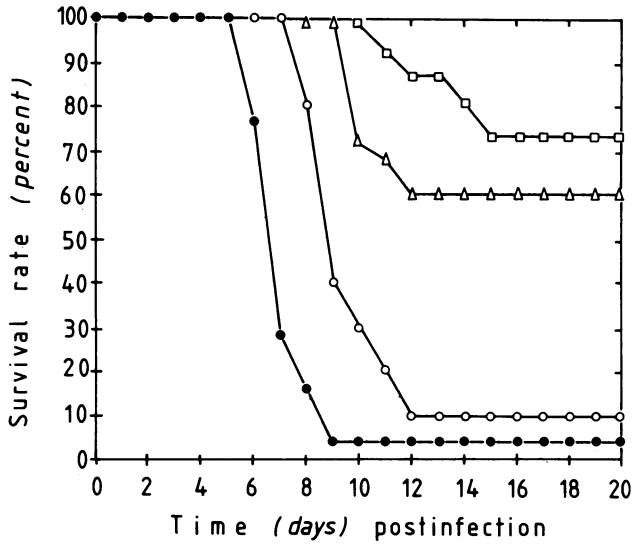

FIG. 2. Survival rate of hairless mice inoculated intracutaneously with HSV-2 (196) and treated intraperitoneally with placebo (25 mice [●]) or xylotubercidin at 5 mg/kg per day (10 mice [○]), 12.5 mg/kg per day (15 mice [△]), or 25 mg/kg per day (15 mice [□]).

toyocamycin and sangivamycin even at lower concentrations, the compounds provoked local erythema and crust formation. This local toxicity was dose dependent, and thus more pronounced with increasing concentrations of the compound; and at 0.25%, toyocamycin and sangivamycin were lethal for the mice (Table 3).

Acyclovir, which was included as the reference compound in the experiments with intracutaneous HSV-2 infection in hairless mice (Table 3), caused, in agreement with previous results (11), a significant increase in the final survival rate, i.e., from 0 to 60 and 90%, when applied topically at 5 or 10%, respectively. When used at 2.5 or 1%, acyclovir offered only transient protection, without affecting the final mortality rate.

When xylotubercidin was administered intraperitoneally, it again proved effective in protecting hairless mice against an intracutaneous HSV-2 infection, as reflected by the increase in the final survival rate from 4% (placebo) to 60% ($P < 0.001$) and 73% ($P < 0.001$) following administration of xylotubercidin at a daily dose of 12.5 and 25 mg/kg, respectively (Fig. 2). At 5 mg/kg per day xylotubercidin only conferred transient protection without a significant change in the final mortality rate, whereas at 50 mg/kg per day it was lethal for about 50% of the mice.

To establish whether xylotubercidin may also be efficacious against a systemic HSV-2 infection, it was administered intraperitoneally to NMRI mice which had been infected with HSV-2 (196) via the intraperitoneal route. Xylotubercidin brought about a significant increase in the survival rate when administered at a dose of 10, 25, or 50 mg/kg per day (Fig. 3A). With these treatment regimens the final survival rate was increased from 14 to 75, 80, and 60%, respectively (all significant at $P < 0.001$). The mortality (35%) observed on days 3 to 5 for the group treated with xylotubercidin at 50 mg/kg per day can be attributed to toxicity of the compound.

When acyclovir was evaluated under the same conditions as xylotubercidin, it did not substantially alter the survival rate of NMRI mice infected intraperitoneally with HSV-2 (Fig. 3B), even if acyclovir was administered at doses up to 250 mg/kg per day. Because acyclovir was lethal for about half (or more) of the mice when administered intraperitoneally at a dose of 500 (or 1,000) mg/kg per day, it was not evaluated against intraperitoneal HSV-2 infection at these dosage regimens.

DISCUSSION

In previous attempts to decrease the cytotoxicity, increase the antiviral activity of tubercidin, or both, a number of C-5-substituted tubercidin analogs were synthesized and examined in a broad variety of antiviral assay systems (3). While substitution of the C-5 hydrogen of tubercidin by a 1-hydroxyethyl, 1-methoxyethyl, or 2-buten-1-yl group made the compounds more selective against reovirus type 1, parainfluenza virus type 3, and coxsackievirus B4, for other viruses, i.e., HSV-1 and HSV-2, the specificity index remained essentially unchanged (3).

