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Post-exposure prophylaxis against western equine encephalitis virus by adenovirus-mediated delivery of interferon-alpha gene

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Defence R&D Canada
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Abstract

Western equine encephalitis virus (WEEV) is an endemic and biowarfare agent which poses a potential threat to the operations of the Canadian Armed Forces (CAF). A vaccine for the prevention of WEEV infection before exposure to the virus has been developed and approved by the U.S. Food and Drug Administration as an investigational new drug. However, very little progress has been made in finding post-exposure prophylaxis for WEEV. Using the mouse WEEV model, DRDC Suffield has tested a novel strategy for developing such medical countermeasures. A modified, harmless human adenovirus has been used to ferry a gene encoding a potent antiviral protein, interferon alpha (IFN-α), into the body. In this study, DRDC Suffield has demonstrated that IFN-α was rapidly produced in mice after intramuscular (IM) injection of the adenovirus and that a single-dose IM injection provided post-exposure protection against the lethal challenge of WEEV. This pilot study provides insights into further development of medical countermeasures for post-exposure prophylaxis against WEEV and other viral biothreat agents.

Résumé

Le virus de l'encéphalite équine de l'Ouest (VEEO) est un micro-organisme endémique utilisé comme agent de guerre biologique qui constitue une menace potentielle pour les opérations des Forces canadiennes (FC). Un vaccin a été mis au point et approuvé par l'Agence américaine des aliments et des médicaments (FDA) à titre de nouveau médicament de recherche pour prévenir l'infection à VEEO avant l'exposition au virus. Cependant, aucune prophylaxie post-exposition n'a encore été établie contre le VEEO. Nous avons utilisé un modèle VEEO chez la souris pour mettre à l'essai une nouvelle méthode d'élaboration de contre-mesures médicales (CM méd). Plus précisément, à l'aide d'un adénovirus humain modifié inoffensif, nous avons transféré un gène codant une protéine antivirale puissante – l'interféron alpha (IFN-α) – dans l'organisme des souris. Notre étude a montré que l'IFN-α était rapidement produit chez la souris à la suite d'une injection intramusculaire de l'adénovirus et qu'une dose intramusculaire unique conférait une protection post-exposition contre une provocation létale par le VEEO. Cette étude pilote aidera à mettre au point des CM méd prophylactiques post-exposition contre le VEEO et d'autres agents de menace biologique d'origine virale.

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Executive Summary

Post-exposure prophylaxis against western equine encephalitis virus by adenovirus-mediated delivery of interferon-alpha gene

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Introduction or background: Western equine encephalitis virus (WEEV) is not only an endemic animal and human pathogen, but also a potential biowarfare and bioterrorism agent. WEEV infection in humans results in a spectrum of clinical symptoms, ranging from flu-like illness to encephalitis with delirium, disorientation and coma, with a case mortality rate of 3–8%. A preventive vaccine based on formalin-killed whole WEEV particles has been approved by the U.S. Food and Drug Administration as an investigational new drug vaccine. However, very little has been done to develop medical countermeasures for post-exposure protection. To fill this gap, DRDC Suffield is developing a method of delivering a broad spectrum antiviral drug, interferon alpha (IFN-α), to exposed personnel. A harmless human adenovirus has been genetically engineered to carry a gene encoding IFN-α. When this modified virus is administered to test subjects, they then produce IFN-α.

Results: In this study, DRDC Suffield found that IFN-α was rapidly produced in mice after intramuscular (IM) injection of the adenovirus and that a single-dose IM injection provided post-exposure protection against a WEEV challenge which otherwise would have been lethal.

Significance: This study shows that the use of adenovirus-mediated expression of IFN-α has significant potential as a potential medical countermeasure for post-exposure protection against WEEV. The successful development of such a medical countermeasure would protect the CAF from both naturally occurring and intentional WEEV infections.

Future plans: IFN-α is a broad-spectrum antiviral agent. Studies are now needed to test whether adenovirus-mediated IFN-α expression could provide generic post-exposure protection against other viruses important for biodefence.

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Post-exposure prophylaxis against western equine encephalitis virus by adenovirus-mediated delivery of interferon-alpha gene

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Introduction ou context : Le virus de l'encéphalite équine de l'Ouest (VEEO) est non seulement un agent pathogène pour l'humain et l'animal, mais aussi un agent potentiel de guerre biologique et de bioterrorisme. Le VEEO cause chez l'humain un éventail de symptômes cliniques qui vont du syndrome grippal à l'encéphalite provoquant des délires, la désorientation et le coma; le taux de mortalité de l'infection est de 3-8 %. Un vaccin préventif à base de particules entières du VEEO inactivées au formol a été approuvé à titre de nouveau médicament de recherche par l'Agence américaine des aliments et des médicaments (FDA). Cependant, très peu de mesures ont été prises pour élaborer des contre-mesures médicales post-exposition contre le VEEO. Pour combler cette lacune, RDDC Suffield a mis au point une plateforme technologique permettant d'injecter un médicament antiviral à large spectre – l'interféron alpha (IFN-α) – dans l'organisme. Cette plateforme repose sur la modification moléculaire d'un adénovirus humain inoffensif de manière qu'il transfère un gène codant l'IFN-α. Dans le présent rapport, nous décrivons l'efficacité de cette méthode pour la prophylaxie post-exposition contre le VEEO.

Résultats : Nous avons observé que l'inoculation d'une dose de l'adénovirus exprimant l'IFN-α a conféré une protection post-exposition contre une provocation létale par le VEEO. Cette protection était liée à la production d'IFN-α immédiatement après l'inoculation de l'adénovirus.

Signification : Cette étude montre que l'utilisation de l'expression de l'IFN-α par médiation adénovirale présente un potentiel considérable comme contre-mesure médicale pour la protection post-exposition contre le VEEO. L'élaboration d'une telle contre-mesure médicale protégerait les FC contre les menaces d'infection à VEEO aussi bien naturelles que délibérées.

Plans futurs : L'IFN-α est un antiviral à large spectre. Des études sont maintenant nécessaires pour déterminer si l'expression de l'IFN-α par médiation adénovirale pourrait conférer une protection post-exposition générique contre d'autres virus importants pour la défense biochimique.

