Vaccine 37 (2019) 4686–4693

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Re-evaluating the effect of Favipiravir treatment on rabies virus infection ☆

Ashley C. Banyard [a], Karen L. Mansfield [a], Guanghui Wu [a], David Selden [a], Leigh Thorne [a], Colin Birch [b], Penelope Koraka [c], Albert D.M.E. Osterhaus [c], Anthony R. Fooks [a,d,*]

[a] Wildlife Zoonoses and Vector-borne Diseases Research Group, OIE Reference Laboratory and WHO Collaborating Centre, Animal and Plant Health Agency (APHA), Addlestone, Surrey KT15 3NB, UK
[b] Biomathematics and Risk Research Group, Animal and Plant Health Agency (APHA), Addlestone, Surrey KT15 3NB, UK
[c] Erasmus Medical Centre (EMC), Rotterdam, The Netherlands
[d] Institute for Infection and Immunity, St. George's Hospital Medical School, University of London, London, UK

ARTICLE INFO

Article history:
Available online 10 November 2017

Keywords:
Rabies
Antiviral
Favipiravir
HRIG
T-705
In vivo

ABSTRACT

There is no antiviral treatment available once clinical disease following rabies virus infection has initiated. Considered a neglected tropical disease, >60,000 human rabies deaths are estimated each year despite the availability of pre- and post-exposure prophylaxis for pre-immunisation or administration following a potential exposure before the onset of clinical disease. Such post-exposure treatments include administration of rabies immunoglobulin (RIG) and vaccination at a distant site to prime a humoral immune response. However, current therapeutic options are limited. Regardless there is a need for molecules that target virus infection following the onset of clinical disease where the outcome of infection is invariably fatal. Numerous molecules have been assessed as potential antivirals against rabies virus (RABV) but with little promise. Favipiravir (T-705) is a broad-spectrum RNA polymerase inhibitor, which has been shown to have antiviral activity against a range of RNA viruses including some against RABV. In the present study, the utility of T-705 has been reassessed *in vitro* as well as *in vivo* in a murine model using intraperitoneal administration to investigate any immune protective effect of the molecule. *In vitro* T-705 effectively reduces RABV replication. However, *in vivo*, following assessment of various applications of the molecule in both pre- and post-exposure scenarios, the effect was limited. T-705 treatment delayed the onset of clinical signs when virus was delivered intramuscularly at a higher dose ($10^{6.8}$ $TCID_{50}$/ml) and reduced the number of mice that developed clinical signs when virus was delivered at a lower dose ($10^{5.8}$ $TCID_{50}$/ml) during the observation period. The day at which treatment commenced did not appear to have a statistically significant effect on the results in either experiment. The use of T-705 as a single biological entity may be limited, however, further work is required to assess the synergistic effect of T-705 as a component of a multi-drug therapy for treating human rabies infections.

1. Introduction

Clinical infection with rabies virus (RABV) is invariably fatal with an estimated >60,000 human deaths each year despite the availability of both pre- and post-exposure tools [1]. Post-exposure prophylaxis (PEP), utilising vaccination and passive immunisation with rabies immunoglobulin (RIG) [2] to neutralise virus present at the infection site, is 100% effective if administered according to defined schedules and within proposed time periods [3]. The major route of human infection with rabies is via a bite from an infected dog with infection disproportionately affecting children in developing countries [4]. Simple guidelines to prevent the establishment of virus infection following exposure, including wound washing, have long been established [2,5]. However, in endemic areas the awareness of rabies, potential transmission routes, and knowledge of actions required following potential exposure is poor, and often the simple WHO guidelines are not followed [6–8]. Furthermore, where there is an awareness of the disease, the availability of medical facilities that stock life-saving

☆ This work was presented at the International Conference on Antiviral Research, Rome, Italy between May 11th–15th 2015, reporting for the 1st time the antiviral effects of T-705 on rabies virus.

* Corresponding author at: Wildlife Zoonoses and Vector-borne Diseases Research Group, OIE Reference Laboratory and WHO Collaborating Centre, Animal and Plant Health Agency (APHA), Addlestone, Surrey KT15 3NB, UK.
E-mail address: Tony.Fooks@apha.gsi.gov.uk (A.R. Fooks).

https://doi.org/10.1016/j.vaccine.2017.10.109
0264-410X/

vaccines and RIG is often low [9]. Issues with use of PEP can stem from a lack of disease awareness and the need for PEP, unavailability of the product in resource limited settings or, where available, high costs of purchase that prohibit affected individuals receiving it [10]. Further, existing PEP is of no use following the onset of clinical disease. Antiviral molecules that may be able to prevent replication in the brain of an infected individual are urgently required [11]. Favipiravir (6-fluoro-3-hydroxy-2-pyrazinecarboxamide) is a pyrazine derivative, more commonly known as T-705. This molecule is a broad-spectrum RNA polymerase inhibitor that causes an indiscriminate accumulation of mutations within coding regions that disrupts efficient virus replication [12]. T-705 has already been shown to have antiviral activity against a wide range of positive and negative sense RNA viruses [12–18]. Importantly, T-705 has also been shown to have an antiviral effect against RABV *in vitro* and *in vivo* when administered soon after virus infection [19,20] . The aims of this study were to investigate the effect of T-705 in a murine model, both in direct comparison with, and in combination with HRIG at different time points to assess the effect of pre- and post-exposure prophylaxis.

2. Materials and methods

2.1. Antiviral compounds

T-705, ribavirin and HRIG were used in antiviral treatment regimes. Ribavirin was used as a positive control for a rabies anti-viral effect [21]. The purity of all compounds tested was previously assessed by the manufacturers and confirmed by the suppliers detailed here as being at least 95% – ribavirin (Sigma, UK) >98%; T-705 *in vitro* (Medchemtronica) >98%; T-705 *in vivo* (BOC Sciences, UK). HRIG was obtained from The National Institute of Biologicals, Standards and Controls (NIBSC, UK) and was used at a concentration of 40 IU/kg.