In our current attempts to improve the therapeutic ratio of tubercidin, chemical modifications were introduced in the sugar moiety, and among the various sugar-modified analogs of tubercidin that were synthesized, xylotubercidin emerged

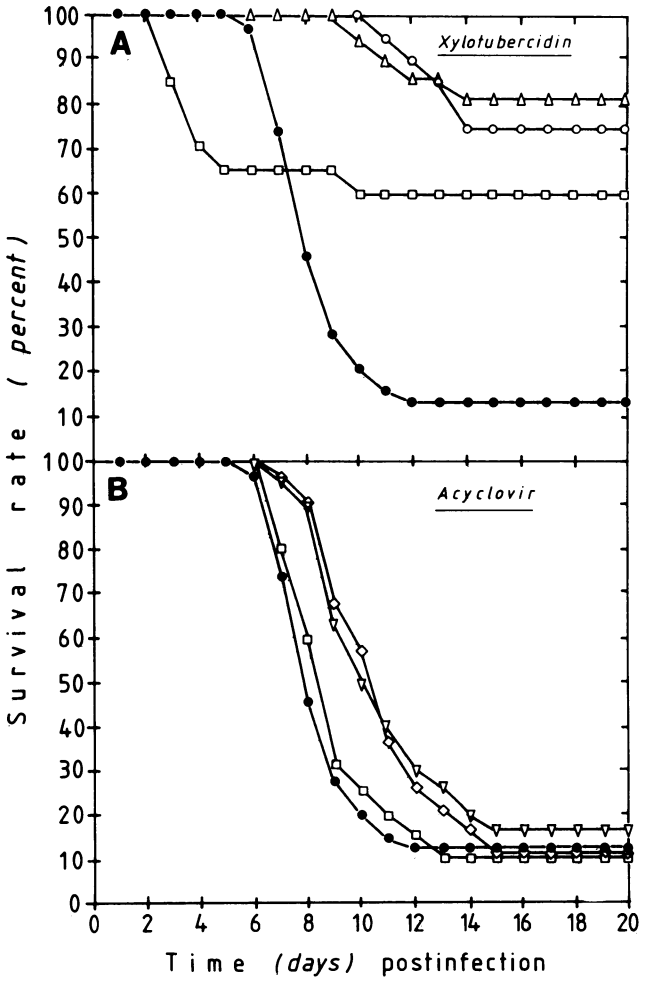

FIG. 3. Survival rate of NMRI mice inoculated intraperitoneally with HSV-2 (196) and treated intraperitoneally with placebo (50 mice [●]); xylotubercidin at 10 mg/kg per day (20 mice [○]), 25 mg/kg per day (20 mice [△]), or 50 mg/kg per day (20 mice [□]) (A); or acyclovir at 50 mg/kg per day (20 mice [□]), 100 mg/kg per day (30 mice [▽]), or 250 mg/kg per day (30 mice [◇]) (B).

as the most selective inhibitor of HSV-1 and HSV-2. As compared with its parent compound, tubercidin, xylotubercidin turned out to be equally potent, but significantly less cytotoxic, thus achieving a specificity index of at least 2 orders of magnitude (Table 1). That xylotubercidin is at least 100-fold less cytotoxic or -static than tubercidin has been confirmed in several cell systems, e.g., murine L-1210 leukemia cells (De Clercq et al., in press) and a subline thereof, L-1210/C2 (6). In the latter system, tubercidin brought about a 50% reduction in cloning efficiency at a concentration of about 0.05 μM, whereas xylotubercidin could be estimated to do so at a concentration of about 5 to 10 μM.

The in vitro results with xylotubercidin indicate that it is a sufficiently selective anti-HSV agent that should be investigated further for its efficacy against HSV infections in animal models. Indeed, when administered at the appropriate dosage regimens (i.e., 0.25, 0.5, or 1%, topically, or 12.5 or 25 mg/kg per day, systemically), xylotubercidin clearly suppressed the clinical manifestations of HSV-2 infection without being toxic for the host (Table 3 and Fig. 2 and 3). However, toxicity became apparent when xylotubercidin was used topically at 2.5% (skin erythema, crust formation, and about 40% mortality) or systemically (intraperitoneally) at 50 mg/kg per day (35 to 50% mortality).

