Effective Delivery of a Lipophilic 6-chloro-2',3'-dideoxyguanosine (6-Cl-ddG) into Rat Lymphoid Tissues

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Abstract: Lipophilic 6-chloro-2',3'-dideoxyguanosine (6-Cl-ddG) was evaluated for its improved lymph node delivery by comparison with the parental nucleoside (ddG) *in vitro* and *in vivo*. The *in vitro* studies with rat plasma, lymph node homogenate and stomach content indicated that 6-Cl-ddG converted to ddG more effectively in the lymph node homogenate and that 6-Cl-ddG was more stable than ddG in the stomach content. In an *in vivo* study, plasma and lymph nodes were collected from rats after a subcutaneous or oral administration of 6-Cl-ddG or ddG. With the subcutaneous administrations of the drugs, the area under the concentration time-curve (AUC) value in the plasma for converted ddG following a 6-Cl-ddG administration was less than half the value for ddG following a ddG administration but the converted ddG AUC values in the lymph nodes due to 6-Cl-ddG administration were 1.4- to 2.0-fold higher than the ddG AUC values due to ddG administration. Moreover, with the oral administrations, the converted ddG AUC value in plasma after a 6-Cl-ddG administration was 3-fold higher than ddG after a ddG administration, and high levels of converted ddG were detected in the lymph nodes, but no ddG was detected in the lymph node following ddG administration. These results suggest that lipophilic 6-Cl-ddG is a useful prodrug for delivering ddG into the lymph nodes by oral administration.

Key words: anti-HIV, 6-chloro-2',3'-dideoxyguanosine, lymph nodes, rat

Introduction

Several reports have suggested that a large viral burden exists in the lymphoid tissue of patients infected with human immunodeficiency virus (HIV) even at an

apparently healthy asymptomatic carrier (AC) stage [1]. In addition, the AIDS dementia complex is caused by the entry and residence of HIV in the central nervous system (CNS) after HIV infection [2]. The lymph nodes and CNS are therefore the ultimate target organs for

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effective anti-HIV therapy.

Lipophilic drugs targeting HIV in the CNS and lymph nodes have been widely synthesized recently [3]. The transport of drugs over the blood-brain barrier or to the lymph nodes is primarily dependent on the lipophilicity of the drugs. Shirasaka *et al.* reported that 6-chloro-2',3'-dideoxyguanosine (6-Cl-ddG), a lipophilic prodrug of ddG, exerted potent antiretroviral activity against HIV *in vitro* [4, 5] and showed improved delivery to the CNS [6]. Moreover, 6-Cl-ddG was effective at the stage of acute infection and in the stages of AC and AIDS-related complex (ARC)/acquired immunodeficiency syndrome (AIDS) in rhesus monkeys infected with simian immunodeficiency virus (SIV) [7–9]. The administration of this drug decreased the virus load to less than 1/7 of the pretreatment level in the cerebrospinal fluid (CSF) and decreased the number of virus productive cells to 1/20–1/30 in the lymph nodes in the AC stage in rhesus monkeys [8, 10].

The purpose of the present study was to evaluate the efficacy of the 6-Cl-ddG delivery in the form of ddG into the lymph nodes after administration of the drug to Wistar rats as a model of primates.

Materials and Methods

Materials: 6-chloro-2',3'-dideoxyguanosine and 2',3'-dideoxyguanosine were synthesized as previously described [11].

In vitro study: The *in vitro* hydrolysis rate of the 6-Cl-ddG or ddG was determined with female Wistar rat plasma, cervical lymph node homogenate and stomach content (pH 3.5). Three rats were used for each *in vitro* test, and the samples were pooled. Blood was collected from the trunk in heparinized tubes and centrifuged (3,000 rpm, 5 min). Lymph node homogenate was prepared in a 1:4 (g:ml of phosphate-buffered saline (PBS)) ratio. The stomach was removed from the rat and the stomach content was collected as the supernatant obtained by centrifugation at 3,000 rpm for 5 min. 450 μ l of each sample was then added to 50 μ l of 2 mM 6-Cl-ddG or ddG solution. The mixtures were incubated at 37°C and withdrawn (20 μ l) at 0, 5, 60 and 120 min after incubation. To block deamination, 180 μ l of 1 nM of 2'-deoxycoforomycin (2'-DCM) was immediately added to all of the samples, and the drug concentrations were then analyzed by high-performance

liquid chromatography (HPLC).

