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Short Communication

Competitive inhibitor of cellular α-glucosidases protects mice from lethal dengue virus infection

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ABSTRACT

Dengue virus infection causes diseases in people, ranging from the acute febrile illness dengue fever, to life-threatening dengue hemorrhagic fever/dengue shock syndrome. We previously reported that a host cellular α-glucosidases I and II inhibitor, imino sugar CM-10–18, potently inhibited dengue virus replication in cultured cells, and significantly reduced viremia in dengue virus infected AG129 mice. In this report we show that CM-10–18 also significantly protects mice from death and/or disease progress in two mouse models of lethal dengue virus infection. Our results thus provide a strong support for the development of CM-10–18 or its derivatives as antiviral agents to treat servere dengue virus infections.

Dengue virus (DENV) is mosquito-borne flavivirus that causes mild dengue fever (DF) or lethal dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) in people (Mackenzie et al., 2004). Four distinct serotypes of DENV have spread throughout the tropical and subtropical world, with an estimated 50–100 million human cases annually and about 2.5 billion people worldwide being at risk of infection (King et al., 2007). Thus far, effective antiviral therapies and vaccines are not yet available to treat or prevent DENV infections in humans (Cassetti et al., 2010).

DHF and DSS are considered to correlate directly with higher titers of viremia and thus, antiviral therapies that lower the viral load in the early phase of infection are anticipated to decrease disease severity and reduce mortality (Vaughn et al., 2000). Currently, several antiviral compounds targeting either DENV-encoded enzymes or host cellular functions required for DENV replication are under development (Noble et al., 2010).

Imino sugars, exemplified by deoxynojirimycin (DNJ), are glucose mimetics with a nitrogen atom in place of a ring oxygen (Dwek et al., 2002) and are competitive inhibitors of ER-resident α-glucosidases I and II. As with other enveloped viruses, proper processing of N-linked glycans on DENV envelope (E) glycoprotein is essential for its proper folding, oligomerization and thus, virion particle assembly and secretion. The ER α-glucosidases I and II catalyze the sequential removal of three terminal glucose residues on the N-linked glycans of glycoproteins and thus are essential host proteins for DENV assembly and secretion (Helenius and Aebi, 2004). Accordingly, we and others have made extensive effort to develop imino sugar α-glucosidase inhibitors as broad-spectrum antiviral agents against enveloped viruses, including DENV (Block et al., 1998; Chang et al., 2011, 2009; Dwek et al., 2002; Gu et al., 2007; Qu et al., 2011; Schul et al., 2007; Whitby et al., 2005; Wu et al., 2002). Recently, we synthesized a group of imino sugar derivatives that demonstrated superior antiviral activity against DENV and other flaviviruses in cultured cells. More importantly, one of the derivatives, CM-10–18, had been shown to reduce viremia in DENV-infected AG129 mice (Chang et al., 2011).

The paramount feature of DENV pathogenesis is the immune response mediated leakage of blood vessels, which is the pathological basis of the hemorrhagic symptoms (Green and Rothman, 2006). Animal models that recapitulate characteristic dengue hemorrhagic symptoms are useful for testing the therapeutic efficacy of investigative antiviral drugs. However, most laboratory strains and even clinical isolates of DENV do not replicate well in immune competent mice, and thus do not cause disease.

AG129 mice are defective in both type I and type II interferon receptors (van den Broek et al., 1995), and support efficient replication of all four serotypes of DENV with tissue/organ tropism similar to that in humans (Williams et al., 2009). Although a transient viremia, peaking between 3 and 5 days post infection, is readily detectable, the mice die from neurological disorders, and not hemorrhagic diseases (Johnson and Roehrig, 1999; Schul et al., 2007).

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Alternatively, two AG129 mice-based DENV infection models, which reproduce a typical viremia as well as severe vascular leakage leading to hemorrhage-related death, are currently available (Shresta et al., 2006; Tan et al., 2010). Taking advantage of these dengue disease models, we set out to test the therapeutic potential of CM-10–18 for treatment of lethal DENV infection and its associated hemorrhagic diseases.