2.2. In vitro assessment of antiviral activity

The cytotoxicity of T-705 was measured using a lactate-dehydrogenase (LDH) assay (Roche) in N2A cells (ATCC; CCL-131). The antiviral activity of T-705 was tested as follows: cell monolayers were infected with the challenge virus standard-11 (CVS-11) strain of RABV (100 $TCID_{50}$/well at a multiplicity of infection (moi) of 0.01 and 0.03, respectively) for one hour at 37 °C/5% CO_2, washed with serum-free medium, and replaced with a dilution series of T-705 (starting at a concentration of 2048 μM) in cell culture media plus 2% fetal calf serum (FCS). Untreated- and ribavirin-treated -infected cells were assessed as negative and positive controls, respectively. After 48 h at 37 °C/5% CO_2 the virus present in supernatants was quantified as described previously [22]. Simultaneously, N2A cell monolayers were fixed in 80% acetone, stained with a Fluorescein isothiocyanate (FITC)-conjugated anti-rabies nucleoprotein monoclonal antibody (Fujirebio, USA) (diluted 1:40) for 30 minutes at 37 °C/5% CO_2, and washed with 0.01 M PBS. The percentage fluorescence/well was assessed independently by two operators to limit subjectivity. The mean percentage fluorescence was calculated for each treatment group. To assess virus release, virus titrations of cell culture supernatant were undertaken using 10-fold serial dilutions (neat to 10^{-6}) with three replicates per dilution. After 3 days, titration plates were fixed and stained as described above and the titre calculated ($TCID_{50}$/ml) according to the Spearman-Karber technique [22].

2.3. In vivo assessment of antiviral activity

Two *in vivo* experiments were performed. All *in vivo* studies were performed under UK Home Office approved licenses (PPL70/7394) following ethical review and statistical review and acceptance at the Animal and Plant Health Agency (APHA). Mice were randomly allocated to groups and within groups to two boxes of 6 mice to allow analysis to take account of interactions among mice sharing boxes and any other differences between boxes. Both experiments utilised 3–4 week old C57/bl6 mice (Charles River, USA) and intramuscular (IM) virus infection (50 μl) with the silver haired bat rabies virus (SHBRV) RABV strain, isolated from a human fatality following infection from an insectivorous bat [23]. All mice were humanely terminated at the onset of clinical rabies to minimise any suffering according to a defined schedule of humane end-points [24], and the effect of the treatment was analysed based on mortality as it is understood that following the onset of clinical disease the mice progress to death if humane end-points are not observed.

Experiment 1: Mice were inoculated intramuscularly (IM) with 50 μl of the SHBRV ($10^{6.8}$ $TCID_{50}$/ml) RABV strain on day 0. Mice (n = 6/box, two boxes for each treatment group) were then treated IP with T-705 (300 mg/kg in 500 μl), staggering the initiation of the treatment: Group 1 – received IP treatments on days 3, 4, 5 and 6; Group 2 – days 4, 5 and 6; and Group 3 – days 5 and 6. Positive control groups (n = 24 in 4 boxes) were treated with PBS alone and acted as controls for the viability of the viral inocula. Mice were monitored and terminated according to pre-defined humane end points [24]. Survivors were terminated after 28 days. The results were analysed using a log-rank (Mantel-Cox) test.

Experiment 2: Mice were inoculated IM with 50 μl SHBRV ($10^{5.8}$ $TCID_{50}$/ml). Treatment (IP) with T-705 was compared with human rabies immunoglobulin (HRIG) as singly and in tandem. Mice (n = 12/group) received PBS only, T-705 alone (300 mg/kg in 500 μl), HRIG alone (40 IU/kg/ml) or T-705 and HRIG at equivalent doses to the individual treatments as a combination treatment. Mice were treated for 10 days with each treatment being initiated either four hours before virus inoculation (−4 h), two days (+2 d) after virus infection or 4 days (+4 d) after virus inoculation. The data were analysed for treatment effects as a full factorial design by applying a multilevel mixed effects generalised linear model to take account of potential correlation among mice in each box (melogit in Stata® 14, treating differences between boxes as random effects). Timing was not expected to influence outcomes in the PBS-only control treatments, so an additional analysis for timing effects excluded controls.

Post-mortem, the left hemisphere of terminated mice was removed and frozen at −80 °C. Following the termination of each experiment each brain was tested for the presence of viral antigen using the fluorescent antibody test (FAT) [25]. Brains were scored as positive or negative for the presence of RABV antigen following fixation and staining with a FITC-conjugated anti Rabies-N antibody (Fujirebio). For the detection of nucleic acid, RNA was extracted from brain homogenates as described previously [26] (data not shown).

2.4. Virus neutralisation tests

Experiment 2 mice that survived infection were bled by cardiac puncture under terminal anaesthesia. Sera were separated from whole blood samples by centrifugation and stored at 4 °C until required. Virus-specific neutralising antibodies (VNA) were determined using the fluorescent antibody neutralisation (FAVN) test [22]. VNAs were determined as IU/ml in accordance with control virus and sera.

