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Antiviral activity and pharmacokinetics of HOE 602, an acyclic nucleoside, in animal models

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Summary

The acyclic nucleoside derivative HOE 602 (2-amino-9-[1,3-bis(isopropoxy)-2-propoxymethyl]purine) was evaluated for its antiviral activity in cell culture and for its therapeutic efficacy in mice infected with herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) or with murine cytomegalovirus (MCMV). HOE 602 was inactive in vitro against a variety of DNA- and RNA-viruses. However it prevented symptoms and mortality in mice systemically infected with HSV-1, HSV-2 or MCMV when administered intraperitoneally or orally at a dosage of 100 μmol/kg twice per day. Pharmacokinetic studies in mice and macaques revealed that HOE 602 was converted via three metabolic steps to ganciclovir, which seemed to be the antivirally active compound. The bioavailability of ganciclovir after oral administration of HOE 602 or ganciclovir was similar in mice, while in rhesus monkeys much higher serum levels of ganciclovir were reached with HOE 602. After intraperitoneal or intravenous administration higher drug levels were obtained with ganciclovir. The excellent therapeutic efficacy in animal models, the high enteral absorption in monkeys, and the favourable physical properties will hopefully lead to an orally active drug against cytomegalovirus and severe herpes infections in man.

Ganciclovir; HOE 602; Herpesvirus; Cytomegalovirus; Animal model

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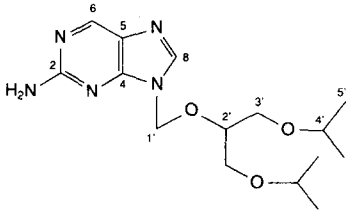
Dedicated to Dr Elmar Schrinner on the occasion of his 60th birthday.

Introduction

Since the discovery of the selective activity of aciclovir against herpes viruses (Elion et al., 1977) much effort has been directed towards the search of nucleoside derivatives with still higher efficacy and a broader spectrum of activity. A number of compounds with improved antiherpes activity has been described (De Clercq 1987, 1988). Amongst these, ganciclovir is one of the most promising compounds due to its broad spectrum of antiviral activity, which covers the herpes viruses pathogenic to humans, including cytomegalovirus (Smee et al., 1983). The clinical development of ganciclovir was confined to severe CMV infections due to toxic effects of the compound (Bowden et al., 1987; Shepp et al., 1985).

Treatment with nucleoside analogs can only inhibit active virus replication while latent herpes viruses in the host are not affected. Reactivation of the virus is frequent, especially in immunocompromised patients, and lifelong therapy in these patients is mandatory. Enteral absorption of ganciclovir is poor (De Miranda et al., 1986) and parenteral administration is obligatory, which is very inconvenient for prolonged treatment. Therefore our efforts have been directed towards the synthesis of an antiviral agent exhibiting a broad spectrum of activity and good oral bioavailability offering the possibility of oral treatment for CMV and other herpes virus diseases (Winkelmann et al., 1988). HOE 602 (Fig. 1) was selected from this series for further development.



HOE 602

colourless crystals, m.p. 91°C

^{1}H-NMR (270 MHz)	δ (ppm)			^{13}C-NMR (67.93 MHz)	δ (ppm)
H-6	8.59	s	1H	C-2	160.63
H-8	8.16	s	1H	C-4	153.06
NH_2	6.52	s	2H	C-5	126.78
H-1'	5.53	s	2H	C-6	148.92
H-2'	3.80	m	1H	C-8	142.71
H-3'	3.30	m	4H	C-1'	71.41
H-4'	3.43	m	2H	C-2'	77.58
H-5'	0.98	d	12H	C-3'	67.36
				C-4'	70.96
				C-5'	21.68

Fig. 1. Characterization of HOE 602 by ^{1}H and ^{13}C NMR spectroscopy in DMSO-d_6.

Materials and Methods

Compounds

HOE 602 and its metabolites 1 and 2 were synthesized by Hoechst AG, Frankfurt, by methods described elsewhere (Winkelmann et al., 1988), and ganciclovir was synthesized according to Ogilvie et al. (1982). The structures of the compounds were confirmed by mass spectroscopy, ^{1}H-NMR, and in the case of HOE 602 (Fig. 1), ^{13}C-NMR spectroscopy. Aciclovir (Zovirax® I.V., Burroughs Wellcome) was purchased from the local pharmacy.

Cell cultures

HeLa and VERO cells were grown in Dulbecco's Minimal Essential Medium (MEM) with 10% fetal calf serum (FCS). Cell passages were performed without the use of antibiotics. All media and sera were purchased from Gibco Deutschland GmbH (Mainz) or Roth (Darmstadt).

