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Short communication

Antiviral activity of ganciclovir and artesunate towards human cytomegalovirus in astrocytoma cells

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ABSTRACT

Antimalarial drug artesunate inhibits cytomegalovirus (HCMV) replication in human fibroblasts. Astrocytes, the major cell type of the brain, support cytomegalovirus (HCMV) replication. The aim of the study was to assess the antiviral activity of artesunate in astrocytoma cell line U373MG in comparison with ganciclovir. The antiviral concentration inhibiting by 50% (IC_{50}) the synthesis of viral DNA was measured by real-time PCR in parallel in U373MG and MRC-5 cells. Reference HCMV strains susceptible and resistant to ganciclovir, and clinical isolates were tested. Ganciclovir and artesunate had similar activity in U373MG cells and MRC-5 fibroblasts. The artesunate IC_{50}s in U373MG cells (1.5–2.25 μM) were at least 36-fold lower than the 50% cytotoxicity concentrations. Then, the anti-HCMV activity of artesunate was demonstrated in a cancer cell line.

Central nervous system (CNS) injuries are life-threatening complications of human cytomegalovirus (HCMV) infection in immunocompromised individuals, and lead to serious neurodevelopment sequelea in infants with congenital infection. In addition, HCMV has been recently associated with brain tumors (Mitchell et al., 2008). These CNS disorders can result from both direct and indirect effects of HCMV infection (Cheeran et al., 2009). However, activity of antiviral drugs in cells originating from CNS is of great concern. The clinical use of current antiviral drugs ganciclovir, foscarnet and cidofovir is limited by severe adverse side effects and the possible emergence of resistance. Artesunate, an antimalarial drug, inhibits in vitro replication of HCMV by mechanisms linked to cellular activation pathways (de Vries and Dien, 1996; Efferth et al., 2002; Arav-Boger et al., 2010). Thus, it has been proposed as an alternative to the currently available antiviral drugs (Shapira et al., 2008). Activation of HCMV replication is differentially regulated according to the cell type (Sadanari et al., 2009). Therefore in vitro artesunate activity has to be assessed in different cell types. Astrocytes, the major cell constituting 70% of the brain, support HCMV replication. The aim of the study was to evaluate the antiviral activity of artesunate in HCMV-infected U373 astrocytoma cell line in comparison with ganciclovir.

Ganciclovir and artesunate were purchased from Sigma–Aldrich (Evry, France). Fourteen HCMV strains were tested in parallel in MRC-5 cells (Argène, Varhiles, France) and U373MG cells (generous gift from Dr. David Boutolleau, Pitié-Salpétrière Hospital, Paris). They included four clinical isolates, the reference strains AD169, Davis, Towne and Toledo, the recombinant viruses Rec545, Rec987, Rec495 and Rec715, and the laboratory strains RCL-1 and ADm773 already described (Schnepf et al., 2009). Artesunate has been shown to inhibit HCMV DNA synthesis in a dose-dependent manner, then its activity was evaluated by measuring the drug concentration inhibiting by 50% (IC_{50}) the viral DNA synthesis using a real-time PCR-based assay as already reported (Schnepf et al., 2009). The test was performed after a four-day incubation in the absence and the presence of the drug to test. The effect of the multiplicity of infection (MOI) on the artesunate IC_{50}s in U373MG cells was determined in parallel experiments in which cell cultures were infected by reference strain AD169 at a range of MOIs from 0.02 to 0.4.

The artesunate IC_{50} values did not vary according to the MOI in the range tested as shown in Fig. 1. To test the drug activity towards the different HCMV strains, cell layers were infected at a MOI of 0.025. The artesunate IC_{50}s determined in U373MG cells were found similar to those obtained in MRC-5 fibroblasts just as ganciclovir had a similar activity in MRC-5 and U373MG cells (Table 1). The results confirmed the absence of cross-resistance to conventional anti-HCMV drugs already reported (Efferth et al., 2002). To test whether the reduction in DNA copy numbers in artesunate treated U373MG cells was due to unspecific cytotoxicity of artesunate, cellular toxicity was measured using the CytoTox96® Non-Radioactive cytotoxicity assay (Promega, Charbonnières, France) as reported by Efferth et al. (2002). The assay

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Table 1
Antiviral activity of ganciclovir and artesunate in HCMV-infected U373 cells and MRC-5 fibroblasts.