3. Results

3.1. In vitro assessment of cytotoxicity and antiviral activity

Although demonstrated previously [19,20], *in vitro* assessment of the ability of T-705 to neutralise RABV *in vitro* was essential as a precursor to *in vivo* studies where the potential toxicity of treatments had to be investigated. T-705 had a 50% cytotoxic concentration (CC_{50}) of >2500 μM, with no cytotoxicity observed at concentrations of <312.5 μM by LDH assay. The antiviral activity of T-705 against RABV in N2A cell monolayers (multiplicity of infection (MOI) of 0.01) was also demonstrated by a decrease in the mean percentage fluorescence observed following an increase in T-705 in a dose-dependent manner (Fig. 1a). A similar trend was observed with ribavirin. The antiviral effect of T-705 on virus release from cells was also demonstrated following treatment across a range of T-705 concentrations (Fig. 1b). This reduction in RABV titre reflected a T-705 dose-dependent effect on virus egress into cell culture supernatant. To further validate this approach a higher dose of RABV (MOI of 0.03) was assessed in the presence and absence of T-705 treatment and both a reduction in infected cells and a reduction in viable virus being released from infected monolayers was observed (Fig. 2a and b). A combination approach was also used to assess if treatment with both T-705 and ribavirin in combination had an additive antiviral effect (Fig. 2) and the combination therapy was effective although the effect on virus infection (Fig. 2a) was more prominent than the effect on virus release (Fig. 2b). However, the combination of T-705 and ribavirin reduced cellular fluorescence relative to ribavirin alone, at a concentration of 1–8 μM (Fig. 2a).

3.2. Assessment of T-705 in vivo as a pre- and post-exposure treatment

In vivo experimentation was undertaken following two different strategies (Fig. 3). Staggered administration of T-705 was initially undertaken as described in Fig. 3a. Administration of T-705 appeared to delay the initiation of clinical disease at all three time periods (Fig. 4a and b). The untreated control group all succumbed by day 9 post-infection; whereas groups of mice treated with T-705 from either day 3 or 5 did not all succumb until day 12. Moreover, the group that were treated from day 4 had 25% survivorship (n = 3/12) (Fig. 4a). However, despite survivorship in the group receiving T-705 on days 4, 5 and 6, there was no statistically significant difference in survival among the three T-705 treatment groups (p = .18); therefore, this study did not demonstrate any effect from the timing of treatment.

A secondary approach was taken to further assess the action, if any, of T-705 on rabies infection *in vivo* by treating a few hours before, 2 days after and 4 days after infection with RABV (Fig. 3b). This strategy was designed to give the molecules assessed the optimal opportunity to exert an effect on RABV replication. Staggering treatment in this way demonstrated that when compared with experiment 1, the onset of clinical disease appeared later and mortality was lower (Fig. 5a–c). Although mortality was lower among treated mice than controls, mice succumbed during a similar time period in all treatments, and as such survival analy-

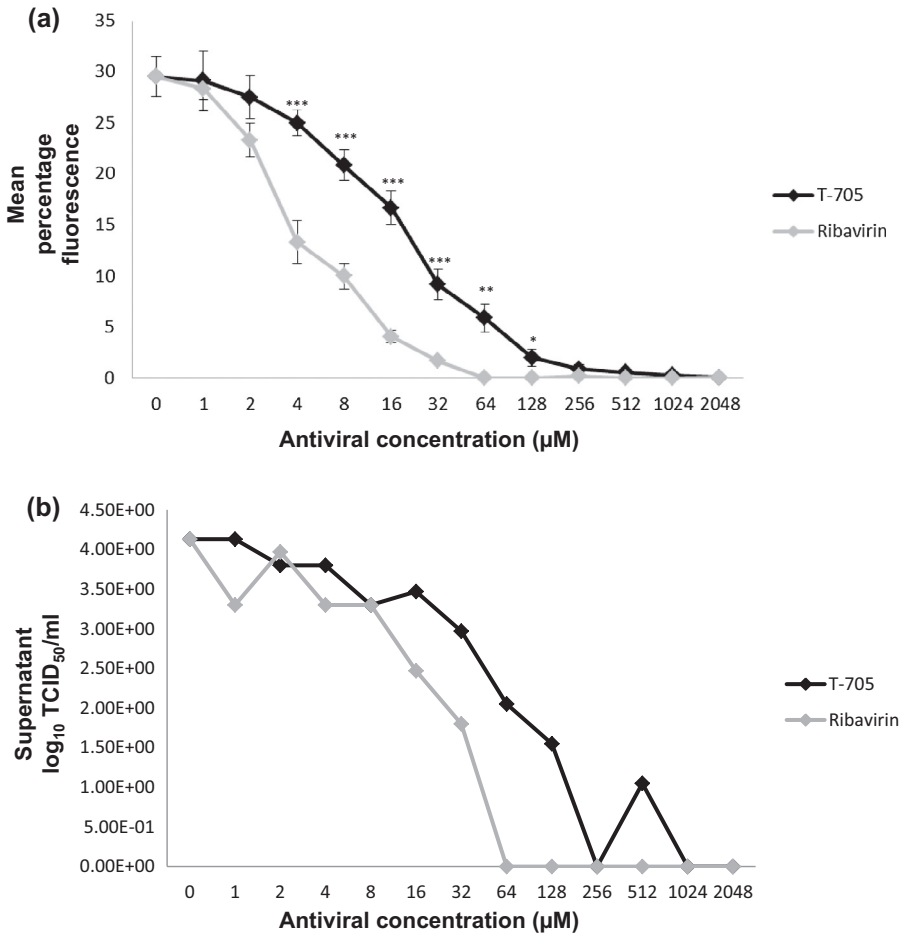

Fig. 1. Antiviral activity of T-705 and ribavirin against rabies virus in N2A cell monolayers (MOI of 0.01), demonstrated by (a) a reduction in the mean percentage of FITC-stain fluorescence, and (b) a reduction in virus titre (expressed as $\log_{10}$ $TCID_{50}$/ml) of infected N2A cell supernatant. Error bars represent the standard error of the mean (SEM).