Viruses

Viruses were obtained from the American Type Culture Collection, Rockville, MD, U.S.A. unless stated otherwise. Stocks of adenovirus 5 (Adenoid 55), vaccinia (P71) (Prof. Haagen, Bundesforschungsanstalt Tübingen) and rhinovirus 2 (HGP) were grown in HeLa cells. HSV-1 (cornea strain) (Prof. Sauer, Deutsches Krebsforschungszentrum Heidelberg) and HSV-2 (MS) were prepared in VERO cells. Influenza A2 (Aichi) virus was grown in the allantoic sac of 11-day-old chick embryos.

Antiviral activity in cell culture

The test compounds were diluted in flat bottom 96-well microtiter plates in a 3-fold serial dilution. After addition of 4×10^4 cells per well the plates were incubated at 37°C under 5% CO_2 for 3 h. For infection a virus suspension was added to achieve a cytopathic effect within 72 h of incubation at 37 or 33°C, respectively, in the case of rhinovirus. The test mixture contained the test compounds from 400 mg/l to 0.18 mg/l in Dulbecco's MEM, supplemented with 2% FCS, 100 U/ml penicillin G, and 100 mg/l streptomycin. All tests were performed in duplicate except for the controls which were only performed once per test plate. The cytotoxicity of the compounds was determined by microscopic examination of the plates after 24 h incubation. The maximal tolerated dose (MTD) was the highest drug concentration which did not cause microscopically detectable destruction of the cells under these conditions. Plates were checked again 48 h later when the infected controls showed a complete cytopathogenic effect (CPE). After microscopic examination the plates were stained with neutral red according to the dye uptake assay by Finter (1966). The antiviral activity of a compound was defined as the minimal inhibitory

concentration (MIC) required to inhibit virus-induced CPE by 50%.

Animals

The mice used throughout the experiments were 5-week-old NMRI or BALB/c mice, weighing 16 to 18 g, which were obtained from our own breeding station (NMRI) or were purchased from WIGA, Sulzfeld, F.R.G. The mice were housed under conventional conditions in groups of 5 or 10 with food and water ad libitum.

Rhesus monkeys (*Macaca mulatta*) were from our own breeding colony. For the experiments the animals were housed separately in steel cages 12 h prior to the experiment. The monkeys used for pharmacokinetic measurements were female animals with 6 to 7 kg bodyweight.

Systemic HSV infection

NMRI mice were infected intraperitoneally with approximately 50 LD_{50} (frozen material from cell culture) HSV-1 or HSV-2. The mice were treated once 3 h after infection and twice daily, 8 hours apart, for the next 4 days with the compound administered intraperitoneally or orally at the indicated doses unless stated otherwise. Controls were treated with saline. Deaths were recorded daily for 2 weeks post-infection.

Systemic MCMV infection

BALB/c mice were infected intraperitoneally with 50 LD_{50} MCMV (SGV), which was obtained as a 10% homogenate of salivary glands from infected NMRI mice. Mice were treated with the compounds dissolved in saline 3 hours after infection and then twice daily for the following 4 days unless stated otherwise. The mortality rate was monitored for 2 weeks post-infection.

Pharmacokinetics

The mice (NMRI and BALB/c) and monkeys (*Macaca mulatta*) were fasted overnight but were allowed water before each experiment. During the experiment the mice had food and water ad libitum, while the monkeys received only water. The drugs were administered intraperitoneally (mice), intravenously (monkeys) or orally in both species. Blood samples from mice were obtained from the jugular vein by bleeding and pooled (3 animals per pool). In monkeys, individual samples were taken from the alternate femoral veins. The samples were allowed to coagulate for 3 to 4 h at 4°C. Serum was obtained by centrifugation and stored frozen at −20°C until analysis. Prior to analysis the serum samples were treated with 2 volumes of 10% trichloroacetic acid and allowed to settle at 0°C for 30 min. After centrifugation (10 min at 12 000 rpm in benchtop centrifuge) the supernatants were analyzed by reversed phase high-pressure liquid chromatography on a Nucleosil® C_{18} microsphere column (pore size 5 μm; dimensions 60 to 120 by 4.6 mm,

Macherey und Nagel, Düren, F.R.G.) and eluted with acetonitrile/0.1 M phosphate buffer pH 7.2 at a flow rate of 1 ml/min. Ganciclovir was detected by fluorescence at 370 nm after excitation at 280 nm. The detection limit was 0.1 mg/l in serum. A detailed description of the assay methods for all the compounds will be published elsewhere (Scholl et al., in preparation).

Statistics

The mean survival time of the deceased animals in the drug treated groups was compared to untreated controls by Student's *t*-test, whereas the death rate was monitored by Wilcoxon rank analysis. Results were considered as statistically significant, if the corresponding *P*-values were ≤ 0.05.