Pharmacokinetics: Eighty-four female Wistar rats weighing 150–180 g were obtained from Charles Rivers Laboratories (Atsugi, Japan) and used for the *in vivo* tests. For the subcutaneous administration, an equimolar concentration (200 μ M/kg) of 6-Cl-ddG (53.75 mg/kg) or ddG (50.25 mg/kg) formulated in 10% ethanol (EtOH) in PBS was injected. For the oral administration by gavage, 6-Cl-ddG or ddG was formulated in 10% EtOH with sodium citrate buffer solution (0.1M). Twenty μ l of plasma obtained from the heparinized blood of the tail vein of each of 3 rats was collected at 0, 5, 10, 20, 40, 60 and 120 min after the drug administration. 180 μ l of 2'-DCM was then added immediately at a concentration of 4 mM in order to inhibit blood adenosine deaminase (ADA) activity giving rise to dehalogenisation of 6-Cl-ddG. Immediately after the collection of blood the rats were euthanized by decapitation under light ether anesthesia. The lymph nodes (neck, axillary and mesenteric) were rapidly removed and weighed. The lymph node homogenate was prepared at a 1:10 (g:ml of 1 nM of 2'-DCM) ratio. The care and use of the rats were in accordance with the Guidelines for animal experiments at the Iwakuni Research Laboratory, Nippon Paper Industries Co., Ltd.

HPLC Analysis: Each metabolite was analyzed in a liquid chromatograph (Shimadzu model LC-10A, Shimadzu Associates, Tokyo, Japan). All blood and lymph node samples were ultrafiltrated with Ultrafree C3LGC (Millipore, Tokyo, Japan) (12,000 rpm, 30 min, 4°C), and the eluate was subjected to HPLC. A YMC-Pack ODS-AQ column (4.6 mm diameter, 150 mm long, YMC Co., Ltd., Japan) protected by a YMC-Guard pack ODS-AQ (4.0 $\times$ 25 mm) was eluted with 50 mM phosphate buffer (pH 7.0)-CH₃CN (92:8, v/v) at a flow rate of 1.1 ml/min. The products were detected by UV absorbance at 254 nm, and the chromatogram was recorded and integrated with a data processor (CR-5A Chromatopac, Shimadzu).

Pharmacokinetic analysis: The area under the concentration time-curve (AUC) in the plasma or lymph nodes was calculated by the trapezoidal rule. The pharmacokinetic parameters were calculated by the software program MULTI for microcomputers [12].

Statistics: The statistical significance of differences between groups was evaluated by Student's *t*-test. Dif-

ferences with probability values less than 5% were considered significant.

Results

In vitro studies

Adenosine deaminase-mediated bioconversion studies and an analysis of the *in vitro* stability of 6-Cl-ddG or ddG were performed with the lymph node homogenate, plasma and stomach content of rats (Fig. 1).

In the lymph node homogenate, 6-Cl-ddG was completely hydrolyzed rather quickly (within 5 min), and 86% of the converted ddG was detected at 60 min. In the plasma, about 90% was still in the 6-Cl-ddG form at 5 min, and 65% of 6-Cl-ddG was detected and the other 35% had been converted to ddG at 60 min. The bioconversion of 6-Cl-ddG to both 6-chloro-guanine (6-Cl-G) and ddG was observed in the acid environment of the stomach content at 5 min, and 10% of the 6-Cl-ddG and 65% of the converted ddG were detected at 60 min. In contrast, when ddG was incubated with the lymph node homogenate, plasma or stomach content, the ddG concentration was decreased by 81% in the lymph nodes, 90% in the plasma and by only 13% in the stomach content at 60 min.