First we used the lethal disease model in which mice are infected with mouse-adapted serotype 2 dengue virus, D2S10 (Shresta et al., 2006). The AG129 mice were challenged with 2×10^7 PFU of the virus intravenously. All control mice treated with PBS showed severe illness, characterized by ruffled fur, hunched and sluggish movement on day 6 post infection and euthanasia conditions were applied. Similarly, all animals treated with ribavirin (40 mg/kg, once daily) also showed severe sickness with euthanasia condition applied on day 5 after infection. On the contrary, animals treated with 75 or 150 mg/kg (oral administrated, twice daily at 12 h interval) of CM-10–18 all maintained a healthy appearance (Fig. 1). This result indicates that CM-10–18, is not only well tolerated at up to 300 mg/kg/day, it also demonstrates a beneficial effect of treatment by preventing disease progression related to lethal DENV infection.

To confirm and extend the above observation, we utilized a newly developed model of severe Dengue infection, in which AG129 mice are infected with a non-mouse-adapted serotype 2 dengue virus stain, D2Y98P-rc (Tan et al., 2010). Intraperitoneal injection of 10^7 PFU of the virus caused death of the animals within 8–13 days in the group of mice treated with PBS only. On the contrary, treatment of the mice with CM-10–18, orally administrated twice daily at a 12 h interval for the first 3 days after infection, resulted in a significant delay of death in a dose-dependent manner (Fig. 2A). Interestingly, 40% of mice treated with 75 mg/kg CM-10–18, administrated twice daily at 12 h interval for the first 3 days after infection, survived from the infection (Fig. 2B). As positive control, a DENV NS5 RNA polymerase inhibitor, NITD008, as previously reported, conferred complete protection of the infected mice (Yin et al., 2009).

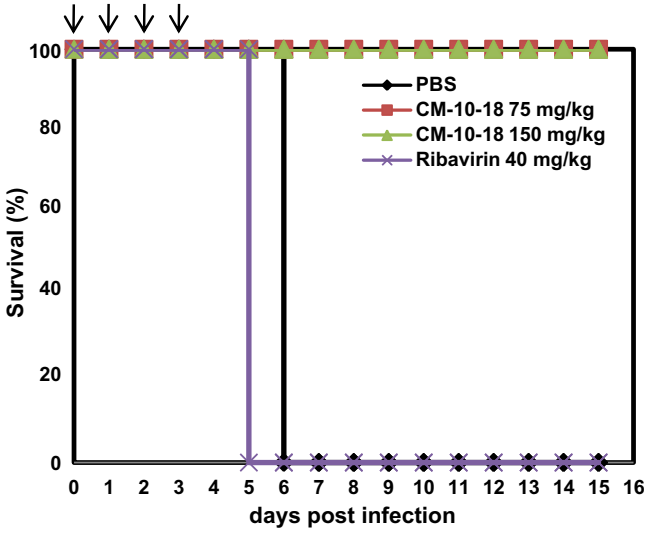

Fig. 1. CM-10–18 protects death of AG129 from lethal DENV infection. AG129 mice were challenged with 2×10^7 PFU DENV (serotype 2, strain D2S10) *via* tail vein injection. In treatment groups, CM-10–18 was given orally at two indicated doses (75 or 150 mg/kg in PBS) twice daily at 12 h interval. As control, one group of mice was treated with ribavirin at 40 mg/kg once daily. Negative control mice were given PBS. The treatment started immediately after infection until 3 days post infection (arrows indicate treatment days). Curves represent percentage of survival in each group that contains five mice. Log rank test $p < 0.003$ for two of the CM-10–18 treated groups.

