EFFICACY OF OXETANOCIN G AGAINST HERPES SIMPLEX VIRUS TYPE 2 AND MURINE CYTOMEGALOVIRUS INFECTIONS IN MICE

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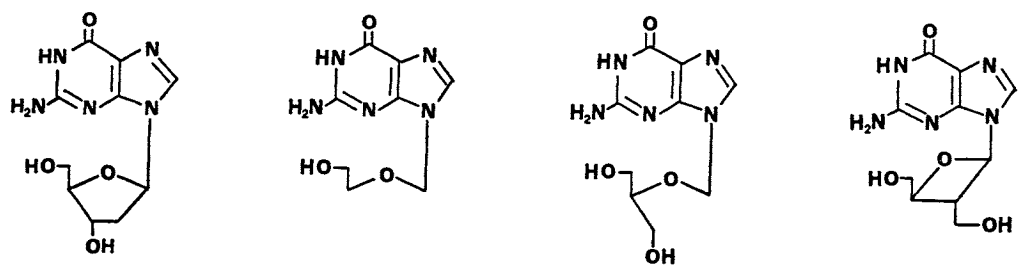
Oxetanocins are a new family of nucleoside analogs which possess an oxetanosyl-*N*-glycoside in the sugar moiety[1]. Recently we have reported that one of the oxetanocins, 9-(2-deoxy-2-hydroxymethyl-β-D-*erythro*-oxetanosyl)guanine (OXT-G), has a potent and selective antiviral activity against human cytomegalovirus (HCMV) *in vitro*[2], and it has been shown that the mode of action of OXT-G is inhibition of viral replication by impairing the viral DNA synthesis[2]. The next step to evaluate this compound as an antiviral agent is to determine whether it is active *in vivo*. In the present study, we examined the activity of OXT-G against herpes simplex virus type 2 (HSV-2) and murine cytomegalovirus (MCMV) infections in mice.

For these experiments, 4- to 8-week-old male mice were obtained from Shizuoka Laboratory Animal Center, Japan. They were kept in groups of about 5 in isolator units. The virus stock of HSV-2 strain 186 was prepared as described previously[3]. The *in vivo* passage Smith strain of MCMV was kindly provided by Y. MINAMISHIMA (Miyazaki Medical College, Miyazaki, Japan), and the virus inoculum was prepared as a 10% (w/v) homogenate of infected salivary gland tissue in EAGLE's minimal essential medium (MEM). The titration of MCMV was performed by plaque assay in mouse embryo fibroblast (MEF) monolayers which were prepared from 15- to 18-day-old embryos of ICR mice by trypsinization[4]. Fig. 1 shows the chemical structures of nucleoside analogs used in this experiment. OXT-G and (9-(2-deoxy-2-hydroxymethyl-β-D-*erythro*-oxetanosyl)-2-amino)-adenine (2-amino-OXT-A) were synthesized as described previously[1,5], and 9-(2-hydroxyethoxymethyl)guanine (ACV) and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) were provided by Burroughs Wellcome Co., Research Triangle Park, N.C., and Syntex Laboratories, Inc., Palo Alto, Calif., respectively.

The therapeutic activity of OXT-G was first evaluated for a systemic HSV-2 infection in mice. The 4-week-old or 8-week-old male ICR mice were inoculated intraperitoneally (ip) with HSV-2 at 2.5×10^5 PFU/0.2 ml/mouse. In this mouse model, HSV-2 replicates relatively well in the ip organs including the liver, kidney and spleen, and almost all mice, when given this quantity of virus, develop encephalitis by 7 days after virus inoculation. OXT-G and the control drug ACV were administrated ip once a day (at 24 hours intervals) with the indicated doses for 5 days starting 6 hours after infection, and deaths were monitored at least for 3 weeks after inoculation. The results are summarized in Table 1. The administration of OXT-G was found to be highly effective against

Fig. 1. Structures of deoxyguanosine, ACV, DHPG and OXT-G.



