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Effect of T-705 treatment on western equine encephalitis in a mouse model

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Summary

A mouse model of western equine encephalitis (WEE) was characterized for use in antiviral studies. Virus was detected in several tissues, most notably an average titer of 9.5 ± 1.1 Log_{10} 50% cell culture infectious doses ($CCID_{50}$)/g tissue in the brains of infected animals. Signs of WEE included limb weakness, paralysis, involuntary spasms or extension of limbs, clenching of paws, hunching, ruffling of fur, and eye exudates, many of which are indicative of neurological disease. The pyrazinecarboxamide derivative, T-705, was found to be active in Vero cells against WEE virus (WEEV) with an 90% effective concentration (EC_{90}) of 49 μg/ml (selective index [SI] >20). Treatment with T-705 in this WEE mouse model resulted in significant improvement in survival and mean day to death after oral treatment administered twice a day for 7-days at a dose of 400 mg/kg/d. Virus titer in the brain was not significantly reduced, despite a 1-log reduction in average brain titer in treated animals on 4 dpi. Signs of disease were relatively mild in treated animals, but were not eliminated. Treatment with T-705 improved morbidity and mortality of WEEV-infected mice, further illustrating the broad-spectrum activity of T-705 in the treatment of RNA viruses.

T-705, a pyrazinecarboxamide derivative, has been shown to have broad-spectrum activity against several RNA viruses. Most notably, this compound is effective against influenza in cell culture as well as in animal models through inhibition of the viral polymerase enzyme (Furuta et al., 2002; Furuta et al., 2005; Sidwell et al., 2007; Takahashi et al., 2003) and is currently undergoing clinical trials for the treatment of influenza infection in man (2007). Other RNA viruses that are effectively treated by T-705 in animal models include yellow fever virus (Julander et al., 2009), West Nile virus (Morrey et al., 2008), and several bunya and arenaviruses (Gowen et al., 2007). With efficacy seen in an encephalitic West Nile virus model, it was anticipated that T-705 might also be effective in the treatment of western equine encephalitis virus (WEEV) infection in cell culture as well as in a mouse model.

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WEEV is an alphavirus that causes occasional outbreaks of human disease (Calisher, 1994) and is listed as a category B agent on the NIH Biodefense and Emerging Diseases list as an agent of bioterrorism concern (Sidwell and Smee, 2003). Emphasis has been placed on finding therapeutic options for viruses on the NIH list (http://www3.niaid.nih.gov/topics/emerging/list.htm), which places value on the discovery of broad-spectrum antiviral agents. The present study is in line with this philosophy- attempting to show efficacy of the broad-spectrum antiviral T-705 against WEEV.

Hamsters, which are very susceptible to WEEV infection, were used in previous antiviral studies, but neurological signs of disease are not observed in infected hamsters, despite relatively high virus titer detected in the brain (Julander et al., 2007). While performing a titration of WEEV (VR-70, American Type Culture Collection) administered intraperitoneally (i.p.) in C57BL/6 mice (18–20 g, Charles River Laboratories) disease signs that were indicative of neurologic involvement were observed, which included limb weakness, paralysis, hunching, and fur ruffling. Challenge doses of $10^{3.9}$ or $10^{4.9}$ pfu/ml given i.p. resulted in high mortality (80–90%), which correlated with observation of the aforementioned disease signs. Infection of mice by s.c. injection with $10^{4.9}$ pfu/ml resulted in low morbidity and mortality (1/7 showed neurologic disease signs and died shortly thereafter). It appeared that route of virus challenge had a large influence on the progression of neurological disease.

Disease parameters of 6–8 week old C57BL/6 mice infected with WEEV were evaluated to determine appropriate markers of morbidity for use in antiviral studies. Virus was detected by infectious cell culture assay in several tissues, including brain, liver, spleen, kidney, and serum of infected mice (Figure 1), but not in sham-infected controls (data not shown). While virus was detected in all of the tissues tested, brain titers reached the highest levels, had a more consistent distribution, and peaked 4 dpi, which was similar to previously published data (Liu et al., 1970). The brain is also the target organ in WEE models (Aguilar, 1970), as well as in human infection (Anderson, 1984), which underlies the potential importance of a therapeutic agent that can lower the titer in the brains of infected animals.