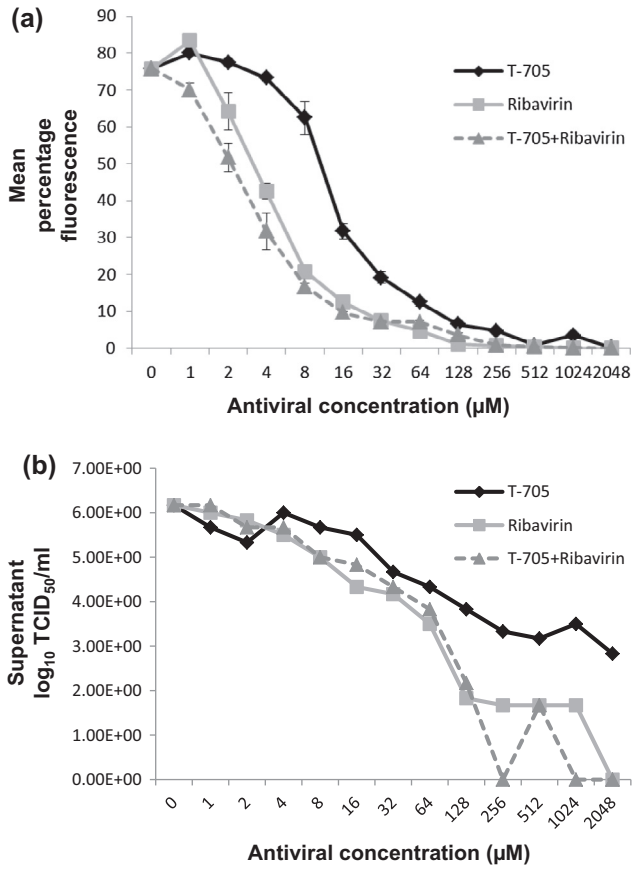

Fig. 2. Antiviral activity of T-705, ribavirin and a combination of T-705 and ribavirin, against RABV in N2A cell monolayers (MOI of 0.03), demonstrated by (a) a reduction in the mean percentage of FITC-stain fluorescence, and (b) a reduction in virus titre (expressed as $\log_{10}$ $TCID_{50}$/ml) of infected N2A cell supernatant. Error bars represent the standard error of the mean (SEM).

sis was inappropriate (Fig. 5a–c). Most mortality occurred before 15 days, but one mouse from a control treatment (23 days) and one mouse from a T-705 treatment (43 days) succumbed significantly later. Both HRIG and T-705 substantially reduced mortality across all treatment timings (Fig. 5a-c, Table 1), although the effect of HRIG was significantly greater (Table 1). Only two mice succumbed in groups treated with HRIG, both with and without T-705, so whether T-705 had an additive effect with HRIG was unclear, as indicated by the broad confidence interval (Table 2) for the odds ratio for the interaction of T-705 and HRIG.

Low mortality in the T-705 and HRIG treatments also left the effect of timing on treatments uncertain (Table 2). Mortality did not significantly change with timing and was low even when treatments were applied 4 d after inoculation (Fig. 5c). There was no significant interaction between treatments and timing (data not shown). All experiment 2 mice that succumbed to infection were positive by FAT (n = 52) whilst all survivors (n = 164) were negative.

The serological response to infection in survivors from experiment 2 suggested that whilst all mice had been infected, few mounted a serological response that was detectable 54 days post infection (Fig. 6a–c). Of the mice treated with T-705 from 4 hours pre-inoculation (Fig. 6a), only one mouse mounted a serological response that was detectable using the method described. From the other treatment groups, several mice seroconverted to the serological titre considered the cut off for protection (>0.5 IU/ml) but few mounted a strong serological response. Of the PBS-treated control mice, both survivorship and seroconversion in survivors was low. However, within these groups one mouse strongly seroconverted, recording a virus neutralising antibody titre of >40 IU/ml (Fig. 5c) on termination (day 54). It is of note that, neither of the mice that developed disease and were terminated outside of the range during which the majority of box mates were terminated (n = 2; PBS −4 h treatment group (day 23) and T-705 4 day treatment group (Day 43)) had seroconverted.

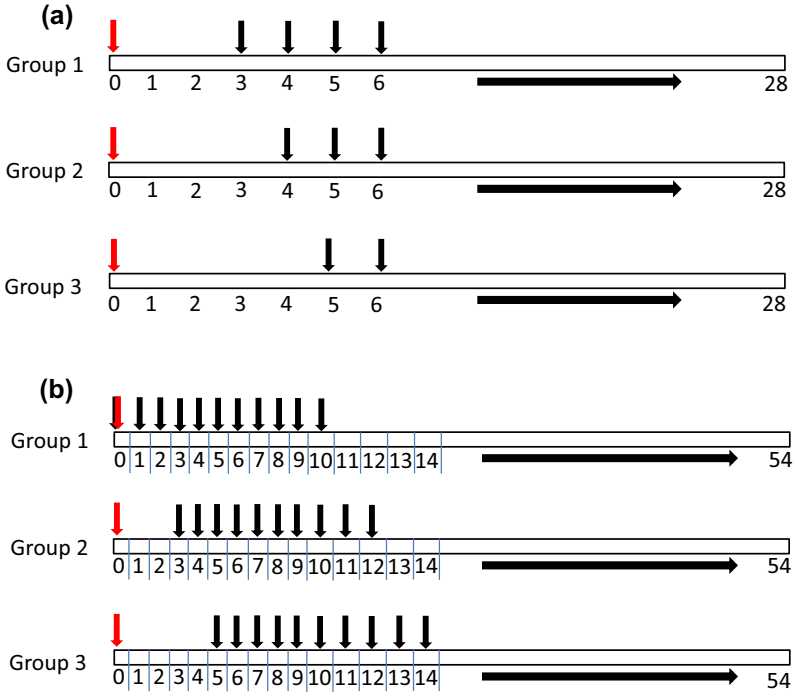

Fig. 3. Staggered *in vivo* application of T-705 used for experiment 1 (a) and experiment 2 (b).