Results

Within a range of 0.18 to 400 mg/l HOE 602 did not suppress replication of DNA- or RNA-viruses in cell culture, whereas ganciclovir and aciclovir were active in inhibiting HSV-1 and HSV-2 in VERO cells (Table 1). None of the compounds showed any effect on the viability of HeLa and VERO cells up to 400 mg/l. HOE 602 was further tested for its efficacy against herpes viruses in mice. When administered intraperitoneally in nine doses of 10, 30 or 100 μmol/kg, death due to systemic HSV-1 infection could be prevented (Fig. 2A). The same result was obtained with ganciclovir, while aciclovir was less active with a mortality rate of 40% at 100 μmol/kg. Oral treatment twice daily gave a similar result (Fig. 2B) as parenteral administration. The protective effect was less with all three compounds when oral treatment was started 24 h after infection with one daily dose of 5 or 10 μmol/kg for 4 days (Table 2). This experimental design showed a higher activity of HOE 602 in comparison to ganciclovir. Only low efficacy was seen with aciclovir in this test. Parenteral or oral treatment of mice, systemically infected with HSV-2, with HOE 602 (Table 3) showed comparable results to experiments with HSV-1-infected mice (Fig. 2).

Murine cytomegalovirus infection in mice is a convenient model of cy-

TABLE 1
Antiviral activity and toxicity of HOE 602 in cell culture compared to ganciclovir and aciclovir

Virus	Cell type	HOE 602	ED_{50} (mg/l)	
			Ganciclovir	Aciclovir
Adeno-5	HeLa	>400	>400	>400
Vaccinia P71	HeLa	>400	>400	>400
HSV-1	VERO	>400	0.55	0.18
HSV-2	VERO	>400	0.55	0.55
Influenza A2	VERO	>400	> 400	>400
Rhino 2	HeLa	>400	>400	>400
			MTD (mg/l)	
	HeLa	>400	>400	>400
	VERO	>400	>400	>400

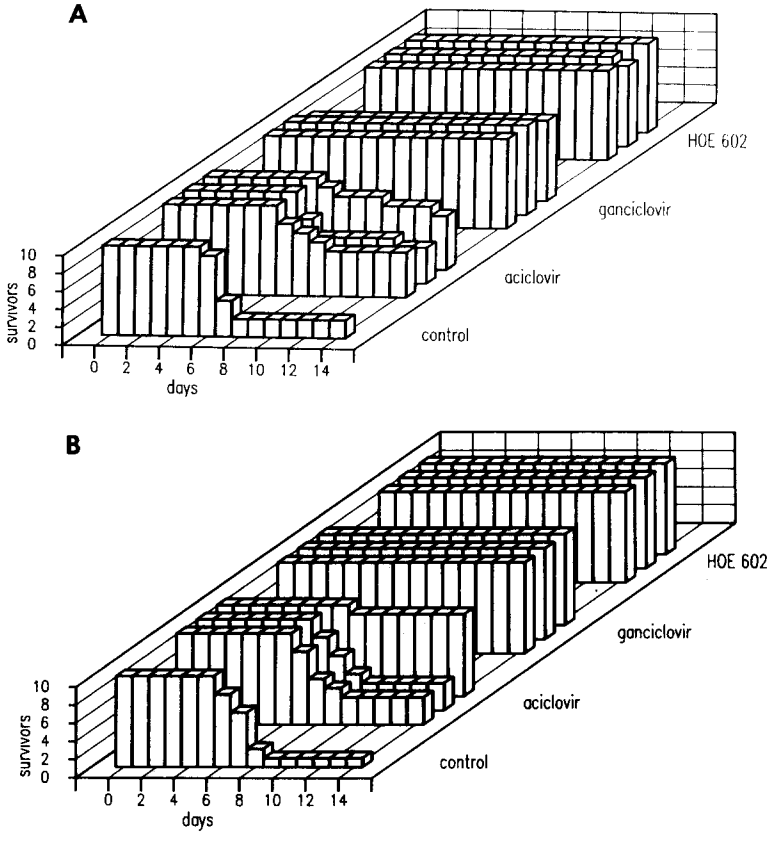


Fig. 2. Therapeutic activity of HOE 602 compared to ganciclovir and aciclovir in mice systemically infected with HSV-1. The compounds were administered intraperitoneally (A) or orally (B) 3 h after infection and then twice daily for 4 days at a dose of 10 μmol/kg (first row), 30 μmol/kg (second row) or 100 μmol/kg (third row of bars).

tomegalovirus infection in man. Adult BALB/c mice were sensitive to systemic MCMV infection and generally died at 4 to 6 days post-infection. Parenteral treatment with HOE 602 (9 × 10, 30 or 100 μmol/kg) partially prevented the fatal outcome of the infection in a dose-dependent manner. In this experiment better results were obtained with ganciclovir than with HOE 602, while oral treatment with the same doses showed comparable therapeutic efficacy for both compounds. Aciclovir again was less active after oral and parenteral administration (Table 4).