An average mortality rate of 78% (range 76–100%) was observed in mice infected with WEEV in four separate experiments. The mean day to death of these animals was 5.6 ± 1.2 dpi, which was around 1 day longer than mean day to death of hamsters infected with the same virus strain (Julander et al., 2007). Significant ($P<0.01$) weight loss was observed beginning 4 days post-virus injection (dpi), which continued through 6 dpi (Figure 1).

Ninety percent of a group of 10 mice showed disease signs, which included limb weakness, paralysis, ruffled fur, and hunching (Table 1). More serious disease signs were generally followed by death, and included lying prone, involuntary extension of limbs, labored breath, lack of motility, and exudates from the eyes (data not shown). With high mortality, weight loss during later disease course, measurable signs of disease, high virus titers in the brain, and a moderate infection interval around 6 days, this mouse model of WEE appeared to be suitable for antiviral studies.

To evaluate the utility of this model for antiviral testing, the broad-spectrum compound T-705 (Toyama Chemical Co., Tokyo, Japan) was selected. Initial tests to determine the activity of this compound against WEEV were conducted with various half-log concentrations of T-705 between 1000 and 3.2 μg/ml in 96-well flat-bottomed microplates plated with Vero cells (ATCC CCL-81) and read after 3 days incubation at 37°C. T-705 had a 90% effective concentration (EC_{90}) against WEEV of 49 μg/ml (312 μM) as determined by virus yield assay. The 50 % cytotoxic concentration (CC_{50}) was > 1000 μg/ml (6.4 mM), generating a selective index of >20. Activity was confirmed in a second cell culture experiment that yielded similar results (data not shown).

To determine the efficacy of this compound in mice, T-705 was administered twice a day (bid) by oral gavage for 8 days beginning 4 h prior to virus challenge. Mice treated with 400 mg/kg/d of T-705 had a significant improvement in survival and mean day to death as compared with that of placebo-treated mice (Figure 2). Despite significant improvement in the survival curve, 60% of mice treated with T-705 died of WEE as compared with 80% mortality in the placebo-treated infection control group.

Treatment with T-705 resulted in a 1-log drop in mean infectious cell culture titers on 4 dpi in the brains of infected mice, although the difference was not statistically significant (Figure 3). A significant 3-log drop in brain titer was observed in hamsters infected with WNV and treated with T-705, suggesting that this compound can cause significant reductions in brain titers of flaviviruses (Morrey et al., 2008), but it is unknown whether this drop is a result of direct inhibition of virus in the brain or from an earlier reduction of peripheral virus that ultimately results in lower levels of virus infecting the brain. The 1-$\log_{10}$, non-significant reduction in virus titer in the brains of treated mice may reflect a more widespread systemic reduction in virus titer. Generally, WEEV-infected mice that display signs of neurological disease would succumb to infection. Several mice treated with T-705, however, showed signs of brain disease, but subsequently recovered, suggesting the possibility of amelioration of disease by T-705 despite neurological involvement.

The immunomodulatory double-stranded RNA, ampligen (HEMISPHERx, Philadelphia, PA), was included as a positive control. A dose of 12 mg/kg of ampligen was administered i.p. −4 h and 2 dpi. Complete survival was observed in mice treated with ampligen (Figure 2). These results are similar to those from previously published studies reporting the treatment of WEE with double stranded RNA, including dsRNA extracted from rice dwarf virus and poly I:C (Takehara, 1977), as well as treatment with both free and liposome encapsulated poly ICLC (Wong et al., 2005). Neurological disease signs were also observed, but were generally mild limb weakness or temporary paralysis and all mice that exhibited signs recovered (data not shown).

From these studies, this mouse model appears to be suitable for antiviral studies in the development of therapies for human WEEV-infection. With significant titers in the brains of infected mice, significant weight change, observable disease signs, and high mortality, antiviral compounds can be evaluated with these parameters to gauge efficacy. T-705 showed moderate efficacy, although treatment was initiated just prior to virus challenge. Future studies, therefore, should be conducted to further evaluate this compound in the treatment of WEE, specifically evaluating the effect of treatment initiated after virus infection.

