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Short Communication

Dose- and schedule-dependent protective efficacy of celgosivir in a lethal mouse model for dengue virus infection informs dosing regimen for a proof of concept clinical trial

Satoru Watanabe [a,1], Abhay P.S. Rathore [a,1], Cynthia Sung [a,1], Fan Lu [b], Yok Moi Khoo [b], John Connolly [c], Jenny Low [d], Eng Eong Ooi [a], How Sung Lee [b], Subhash G. Vasudevan [a,*]

[a] Program in Emerging Infectious Diseases, Duke-NUS Graduate Medical School, 8 College Road, Singapore 169857, Singapore
[b] Department of Pharmacology, Yong Loo Lin School of Medicine, Clinical Research Centre 10 Medical Drive, Singapore 117597, Singapore
[c] Singapore Immunology Network (SIgN), 8A Biomedical Grove, Immunos Building, Level 4, Singapore 138648, Singapore
[d] Department of Infectious Diseases, Singapore General Hospital, Singapore 169608, Singapore

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ABSTRACT

Celgosivir (6-O-butanoyl castanospermine), a pro-drug of the naturally occurring castanospermine, is an inhibitor of α-glucosidase I and II that is found to be a potent inhibitor of several enveloped viruses including all four serotypes of dengue virus. We showed previously that the compound fully protected AG129 mice from lethal infection with a mouse adapted dengue virus at a dose of 50 mg/kg twice daily (BID) for 5 days and was effective even after 48 h delayed treatment. Here we show that the protection by celgosivir is dose- and schedule-dependent and that a twice-a-day regimen of 50, 25 or 10 mg/kg is more protective than a single daily dose of 100 mg/kg. Treatment with 50 mg/kg BID castanospermine had comparable efficacy as 25 mg/kg BID celgosivir, suggesting that celgosivir is approximately twice as potent as castanospermine with respect to *in vivo* antiviral efficacy. Pharmacokinetics (PK) studies of celgosivir in mice showed that it rapidly metabolized to castanospermine. Simulation of the PK data with the survival data for the various doses of celgosivir tested suggests that the steady-state minimum concentration is a critical parameter to note in choosing dose and schedule. These results influenced the selection of the dose regimen for a proof-of-concept clinical trial of celgosivir as a treatment against dengue fever.

Dengue is a global public health threat caused by infection with any of the 4 related viral serotypes (DENV1–4). Clinical manifestations range from self-limiting febrile illness, known as dengue fever (DF), to the life-threatening severe diseases, such as dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) (Gubler, 2006; Halstead, 2007). Severe diseases are often accompanied with secondary heterotypic infections, probably due to the phenomenon that is described as antibody-dependent enhanced (ADE) infection (Halstead, 2003). Annually, there are more than 50 million human infections and several hundred thousand cases of DHF/DSS (Halstead, 2007). At present, however, there are no approved preventive vaccines or antiviral drugs against DENV infection.

Celgosivir, also known as 6-O-butanoyl castanospermine (Bu-Cast), is a butyl ester derivative of castanospermine (Cast), a natural product derived from the Moreton Bay chestnut tree (*Castanospermum australae*) (Molyneux et al., 1986). It readily crosses cell membranes and is rapidly converted to Cast (Kang, 1996), which acts as an α-glucosidase I and II inhibitor in the endoplasmic reticulum (ER). Celgosivir or Cast have been shown to have broad antiviral effects in cell culture systems and/or *in vivo* mouse models such as Sindbis virus, CMV, influenza virus, several flaviviruses, HSV, HIV-1 and HBV (Ahmed et al., 1995; Block et al., 1994; Bridges et al., 1995; Saito and Yamaguchi, 2000; Schlesinger et al., 1985; Schul et al., 2007; Sunkara et al., 1989; Taylor et al., 1988; Whitby et al., 2005; Zitzmann et al., 1999). Since the drug target is a host enzyme required for viral maturation, the potential for development of drug resistance is expected to be lower than a drug directed against a viral target. In a previous study, we demonstrated that celgosivir drastically inhibits the replication of clinical dengue strains of DENV1–4 by impairing proper folding of viral glycoproteins such as NS1, E and prM (Courageot et al., 2000; Rathore et al., 2011). In a lethal DENV infection mouse model using AG129 mice (Sv/129 mice deficient in type I and II IFN receptors), treatment of 50 mg/kg of celgosivir given twice daily resulted in 100% survival by day 10 after infection (Rathore et al., 2011). These results encouraged further development of celgosivir as a possible

* Corresponding author. Tel.: +65 6516 6718; fax: +65 6221 2529.
E-mail address: subhash.vasudevan@duke-nus.edu.sg (S.G. Vasudevan).
[1] These authors made equal contribution in this study.