The pharmacokinetic data of HOE 602 in rhesus monkeys (Fig. 3) showed that the compound is converted to ganciclovir via two metabolites which proved to be inactive against HSV-1 and HSV-2 in vitro (data not shown), but exhibited excellent activity against HSV-1 in mice (Table 5). In mice HOE 602 and ganciclovir were well absorbed after oral administration but the amount of ganciclovir obtained from HOE 602 was considerably higher than with oral ganciclovir itself (Fig. 4). In rhesus monkeys only low levels of ganciclovir were achieved after oral administration

TABLE 2
Therapeutic efficacy of HOE 602 compared to ganciclovir and aciclovir in systemically HSV-1-infected NMRI mice; oral treatment was started 24 h after infection and continued once daily for 3 days

Compound	Dose (p.o.) μmol (mg)/kg	Survival time days	Survivors/ group
Control	4 × 0	7.4 ± 0.7	2/10
HOE 602	4 × 10 (3.2)		10/10[b]
	4 × 5 (1.6)	10.3 ± 0.6[a]	7/10[b]
Ganciclovir	4 × 10 (2.6)	7.0	9/10[b]
	4 × 5 (1.3)	7.7 ± 0.8	4/10
Aciclovir	4 × 10 (2.3)	9.0 ± 2.8[a]	4/10
	4 × 5 (1.1)	7.6 ± 0.5	3/10

[a]Statistically significant ($P \leq 0.05$) by Student's *t*-test.
[b]Statistically significant ($P \leq 0.05$) by Wilcoxon rank test.

of ganciclovir at 25 mg/kg (c_{max}: 0.6 mg/l). However, a dose of 25 mg/kg of oral HOE 602 resulted in maximum peak levels of ganciclovir up to 6 mg/l in the serum (Fig. 5). The bioavailability of ganciclovir after intravenous HOE 602, calculated from the AUDC-values (Table 6), was only 13 to 22% of intravenous ganciclovir depending on the applied dose, while the amount of ganciclovir in monkeys after oral administration of HOE 602 was 4 to 21 times higher than after oral administration of ganciclovir itself.

Discussion

HOE 602 is a nucleoside analog with high therapeutic efficacy against HSV (Winkelmann et al., 1987) and MCMV (Helsberg et al., 1988) infections in mice. It lacks antiviral activity in cell cultures. The serum of HOE-602-treated mice, however, was active against HSV-1 and HSV-2 in vitro (results not shown). It can be assumed therefore that HOE 602 is a prodrug which is not metabolized to its active form in HeLa or VERO cells.

Pharmacokinetic studies in mice and rhesus monkeys revealed that HOE 602 is metabolically activated to ganciclovir in a three-step pathway, as confirmed by HPLC. From these data a schematic metabolic pathway of HOE 602 was constructed (Fig. 6). The first step, oxidation of the 6-aminopurine moiety to guanine by xanthine oxidase could be demonstrated in vitro. Xanthine oxidase from rat liver converted HOE 602 to 9-[1,3-bis(isopropoxy)-2-propoxymethyl]guanine (data not shown).

HOE 602 is only one of a series of compounds carrying halogen-, ester-, or ether groups instead of the two hydroxy groups of ganciclovir which were synthesized (Jähne et al., 1988) and tested for their antiviral activity in vitro and in vivo (Winkelmann et al., 1988). The results showed that replacement of only one of the hydroxy groups of ganciclovir by an organic side chain abolished the antiviral

TABLE 3
Therapeutic efficacy of HOE 602 compared to ganciclovir and aciclovir in systemically HSV-2-infected NMRI mice; intraperitoneal or oral treatment was started 3 h after infection and continued twice daily for 4 days