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Acknowledgements

The authors wish to thank the staff at the animal care facility of DRDC Suffield for providing mice for the challenge and protection study.

This study was supported by the Technology Investment Fund of DRDC.

Introduction

Western equine encephalitis virus (WEEV) is endemic in western North America, Central America, and South America [1]. Humans and other species are infected by WEEV after being bitten by a mosquito carrying the virus. Symptoms of WEEV infection in humans include fever, headache, delirium, disorientation and coma. The overall case fatality rate ranges from 3 to 8%. A high mortality rate in humans has been observed in laboratory-acquired WEEV infections in which two out of five laboratory workers died after being accidently exposed to the aerosolized WEEV [2]. Currently, no licensed vaccine or antiviral drug is available for prevention and treatment of WEEV infection. The high mortality rate after aerosol transmission and the lack of medical countermeasures make WEEV a potential biowarfare and bioterrorism agent [3].

DRDC Suffield has developed a novel molecular technology which can be used to deliver an antiviral gene against WEEV. This technology is based on a human adenovirus type 5 (HAd5) vector, which has been extensively used for developing genetic vaccines in humans [4] (Figure 1). The antiviral gene chosen encodes interferon alpha (IFN-α). IFN-α is a potent antiviral protein which is secreted by host cells upon viral infection [5]. After secretion, IFN-α binds to target cells, which produces antiviral proteins, such as double-stranded RNA-dependent protein kinase, MxA GTPase, and 2′-5′ oligoadenylate synthetase. IFN-α has been used for the treatment of hepatitis B and C. Studies have demonstrated that IFN-α therapy is able to completely clear all residual viruses from patients and to prolong the survival of patients with chronic hepatitis B virus infection. Because of the short half-life of IFN-α, direct injection of purified IFN-α protein for the treatment requires multiple doses for a long period of time, which could cause serious side-effects. To overcome this problem, a gene delivery method involving an HAd5 vector was tested as an alternative approach for the IFN-α treatment.

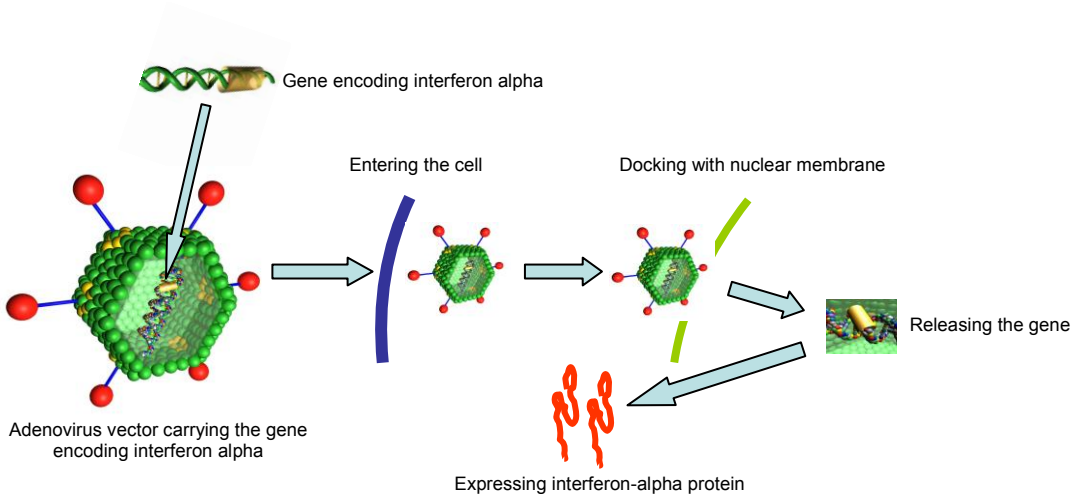

Figure 1. Schematic illustration of adenovirus-mediated delivery of the gene encoding interferon alpha.

In a previous study, an HAd5 vector (Ad5-mIFNα) expressing mouse IFN-α (mIFN-α) was constructed and characterized [6]. Extensive studies on pre-exposure protection against WEEV by Ad5-mIFNα were conducted and it was found that a single inoculation of Ad5-mIFNα before the WEEV challenge provides a complete protection against the WEEV infection [7]. Because limited knowledge is available regarding the efficacy of Ad5-mIFNα for post-exposure protection against WEEV, the current study examined whether Ad5-mIFNα given immediately after or at 24 h after the WEEV exposure would provide protection. How fast mIFN-α could be produced after a single-dose inoculation of Ad5-mIFNα was also evaluated.

Materials and methods

Recombinant adenoviruses

Three recombinant human adenoviruses were used throughout the study, Ad5-mIFNα, Ad5-EGFP (a recombinant human adenovirus expressing the enhanced green fluorescent protein), and Ad5-Empty (a recombinant human adenovirus containing no foreign gene insertion). These recombinant viruses were constructed previously [7–9] and purified by double cesium chloride gradient centrifugation or by Adeno-X Mega Purification Kit (Clontech, Mountain View, CA). The titers of the purified recombinant adenoviruses were determined by a 50% tissue culture infectious dose assay [10].

Mice

Female BALB/c mice (17-20 g) were used in this study. They were obtained from the pathogen-free mouse breeding colonies at DRDC Suffield, with the original breeding pairs from Charles River Canada (St. Constant, Quebec, Canada). The use of the mice was approved by the Animal Care Committee of DRDC Suffield. The Canadian Council on Animal Care guidelines for caring and handling mice were followed.

Time course of mIFN-α production in mice given Ad5-mIFNα injection

A total of 72 mice were divided into 2 groups of 36 mice each. In group I, each mouse was given a single dose, IM injection of 10^7 plaque-forming units (PFU) of Ad5-mIFNα. Inoculation was done by injecting 50 µl of phosphate buffered saline (PBS) containing Ad5-mIFNα into the right thigh muscle of each mouse with 1mL Syringes (Becton Dickinson). In Group II, each mouse was given a single dose, IM injection of 10^7 PFU of Ad5-EGFP control. Immediately after the injection and at 1, 3, 5, 72 and 120 h (6 mice per time point) after the injection, 150 µL of blood was collected from each mouse by tail-vein bleeding into a Microtainer® Brand Serum Separator Tube (Becton Dickinson). The blood in the tube was incubated at room temperature (RT) for 30 min and centrifuged at 10,000 rpm (1,500 × *g*) for 8 min. Serum was collected and pooled for the same time point group.