Thus, the concentration margin (or "window") at which xylotubercidin exhibited a protective activity against HSV-2 infection may seem relatively small, but it should be recognized that when acyclovir was compared with xylotubercidin under the same experimental conditions, its activity window for topical application was also narrow (only 5 to 10%), and when acyclovir was used intraperitoneally (against intraperitoneal HSV-2 infection) no activity window was apparent, because acyclovir was not effective up to doses of 250 mg/kg per day and toxic (lethal) from a dose of 500 mg/kg per day upward.

It should also be recognized that intracutaneous HSV-2 infection of hairless mice, as well as intraperitoneal HSV-2 infection of NMRI mice, represent rather stringent models for establishing the efficacy of anti-HSV-2 agents. In the latter model, acyclovir was found to be effective only when given perorally at a dose greater than 500 mg/kg per day (41). In the intracutaneous HSV-2 infection model in hairless mice, only phosphonoacetate and acyclovir have previously been found to be effective when used topically at 5 or 10% (11). Various other antiviral drugs such as EDU and arabinosylthymine, which are known to be active against HSV-2 (14) and which have shown efficacy in a variety of animal models (2, 10, 26), failed to suppress cutaneous HSV-2 lesions in the *hr/hr* mouse model (11). Similarly, arildone (Win 38020) did not show any effect on cutaneous HSV-2 lesions in hairless mice when applied at 5% in DMSO (data not shown), although the structurally related pyrazole derivative Win 41258-3 has proven effective against vaginal HSV-2 infections in mice and intradermal HSV-1 infections in guinea pigs (31).

These considerations make the efficiency of xylotubercidin in the treatment of local and systemic HSV-2 infections all the more interesting. Xylotubercidin should be explored further for its potential in the treatment of genital HSV-2 infections, i.e., both primary and recurrent genital herpes in mice, guinea pigs, or both. In addition, xylotubercidin should be explored for its effects on TK^- HSV infections, because its antiviral activity is not dependent on phosphorylation by the viral thymidine kinase. An animal model based on intracutaneous infection of nude (*nu/nu*) mice with TK^- HSV-1 (VMW-1837) has been recently established in our laboratory and should be useful in determining the efficacy of xylotubercidin against TK^- HSV infections in vivo.

The mechanism of action of xylotubercidin and the basis for its selectivity remain to be determined. Considering the activity spectrum of xylotubercidin (Table 2) in comparison with the activity spectrum of those adenosine analogs, i.e., carbocyclic 3-deazaadenosine (16) and neplanocin A (12), that are assumed to be targeted at *S*-adenosylhomocysteine hydrolase, a feedback inhibitor of transmethylation reactions, it is obvious that xylotubercidin does not act via such a mechanism. Where it precisely acts and whether it needs to be phosphorylated (i.e., by adenosine kinase) to exert its antiviral activity are interesting questions prompting further research.

ACKNOWLEDGMENTS

This work was supported by grants from the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (Krediet no. 3.0040.83), the Belgian Geconcerteerde Onderzoeksacties (Conventie no. 85/90-79), the National Cancer Institute of Canada, and the Natural Sciences and Engineering Research Council of Canada.

We thank Anita Van Lierde, Frieda De Meyer, Willy Zeegers, and Danuta Madej for excellent technical assistance and Christiane Callebaut for fine editorial help.