Compound	Dose μmol (mg)/kg	Survival time days	Survivors/ group
Intraperitoneal			
Control	9 × 0	6.9 ± 0.7	0/10
HOE 602	9 × 100 (32 0)		10/10[b]
	9 × 30 (9.6)		10/10[b]
	9 × (3.2)	13.0 ± 1.0[a]	7/10[b]
Ganciclovir	9 × 100 (25.5)		10/10[b]
	9 × 30 (7.7)	10.0[a]	9/10[b]
	9 × 10 (2.6)		10/10[b]
Aciclovir	9 × 100 (22.5)	11.0 ± 2.5[a]	6/10[b]
	9 × 30 (6.8)	9.6 ± 1.9[a]	2/10
	9 × 10 (2.3)	9.4 ± 1.1[a]	2/10
Oral			
Control	9 × 0	7.0 ± 0.7	0/10
HOE 602	9 × 100 (32.0)	6.5 ± 0.7	8/10[b]
	9 × 30 (9.6)		10/10[b]
	9 × 10 (3.2)	11.0[a]	9/10[b]
Ganciclovir	9 × 100 (25.5)		10/10[b]
	9 × 30 (7.7)	13.0	9/10[b]
	9 × 10 (2.6)	9.5 ± 0.7[a]	8/10[b]
Aciclovir	9 × 100 (22.5)	10.0 ± 2.5[a]	5/10[b]
	9 × 30 (6.8)	10.0 ± 2.2[a]	0/10
	9 × 10 (2.3)	8.6 ± 1.7[a]	1/10

[a]Statistically significant ($P \leq 0.05$) by Student's t-test.
[b]Statistically significant ($P \leq 0.05$) by Wilcoxon rank test.

activity in cell culture while halogenated derivatives exhibited diminished activity in vitro.

Remarkable activities against HSV-1 infections in mice were achieved with isopropylether derivatives of ganciclovir (Winkelmann et al., 1988). These results were unexpected, as ether derivatives of nucleosides are totally unknown as prodrugs of antivirals, whereas ester derivatives are commonly used to achieve better enteral resorption compared to the parent compound (Canonico et al., 1988; Harnden, 1989). Diesters of ganciclovir were screened for enteral absorption in an in vitro assay (Benjamin et al., 1987), and their bioavailability was tested in mice systemically infected with HSV-2 (Martin et al., 1987). No pharmacokinetic data of these compounds have been published so far.

Although the enzymes responsible for the metabolic activation of the ether derivatives have not yet been determined, it can be assumed that cytochrome P_{450} containing enzymes in the liver are involved (Johanson et al., 1986; Brady et al., 1988). The 6-deoxy derivative of aciclovir, desciclovir, has been described as a

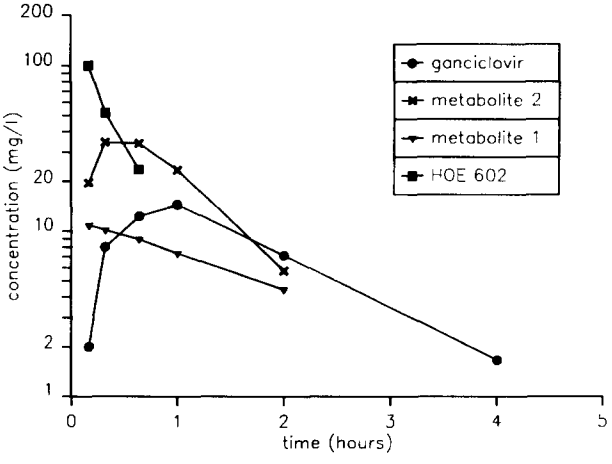


Fig. 3. Serum levels of HOE 602 and its metabolites in a rhesus monkey after intravenous administration of a single dose of 100 mg HOE 602/kg.

TABLE 4
Therapeutic efficacy of HOE 602 compared to ganciclovir and aciclovir in systemically MCMV-infected BALB/c mice; intraperitoneal or oral treatment was started 3 h after infection and continued twice daily for 4 days

Compound	Dose μmol (mg)/kg	Survival time days	Survivors/ group
Intraperitoneal			
Control	9 × 0	5.8 ± 2.0	4/10
HOE 602	9 × 100 (32.0)		10/10[b]
	9 × 30 (9.6)	6.8 ± 0.4[a]	4/10[b]
	9 × 10 (3.2)	5.4 ± 0.5	1/10[b]
Ganciclovir	9 × 100 (25.5)		10/10[b]
	9 × 30 (7.7)		10/10[b]
	9 × 10 (2.6)	6.3 ± 0.6	7/10
Aciclovir	9 × 100 (22.5)		10/10[b]
	9 × 30 (6.8)	5.5 ± 0.9	2/10
	9 × 10 (2.3)	5.2 ± 1.1	1/10
Oral			
Control	9 × 0	5.9 ± 1.0	2/10
HOE 602	9 × 100 (32.0)		10/10[b]
	9 × 30 (9.6)	10.0[a]	9/10[b]
	9 × 10 (3.2)	5.9 ± 0.4	2/10
Ganciclovir	9 × 100 (25.5)		10/10[b]
	9 × 30 (7.7)	8.3 ± 1.2[a]	7/10[b]
	9 × 10 (2.6)	6.5 ± 0.5	2/10
Aciclovir	9 × 100 (22.5)	9.0[a]	9/10[b]
	9 × 30 (6.8)	5.8 ± 0.4	1/10
	9 × 10 (2.3)	6.7 ± 1.3	0/10

[a]Statistically significant ($P \leq 0.05$) by Student's t-test.
[b]Statistically significant ($P \leq 0.05$) by Wilcoxon rank test.