Deoxyguanosine ACV DHPG OXT-G

Table 1. Effect of treatment with OXT-G and ACV against a systemic HSV-2 infection in mice.

Mice (ICR male)	Treatment[a]	Dose (mg/kg/day)	Survivors/total[b] (%)	Mean survival time (days)
4-week-old	—	0	0/20(0)	7.8
	OXT-G	5	2/10(20)	8.3
	OXT-G	10	2/10(20)	12
	OXT-G	20	4/10(40)	11.3
	ACV	20	0/10(0)	8.3
	ACV	50	0/10(0)	9.9
8-week-old	—	0	0/15(0)	8.1
	OXT-G	5	3/10(30)	12.1
	OXT-G	10	8/10(80)	13.5
	OXT-G	20	10/10(100)	
	2-Amino-OXT-A	10	7/10(70)	12.6
	ACV	10	0/10(0)	10.3
	ACV	50	0/10(0)	12.2

[a] Mice received drugs ip 6 hours after virus inoculation and thereafter once a day every 24 hours for 5 days.

[b] Calculated on days 21.

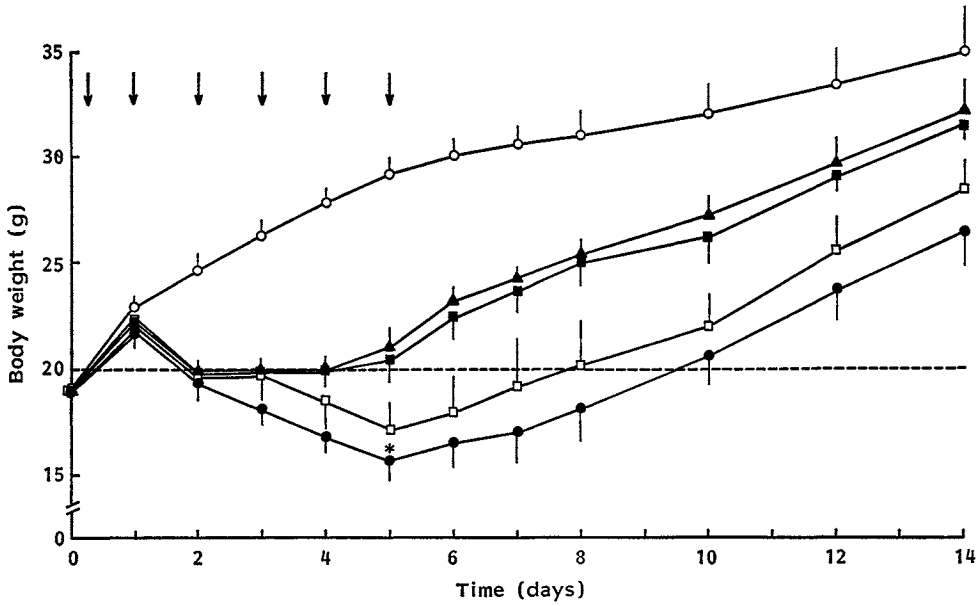
the systemic HSV-2 infection. When tested in 8-week-old mice, OXT-G at a dose of 20 mg/kg/day reduced the mortality rate from 100 to 0%, and 50% effective dose was about 7 mg/kg/day. However, much higher doses of OXT-G were required to reduce the mortality rate in 4-week-old mice. 2-Amino-OXT-A, which can be considered a prodrug of OXT-G, was also very effective in reducing the mortality rate by ip route, whereas ACV even at a dose of 50 mg/kg/day had no effect on the mortality rate although the administration of this dose of ACV resulted in a significant extension of survival time. Since ACV is effective at lower doses against HSV-2 than OXT-G in cell cultures including MEF (unpublished data), the reasons for the low efficacy of ACV in this mouse model are at present unclear. However, the relatively low ability of ACV to penetrate the blood-brain barrier might explain it[6,7].