Lymph nodes and plasma delivery of 6-Cl-ddG after subcutaneous administration in the rat

Pharmacokinetic studies were performed in rats subcutaneously administered ddG or an equimolar dose of 6-Cl-ddG. The concentration values after the 6-Cl-ddG or ddG subcutaneous administration in the lymph nodes and plasma are shown in Table 1.

In the plasma, the peak concentration of 6-Cl-ddG (15.5 μM) or converted ddG (19.0 μM) was observed at 10 min after the 6-Cl-ddG administration. The peak concentration of ddG was detected at 5 min (56.6 μM) after the ddG administration. The converted ddG levels after the 6-Cl-ddG injection were significantly lower than the ddG levels after the ddG injection at 5, 10 and 20 min ($p < 0.05$). Low levels of converted ddG (3.4 μM) and ddG (8.0 μM) were detected at 120 min after the 6-Cl-ddG and ddG administration, respectively.

In the lymph nodes, 6-Cl-ddG was detected only in the neck lymph nodes at 10 min (0.5 μM). The concentration of converted ddG after the 6-Cl-ddG injection was higher than that after the ddG injection at all times

Fig. 1. Hydrolysis of 6-Cl-ddG or ddG in rat lymph node homogenate, plasma and stomach content at 0, 5 and 60 min after the incubation. In each column, the drug percentages of ■: 6-Cl-ddG, ▨: 6-Cl-G and □: ddG are shown.

in the neck, axillary and mesenteric lymph nodes, and a significant difference was observed in the mesenteric lymph nodes at 20 min ($p < 0.05$) so that the plasma AUC value for converted ddG after the 6-Cl-ddG administration was less than half the value for ddG after the ddG administration. Nevertheless, the AUCs for converted ddG in the lymph nodes after the 6-Cl-ddG injection were 1.4- to 2-fold greater than the AUCs for ddG after the ddG injection (Fig. 2).

These results indicated that the 6-Cl-ddG form is less effectively transferred into plasma than ddG by subcutaneous injection, whereas the distribution and conversion of 6-Cl-ddG in the lymph nodes were more effective than those of ddG.

Lymph nodes and plasma delivery of 6-Cl-ddG after oral administration in the rat

The concentrations of 6-Cl-ddG, converted ddG and

Table 1. Pharmacokinetic analysis of 6-Cl-ddG and ddG in the plasma and lymph nodes of rats after subcutaneous injection

Sample	Time (min)	6-Cl-ddG		ddG
		6-Cl-ddG	Converted ddG	ddG
Plasma (μM)	0	n.d. ^{a)}	n.d.	n.d.
	5	15.2 ± 5.5	$16.3 \pm 0.6^{\text{b)}$	56.6 ± 4.8
	10	15.5 ± 5.0	$19.0 \pm 4.1^{\text{b)}$	55.2 ± 3.8
	20	8.1 ± 0.3	$16.0 \pm 3.3^{\text{b)}$	40.0 ± 4.5
	40	4.9 ± 0.6	12.1 ± 0.6	18.2 ± 1.3
	60	1.3 ± 0.2	5.6 ± 0.4	8.5 ± 1.3
	120	0.2 ± 0.1	3.4 ± 0.8	8.0 ± 2.9
Neck (nM/g)	0	n.d.	n.d.	n.d.
	5	n.d.	28.5 ± 5.8	19.6 ± 0.9
	10	0.5 ± 0.2	14.3 ± 6.9	13.4 ± 0.6
	20	n.d.	17.1 ± 1.9	12.8 ± 0.1
	40	n.d.	9.3 ± 1.9	5.4 ± 0.5
	60	n.d.	5.8 ± 3.3	4.0 ± 0.6
	120	n.d.	n.d.	n.d.
Axillary (nM/g)	0	n.d.	n.d.	n.d.
	5	n.d.	12.7 ± 0.6	11.2 ± 1.7
	10	n.d.	10.9 ± 3.9	8.9 ± 0.5
	20	n.d.	12.1 ± 1.1	11.2 ± 2.2
	40	n.d.	7.9 ± 1.1	6.3 ± 1.6
	60	n.d.	5.0 ± 1.6	4.0 ± 0.7
	120	n.d.	n.d.	n.d.
Mesenteric (nM/g)	0	n.d.	n.d.	n.d.
	5	n.d.	22.4 ± 4.4	16.6 ± 1.2
	10	n.d.	17.6 ± 4.5	10.7 ± 0.7
	20	n.d.	$15.7 \pm 1.0^{\text{b)}$	12.3 ± 1.2
	40	n.d.	8.8 ± 2.1	5.3 ± 0.3
	60	n.d.	7.5 ± 2.0	3.2 ± 0.8
	120	n.d.	n.d.	n.d.