LITERATURE CITED

1. **Alenius, S., and H. Nordlinder.** 1979. Effect of trisodium phosphonoformate in genital infection of female guinea pigs with herpes simplex virus type 2. Arch. Virol. **60**:197–206.
2. **Aswell, J. F., G. P. Allen, A. T. Jamieson, D. E. Campbell, and G. A. Gentry.** 1977. Antiviral activity of arabinosylthymine in herpesviral replication: mechanism of action in vivo and in vitro. Antimicrob. Agents Chemother. **12**:243–254.
3. **Bergstrom, D. E., A. J. Brattesani, M. K. Ogawa, P. A. Reddy, M. J. Schweickert, J. Balzarini, and E. De Clercq.** 1984. Antiviral activity of C-5 substituted tubercidin analogues. J. Med. Chem. **27**:285–292.
4. **Bryson, Y. J., M. Dillon, M. Lovett, G. Acuna, S. Taylor, J. D. Cherry, B. L. Johnson, E. Wiesmeier, W. Growdon, T. Creagh-Kirk, and R. Keeney.** 1983. Treatment of first episodes of genital herpes simplex virus infection with oral acyclovir. N. Engl. J. Med. **308**:916–921.
5. **Burns, W. H., R. Saral, G. W. Santos, O. L. Laskin, P. S. Lietman, C. McLaren, and D. W. Barry.** 1982. Isolation and characterisation of resistant herpes simplex virus after acyclovir therapy. Lancet **i**:421–423.
6. **Cass, C. E., M. Selner, T. H. Tan, W. H. Muhs, and M. J. Robins.** 1982. Comparison of the effects on cultured L1210 leukemia cells of the ribosyl, 2′-deoxyribosyl, and xylosyl homologs of tubercidin and adenosine alone or in combination with 2′-deoxycoformycin. Cancer Treat. Rep. **66**:317–326.
7. **Corey, L., K. H. Fife, J. K. Benedetti, C. A. Winter, A. Fahnlander, J. D. Connor, M. A. Hintz, and K. K. Holmes.** 1983. Intravenous acyclovir for the treatment of primary genital herpes. Ann. Intern. Med. **98**:914–921.
8. **Corey, L., A. J. Nahmias, M. E. Guinan, J. K. Benedetti, C. W. Critchlow, and K. K. Holmes.** 1982. A trial of topical acyclovir in genital herpes simplex virus infections. N. Engl. J. Med. **306**:1313–1319.
9. **Crumpacker, C. S., L. E. Schnipper, S. I. Marlowe, P. N. Kowalsky, B. J. Hershey, and M. J. Levin.** 1982. Resistance to antiviral drugs of herpes simplex virus isolated from a patient treated with acyclovir. N. Engl. J. Med. **306**:343–346.
10. **Davis, W. B., J. E. Oakes, and J. A. Taylor.** 1978. Effect of treatment with 5-ethyl-2′-deoxyuridine on herpes simplex virus encephalitis in normal and immunosuppressed mice. Antimicrob. Agents Chemother. **14**:743–748.

