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Antiviral activity of berberine and related compounds against human cytomegalovirus

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Abstract—Berberine chloride (**1**) and the structurally related compounds were assessed for the anti-human cytomegalovirus (HCMV) activity using the plaque assay. The anti-HCMV activity (IC_{50} 0.68 μM) of **1** was equivalent to that (IC_{50} 0.91 μM) of ganciclovir (GCV). The mechanism of action by which **1** inhibits the replication of HCMV is presumed to be different from that of GCV; **1** would interfere with intracellular events after virus penetration into the host cells and before viral DNA synthesis.

Human cytomegalovirus (HCMV) belongs to herpes virus. HCMV generally does not significantly influence the health of persons with normal body. However, its infection sometimes threatens to cause serious diseases including retinopathy and acute hepatitis for immunocompromised patients such as AIDS and organ-transplanted patients. Although DNA polymerase inhibitors such as ganciclovir (GCV) and foscarnet are used for its treatment, their increased and prolonged use has led to the emergence of a resistant virus, which is remarkable for the immunocompromised patients.[1] Additionally, these medicines are reported to show side effects such as neutropenia[2] and nephrotoxicity.[3] Thus, it is required to develop a new type of antiviral drugs possessing different mechanism of action from those of these drugs. In the course of search for anti-HCMV agents, we paid attention to a benzylisoquinoline alkaloid, berberine chloride (**1**), and its related compounds, since papaverine hydrochloride (**2**) of the same group was reported to have the anti-HCMV activity.[4] The present work deals with the investigation on the anti-HCMV activity of **1** and the related compounds. Furthermore, the work involves the discussion on the structure–activity relationship of these compounds as well as on the mechanism of antiviral action of **1**.

In the first experiment, we examined the anti-HCMV activity of berberine chloride (**1**), as well as papaverine hydrochloride (**2**) and GCV as positive controls, by the plaque yield reduction assay.[5] Table 1 exhibits the anti-HCMV activity (50% inhibitory concentration, IC_{50}), cytotoxicity (50% cytotoxic concentration, CC_{50}) to MRC-5 cells, and resulting selectivity index (SI, CC_{50}/IC_{50}) for each compound. Compound **1** had ca. 30 times lower IC_{50} (0.68 μM) than did **2**. Although its IC_{50} value was equivalent to that (0.91 μM) of GCV, the SI of **1** (110) was 10 times inferior to that of GCV (1100).

Table 1. Anti-HCMV activities of **1**, **2**, and GCV

Sample	Cytotoxicity[a] (CC_{50}, μM)	Antiviral activity[a] (IC_{50}, μM)	Selectivity index[a] (SI, CC_{50}/IC_{50})
1	74	0.68	110
2	610	21	29
GCV	970	0.91	1100

[a] Values are means of three experiments.

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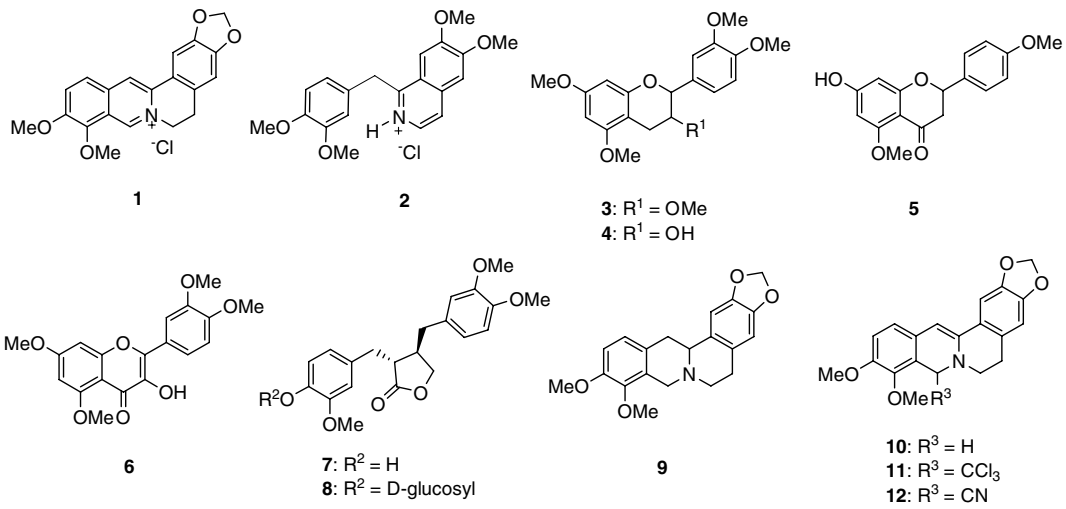