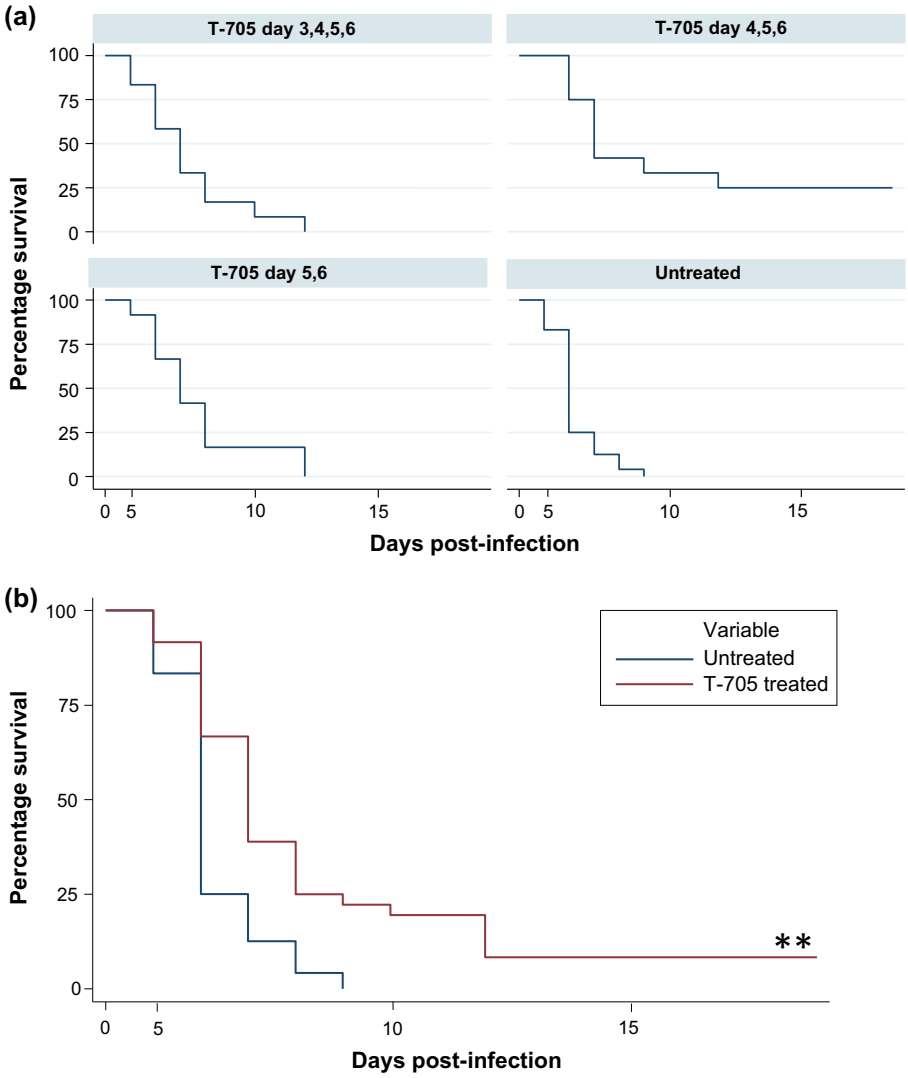

Fig. 4. Effect of T-705 administration across different days (a) demonstrated that when comparing all three T-705 treatment groups and the untreated control group, there was a significant effect on survivorship (log rank test for survivor function (p = .003)). (b) an amalgamation of all treatment groups in comparison with the control group demonstrated a statistically significant effect on survivorship (log rank test for survivor function; denoted as ** p=<.001).

4. Discussion

In vitro, the administration of T-705 following cell infection was clearly able to inhibit RABV replication [27]. The observed *in vitro* cytotoxicity of T-705 was lower than many other antiviral candidates tested (data not shown) and most likely reflected the lack of interference with cellular DNA and RNA as previously reported for T-705 [28]. The observed lack of synergy when assessing ribavirin in combination with T-705 may reflect a similarity in the action of these molecules that as yet is undefined. Regardless, whilst numerous molecules have been shown to have neutralising activity against lyssaviruses *in vitro,* this neutralisation activity has not translated to *in vivo* efficacy [29–31].

The *in vivo* studies presented here indicated that T-705 treatment was able to delay the onset of clinical disease when mice were challenged with a higher dose of rabies (experiment 1) and that T-705 treatment decreased the mortality rates when mice were challenged with a lower dose of rabies (experiment 2). The day at which treatment commenced did not appear to have a significant effect on results in either *in vivo* experiment. It is interesting to note that an effect of T-705 was demonstrated when the treatment was started 5 days post infection when the clinical symptoms had appeared in some mice in experiment 1.

These data add to our knowledge of previously published studies [19,20] that have indicated that T-705 may be of utility as an antiviral molecule for the treatment of rabies. However, the studies presented here demonstrate that when used alone *in vitro* the effect of treatment with T-705 is limited. Previously, T-705 was shown to be superior to HRIG [20], whereas the reverse was true in our study. It is not clear whether the differences were attributable to the different RABV strains used in these two studies, the different challenge doses or the different routes of administration. Other studies utilising oral dosing of T-705 demonstrated a low degree of survival although again the timing of disease onset was not statistically different between the treatment and control groups; however, some treated mice had a delayed time to onset of clinical disease [20]. Key to the outputs from all of these studies is the difficulty in performing an assessment of antiviral molecules against RABV. Whilst the data presented here do not strongly support the use of T-705 as an antiviral against RABV, its use may be worth assessing in a human setting. In human infection, where clinical disease represents a death sentence, such molecules may be of utility even where use is controversial. Alternative routes