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treatment for DENV infection. In this study, we examined the dose effect and pharmacokinetics (PK) *in vivo* in order to gain a better understanding of celgosivir as a potential anti-dengue drug in clinical settings.

To evaluate the effect of dose and schedule of celgosivir treatment, four drug regimens were studied in the ADE mouse model: 10, 25 and 50 mg/kg twice a day (BID) and 100 mg/kg once a day (QD) for 5 days. ADE was induced in AG129 mice by first injecting intraperitoneally (i.p.) 20 μg of mouse monoclonal antibody against DENV E protein (4G2 clone) one day prior to infection (Zellweger et al., 2010) followed by infection (i.p.) with 2×10^5 pfu of mouse-adapted DENV2 strain S221. In this model vehicle control mice that did not receive celgosivir treatment died from severe disease on day 4 or 5 after infection. For the BID treatment schedule, survival rates of 13%, 63% and 100% were observed for 10, 25, and 50 mg/kg BID ($P = 0.13$, 0.003, 0.0002, compared to untreated mice, respectively) at day 10 (Fig. 1A). Surprisingly, although 50 mg/kg BID dosing achieved complete protection, the same total dose of 100 mg/kg given as a single daily dose for 5 days failed to protect mice from death, with none surviving past day 6, which is comparable to mice given vehicle control ($P = 0.4$) but is significantly different to mice given 50 mg/kg BID ($P = 0.0002$) (Fig. 1A). Even 25 mg/kg given BID was more effective than 100 mg/kg QD ($P = 0.0042$ between the 2 groups) (Fig. 1A). Viremia on day 3 also showed significant reduction in mice given 50 and 25 mg/kg BID, but not in mice with 100 mg/kg QD (Fig. 1B), indicating that the drug, when divided into two doses, is more effective than giving the same total dose once a day.

Celgosivir has been shown to be 100 times more potent than the naturally occurring parent molecule (Cast) at inhibiting the replication of DENV (Rathore et al., 2011) as well as HIV-1 (Taylor et al., 1991) and Rauscher murine leukemia virus (RLV) (Ruprecht et al., 1991) at a cellular level. This greater antiviral activity of celgosivir is thought to be due to improved cellular uptake (Kang, 1996). However, celgosivir did not show any advantage in antiviral efficacy over the Cast *in vivo* in the RLV mouse system (Ruprecht et al., 1991). Upon DENV infection, Cast was also shown to be effective *in vivo* in the encephalitis mouse model using A/J mice (Whitby et al., 2005). We therefore tested the antiviral effect of Cast in our ADE mouse system for a direct comparison with celgosivir. Treatment with 50 mg/kg BID dosing of Cast showed significant protective effect ($P = 0.004$) with survival rate of 60% at day 10 after infection (Fig. 1C); however, the protective efficacy was less prominent compared to the complete protection of celgosivir at 50 mg/kg BID (Fig. 1A), demonstrating the improvement in the antiviral efficacy of celgosivir over Cast against DENV. The treatment effect of 50 mg/kg BID dosing of Cast was comparable to that achieved by 25 mg/kg BID of celgosivir. Thus, although the *in vitro* potency of Cast is 100-fold lower than celgosivir (Rathore et al., 2011), *in vivo* potency appears to be only twofold lower. This observation needs to be thoroughly examined in future studies.