Measurement of serum mIFN-α

Mouse Interferon Alpha ELISA kit purchased from PBL Biomedical Labs (Piscataway, NJ) was used to measure serum concentrations of mIFN-⟨ at different

time points. The detection limit of the ELISA kit is 12 pg/mL. Sera were 1:5 or 1:10 diluted in Dilution Buffer. Each plate contained duplicates of the mIFN-α standards and duplicates of the negative control containing Dilution Buffer only. Each serum sample was assayed in triplicates. A total of 100 µL of the diluted serum was loaded into the individual wells of the microplate. The samples were incubated at RT for 1 h and washed once with Final Wash Solution using an ELx50 Auto Strip Washer (Bio-Tek Instruments Inc., Winooski, VT). Next, 100 µL of an antibody solution was added to each well and incubated at RT for 24 h. After incubation, the wells were washed 3 times with Final Wash Solution. A total of 100 µL of horseradish peroxidase (HRP) conjugate solution was added to each well and incubated at RT for 1 h. After incubation with HRP conjugate, the wells were washed 4 times with Final Wash Solution. A total of 100 µL of 3,3′,5,5′-tetramethylbenzidine substrate was added to each well and incubated at RT for 15 min in the dark. The reaction was stopped by adding 100 µL of Stop Solution to each well. The optical absorbance of the plates were read at 450nm with a VersaMax™ Microplate Reader (Molecular Devices, Sunnyvale, CA). Standard curves were constructed with GraphPad Prism® 4 software (GraphPad Software Inc.) by entering known mIFN-α standard concentrations in the X-axis and its absorbance readings on the Y-axis. Serum mIFN-α concentrations were determined by extrapolation from the standard curve.

WEEV strains

Two WEEV strains were used throughout the study. 71V-1658 strain was originally provided as a 10% suckling mouse brain suspension by Dr Nick Karabatsos (U.S. Centers for Disease Control and Prevention, Fort Collins, CO, USA). The Fleming strain was purchased from American Type Culture Collection (ATCC), Manassas, VA. Seed stocks of WEEV were made from the original vials by the inoculation of Vero cells (ATCC) with the viruses at a multiplicity of infection (MOI) of less than 0.1. The supernatant of the infected cells was collected, aliquoted, and stored at −70 °C for further use in animal challenge studies. The titers of the WEEV stocks were determined by plaque assay in Vero cells. All the experiments with WEEV were carried out in the Biosafety Level 3 laboratory at Defence Research and Development Canada – Suffield (DRDC Suffield) in compliance with the guidelines of Health Canada and the Canadian Food Inspection Agency.

Post-exposure protection study in mice against the WEEV 71V-1658 strain

Female BALB/c mice (17–20 g) were used for the post-exposure protection study. The experimental protocol was approved by the Animal Care Committee of DRDC Suffield. The guidelines of the Canadian Council on Animal Care were followed for animal handling. The efficacy of Ad5-mIFNα against 71V-1658 when Ad5-mIFNα was given immediately after the challenge was tested. A total of 16 mice were divided

into two groups of eight mice each. Mice were challenged intranasally (IN) with 25 lethal dose 50 (LD_{50}) doses of 71V-1658. Immediately after the challenge, each mouse in Group I was given an IM injection of 10^7 PFU of Ad5-mIFNα and each mouse in Group II was given an IM injection of 10^7 PFU of Ad5-Empty vector control. The mice were monitored daily for up to 14 days for survival. The progression of the infection were measured using the following scoring system: 0, normal; 1, slightly ruffled hair, very active, no visible signs of infection; 2, very ruffled hair, definite signs of infection, not as active, but still fairly mobile; 3, very ruffled hair, hunched posture, reduced mobility; and 4, very ruffled hair, hunched posture, little or no mobility, rapid breathing. Mice scored at the scale of 4 were considered terminally ill and were euthanized.

Post-exposure protection study in mice against the WEEV Fleming strain

Three experiments on the efficacy of Ad5-mIFNα for post-exposure protection against Fleming were carried out. In the first experiment, mice (8 per group) were first challenged IN with 25 LD_{50} doses of Fleming. Immediately after the challenge, each mouse was given an IM injection of 10^7 PFU of Ad5-mIFNα or 10^7 PFU of Ad5-EGFP control. The treated mice were monitored daily for 14 days for survival and the severity of the infection. In the second experiment, mice (8 per group) were first challenged IN with 25 LD_{50} doses of Fleming and at 24 h after the challenge, they were given an IM injection of 10^7 PFU of Ad5-mIFNα or 10^7 PFU of Ad5-EGFP. In the third experiment, a total of 32 mice were divided into four groups of eight mice each. All the mice were first challenged IN with 50 LD_{50} doses of Fleming. Immediately after the challenge, each mouse in Groups I and II were treated with an IM injection of 10^7 PFU of Ad5-mIFNα or 10^7 PFU of Ad5-EGFP. At 24 h after the challenge, each mouse in Groups III and IV were treated with the same dose of Ad5-mIFNα or Ad5-EGFP. The treated mice were monitored daily for survival and the severity of the infection.

Statistics analysis

PRISM® 4 program (GraphPad Software Inc.) was used for statistical analysis. A *P* value of less than 0.05 is considered to be significant. Differences in mean survival time between the Ad5-mIFNα-treated mice and the control mice were compared by a two-tailed paired *t* test.

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Results

Rapid production of mIFN-α in mice given a single-dose injection of Ad5-mIFNα

In a previous DRDC Suffield study [7], it was found that serum mIFN-α was detected in mice as early as 6 h after a single-dose, intramuscular (IM) inoculation of Ad5-mIFNα. In addition, the production of mIFN-α lasted for at least 48 h. This study extends the previous study on time course by monitoring the mouse serum mIFN-α concentrations at 1, 3, 5, 72 and 120 h after the injection. As shown in Figure 2, serum mIFN-α was detected as early as 3 h after a single-dose, IM injection of Ad5-mIFNα and quickly reached a high level at 5 and 72 h after injection. The level of mIFN-α production waned by 120 h (day 5) after injection but was still measurable. The results from DRDC Suffield's previous and current studies indicate that the production of mIFN-α in mice after a single-dose, IM injection of Ad5-mIFNα is rapid and long lasting.