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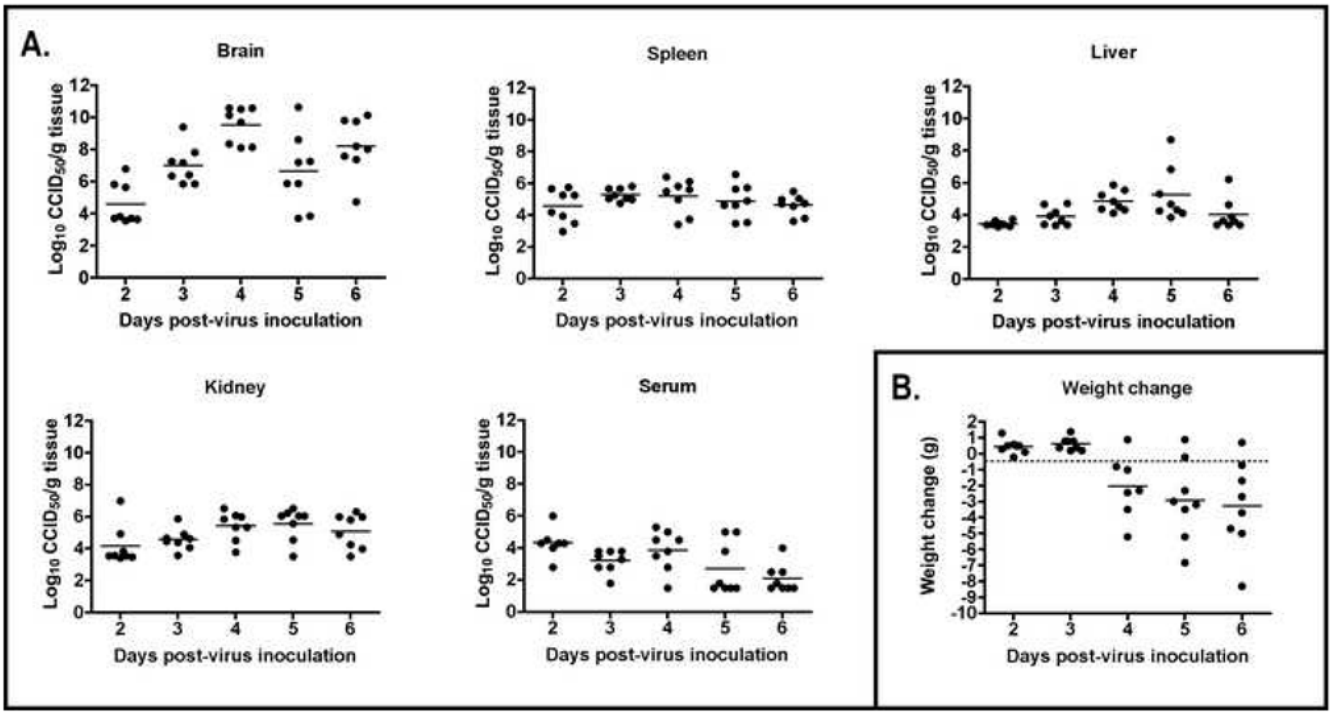

Figure 1.

A) Titer, measured as $\log_{10}$ 50% cell culture infectious doses ($CCID_{50}$)/g tissue or ml serum, of WEEV in various tissues taken from mice on 2, 3, 4, 5, and 6 days post-virus infection (dpi) with $10^{3.9}$ pfu/ml WEEV. B) Time-course weight change (g) of mice infected with WEEV.