11. **De Clercq, E.** 1984. Topical treatment of cutaneous herpes simplex virus infection in hairless mice with (*E*)-5-(2-bromovinyl)-2′-deoxyuridine and related compounds. Antimicrob. Agents Chemother. **26**:155–159.
12. **De Clercq, E.** 1985. Antiviral and antimetabolic activities of neplanocins. Antimicrob. Agents Chemother. **28**:84–89.
13. **De Clercq, E., J. Balzarini, P. F. Torrence, M. P. Mertes, C. L. Schmidt, D. Shugar, P. J. Barr, A. S. Jones, G. Verhelst, and R. T. Walker.** 1981. Thymidylate synthetase as target enzyme for the inhibitory activity of 5-substituted 2′-deoxyuridines on mouse leukemia L1210 cell growth. Mol. Pharmacol. **19**:321–330.
14. **De Clercq, E., J. Descamps, G. Verhelst, R. T. Walker, A. S. Jones, P. F. Torrence, and D. Shugar.** 1980. Comparative efficacy of antiherpes drugs against different strains of herpes simplex virus. J. Infect. Dis. **141**:563–574.
15. **De Clercq, E., M. Luczak, J. C. Reepmeyer, K. L. Kirk, and L. A. Cohen.** 1975. Fluoroimidazoles as antiviral agents and inhibitors of polynucleotide biosynthesis. Life Sci. **17**:187–194.
16. **De Clercq, E., and J. A. Montgomery.** 1983. Broad-spectrum antiviral activity of the carbocyclic analog of 3-deazaadenosine. Antiviral Res. **3**:17–24.
17. **Douglas, J. M., C. Critchlow, J. Benedetti, G. J. Mertz, J. D. Connor, M. A. Hintz, A. Fahnlander, M. Remington, C. Winter, and L. Corey.** 1984. A double-blind study of oral acyclovir for suppression of recurrences of genital herpes simplex virus infection. N. Engl. J. Med. **310**:1551–1556.
18. **Fraser-Smith, E. B., D. A. Eppstein, Y. V. Marsh, and T. R. Matthews.** 1984. Enhanced efficacy of the acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl)guanine in combination with beta-interferon against herpes simplex virus type 2 in mice. Antimicrob. Agents Chemother. **25**:563–565.
19. **Fraser-Smith, E. B., D. A. Eppstein, Y. V. Marsh, and T. R. Matthews.** 1984. Enhanced efficacy of the acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl)guanine in combination with alpha-interferon against herpes simplex virus type 2 in mice. Antimicrob. Agents Chemother. **26**:937–938.
20. **Fraser-Smith, E. B., D. A. Eppstein, Y. V. Marsh, and T. R. Matthews.** 1985. Enhanced efficacy of the acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl)guanine in combination with gamma interferon against herpes simplex virus type 2 in mice. Antiviral Res. **5**:137–144.
21. **Fraser-Smith, E. B., D. F. Smee, and T. R. Matthews.** 1983. Efficacy of the acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl)guanine against primary and recrudescent genital herpes simplex virus type 2 infections in guinea pigs. Antimicrob. Agents Chemother. **24**:883–887.
22. **Hansske, F., D. Madej, and M. J. Robins.** 1984. 2′ and 3′-Ketonucleosides and their *arabino* and *xylo* reduction products: convenient access *via* selective protection and oxidation of ribonucleosides. Tetrahedron **40**:125–135.
23. **Kern, E. R., L. A. Glasgow, J. C. Overall, Jr., J. M. Reno, and J. A. Boezi.** 1978. Treatment of experimental herpesvirus infections with phosphonoformate and some comparisons with phosphonoacetate. Antimicrob. Agents Chemother. **14**:817–823.
24. **Kern, E. R., J. T. Richards, J. C. Overall, Jr., and L. A. Glasgow.** 1981. A comparison of phosphonoacetic acid and phosphonoformic acid activity in genital herpes simplex virus type 1 and type 2 infections in mice. Antiviral Res. **1**:225–235.
25. **Kern, E. R., J. T. Richards, J. C. Overall, Jr., and L. A. Glasgow.** 1983. Acyclovir treatment of experimental genital herpes simplex virus infections. I. Topical therapy of type 2 and type 1 infections of mice. Antiviral Res. **3**:253–267.
26. **Machida, H., M. Ichikawa, A. Kuninaka, M. Saneyoshi, and H. Yoshino.** 1980. Effect of treatment with 1-β-D-arabinofuranosylthymine of experimental encephalitis induced by herpes simplex virus in mice. Antimicrob. Agents Chemother. **17**:109–114.
27. **Mayo, D. R., and G. D. Hsiung.** 1984. Treatment of primary acute genital herpes in guinea pigs by intraperitoneal administration of fluoropyrimidines. Antimicrob. Agents Chemother. **26**:354–357.