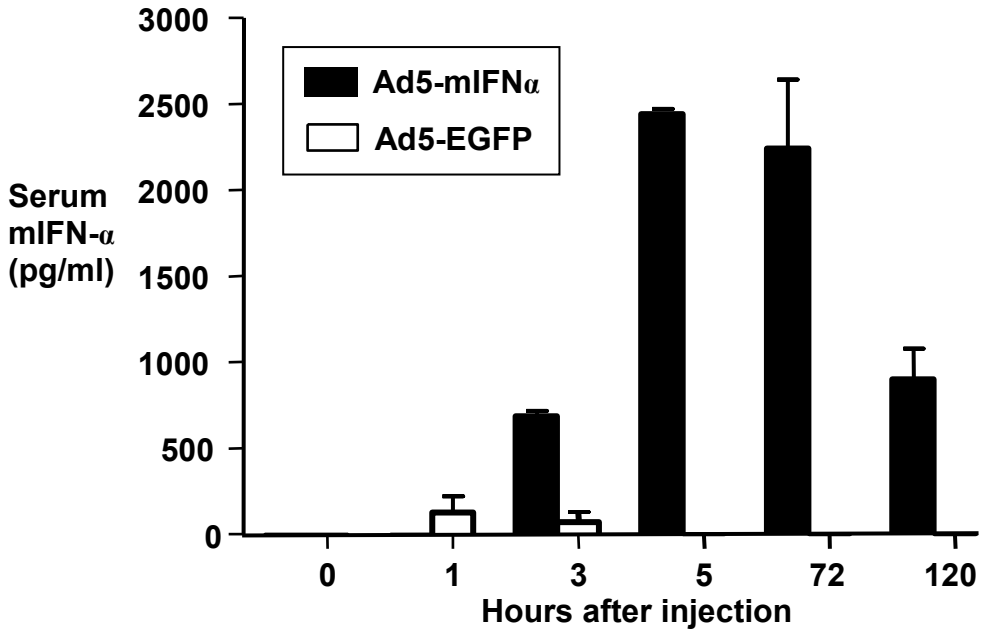

Figure 2. Rapid production of mIFN-α in mice injected with Ad5-mIFNα. *Mice were each given intramuscular injection of 10^7 PFU of Ad5-mIFNα. At 0 (before the injection) and at 1, 3, 5, 72, and 120 h after injection (6 mice for each time point), serum was collected and pooled for each time point group. Control mice were each injected with the same dose of Ad5-EGFP. Serum concentrations of mIFN-α were measured in triplicates by ELISA and shown as mean ± SE.*

Complete protection against the 71V-1658 strain of WEEV when given Ad5-mIFNα immediately after the challenge

The rapid production of mIFN-α by Ad5-mIFNα makes Ad5-mIFNα suitable for post-exposure prophylaxis against WEEV. Indeed, in its previous study [7], DRDC Suffield demonstrated that 60% of mice were protected from lethal challenge of the WEEV Fleming strain when given Ad5-mIFNα at 6 h after exposure to the virus.

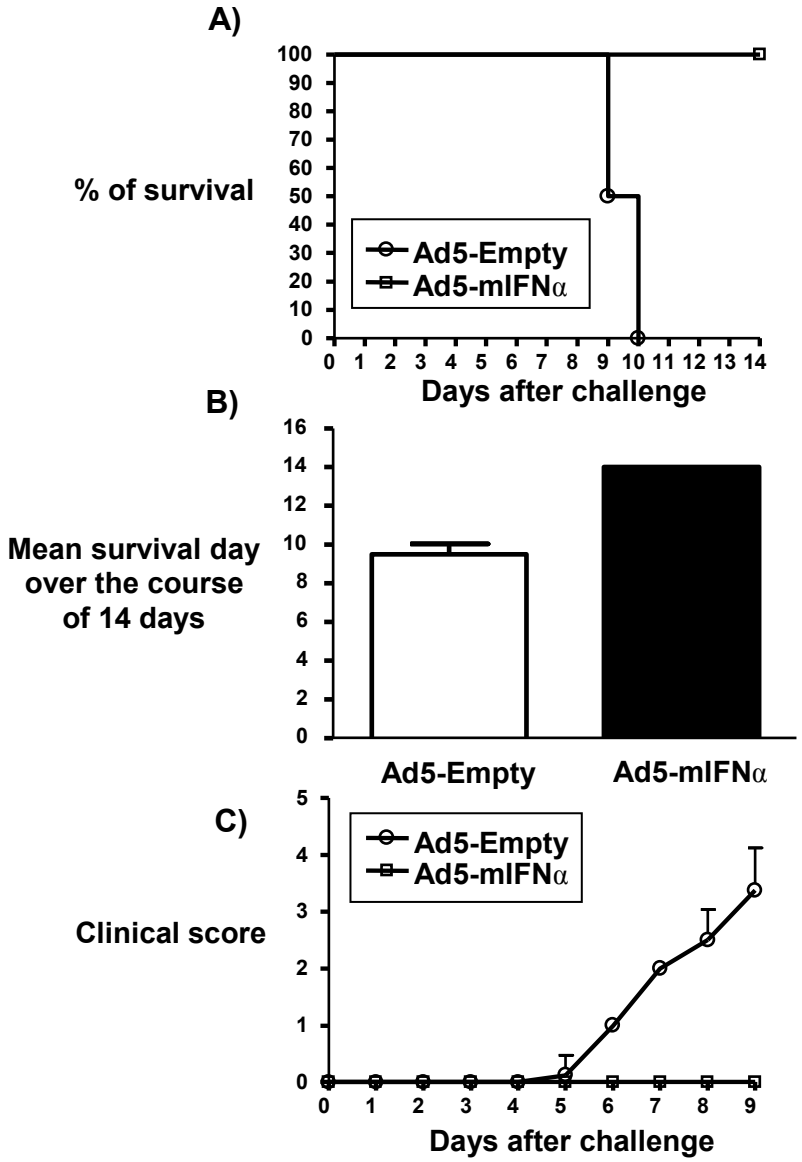

Figure 3. Post-exposure protection against the 71V-1658 strain of WEEV by Ad5-mIFNα. *A) Kaplan-Meier survival curves of the mice treated with Ad5-mIFNα or Ad5-Empty (8 mice per group) immediately after exposure to 71V-1658. B) Mean day of survival of the treated mice. Error bar represents mean ± SD. C) Severity of infection in treated mice. Mice were scored daily for the severity of the infection using the scoring system described in Materials and Methods. Error bars represent mean ± SD.*