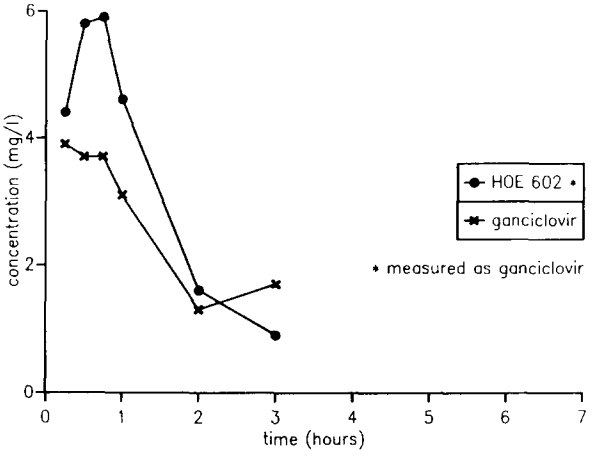


Fig. 4. Serum levels of ganciclovir in mice after a single oral administration of 100 mg/kg HOE 602 or ganciclovir. Each point represents the pooled serum from 3 mice.

TABLE 5
Therapeutic efficacy of HOE 602 and its metabolites in systemically HSV-1-infected NMRI mice; oral treatment was started 3 h after infection and continued twice daily for 4 days

Compound	Dose μmol (mg)/kg	Survival time days	Survivors/ group
Control	9 × 0	6.4 ± 0.5	1/10
2-amino-9-[1,3-bis(isopropoxy)-2-propoxymethyl]purine			
HOE 602	9 × 100 (32.0)		10/10[b]
	9 × 30 (9.6)		10/10[b]
	9 × 10 (3.2)		10/10[b]
9-[1,3-bis(isopropoxy)-2-propoxymethyl]guanine			
Metabolite 1	9 × 100 (34.0)		10/10[b]
	9 × 30 (10.2)	9.5 ± 0.7[a]	8/10[b]
	9 × 10 (3.4)	8.0 ± 1.7[a]	7/10[b]
9-(1-isopropoxymethyl-2-hydroxyethoxymethyl)guanine			
Metabolite 2	9 × 100 (29.7)		10/10[b]
	9 × 30 (8.9)	8.5	9/10[b]
	9 × 10 (3.0)	9.5 ± 0.6[a]	6/10[b]
9-[2-hydroxy-1-(hydroxymethyl)ethoxymethyl]guanine			
Ganciclovir	9 × 100 (25.5)		10/10[b]
	9 × 30 (7.7)		10/10[b]
	9 × 10 (2.6)		10/10[b]

[a]Statistically significant ($P \leq 0.05$) by Student's t-test.
[b]Statistically significant ($P \leq 0.05$) by Wilcoxon rank test.

potent prodrug of aciclovir, and showed improved enteral absorption in humans compared to the parent compound. It was shown that desciclovir is converted to

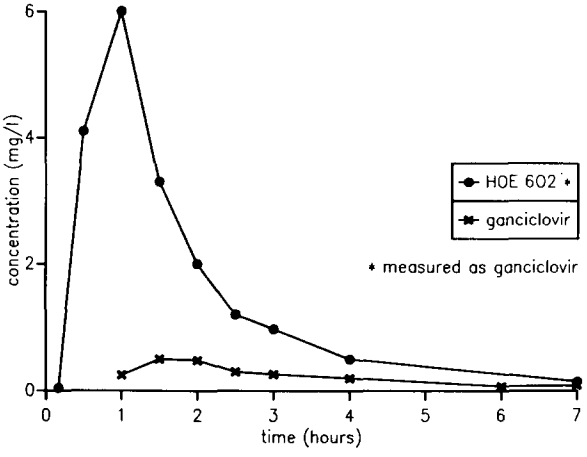


Fig. 5. Serum levels of ganciclovir in a rhesus monkey after a single oral administration of HOE 602 or ganciclovir at 25 mg/kg.