PK in the mouse after a single i.p. dose of celgosivir or Cast at a dose of 50 mg/kg was examined using a validated LC/MS/MS assay. Celgosivir was rapidly converted to Cast and measurable in serum only up to 1 h post-dosing at the lower limit of quantification (LLOQ) of 10 ng/ml (Fig. 2). This result corresponds to a previous

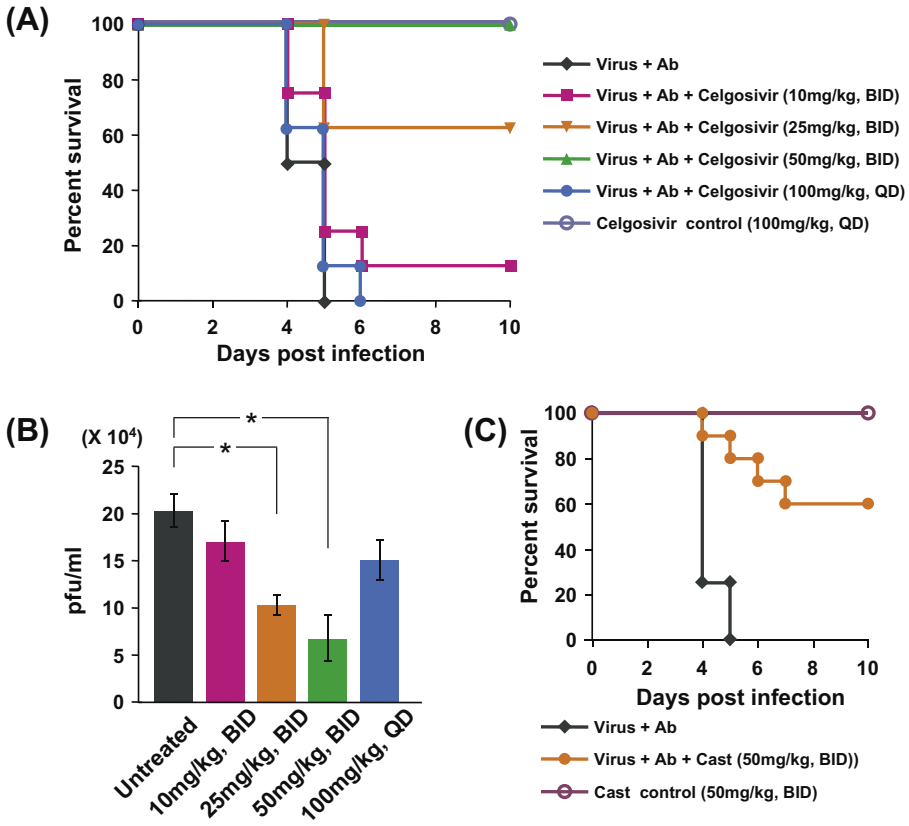

Fig. 1. Dose and schedule effects of celgosivir against lethal DENV infection. AG129 mice were inoculated i.p. with 2×10^5 pfu of S221 in the presence of 20 μg of DENV anti-E Ab (4G2). (A and B) Mice were treated with saline vehicle or different concentrations and doses of celgosivir at the time of infection and daily for 5 days. Mouse survival rates were monitored until day 10 post infection (A) and survival significance was evaluated using log-rank test. Viremia on day 3 post infection was measured by standard plaque assay using BHK-21 cells (B). Significant differences between data groups were determined by 2-tailed Student *t* test analysis and *P* value less than 0.05 was considered significant ($^*P < 0.05$). The numbers of mice per group are 7 (50 mg/kg BID) or 8 (vehicle control, 10, 25 mg/kg BID and 100 mg/kg QD). (C) Mice were treated with saline vehicle or 50 mg/kg of Cast twice a day for 5 consecutive days, and their survival rate was monitored until day 10 post infection. Survival significance was evaluated using log-rank test. The numbers of mice per group are 8 (vehicle control) or 10 (50 mg/kg BID).