This study was undertaken to determine whether the protection rate could be improved when Ad5-mIFNα was given immediately after the virus exposure. To do this, mice were challenged IN with 71V-1658 and given a single-dose, IM injection of 10^7 PFU of Ad5-mIFNα or 10^7 PFU of Ad5-Empty immediately after the challenge. As shown in Figure 3, all of the mice given Ad5-mIFNα survived challenge (panel A) and remained healthy throughout the 14-day observation period (panel C). In contrast, all the control mice died from the challenge with the mean survival time of 10 days (panel B). These control mice showed signs of infection as early as day 6 and became serious ill by day 9 (panel C). Taken together, the data demonstrate that complete post-exposure protection against 71V-1658 is achieved when Ad5-mIFNα treatment was given immediately after the virus exposure.

Partial protection against the Fleming strain of WEEV when given Ad5-mIFNα immediately and 24 h after the challenge

The result of the complete post-exposure protection against 71V-1658 when given Ad5-mIFNα immediately after the challenge prompted DRDC Suffield to test whether the same outcome would be achieved for the Fleming strain, which is more pathogenic than 71V-1658. DRDC Suffield demonstrated previously that Ad5-mIFNα partially protects mice from the Fleming challenge when given at 6 h after the challenge [7]. This study shows that up to 50% of the mice given Ad5-mIFNα survived to day 14 post challenge, while all of the control mice had died by day 5 (Figure 4, panel A). The protection rate is similar to that observed for the Ad5-mIFNα treatment at 6 h after challenge [7]. The mean survival time for the mice treated with Ad5-mIFNα is almost doubled when compared to that for the control mice (panel B; $P < 0.01$; Ad5-mIFNα- vs Ad5-EGFP-treated mice). In addition, four out of eight mice treated with Ad5-mIFNα did not show any signs of infection throughout the 14-day observation period while all the control mice showed signs of infection as early as day 3 after the challenge (panel C). These results suggest a partial protection against the highly virulent strain Fleming is achieved when Ad5-mIFNα was given immediately after the challenge.

The effectiveness of Ad5-mIFNα against the Fleming strain when Ad5-mIFNα was given at 24 h after the challenge was determined next. As illustrated in Figure 5, the Ad5-mIFNα-treated mice had longer survival time than the mice given Ad5-EGFP control (panel B; $P < 0.01$). However, by day 14 after the challenge, less than 20% of the Ad5-mIFNα-treated mice survived (panel A) and all the mice showed the signs of infection (panel C). The data demonstrate that when Ad5-mIFNα was given at 24 h after the Fleming challenge, it extends the survival time of the mice but does not prevent them from the death.

Finally, the efficacy of Ad5-mIFNα for post-exposure protection against a high-dose challenge of the Fleming strain was tested. Mice were first IN challenge with 50 LD50 doses of the Fleming strain and then treated with Ad5-mIFNα either immediately or 24 h after the challenge. Figure 6 shows that up to 40% of the mice given Ad5-mIFNα immediately after the challenge survived. The survival rate was similar to that shown for the low-dose challenge (Figure 4, panel A). In addition, two out of eight mice that survived the challenge showed no signs of infection by day 14. However, as also shown in Figure 6, all the mice given Ad5-mIFNα at 24 h after a high-dose challenge were dead by day 7. These data demonstrate that Ad5-mIFNα gives partial protection against the high-dose challenge of Fleming when given immediately after the virus exposure and gives no protection when given at 24 h after the exposure.