TABLE 6
Pharmacokinetic data of ganciclovir in rhesus monkeys (n=3) after administration of HOE 602 or ganciclovir

Compound administered	Dose (mg/kg)	Maximum serum concentration (mg/l)	AUDC[a] (h × mg/l)
HOE 602	10 i.v.[b]	1.6	3.1
	25 i.v.[b]	2.9	5.5
	50 i.v.[b]	12.0	26.3
	100 i.v.	13.6 ± 2.6	36.2 ± 14.5
Ganciclovir	50 i.v.	86.6[c] ± 17.5	136.5 ± 53.3
HOE 602	25 p.o.	5.3 ± 1.1	9.4 ± 1.5
	100 p.o.	23.5 ± 8.0	203.3 ± 91.1
Ganciclovir	25 p.o.	0.6 ± 0.2	2.4 ± 0.5

[a]Area under the data-curve extrapolated.
[b]n = 1.
[c]t = 10 min. (first time point of blood sampling).

aciclovir by xanthine oxidase (Whiteman et al., 1984; Petty et al., 1987). Although the 6-deoxy derivative of penciclovir (BRL 39123) was absorbed only moderately in mice, the diacetyl and dipropionyl esters were quite well absorbed orally (Vere Hodge et al., 1989).

It is not yet known which other tissues are also capable of converting HOE 602 or its two metabolites to ganciclovir. The serum levels of ganciclovir after administration of HOE 602 can only partly be predictive for the calculation of an effective therapeutic dose. The oral treatment of mice infected with herpes virus using HOE 602 demonstrated comparative efficacy to ganciclovir. The therapeutic efficacy of HOE 602 has only been demonstrated in mice. In these animals the

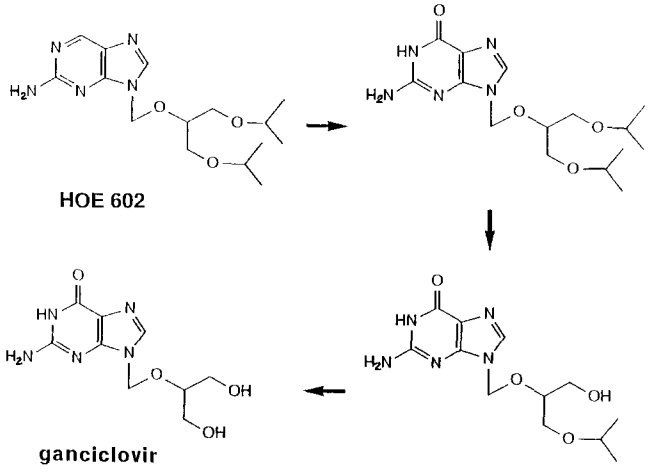


Fig. 6. Schematic metabolic pathway of HOE 602 deduced from pharmacokinetic data in mice and rhesus monkeys.

bioavailability of oral ganciclovir is much higher than in monkeys or in humans (De Miranda et al., 1986; Fletcher et al., 1986). Thus the comparison of the efficacy of HOE 602 and ganciclovir after oral administration in mice is not predictive of the situation in humans. Although maintenance therapy with oral ganciclovir has been discussed (Jacobson et al., 1987), no such clinical studies have been reported. HOE 602 offers the opportunity of an oral treatment of CMV infections if the pharmacokinetics of HOE 602 in humans parallel the situation in monkeys, as is the case for ganciclovir. These investigations are presently under evaluation in our laboratories.

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References

Benjamin, E.J., Firestone, B.A, Bergstrom, R., Fass, M., Massey, I., Tsina, I. and Lin, T. (1987) Selection of a derivative of the antiviral agent 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG) with improved oral absorption. Pharmaceut. Res. 4, 120–125.

Bowden, R.A., Digel, J., Reed, E.C. and Meyers, J.D. (1987) Immunosuppressive effects of ganciclovir on in vitro lymphocyte responses. J. Infect. Dis. 156, 899–903.

Brady, J.F., Lee, M.J., Li, M., Ishizaki, H. and Yang, C.S. (1988) Diethyl ether as a substrate for acetone/ethanol-inducible cytochrome P-450 and as an inducer for cytochrome(s) P-450. Mol. Pharmacol. 33, 148–154.

Canonico, P.G., Kende, M. and Gabrielsen, B. (1988) Carrier-mediated delivery of antiviral agents.