^{a)} Not detected. ^{b)} Significantly different for converted ddG and ddG at $p < 0.05$.

ddG in the plasma and lymph nodes after oral administration of 6-Cl-ddG or ddG are summarized in Table 2.

In the plasma, after the oral administration of 6-Cl-ddG, the peaks of both 6-Cl-ddG and converted ddG were detected at 5 to 10 min (that of 6-Cl-ddG was $5.7 \mu\text{M}$ at 5 min, and that of converted ddG was $14.5 \mu\text{M}$ at 10 min). At 40 min after the oral administration of 6-Cl-ddG, the native form was not detected, but converted ddG was detected at 120 min ($2.2 \mu\text{M}$). In contrast, a very low level of ddG (1 to $3 \mu\text{M}$) was detected in the plasma after the ddG oral administration.

In the lymph nodes, relatively slow increases in the concentrations of 6-Cl-ddG and converted ddG were

Fig. 2. AUC values for ddG in the plasma and lymph nodes (axillary, mesenteric and neck) following subcutaneous and oral administration to rats. The columns represent the AUC of □: converted ddG after 6-Cl-ddG administration and ■: ddG after ddG administration.

observed after oral administration of 6-Cl-ddG. These concentrations reached a peak in the neck lymph nodes at 20 to 40 min ($12.2 \mu\text{M}$ for 6-Cl-ddG and $9.2 \mu\text{M}$ for converted ddG) and in the mesenteric lymph nodes at 20 min ($8.7 \mu\text{M}$ for 6-Cl-ddG and $14.8 \mu\text{M}$ for converted ddG). Surprisingly, after the ddG oral administration, the ddG level was undetectable at any time in any of the lymph nodes.

The converted ddG AUC value in plasma was three times the AUC value for ddG. The converted ddG AUC values in the lymph nodes were 227 in the neck, 86 in the axillary and 417 $\text{nM/g} \cdot \text{min}$ in the mesenteric lymph nodes after the oral 6-Cl-ddG administration but ddG AUC was undetectable in any of the lymph nodes after ddG oral administration.

These results indicated that 6-Cl-ddG is a useful drug, especially for delivery into the lymph nodes as converted ddG by oral administration, compared with the parental drug ddG.

Table 2. Pharmacokinetic analysis of 6-Cl-ddG and ddG in the plasma and lymph nodes of rats after oral administration

Sample	Time (min)	6-Cl-ddG		ddG
		6-Cl-ddG	Converted ddG	ddG
Plasma (μM)	0	n.d. ^{a)}	n.d.	n.d.
	5	5.7 ± 3.2	$13.3 \pm 5.8^{\text{b)}}$	3.1 ± 1.0
	10	4.3 ± 0.5	$14.5 \pm 3.0^{\text{c)}}$	1.3 ± 0.3
	20	3.1 ± 1.5	$14.0 \pm 0.9^{\text{b)}}$	2.5 ± 0.3
	40	n.d.	4.6 ± 1.5	1.4 ± 0.4
	60	n.d.	3.0 ± 1.3	1.0 ± 0.1
	120	n.d.	2.2 ± 1.4	1.0 ± 0.3
Neck (nM/g)	0	n.d.	n.d.	n.d.
	5	n.d.	0.2 ± 0.2	n.d.
	10	n.d.	1.0 ± 0.5	n.d.
	20	10.3 ± 2.3	9.2 ± 2.4	n.d.
	40	12.2 ± 0.4	3.8 ± 1.8	n.d.
	60	n.d.	0.2 ± 0.2	n.d.
	120	n.d.	n.d.	n.d.
Axillary (nM/g)	0	n.d.	n.d.	n.d.
	5	n.d.	0.9 ± 0.3	n.d.
	10	n.d.	1.7 ± 0.6	n.d.
	20	3.4 ± 3.1	1.8 ± 1.0	n.d.
	40	0.6 ± 0.3	0.8 ± 0.1	n.d.
	60	1.2 ± 1.2	0.6 ± 0.2	n.d.
	120	n.d.	n.d.	n.d.
Mesenteric (nM/g)	0	n.d.	n.d.	n.d.
	5	0.6 ± 0.6	2.3 ± 1.2	n.d.
	10	1.3 ± 1.3	11.9 ± 6.4	n.d.
	20	8.7 ± 4.5	14.8 ± 8.4	n.d.
	40	1.8 ± 0.9	3.6 ± 2.5	n.d.
	60	2.4 ± 2.4	0.9 ± 0.4	n.d.
	120	n.d.	n.d.	n.d.