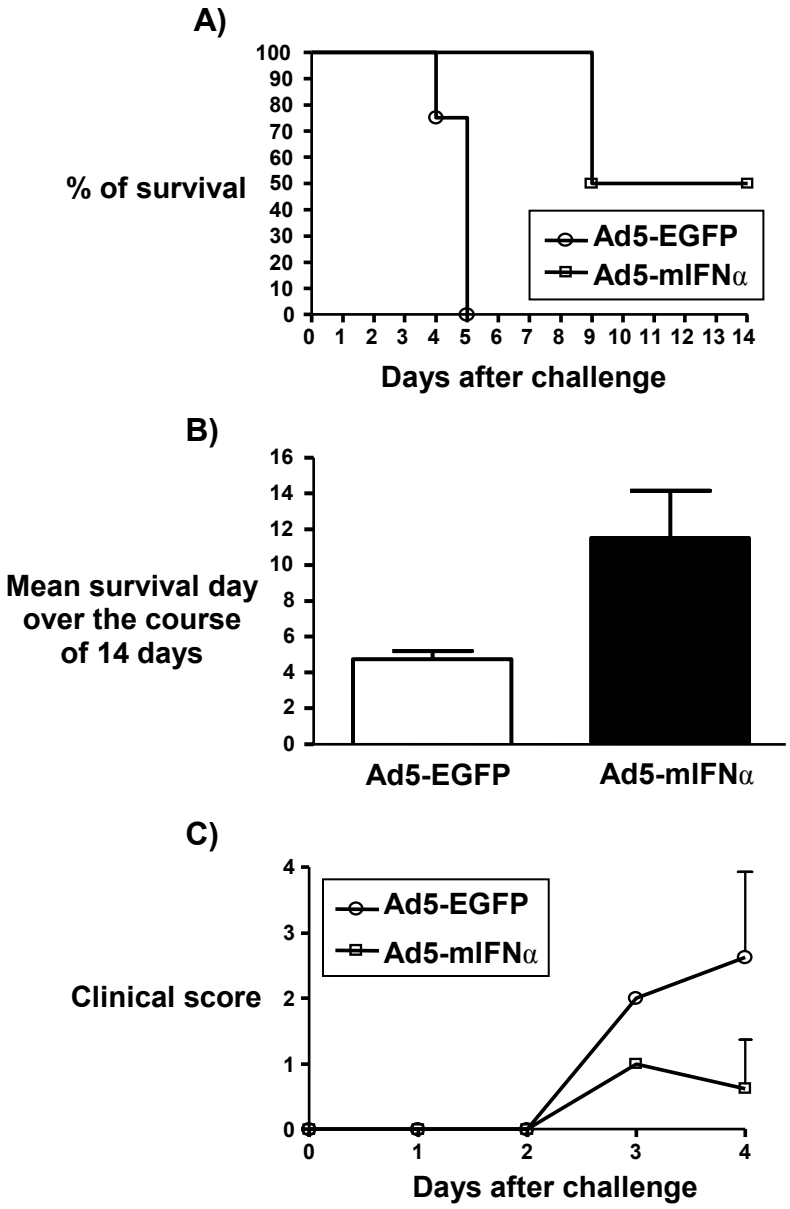

Figure 4. Post-exposure protection against the Fleming strain of WEEV by Ad5-mIFNα given immediately after virus exposure. *A) Kaplan-Meier survival curves of the mice treated with Ad5-mIFNα or Ad5-EGFP (8 mice per group). B) Mean day of survival of the treated mice. Error bar represents mean ± SD. C) Severity of infection in treated mice. Mice were scored daily for the severity of the infection. Error bars represent mean ± SD.*

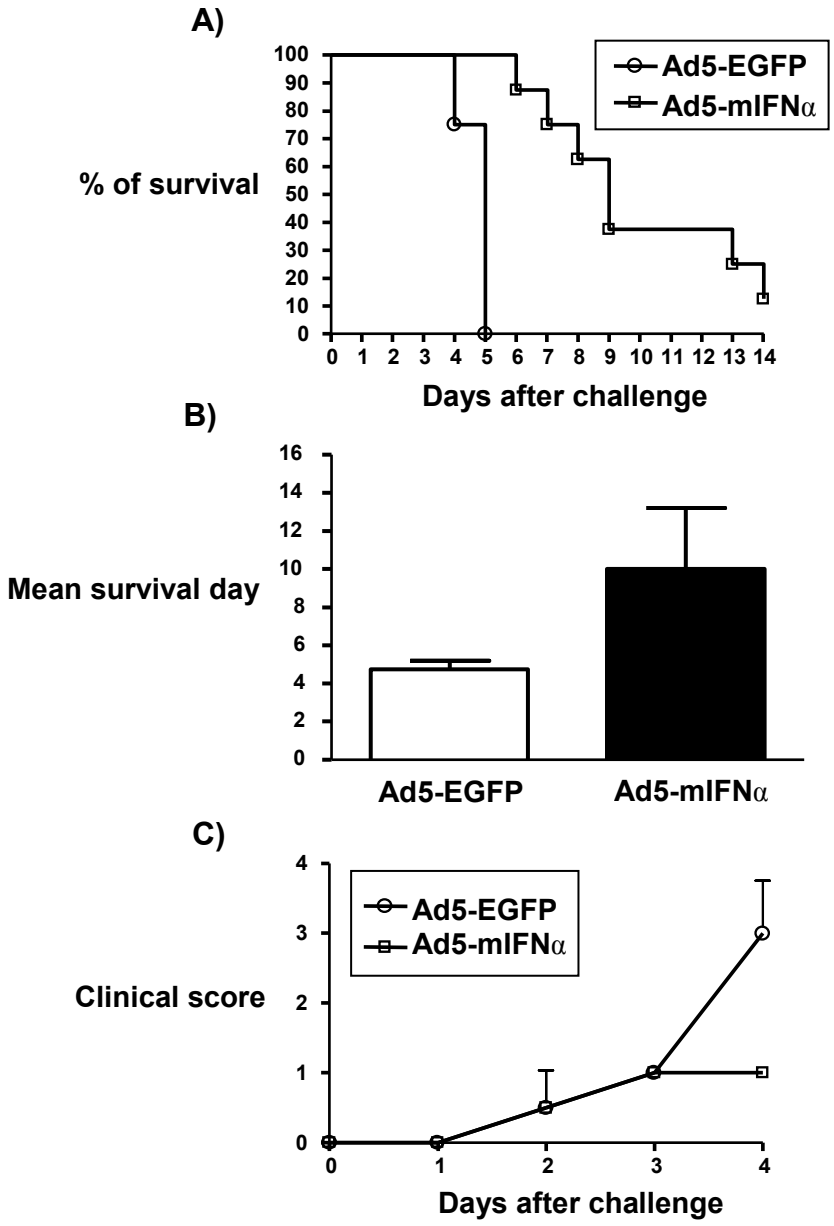

Figure 5. Post-exposure protection against the Fleming strain of WEEV by Ad5-mIFNα given 24 h after virus exposure. *A) Kaplan-Meier survival curves of the mice treated with Ad5-mIFNα or Ad5-EGFP (8 mice for each treatment group). B) Mean day of survival of the treated mice. Error bar represents mean ± SD. C) Severity of infection in treated mice. Mice were scored daily for the severity of the infection. Error bars represent mean ± SD.*