Adv. Virus Res. 35, 271–312.
De Clercq, E. (1987) Antiviral nucleoside analogs. ISI Atlas of Science®: Pharmacology Vol. 1, 20–24.
De Clercq, E. (1988) Recent advances in the search for selective antiviral agents. Adv. Drug Res. 17, 1–59.
De Miranda, P., Burnette, T., Cederberg, D., Blum, M.R., Brodie, H.R. and Mills, J. (1986) Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abst. no. 566.
Elion, G.B., Furman, P.A., Fyfe, J.A., de Miranda, P., Beauchamp, L. and Schaeffer, H.J. (1977) Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl)guanine. Proc. Natl. Acad. Sci. 74, 5716–5720.
Finter, N.B. (1969) Dye uptake methods for assessing viral cytopathogenicity and their application to interferon assays. J. Gen. Virol. 5, 419–427.
Fletcher, C., Sawchuk, R., Chinnock. B., de Miranda, P. and Balfour, H.H. (1986) Human pharmacokinetics of antiviral drug DHPG. Clin. Pharmacol. Ther. 40, 281–286.
Harnden, M.R. (1989) Development of 9-[4-hydroxy-3-(hydroxymethyl)but-1-yl] purines as potential therapeutic agents for treatment of human herpes virus infections. Drugs Fut. 14, 347–358.
Helsberg, M., Winkler, I., Meichsner, Ch., Rolly, H., Winkelmann, E., Jähne, G., Hilpert, Th., Hertzsch, W., Rösner, M. and Sinharay, A. (1988) Antiviral Activity of HOE 602 and Analogues against Herpes Simplex Virus Infections (Abstract II-52: Second International Conference on Antiviral Research, Williamsburg, U.S.A., 10–14 April 1988). Antiviral Res. 9, 153.
Jacobson, M.A., De Miranda, P., Cederberg, D.M., Burnette, T., Cobb, E., Brodie, H.R. and Mills, J. (1987) Human pharmacokinetics and tolerance of oral ganciclovir. Antimicrob. Agents Chemother. 31, 1251–1254.
Jähne, G., Winkelmann, E., Hilpert, Th., Hertzsch, W., Rösner, M., Sinharay, A., Winkler, I., Helsberg, M., Meichsner, Ch. and Rolly, H. (1988) Synthesis of HOE 602 and Analogues. New Acyclic Nucleoside Derivatives with Antiviral Activity (Abstract I-38: Second International Conference on Antiviral Research, Williamsburg, U.S.A., 10–14 April 1988). Antiviral Res. 9, 109.
Johanson, G., Wallen, M. and Nordqvist, M.B. (1986) Elimination kinetics of 2-butoxyethanol in the perfused rat liver: dose dependence and effect of ethanol. Toxicol. Appl. Pharmacol. 83, 315–320.
Martin, J.C., Tippie, M.A., McGee, D.P.C. and Verheyden, J.P.H. (1987) Synthesis and antiviral activity of various esters of 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine. J. Pharm. Sci. 76, 180–184.
Ogilvie, K.K., Cheriyan, O.O., Radatus, B.K., Smith, K.O., Galloway, K.S. and Kennell, W.L. (1982) Biologically active acyclonucleoside analogues. II. The synthesis of 9-[[2-hydroxy-1-(hydroxymethyl)-ethoxy]methyl]guanine (BIOLF-62). Can. J. Chem. 60, 3005–3100.
Petty, B.G., Whitley, R.J., Liao, S., Krasny, H.C., Rocco, L.E., Davis, L.G. and Lietman, P.S. (1987) Pharmacokinetics and tolerance of desciclovir, a prodrug of acyclovir, in healthy volunteers. Antimicrob. Agents Chemother. 31, 1317–1322.
Shepp, D.H., Dandliker, P.S. and de Miranda, P. (1985) Activity of 9-[2-hydroxy-1-(hydroxymethyl)ethoxymethyl]guanine in the treatment of cytomegalovirus pneumonia. Ann. Intern. Med. 103, 368–373.
Smee, D.F., Martin, J.C., Verheyden, J.P.H. and Matthews, T.R. (1983) Antiherpesvirus activity of the acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl)guanine. Antimicrob. Agents Chemother. 23, 676–682.
Vere Hodge, R.A., Sutton, D., Boyd, M.R., Harnden, M.R. and Jarvest, R.L. (1989) Selection of an oral prodrug (BRL 42810; Famciclovir) for the antiherpesvirus agent BRL 39123 (9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine; penciclovir). Antimicrob. Agents Chemother. 33, 1765–1773.
Whiteman, P.D., Bye, A., Fowle, A.S.E., Land, S.J.G. and Posner, J. (1984) Tolerance and pharmacokinetics of A515U, an acyclovir analogue, in healthy volunteers. Eur. J. Clin. Pharmacol. 27, 471–475.
Winkelmann, E., Winkler, I. and Rolly, H. (1987) In 6- und 9-Stellung substituierte 2-Aminopurine, ihre Verwendung, diese Purine enthaltende Arzneimittel und Verfahren zur Herstellung der Purine. Eur. Patent Appl. 0.217.207.
Winkelmann, E., Winkler, I., Rolly, H., Rösner, M. and Jähne, G. (1988) New prodrugs of acyclic nucleosides with antiviral activity. Drug Res. 38, 1545–1548.