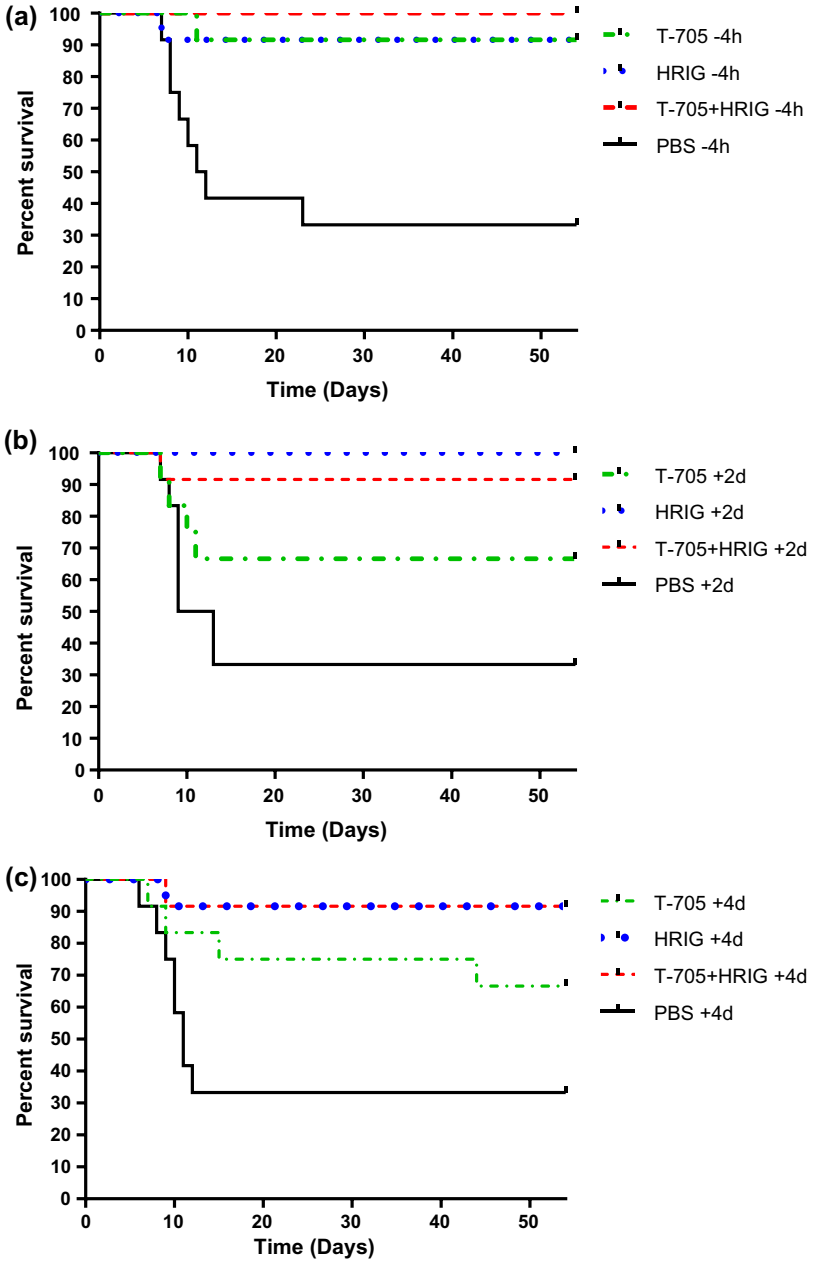

Fig. 5. Survival following treatments at 4 hours before (a), 2 day post (b) and 4 day-post infection (c) versus PBS controls.

Table 1
Effect of HRIG and T-705 on mortality relative to PBS only controls across 3 different timings of treatment following inoculation with RABV.

Treatment	Odds ratio	P for ratio = 1.00	95% confidence interval	
HRIG vs control	0.028	<0.001	0.006	0.139
T-705 vs control	0.161	0.001	0.057	0.455
T-705 vs HRIG	5.75	0.034	1.14	29.0
Interaction HRIGxT-705	6.20	0.115	0.640	60.1
Timing 2 d vs −4 h	1.66	0.387	0.527	5.23
Timing 4 d vs −4 h	1.94	0.255	0.619	6.10

such as intravenous or intrathecal routes may enable the delivery of such molecules to the brain where they are needed. With this in mind, both published reports and the data presented here indicate that further studies with T-705 are warranted. From a clinical perspective, the oral administration of a molecule to treat RABV infection would not appear to be the most suitable route where treatment uptake may be critical. Further, the previous studies utilised a fixed rabies strain (CVS) for challenge, whilst the present study challenged animals with a wildtype American bat RABV strain. The disease progression observed in the present study may reflect the use of a street strain of rabies as a challenge model.

Table 2
Effect of timing on mortality in treatments including HRIG, T-705 and HRIG + T-705.

Treatment	Odds ratio	P for ratio = 1.00	95% confidence interval	
+2 d vs −4 h	2.94	0.231	0.504	17.2
+4 d vs −4 h	3.74	0.136	0.661	21.1