28. **Mayo, D. R., H. L. Lucia, and G. D. Hsiung.** 1983. Effect of phosphonoformate on symptomatic genital herpes simplex virus type 2 infection of guinea pigs. Intervirology **19**:26–32.
29. **Mindel, A., M. W. Adler, S. Sutherland, and A. P. Fiddian.** 1982. Intravenous acyclovir treatment for primary genital herpes. Lancet **i**:697–700.
30. **Nilsen, A. E., T. Aasen, A. M. Halsos, B. R. Kinge, E. A. L. Tjøtta, K. Wikström, and A. P. Fiddian.** 1982. Efficacy of oral acyclovir in the treatment of initial and recurrent genital herpes. Lancet **ii**:571–573.
31. **Pancic, F., B. A. Steinberg, G. D. Diana, P. M. Carabateas, W. G. Gorman, and P. E. Came.** 1981. Antiviral activity of Win 41258-3, a pyrazole compound, against herpes simplex virus in mouse genital infection and in guinea pig skin infection. Antimicrob. Agents Chemother. **19**:470–476.
32. **Reichman, R. C., G. J. Badger, M. E. Guinan, A. J. Nahmias, R. E. Keeney, L. G. Davis, T. Ashikaga, and R. Dolin.** 1983. Topically administered acyclovir in the treatment of recurrent herpes simplex genitalis: a controlled trial. J. Infect. Dis. **147**:336–340.
33. **Reichman, R. C., G. J. Badger, G. J. Mertz, L. Corey, D. D. Richman, J. D. Connor, D. Redfield, M. C. Savoia, M. N. Oxman, Y. Bryson, D. L. Tyrrell, J. Portnoy, T. Creigh-Kirk, R. E. Keeney, T. Ashikaga, and R. Dolin.** 1984. Treatment of recurrent genital herpes simplex infections with oral acyclovir. J. Am. Med. Assoc. **251**:2103–2107.
34. **Renis, H. E.** 1977. Chemotherapy of genital herpes simplex virus type 2 infections of female hamsters. Antimicrob. Agents Chemother. **11**:701–707.
35. **Robins, M. J., Y. Fouron, and W. H. Muhs.** 1977. Nucleic acid related compounds. 25. Syntheses of arabino, xylo, and lyxo-anhydro sugar nucleosides from tubercidin ribo-epoxide. Can. J. Chem. **55**:1260–1267.
36. **Robins, M. J., J. R. McCarthy, Jr., R. A. Jones, and R. Mengel.** 1973. Nucleic acid related compounds. 5. The transformation of formycin and tubercidin into 2′- and 3′-deoxynucleosides. Can. J. Chem. **51**:1313–1321.
37. **Robins, M. J., J. S. Wilson, and F. Hansske.** 1983. Nucleic acid related compounds. 42. A general procedure for the efficient deoxygenation of secondary alcohols. Regiospecific and stereoselective conversion of ribonucleosides to 2′-deoxynucleosides. J. Am. Chem. Soc. **105**:4059–4065.
38. **Schinazi, R. F., J. Peters, M. K. Sokol, and A. J. Nahmias.** 1983. Therapeutic activities of 1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)-5-iodocytosine and -thymine alone and in combination with acyclovir and vidarabine in mice infected intracerebrally with herpes simplex virus. Antimicrob. Agents Chemother. **24**:95–103.
39. **Schinazi, R. F., R. T. Scott, J. Peters, V. Rice, and A. J. Nahmias.** 1985. Antiviral activity of 5-ethyl-2′-deoxyuridine against herpes simplex viruses in cell culture, mice, and guinea pigs. Antimicrob. Agents Chemother. **28**:552–560.
40. **Sibrack, C. D., L. T. Gutman, C. M. Wilfert, C. McLaren, M. H. St. Clair, P. M. Keller, and D. W. Barry.** 1982. Pathogenicity of acyclovir-resistant herpes simplex virus type 1 from an immunodeficient child. J. Infect. Dis. **146**:673–682.
41. **Smee, D. F., J. C. Martin, J. P. H. Verheyden, and T. R. Matthews.** 1983. Anti-herpesvirus activity of the acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl)guanine. Antimicrob. Agents Chemother. **23**:676–682.
42. **Straus, S. E., H. E. Takiff, M. Seidlin, S. Bachrach, L. Lininger, J. J. DiGiovanna, K. A. Western, H. A. Smith, S. Nusinoff Lehrman, T. Creagh-Kirk, and D. W. Alling.** 1984. Suppression of frequently recurring genital herpes. N. Engl. J. Med. **310**:1545–1550.
43. **Vince, R., S. Daluge, H. Lee, W. M. Shannon, G. Arnett, T. W. Schafer, T. L. Nagabhushan, P. Reichert, and H. Tsai.** 1983. Carbocyclic arabinofuranosyladenine (cyclaradine): efficacy against genital herpes in guinea pigs. Science **221**:1405–1406.
44. **Wade, J. C., C. McLaren, and J. D. Meyers.** 1983. Frequency and significance of acyclovir-resistant herpes simplex virus isolated from marrow transplant patients receiving multiple courses of treatment with acyclovir. J. Infect. Dis. **148**:1077–1082.