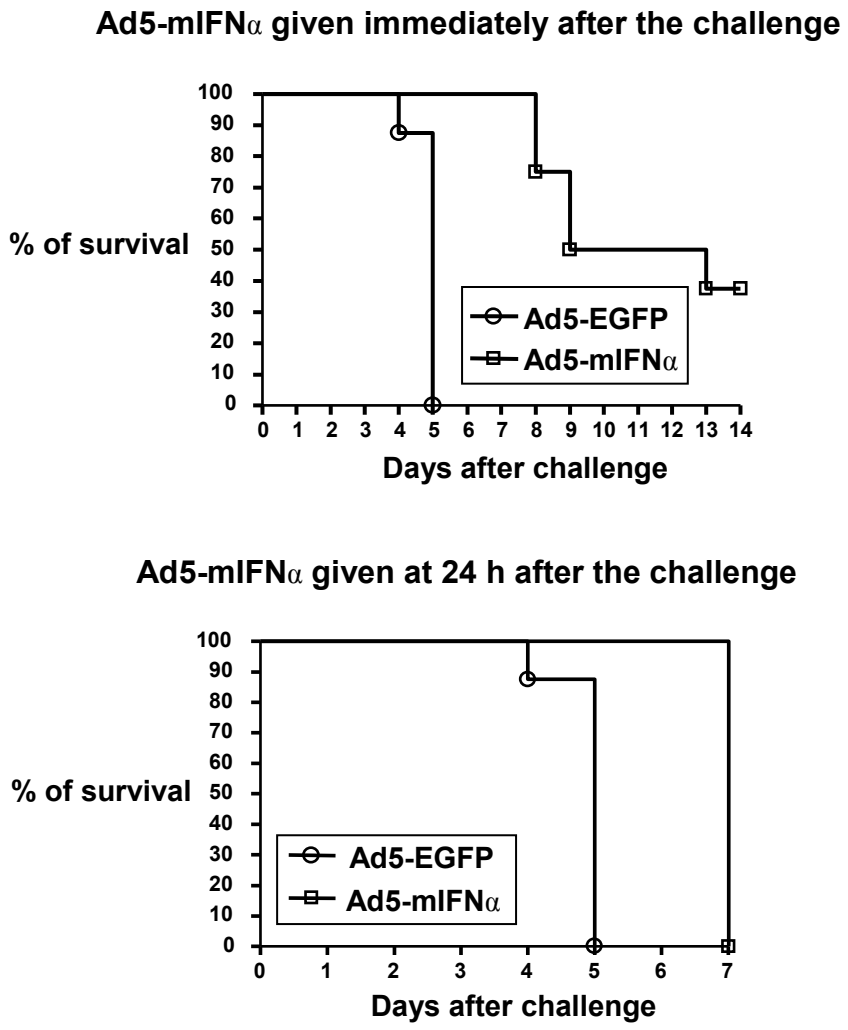

Figure 6. Kaplan-Meier survival curves of the mice treated with Ad5-mIFNα at 0 or 24 h after exposure to a high-dose of the Fleming strain of WEEV.

Discussion

Viral vector derived from human adenovirus type 5 (HAd5) has been used to express IFN-α against viral diseases. A study by Chinsangarm et al. first demonstrated that swine were completely protected against the infection by foot-and-mouth disease virus when given HAd5 vectors expressing porcine IFN-α at 24 h before exposure to the virus [11]. DRDC Suffield has shown previously that complete, pre-exposure protection can be achieved against various WEEV strains when mice were given Ad5-mIFNα at different time points before WEEV challenge [7]. However, only limited data has been available regarding the efficacy of post-exposure protection by HAd5-vector-mediated expression of IFN-α against viral diseases.

The results from the current study demonstrate that a single-dose inoculation of Ad5-mIFNα provides post-exposure protection against a lethal-dose challenge of WEEV. The ability of Ad5-mIFNα to provide such protection is related to the rapid production of mIFN-α after inoculation (Figure 2). It was found that the efficacy of protection is determined by which WEEV strain is used for challenge and when the inoculation is given. When Ad5-mIFNα was inoculated immediately after the challenge with a low-virulence strain of WEEV (71V-1658) [12], all the mice were protected (Figure 3); However, when Ad5-mIFNα was inoculated immediately after the challenge with a high-virulence strain of WEEV (Fleming), only half of the mice were protected (Figure 4). The survival rate dropped to close to 10% when Ad5-mIFNα was given to mice at 24 h after the Fleming strain challenge although the overall survival time was extended in these mice (Figure 5).

There are several limitations in this study which could point out future directions for further research. The first was that only one dosage of Ad5-mIFNα (10^7 PFU per mouse) was tested in the study. Increasing the amount of Ad5-mIFNα given to mice might improve the protection. The second was that protection against 71V-1658 in mice was complete when mice were given Ad5-mIFNα immediately after exposure to the virus and no signs of infection were observed; however, whether the virus was still present in different tissues and organs was not investigated. This data could point out if Ad5-mIFNα can completely clear the existing infection and inhibit the production of progeny viruses. In addition, it was not determined whether the complete protection conferred by Ad5-mIFNα against 71V-1658 is also true if Ad5-mIFNα is given at the late stage of infection, such as 6 or 24 h after the challenge. The data from this study and a previous one [7] showed that at these time points, only partial protection was achieved by Ad5-mIFNα against the Fleming strain. Also, another experiment should be done to test if Ad5-mIFNα could improve the survival of the mice which already display signs of infection. This will demonstrate whether Ad5-mIFNα has potential for the treatment of WEEV infection at later stages when symptoms are evident. The third limitation of this study was that mIFN-α production in the mouse brain after the

IM injection of Ad5-mIFNα, which could indicate whether the protection is through the direct inhibition of WEEV replication by mIFN-α in the brain or through other indirect mechanisms, was not monitored.

The peak production of mIFN-α lasted at least 3 days (Figure 2), which was not sufficient to give protection against the Fleming challenge when Ad5-mIFNα was given at 24 h after the virus exposure (Figure 5). Based on the survival curve (Figure 5, panel A), the progression of the infection in mice treated with Ad5-mIFNα was much slower than that for control mice; however, the majority of the mice died of infection by day 14. This suggests the presence of mIFN-α in mice could block WEEV replication at the early stage of infection; but mIFN-α could not inhibit the spread of WEEV at the late stage of infection. This may be due to virulent factor(s) associated with the Fleming strain that disrupt the antiviral activity of mIFN-α. A recent study showed that Venezuelan equine encephalitis virus, which is closely related to WEEV, shuts off the signal transduction required for IFN-α activity [13]. It will be an important study for therapy to see whether the high virulence of Fleming is due to its interference of the IFN system.

Conclusion

The data support the use of adenovirus-mediated expression of IFN-α as a potential medical countermeasure for post-exposure protection against WEEV. The successful development of such a medical countermeasure would not only protect the CAF in the event of biological warfare, but would also deter the development WEEV as a biological weapon. Due to the broad-spectrum antiviral activity of IFN-α, studies are now needed to test whether adenovirus-mediated IFN-α expression could provide post-exposure protection against other viral agents.