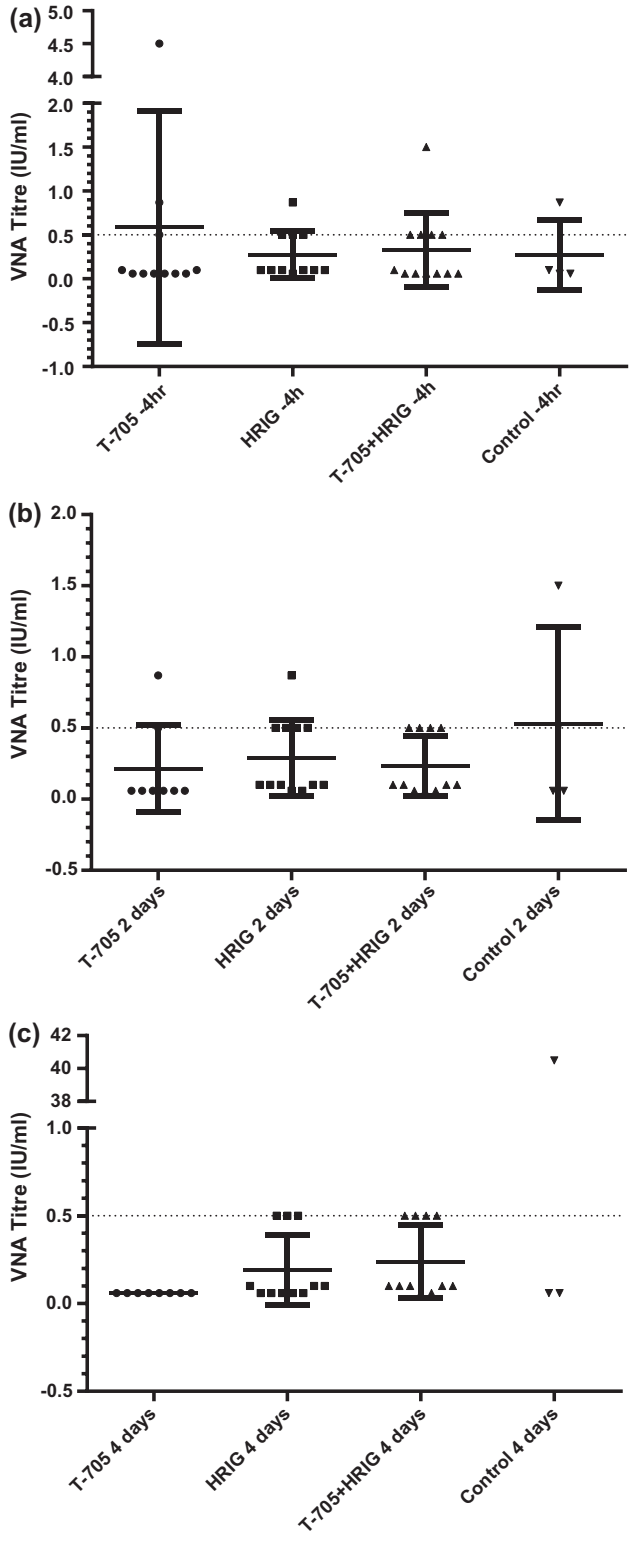

Fig. 6. Assessment of serological response to infection in mice that survived the 54 day duration of experimentation following treatments at 4 h before (a), 2 day post (b) and 4 day-post infection (c) versus PBS controls.

The schedules chosen for treatment described for experiment 2 were dictated by existing knowledge surrounding the treatment of potential exposures following the bite of an infected dog. These time periods were chosen as attempts to initiate treatment either before infection (−4 h), before hypothesised infection of the peripheral (PNS) or central nervous system (CNS) (2 days) and after 4 days when it was hypothesised that the virus may have entered the CNS to establish a productive infection. Whilst this study was most interested in studying the effect of antiviral treatment following infection, ethical input required that treatment shortly before infection was assessed and this served a control for adverse effect of drug administration. Certainly, both scientifically and ethically these experiments are difficult to perform as they require that the presence of virus in the CNS has been confirmed before treatment is initiated to ensure that the outputs can be correctly interpreted. In experiment 2, it was predicted that at 4 days post infection the virus would be present within the CNS and as such a low level of survivorship was expected, even within the HRIG group. This was predicted, since if HRIG is to be effective it must act in the periphery as it is not believed to cross the blood brain barrier (BBB) [3]. The high survival rates in the HRIG treatment groups indicated that virus may not have sufficiently established infection of CNS by 4 days post infection. Ultimately, the mechanisms by which RIG facilitates clearance of virus from the periphery following exposure remain unclear. Certainly, the ability of RIG to bind to and neutralise virus is clear, but how this can be effective for a time period following potential exposure is unclear.

In summary, these data add to published reports on the potential utility of T-705 as a post exposure antiviral treatment for rabies [19,20]. Despite differences in experimental approach, these data presented have demonstrated proof-of-principle for T-705 as an antiviral molecule against RABV although whilst *in vitro* the effect of T-705 on rabies virus replication seems clear, *in vivo* the effect appears limited. However, further work is required to evaluate any effect of the molecule on rabies infection of the CNS and likely a better model is required to assess this effect. Certainly, the utility of T-705 as an antiviral molecule for treatment of human cases of rabies in a therapeutic coma needs further evaluation.

Acknowledgements

This study was funded through EU FP7 project ASKLEPIOS – Advanced Studies towards Knowledge on Lyssavirus Encephalitis Pathogenesis Improving Options for Survival (grant 602825) and Defra grant (SE0431). We wish to thank all members of the Asklepios consortium for their support. We thank Ian Mawhinney for statistical support. We thank Mrs Samantha Watson and Mr Nick Rose for extensive assistance with *in vivo* studies. The authors acknowledge extensive discussions with both Dr Thomas Muller, Professor Noel Tordo and Professor Charles Rupprecht in the design of the experiments and interpretation of outputs.

References

[1] Fooks AR, Banyard AC, Horton DL, Johnson N, McElhinney LM, Jackson AC. Current status of rabies and prospects for elimination. Lancet 2014;384:1389–99.
[2] WHO. Human and dog rabies prevention and control. Report of the WHO/Bill & Melinda Gates Foundation Consultation. Annecy, France; 2010.
[3] WHO. WHO Expert Consultation on Rabies, Second report. WHO technical report series; no. 982. Geneva, Switzerland: World Health Organization; 2013.
[4] Warrell MJ. Emerging aspects of rabies infection: with a special emphasis on children. Curr Opin Infect Dis 2008;21:251–7.
[5] Warrell DA. The clinical picture of rabies in man. Trans R Soc Trop Med Hyg 1976;70:188–95.
[6] Moran D, Juliao P, Alvarez D, Lindblade KA, Ellison JA, Gilbert AT, et al. Knowledge, attitudes and practices regarding rabies and exposure to bats in two rural communities in Guatemala. BMC Res Notes 2015;8:955.