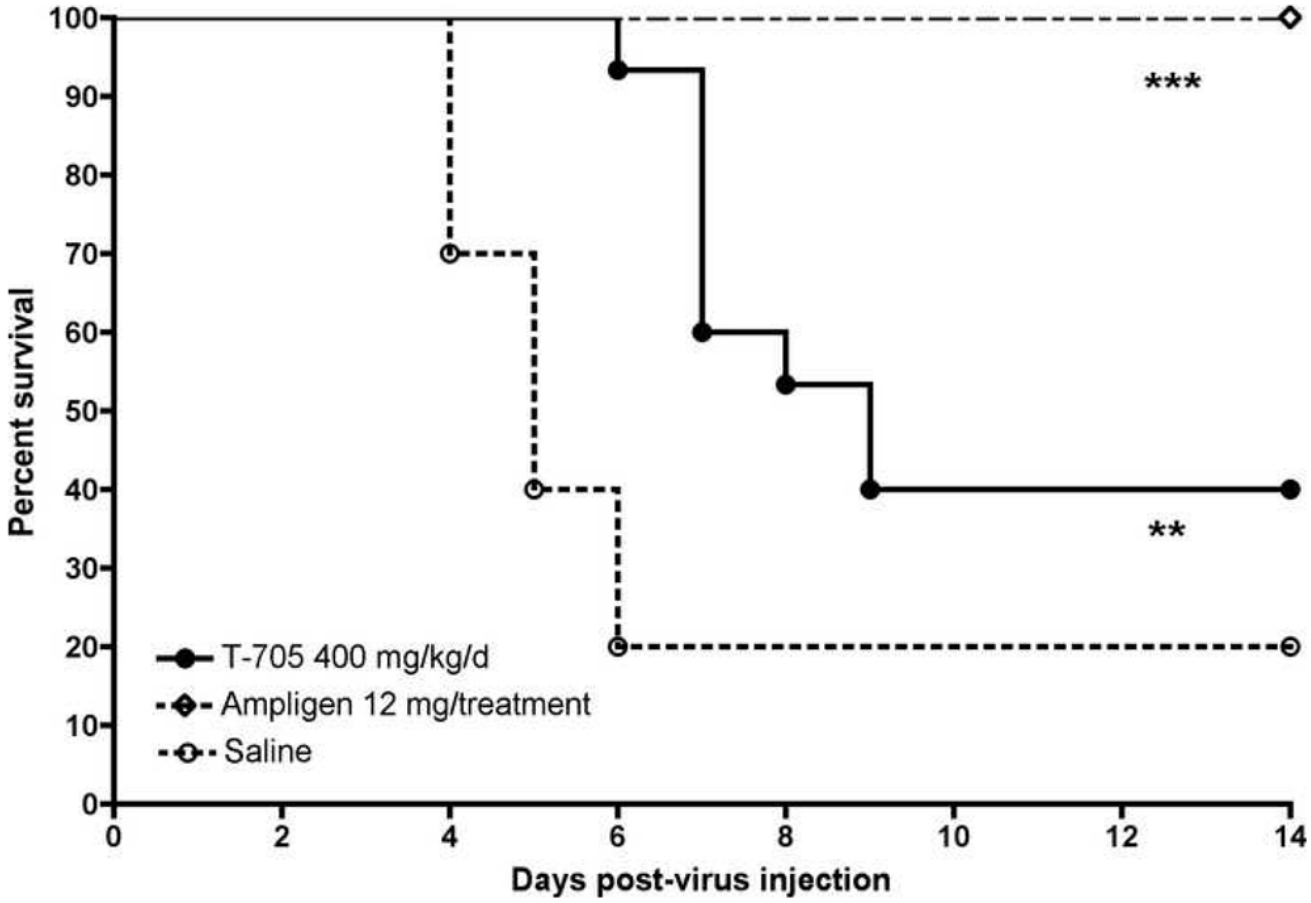

Figure 2.

Survival of C57BL/6 mice infected with WEEV and treated twice a day for 8 days with T-705 given p.o [n=15], or −4 h and 2 dpi with ampligen given i.p. [n=10] (***P<0.001, **P<0.01, as compared with placebo-treated controls [n=20], as determined by Wilcoxon log-rank survival analysis).