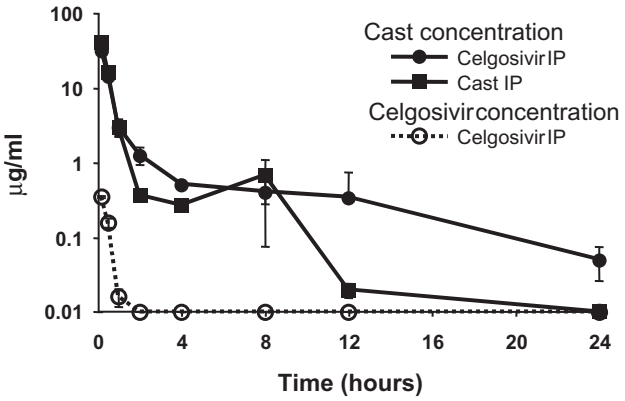

Fig. 2. Pharmacokinetic profile of celgosivir and castanospermine (Cast) after a single administration of 50 mg/kg dose. AG129 mice were injected i.p. with 50 mg/kg of celgosivir or Cast, and blood samples were collected at 10, 30 min, 1, 2, 4, 8, 12 and 24 h after injection. The serum concentration of celgosivir and Cast were measured by LC/MS/MS assay as described in Supplementary materials and methods. The graphs show the average concentrations with standard deviations. The number of mice per group is 3.

report that only Cast was detected at 5 min post-dosing in the plasma of mice given 25 mg/kg of celgosivir orally (Kang, 1996). The maximal Cast concentration (Cmax) of 31.6 µg/mL occurs at the first sampling time of 10 min post-dosing (Fig. 2). Concentration curves were analyzed by noncompartmental analysis with WinNonlin Professional 5.2.1 (Table 1A). Clearance was 2.2 L/h/kg, terminal half-life and mean residence time were 5.0 and 3.6 h, respectively. Dosing with castanospermine yielded a somewhat higher Cmax at 10 min, shorter half-life, but comparable area under the concentration curve (AUC) (Table 1A).

Celgosivir pharmacokinetics in humans is linear over doses ranging from 10 to 450 mg (Sorbera et al., 2005). On the assumption that PK in the mouse is linear as well, the concentration profiles of castanospermine at the dosing regimens used to treat dengue infected mice (Fig. 1) were simulated by nonparametric superposition to predict steady-state Cmax, Cmin and AUC (Table 1B). Interestingly, the parameter that best correlates with the survival rate is steady-state Cmin.

Celgosivir has been evaluated in human clinical trials in over 600 patients with HIV or HCV (Durantel, 2009). The maximally tolerated dose in humans was 400 mg QD, and comparable tolerability was observed at 240 and 300 mg BID (Roth et al., 1996). Although celgosivir exhibited antiviral activity at 400 mg QD for 12 weeks, it was not superior to existing treatments for HIV and HCV (Durantel, 2009). PK simulations of various dosing regimens in humans indicate that 200 mg BID will achieve steady-state Cmin similar to levels attained at 50 mg/kg BID in the mouse. A loading dose of 400 mg will achieve steady state levels after the second dose. Hence the dosing regimen selected for clinical testing is 400 mg loading dose with 200 mg BID. In conclusion, the mouse pharmacology and PK results, along with the previously published human PK, have guided selection of the dosing regimen to be tested in a Phase 1b clinical trial (http://clinicaltrials.gov/ct2/show/NCT01619969) to evaluate the antiviral efficacy of celgosivir in patients with dengue fever.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.antiviral.2012.07.008.

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Table 1
(A). Pharmacokinetic parameters of castanospermine after celgosivir or castanospermine dosing at 50 mg/kg. (B). Steady state castanospermine concentrations at different doses and schedules and corresponding survival in dengue-infected mice.

Drug	Route	Tmax[a] (min)	Cmax[b] (µg/mL)	AUC[c] (µg/mL•h)	CL/F[d] (L/h/kg)	Terminal half-life (h)	Mean residence time (h)
Celgosivir	i.p.	10	31.6	22.8	2.19	5.0	3.6
Castanospermine	i.p.	10	40.9	20.8	2.40	2.3	1.5

Steady-state PK parameter	Dose in mouse			
	10 mg/kg bid	25 mg/kg bid	50 mg/kg bid	100 mg/kg qd
Cmin[e] (µg/ml)	0.08	0.20	0.40	0.10
Cmax (µg/ml)	6.4	16.0	32.0	64.0
AUC per day (µg/mL•h)	9.1	22.8	45.6	45.6
% Survival	12.5	62.5	100	0

[a] Time to maximal concentration.
[b] Maximum concentration.
[c] Area under the plasma curve.
[d] Total body clearance.
[e] Minimum concentration.

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