OXT-G was further tested for its efficacy against a systemic MCMV infection. The 4-week-old male ICR mice were inoculated ip with MCMV at 1×10^5 PFU/0.2 ml and treated with the indicated doses of OXT-G or DHPG once a day for 5 days, starting 6 hours after infection. The mortality and changes in body weight were monitored every day. As shown in Fig. 2, the body weight of infected mice began to decrease 2 days after infection, while uninfected control mice constantly gained weight. At 5 days after inoculation, 2 of 5 infected mice died, but thereafter the weight of the surviving mice began to increase. When virus-inoculated mice were treated with OXT-G or DHPG, all of them survived. The mice receiving OXT-G at the dose of 10 mg/kg/day maintained their body weight on the initial level for several days and gradually gained weight after day-4. Almost the same pattern of changes in body weight could be observed in infected mice treated with DHPG at the dose of 5 mg/kg/day. In this model, the efficacy of OXT-G appeared to be about half of that of DHPG.

We next measured the titers of MCMV in various tissues of the infected mice treated or untreated with OXT-G (10 mg/kg/day). Infection with MCMV and treatment with OXT-G were performed as described above, and mice were sacrificed 3 and 6 days after virus inoculation. Tissues from mice were made into 10% (w/v) homogenate and assayed for MCMV in MEF monolayers. As shown in Table 2, MCMV was recovered from all the tissues examined of untreated mice, including the spleen, liver, kidney, lung and salivary gland; at both 3 and 6 days after challenge the spleen was the organ with the highest virus titers. There was a significant reduction in the quantity of MCMV recovered from the tissues of OXT-G-treated mice. The differences between treated and untreated mice in MCMV titers were more obvious at day-6 than at day-3. However, there was no significant difference in virus titers from the salivary glands.

This *in vivo* study has shown that OXT-G and

Fig. 2. Effect of treatment with OXT-G and DHPG on the body weight of MCMV-infected mice.



Groups of 5 mice were inoculated ip with MCMV (1×10^5 PFU) and treated ip with PBS (●), or OXT-G at 5 mg/kg/day (□), 10 mg/kg/day (■), or DHPG at 5 mg/kg/day (▲) for 5 days. As control (○), mice were injected ip with 10% homogenate of normal salivary gland and 0.2 ml of PBS ip for 5 days. Each point represents the average weight of 3 to 5 mice±standard deviation.

* Two mice died at day-5.

Table 2. Titers of MCMV in various tissues of mice treated or untreated with OXT-G.

Organs	Virus titer[a] (PFU/0.1 g tissue)							
	3 days after infection				6 days after infection			
	Nontreated		OXT-G-treated[b]		Nontreated		OXT-G-treated[b]	
Spleen	6.4	5.7	5.0	5.0	5.1	4.9	<2	<2
Liver	3.4	3.0	2.9	2.3	4.4	2.8	<2	<2
Kidney	4.1	3.3	3.0	2.7	3.7	3.4	<2	<2
Lung	2.3	2.2	2.0	<2	4.0	2.8	<2	<2
Salivary gland	2.3	2.0	<2	<2	3.2	3.0	3.5	3.7

[a] Two mice from each group were sacrificed at day-3 and day-6. MCMV titers were determined from 10% tissue homogenate and given as $\log_{10}$ PFU per 0.1 g tissue.

[b] OXT-G (10 mg/kg/day) was given ip 6 hours after virus inoculation and thereafter once a day every 24 hours for 5 days.

the prodrug 2-amino-OXT-A are highly effective against herpesvirus infections *in vivo* as well as *in vitro*. In our mouse models OXT-G was much more active against HSV-2 infection than ACV. Since the efficacy of antiviral agents is dependent on various factors such as 1) virus strains, 2) animal species, 3) route of infection or treatment *etc.*[8~10], we can not always apply the evaluation of drugs in experimental animal models to humans. From our results, however, it appears that OXT-G is a promising agent for the treatment of HCMV infection.

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