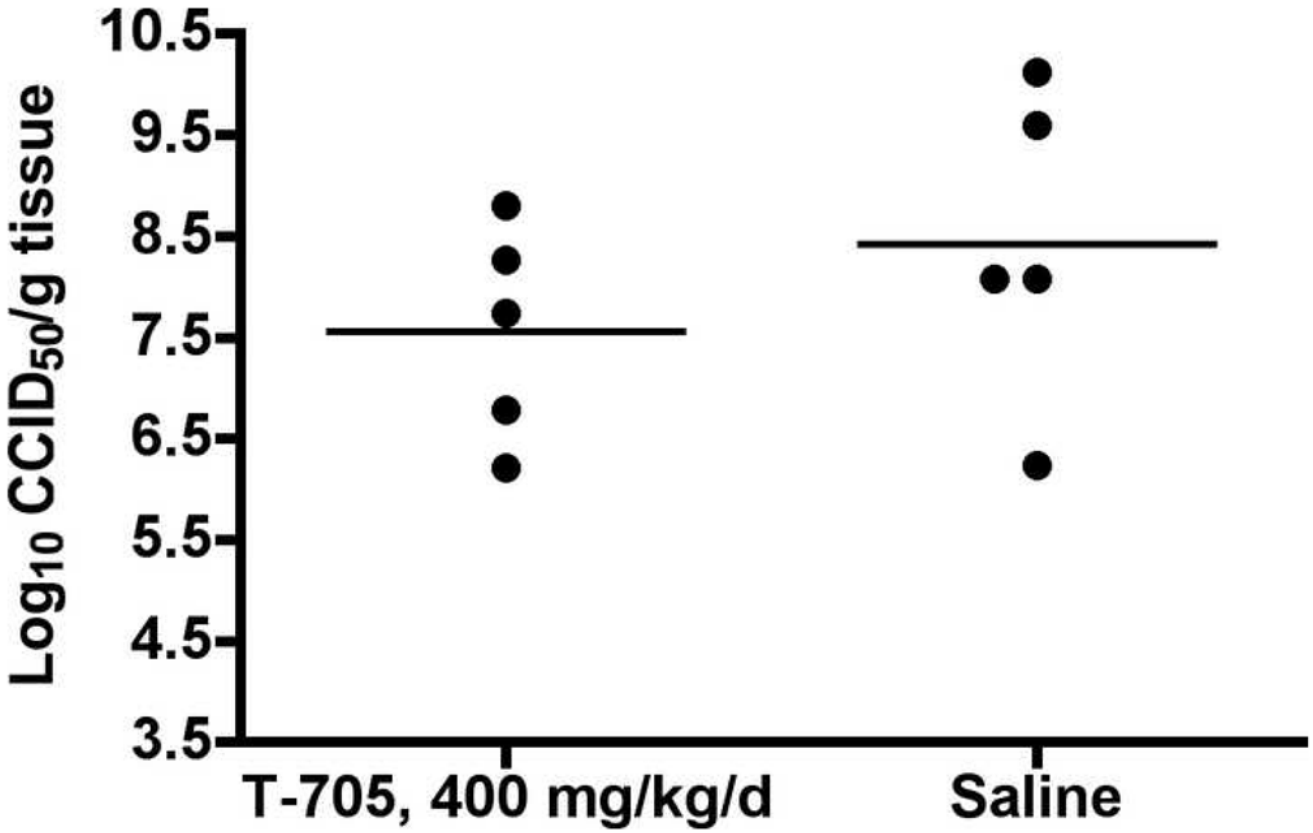

Figure 3.
Brain titers of C57BL/6 mice infected with WEEV and treated twice a day for 7 days with T-705 administered orally at a dose of 400 mg/kg/d. Lines represent mean titers for each group (P = 0.3287 as determined by one-way Student's t-test).

Table 1

Occurrence of major disease signs in mice infected with western equine encephalitis virus.

Symptom	Timing[a]	Affected/total[b]	# multiple symptoms/# affected[c]
Limb weakness	4 dpi	8/10	6/8
Paralysis	3–7 dpi	7/10	7/7
Lacrimation	5–7 dpi	3/10	3/3
Lying prone	3–7 dpi	6/10	6/6
Death	4–8 dpi	10/10	N/A

[a] Time when disease parameter was observed

[b] Number of animals displaying the disease sign per total animals infected

[c] Number of animals that displayed other signs of disease per number affected with the original symptom