^{a)} Not detected. ^{b)} Significantly different for converted ddG and ddG at $p < 0.05$. ^{c)} Significantly different for converted ddG and ddG at $p < 0.01$.

Discussion

Lipophilic halogenated ddG and 6-Cl-ddG have an anti-retroviral activity against HIV *in vitro*, after being catalyzed by ADA and converted to the active nucleoside form as ddG [13]. In the present study, ADA-catalyzed hydrolysis was detected for 6-Cl-ddG *in vitro* and *in vivo*. 6-Cl-ddG was completely hydrolyzed in the lymph node homogenate within 5 min, but 35% of 6-Cl-ddG was converted to ddG and 65% of the 6-Cl-ddG was detected in plasma at 60 min. These results indicated that 6-Cl-ddG is slowly converted to

ddG in blood, but is immediately converted to ddG in lymph nodes. This is because the ADA concentration in lymph nodes is higher than that in blood. In the stomach content, 20% of 6-Cl-ddG was converted to ddG, 7% of 6-Cl-ddG was converted to 6-Cl-G, and 73% of the 6-Cl-ddG was detected at 5 min. Normally dideoxy nucleosides are quite unstable in the acidic conditions encountered in the stomach [14], producing inactive products through glycosic cleavage. 6-Cl-ddG is also unstable in acidic conditions, and is converted to 6-Cl-G. Therefore 6-Cl-ddG or ddG should be formulated with an anti-acidic agent for oral administration.

After the administration of either 6-Cl-ddG or ddG subcutaneously, the peak ddG concentrations in the plasma were found at 5 to 10 min. The converted ddG concentration ($19.0 \mu\text{M}$) after the 6-Cl-ddG administration was lower than the concentration of ddG ($56.6 \mu\text{M}$) after the ddG administration, and the AUC of the converted ddG ($1217 \mu\text{M}\cdot\text{min}$) was lower than the AUC of ddG ($2533 \mu\text{M}\cdot\text{min}$). Nevertheless, the concentrations of converted ddG in all lymph nodes were higher at all times after 6-Cl-ddG administration than the ddG concentration after subcutaneous ddG administration. A 1.4- to 2-fold greater AUC value for converted ddG was therefore produced by the 6-Cl-ddG subcutaneous injection than the ddG AUC value obtained by the administration of ddG. These results indicate that the administration of the prodrug yielded greater AUC values for the ddG concentration in the lymph nodes after subcutaneous administration.

The converted ddG AUC value in the plasma after the 6-Cl-ddG administration was three times that after ddG administration by the oral route. The converted ddG AUC values in the lymph nodes were clearly detected after the 6-Cl-ddG oral administration. In contrast, ddG was not detected in the lymph nodes after the ddG oral administration. This evidence agrees with the results showing that 6-Cl-ddG is more stable than ddG *in vitro* stomach content (pH 3.5). These results provide evidence that the oral administration, and even the subcutaneous administration, of 6-Cl-ddG yields higher concentrations of ddG in the lymph nodes, where lentiviruses including HIV and SIV constantly replicate.

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