[7] Widyastuti MD, Bardosh KL, Sunandar Basri C, Basuno E, Jatikusumah A, et al. On dogs, people, and a rabies epidemic: results from a sociocultural study in Bali, Indonesia. Infect Dis Poverty 2015;4:30.
[8] Matibag GC, Ohbayashi Y, Kanda K, Yamashina H, Kumara WR, Perera IN, et al. A pilot study on the usefulness of information and education campaign materials in enhancing the knowledge, attitude and practice on rabies in rural Sri Lanka. J Infect Dev Ctries 2009;3:55–64.
[9] Bourhy H, Dautry-Varsat A, Hotez PJ, Salomon J. Rabies, still neglected after 125 years of vaccination. PLoS Negl Trop Dis 2010;4:e839.
[10] Both L, Banyard AC, van Dolleweerd C, Horton DL, Ma JK, Fooks AR. Passive immunity in the prevention of rabies. Lancet Infect Dis 2012;12:397–407.
[11] Rupprecht CE, Nagarajan T, Ertl H. Current status and development of vaccines and other biologics for human rabies prevention. Expert Rev Vaccines 2016;1–19.
[12] Arias A, Thorne L, Goodfellow I. Favipiravir elicits antiviral mutagenesis during virus replication in vivo. eLife 2014;3.
[13] Caroline AL, Powell DS, Bethel LM, Oury TD, Reed DS, Hartman AL. Broad spectrum antiviral activity of favipiravir (T-705): protection from highly lethal inhalational rift valley fever. PLoS Negl Trop Dis 2014;8.
[14] Buys KK, Jung KH, Smee DF, Furuta Y, Gowen BB. Maporal virus as a surrogate for pathogenic New World hantaviruses and its inhibition by favipiravir. Antiviral Chem Chemother 2011;21:193–200.
[15] Morrey JD, Taro BS, Siddharthan V, Wang H, Smee DF, Christensen AJ, et al. Efficacy of orally-administered T-705 pyrazine analog on lethal West Nile virus infection in rodents. Antiviral Res 2008;80:377–9.
[16] Kiso M, Takahashi K, Sakai-Tagawa Y, Shinya K, Sakabe S, Le QM, et al. T-705 (favipiravir) activity against lethal H5N1 influenza A viruses. Proc Natl Acad Sci USA 2010;107:882–7.
[17] Oestereich L, Lüdtke A, Wurr S, Rieger T, Muñoz-Fontela C, Günther S. Successful treatment of advanced Ebola virus infection with T-705 (favipiravir) in a small animal model. Antiviral Res 2014;105:17–21.
[18] Mendenhall M, Russell A, Smee DF, Hall JO, Skirpstunas R, Furuta Y, et al. Effective oral favipiravir (T-705) therapy initiated after the onset of clinical disease in a model of arenavirus hemorrhagic fever. PLoS Negl Trop Dis 2011;5.
[19] Yamada K, Noguchi K, Komeno T, Furuta Y, Nishizono A. Efficacy of Favipiravir (T-705) in rabies postexposure prophylaxis. J Infect Dis 2016;213:1253–61.
[20] Virojanapirom P, Lumlertdacha B, Wipattanakitchareon A, Hemachudha T. T-705 as a potential therapeutic agent for rabies. J Infect Dis 2016;213:1253–61.
[21] Appolinario CM, Jackson AC. Antiviral therapy for human rabies. Antiviral Ther 2015;20:1–10.
[22] Cliquet F, Aubert M, Sagne L. Development of a fluorescent antibody virus neutralisation test (FAVN test) for the quantitation of rabies-neutralising antibody. J Immunol Methods. 1998;212:79–87.
[23] Morimoto K, Patel M, Corisdeo S, Hooper DC, Fu ZF, Rupprecht CE, et al. Characterization of a unique variant of bat rabies virus responsible for newly emerging human cases in North America. Proc Natl Acad Sci U S A. 1996;93:5653–8.
[24] Healy DM, Brookes SM, Banyard AC, Nunez A, Cosby SL, Fooks AR. Pathobiology of rabies virus and the European bat lyssaviruses in experimentally infected mice. Virus Res 2013;172:46–53.
[25] Dean DJ, Abelseth MK. Laboratory techniques in rabies: the fluorescent antibody test. Monograph Series of the World Health Organisation; 1973. pp. 73–84.
[26] Hayman DT, Banyard AC, Wakeley PR, Harkess G, Marston D, Wood JL, et al. A universal real-time assay for the detection of Lyssaviruses. J Virol Methods 2011;177:87–93.
[27] Hodge ARV. Meeting report: 28th International Conference on Antiviral Research in Rome, Italy. Antiviral Res 2015;123:172–87.
[28] Furuta Y, Takahashi K, Shiraki K, Sakamoto K, Smee DF, Barnard DL, et al. T-705 (favipiravir) and related compounds; novel broad-spectrum inhibitors of RNA viral infections. Antiviral Res 2009;82:95–102.
[29] Jackson AC. Why does the prognosis remain so poor in human rabies? Expert Rev Anti Infect Ther 2010;8:623–5.
[30] Jackson AC. Current and future approaches to the therapy of human rabies. Antiviral Res 2013;99:61–7.
[31] Castel G, Ben-Mechlia M, Jallet C, Tordo N. Rabies therapeutics: development of anti-viral approaches. In: Nagarajan CRT, editor. Current Laboratory Techniques in Rabies Diagnosis, Research and Prevention; 2015.