HCMV strain	Mutation		Ganciclovir IC_{50} (μM)		Artesunate IC_{50} (μM)	
	UL97	UL54	MRC-5	U373	MRC-5	U373
AD169	–	–	1.2 ± 0.5	1.17 ± 0.15	3.0 ± 1.0	2.22 ± 0.48
Davis	–	–	1.2 ± 0.3	1.6 ± 0.3	1.9 ± 0.14	1.5 ± 0.6
Toledo	–	–	1.8 ± 0.3	1.5 ± 0.3	3.1 ± 0.14	2.25 ± 1.0
Towne	–	–	3.2 ± 0.3	1 ± 0.4	4 ± 0.16	2.3 ± 0.42
Rec495	–	N495K	2.1 ± 0.2	1.1 ± 0.3	2.9 ± 0.5	1.42 ± 0.38
Rec987	–	A987G	**7.0 ± 1.4**	**6.7 ± 1.4**	2.33 ± 1.0	1.65 ± 0.92
Rec545	–	L545S	**9.35 ± 1.2**	**5.1 ± 0.8**	2.64 ± 0.51	2.25 ± 0.91
Rec715	–	V715M	1.3 ± 0.14	1.2 ± 0.22	1.7 ± 0.22	1.7 ± 0.30
ADm773	–	L773V	3.4 ± 0.5	2.9 ± 0.25	2.9 ± 1.0	1.75 ± 0.35
RCL-1	A494P	L545S	**14.7 ± 1.0**	**11.6 ± 3.5**	2.45 ± 0.6	1.95 ± 0.6
Iso1	L595S	–	**16.5 ± 0.2**		2.6 ± 0.5	1.7 ± 0.4
Iso2	C592G	P522S T838A	**37.5 ± 3.5**	**25.3 ± 3.6**	2.75 ± 0.35	2.2 ± 0.14
Iso3	–	–	2.5 ± 0.25		2.1 ± 0.19	
Iso4	–	–	1.9 ± 0.3		3.2 ± 0.25	

IC_{50}: antiviral concentration inhibiting by 50% the synthesis of viral DNA. The IC_{50} values are expressed as the mean of at least 2 independent experiments. In bold: IC_{50} values for strains resistant to ganciclovir.

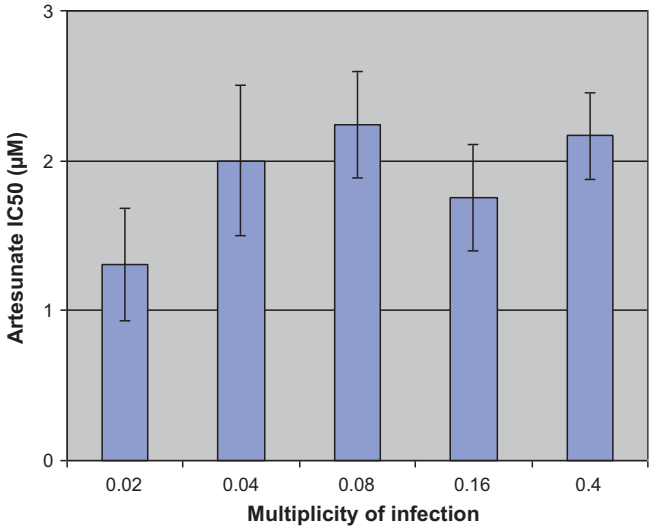

Fig. 1. Antiviral drug concentrations inhibiting by 50% (IC_{50}s) the DNA synthesis of strain AD169 according to the multiplicity of infection (MOI). Mean IC_{50} values (1.3–2.23 μM) and standard deviations were indicated. They were calculated from three to five independent experiments.

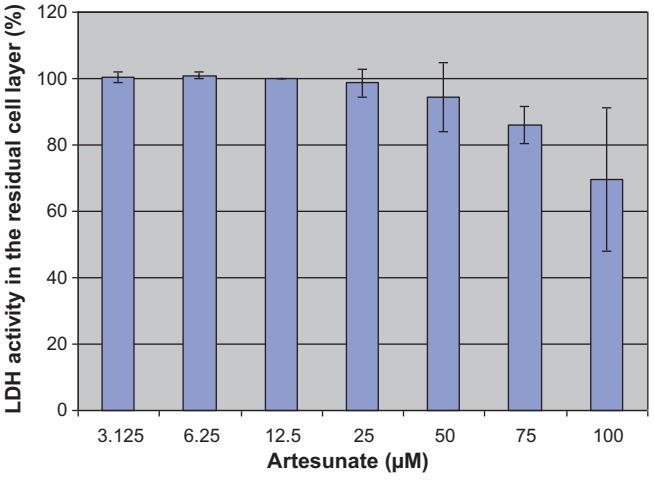

Fig. 2. Cytotoxicity profiles of artesunate for U373MG cells. The cells were cultivated in the presence of different concentrations of the drug. After four days the lactate dehydrogenase (LDH) activity was determined. Mean values (±standard deviations) of three independent experiments are shown.

determines the lactate dehydrogenase (LDH) activity in the residual cells after incubation with the drug to test. Briefly, U373MG were cultivated in 48-well plates until reaching confluency as for the antiviral assays. Serial dilutions of artesunate were then incubated in the medium at 37 °C for four days. A reduction in LDH activity was not found in the relevant range of artesunate concentrations under 50 μM (Fig. 2). The mean artesunate concentration leading to 50% cytotoxicity (CC_{50}) was 108 ± 13.12 μM, indicating the selectivity index (ratio between CC_{50} and IC_{50}) above 36. The cytotoxicity assay was also performed after a seven-day incubation period and gave similar results.

Antiviral activity of ganciclovir in U373MG cells was expected as ganciclovir targets the viral DNA polymerase, enzyme essential for replication. Molecular mechanisms of artesunate antiviral activity are not yet fully understood. Efferth et al. (2002) showed a diminished DNA-binding activity of NF-κB and Sp1 in the presence of artesunate in HCMV-infected fibroblasts, leading to suppression of major immediate early gene expression. However, variations in expression of the cellular transcription factors among different cell types lead to differences in the regulation of the HCMV major immediate early promoter. Here, artesunate was demonstrated to be able to inhibit HCMV replication in a cancer cell line (U373MG) to the same extent as in human fibroblasts.

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