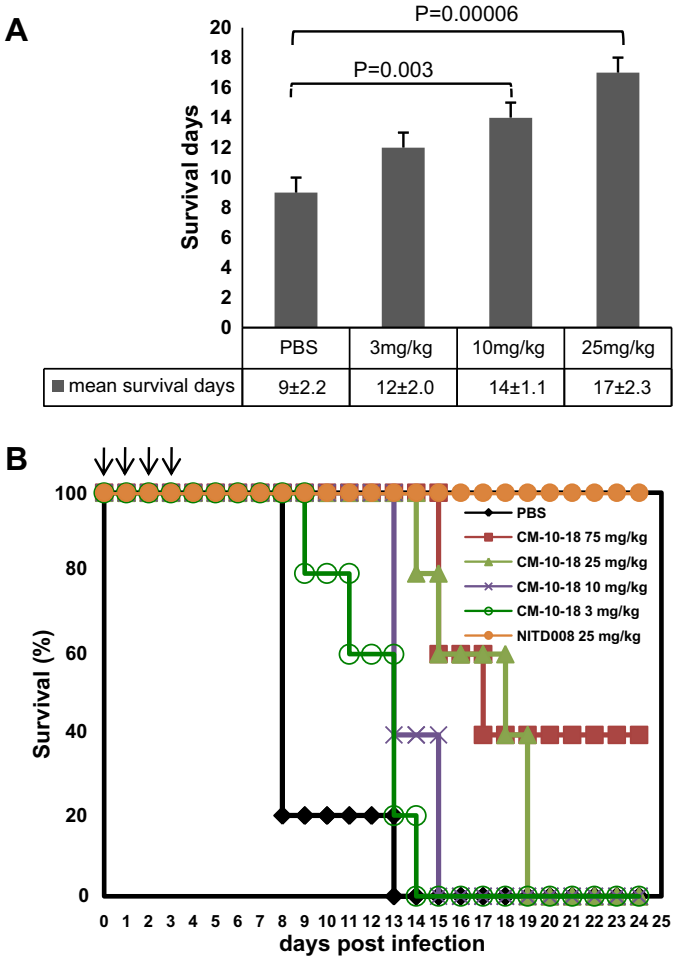

Fig. 2. CM-10–18 delays disease progress and partially protects animal death from lethal DENV infection of AG129. AG129 mice were challenged with 10^7 PFU DENV (serotype 2, strain D2Y98P-rc) *via* intraperitoneal injection. CM-10–18 was given orally at indicated doses (3, 10, 25, or 75 mg/kg) twice daily at 12 h interval, starting immediately after infection until 3 days post infection. Negative control mice were given PBS. As positive control, NITD008 was given at 25 mg/kg twice daily. Each group contains five mice. (A) Mean survival days post infection. (B) Survival curves post infection. Log rank test $p < 0.003$ for 25 mg/kg and 75 mg/kg group; $p = 0.013$ for 10 mg/kg group; $p = 0.079$ for 3 mg/kg group.

Although CM-10–18 treatment at 75 mg/kg, significantly increased the survival rates (log rank test $p < 0.003$) in both models, the effect was less profound in nonmouse adapted virus infection model. This is possibly due to the single amino acid mutation in NS4B, which confers higher virulence to nonmouse adapted strains of dengue virus (D2Y98p-rc) in mice, through enhancement of viral RNA synthesis (Grant et al., 2011).

Previously we have reported that CM-10–18 treatment increased specific serum FOS (Glc1Man4GlcNAc1, a marker for glucosidase inhibition) as well as efficiently reduced viremia in dengue virus infected AG129 mice (Chang et al., 2011). In this report, we further demonstrate that CM-10–18 treatment alleviated the hemorrhagic diseases caused by DENV infection. Taken together, these results suggest CM-10–18 treatment confers increase in survival rate in lethal dengue infection mice models through the proposed host-targeting antiviral effect. Since the infected animals were only treated for the first 3 days post infection, the antiviral efficacy of CM-10–18 observed under these experimental conditions might even be underestimated. Practically, treatment can only be applied once the patient developed viremia. Therefore, in further studies, CM-10–18 will be tested with treatment starting not only immediate after infection, but also with several days delay. Nevertheless,

the work reported herein encourages the further development of imino sugars as antiviral agents for treatment of lethal DENV infection and establishes a solid foundation for in vivo antiviral efficacy study of CM-10–18 and derivatives in the future.

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