Superimposing the structures of **1** and **2** led us to assume that a polar function including a nitrogen atom in the center of molecule as well as a dimethoxybenzo- or [1,3]dioxolobenzo-group at its both ends would be a pharmacophore required for the expression of the anti-HCMV activity. In the second experiment, we thus investigated the anti-HCMV activities of the following compounds structurally related to **1**: 3,5,7,3′,4′-pentamethyl-(+)-catechin (**3**) and 5,7,3′,4′-tetramethyl-(+)-catechin (**4**),[6] 6,4′-dimethyl-naringenin (**5**),[7,8] 3,7,3′,4′-tetramethyl-quercetin (**6**),[9,10] arctigenin (**7**),[11] arctiin (**8**),[11] and berberine derivatives **9**–**12** (Table 2). Of these compounds, arctigenin (**7**), the aglucone of arctiin (**8**), had the relatively potent anti-HCMV activity (IC_{50} 2.1 μM), and its SI (36) was equivalent to the corresponding value (29) of papaverine hydrochloride (**2**). Arctiin (**8**) showed 8-fold greater IC_{50} (16 μM) than its aglucone **7**. Reduction of the quaternary amine of **1** to the tertiary amines **9**[12] and **10**[13] caused the serious lowering of antiviral activities (IC_{50} 12 and 93 μM, respectively). Although both 8-trichloromethyl-(**11**)[14] and 8-cyano-derivative (**12**)[15] showed the anti-HCMV activities (IC_{50} 0.52 and 0.88 μM, respectively) comparable with that of **1**, their SI values (16 and 18) were lower than that of **1** because of higher cytotoxicities. We speculate that the CCl_3 and CN groups of these compounds might have functioned as toxicants to the host MRC-5 cells after a release from the molecule. Thus, it is likely that a polar function such as the quaternary amine and lactone in the center of molecule as well as a methoxybenzo-, 1,2-dimethoxybenzo- or [1,3]dioxolobenzo-group at its both ends might be necessary for the anti-HCMV activity. Berberine chloride (**1**) seems to be a potent anti-HCMV candidate and worthy to examine further their possibility.

We therefore focused on the problem as to whether the antiviral target of **1** in HCMV replication is different from that of GCV, an inhibitor of viral DNA polymerase, in the following way. Berberine chloride (**1**) and GCV were separately administered to MRC-5 cell cultures at different points of time during a period from 0 to 120 h after virus infection, and the virus titer was measured by the plaque assay. Figure 1 shows the virus titer (percentage plaque numbers to that of control) versus time-of-addition of the compound tested.[16] It is clearly indicated that **1** inhibited the HCMV proliferation to approximately the same extent as GCV when added at 120 h post-infection. Furthermore, GCV inhibited extensively the virus proliferation when added at 54 h post-infection, whereas **1** did so only when added at 24 h post-infection, suggesting the different mode of action for these agents. It is presumed that compound

Table 2. Anti-HCMV activities of related compounds

Sample	Cytotoxicity[a] (CC_{50}, μM)	Antiviral activity[a] (IC_{50}, μM)	Selectivity index[a] (SI, CC_{50}/IC_{50})
3	68	17	4
4	190	>200	<1
5	12	92	0.13
6	20	26	0.77
7	76	2.1	36
8	240	16	15
9	125	12	10
10	155	93	1.7
11	8.4	0.52	16
12	16	0.88	18

[a] Values are means of three experiments.

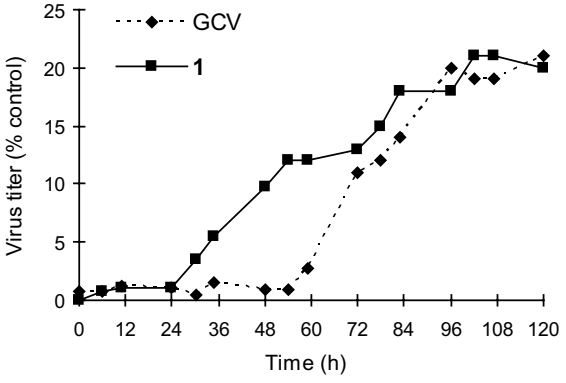

Figure 1. Time-of-addition study comparing the effect of **1** to that of GCV on HCMV replication. Data are expressed as means of duplicate assays.

1 would interact with earlier stage(s) of viral replication than that inhibited by GCV.

In order to estimate the sensitive target(s) of HCMV replication to **1**, we examined the effect of **1** on virus adsorption and penetration. The inhibition of virus adsorption was tested by evaluating directly the number of cells binding the virus particles in the presence of **1** through the infectious center assay.[17] As a result, the virus adsorption to the cells at 4 °C was not inhibited at the concentrations (0.2, 0.5, 1, and 2 μM) around the IC_{50} of **1**. Subsequently, a possibility was investigated as to whether or not **1** inhibits the virus internalization, the next stage of HCMV replication after attachment to host cells. The virus was adsorbed at 4 °C in the absence of **1** without penetration into the cells as confirmed by the plaque assay, and then the temperature was raised to 37 °C to allow the bound virus to penetrate either in the absence or presence of **1**.[18] The compound **1** did not inhibit the virus penetration at the concentrations of 0.2, 0.5, 1, and 2 μM. It is therefore likely that **1** interferes with intracellular events after virus penetration into the host cells and before viral DNA synthesis.

Berberine chloride (**1**) has a variety of pharmacological properties including antibacterial effect,[19,20] hypotensive effect,[21] antidiarrheal effect,[22,23] cholesterol-lowering effect,[24] hypoglycemic effect,[25] and antiviral activity.[26] Additionally, it is generally considered that **1** has neither genotoxic activity, significant cytotoxicity, mutagenicity nor recombinogenicity at doses used in clinical situations.[27]

The present work demonstrates that **1** had the anti-HCMV activity comparable with that of GCV. Furthermore, it was suggested that **1** inhibits the HCMV replication in a different mode of action from that of GCV. Thus, a single application of **1** or combination therapy with GCV may form an epoch in the treatment of HCMV infectious diseases since it could reduce the GCV-induced toxicity and resistant mutants.

Further study is under way to establish the detailed anti-HCMV mechanism of berberine chloride (**1**).

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