References

1. Griffin, D.E. (2007), Alphaviruses. In D.M. Knipe and P.M. Howley (eds), *Fields Virology*, 5th ed., Volume 1, Lippincott Williams & Wilkins, Philadelphia, pp. 1023–1067.

2. Hanson, R.P.; Sulkin, S.E.; Beuscher, E.L.; Hammon, W.M.; McKinney, R.W.; and Work, T.H. (1967), Arbovirus infections of laboratory workers. Extent of problem emphasizes the need for more effective measures to reduce hazards, *Science*, 158, 1283–1286.

3. Sidwell, R.W. and Smee, D.F. (2003), Viruses of the Bunya- and Togaviridae families: potential as bioterrorism agents and means of control, *Antiviral Res*., 57, 101–111.

4. Lasaro, M.O. and Ertl, H.C. (2009), New insights on adenovirus as vaccine vectors, *Mol. Ther*., 17, 1333–1339.

5. Borden, E.C.; Sen, G.C.; Uze, G.; Silverman, R.H.; Ransohoff, R.M.; Foster, G.R.; and Stark, G.R. (2007), Interferons at age 50: past, current and future impact on biomedicine, *Nat. Rev. Drug Discov.*, 6, 975–990.

6. Wu, J.Q.H. (2006), Construction and characterization of a recombinant adenovirus expressing interferon-alpha, (DRDC Suffield TM 2006-024) Defence R&D Canada – Suffield.

7. Wu, J.Q.; Barabe, N.D.; Huang, Y.M.; Rayner, G.A.; Christopher, M.E.; and Schmaltz, F.L. (2007). Pre- and post-exposure protection against Western equine encephalitis virus after single inoculation with adenovirus vector expressing interferon alpha, *Virology*, 369, 206–213.

8. Wu, J.Q.; Barabe, N.D.; Chau, D.; Wong, C.; Rayner, G.R.; Hu, W.G.; and Nagata, L.P. (2007), Complete protection of mice against a lethal dose challenge of western equine encephalitis virus after immunization with an adenovirus-vectored vaccine, *Vaccine*, 25, 4368–4375.

9. Wu, J.Q.H., Barabe, N.D., and Swayze, R.D. (2009), Identification of the 6K-E1 protein of western equine encephalitis virus as a vaccine target, (DRDC Suffield TM 2009-201), Defence R&D Canada – Suffield.

10. Mahy, B.W.J. and Kangro, H.O. (1996). *Virology Methods Manual*, Academic Press, San Diego, CA.

11. Chinsangaram, J.; Moraes, M.P.; Koster, M.; and Grubman, M.J. (2003), Novel viral disease control strategy: adenovirus expressing alpha interferon rapidly protects swine from foot-and-mouth disease, *J. Virol.*, 77, 1621–1625.

12. Nagata, L.P.; Hu, W.G.; Parker, M.; Chau, D.; Rayner, G.A.; Schmaltz, F.L.; and Wong, J.P. (2006), Infectivity variation and genetic diversity among strains of Western equine encephalitis virus, *J. Gen. Virol.*, 87, 2353–2361.

13. Simmons, J.D.; White, L.J.; Morrison, T.E.; Montgomery, S.A.; Whitmore, A.C.; Johnston, R.E.; and Heise, M.T. (2009), Venezuelan equine encephalitis virus disrupts STAT1 signaling by distinct mechanisms independent of host shutoff, *J. Virol.*, 83, 10571–10581.

List of symbols/abbreviations/acronyms/initialisms

ATCC	American Type Culture Collection
CAF	Canadian Armed Forces
HAd5	Human adenovirus type 5
HRP	Horseradish peroxidase
IFN-α	Interferon alpha
IM	Intramuscular
IN	Intranasally
LD_{50}	Lethal dose 50
MC	Medical countermeasure
mIFN-α	Mouse interferon alpha
MOI	Multiplicity of infection
PBS	Phosphate Buffered Saline
PFU	Plaque forming unit
RT	Room temperature
WEEV	Western equine encephalitis virus

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Western equine encephalitis virus (WEEV) is an endemic and biowarfare agent which poses a potential threat to the operations of the Canadian Armed Forces (CAF). A vaccine for the prevention of WEEV infection before exposure to the virus has been developed and approved by the U.S. Food and Drug Administration as an investigational new drug. However, very little progress has been made in finding post-exposure prophylaxis for WEEV. Using the mouse WEEV model, DRDC Suffield has tested a novel strategy for developing such medical countermeasures. A modified, harmless human adenovirus has been used to ferry a gene encoding a potent antiviral protein, interferon alpha (IFN-α), into the body. In this study, DRDC Suffield has demonstrated that IFN-α was rapidly produced in mice after intramuscular (IM) injection of the adenovirus and that a single-dose IM injection provided post-exposure protection against the lethal challenge of WEEV. This pilot study provides insights into further development of medical countermeasures for post-exposure prophylaxis against WEEV and other viral biothreat agents.

Le virus de l'encéphalite équine de l'Ouest (VEEO) est un micro-organisme endémique utilisé comme agent de guerre biologique qui constitue une menace potentielle pour les opérations des Forces canadiennes (FC). Un vaccin a été mis au point et approuvé par l'Agence américaine des aliments et des médicaments (FDA) à titre de nouveau médicament de recherche pour prévenir l'infection à VEEO avant l'exposition au virus. Cependant, aucune prophylaxie post-exposition n'a encore été établie contre le VEEO. Nous avons utilisé un modèle VEEO chez la souris pour mettre à l'essai une nouvelle méthode d'élaboration de contre-mesures médicales (CM méd). Plus précisément, à l'aide d'un adénovirus humain modifié inoffensif, nous avons transféré un gène codant une protéine antivirale puissante – l'interféron alpha (IFN-α) – dans l'organisme des souris. Notre étude a montré que l'IFN-α était rapidement produit chez la souris à la suite d'une injection intramusculaire de l'adénovirus et qu'une dose intramusculaire unique conférait une protection post-exposition contre une provocation létale par le VEEO. Cette étude pilote aidera à mettre au point des CM méd prophylactiques post-exposition contre le VEEO et d'autres agents de menace biologique d'origine virale.

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Western equine encephalitis virus; WEEV; post-exposure; prophylaxis