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In mice the efficiency of immunization with Venezuelan Equine Encephalomyelitis virus TC-83 is transiently increased by dehydroepiandrosterone.

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Abstract. To determine whether treatment with dehydroepiandrosterone (DHEA) improves the efficiency of immunization against the Venezuelan Equine Encephalomyelitis (VEE) virus, mice were vaccinated with the TC-83 VEE virus. DHEA (10 mg/kg) was administered in a single dose, 4 hours before vaccination. IgM antibody titers were determined at days 7, 14 and 21 post-immunization. Treatment with DHEA increased antibody titers at day 14 after immunization. Mice were challenged with live VEE virus at day 21, and viral titers were plaque assayed in chicken embryo fibroblasts from days 2 to 5 post-infection. After the challenge, viremia decreased on day 2 and brain virus levels were reduced at day 4 in mice treated with DHEA. These results suggest that DHEA treatment could enhance the efficiency of immunization against VEE virus in mice.

La eficiencia de la inmunización de ratones con el virus TC-83 de la Encefalomielitis Equina Venezolana, aumenta transitoriamente con la dehidro-epiandrosterona.

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Palabras clave: Dehidroepiandrosterona, encefalomielitis, virus TC-83, equino.

Resumen. Con el objeto de determinar si el tratamiento con la dehidroepiandrosterona (DHEA) mejora la eficiencia de la inmunización contra el virus de la encefalomielitis equina venezolana (EEV), se vacunaron ratones con el virus TC-83 de la EEV. La DHEA (10 mg/kg), se administró, en una sola dosis, 4 horas antes de la vacunación. Los títulos de anticuerpos IgM se determinaron los días 7,14 y 21 después de la vacunación. El tratamiento con DHEA incrementó los títulos de anticuerpos el día 14 post-vacunación. Los ratones fueron retados con el virus vivo de la EEV el día 21 y los títulos virales se ensayaron en placas con fibroblastos de embrión de pollo los días 2 a 5 post-infección. Después de la administración del virus vivo disminuyó la viremia post-infección el día 2 y se redujeron los títulos virales en el cerebro el día 4 post-infección. Estos resultados sugieren que el tratamiento con DHEA podría aumentar la eficiencia de la inmunización de los ratones contra el virus de la EEV.

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INTRODUCTION

Venezuelan Equine Encephalomyelitis (VEE) is a viral disease caused by a mosquito born virus (family *Togaviridae*, genus *Alphavirus*) (1). Outbreaks have occurred in northern South America since 1920, and thousands of people and equines have been affected (2, 3, 4). Three vaccines currently exist. The live, attenuated, VEE virus vaccine TC-83 is produced by serial passages of the wild virus (Trinidad Donkey strain) in guinea pig fetal heart cell cultures. It has proved to be efficacious in providing long term immunity (5). However, up to 25% of the vaccinated individuals develop the illness with a low grade of viremia (5). Even though TC-83 gives a longer immunity than the other two vaccines, it suffers from reactogenicity in some individuals and non-immunogenicity in others (6).

Dehydroepiandrosterone (DHEA) is a native steroid hormone, produced by the adrenal cortex, with immunomodulating activity (7, 8). Treatment with DHEA was found to be effective in augmenting the response to immunization of old mice injected with recombinant Hepatitis B and Influenza vaccines (8, 9). DHEA protected mice against lethal viral infections of the central nerv-

ous system (CNS) such as West Nile virus (WNV), Sindbis virus neurovirulent (SVNI) and SemLiki Forest virus (SFV). DHEA reduced viremia and death rate, delayed the onset of the disease and increased the ability of the host, through various immune mechanisms, to control virus replication and neuroinvasiveness (10).

The aim of this research is to determine the effect of DHEA treatment on the efficiency of mice immunization with TC-83 VEE virus, as measured by the antibody titers, and the response against the challenge with live VEE virus injected after the immunization.

MATERIALS AND METHODS

Male albino mice, NMRI-IVIC strain from the Instituto Venezolano de Investigaciones Cientificas (IVIC), weighing 25-30 g were fed ad libitum with mice chow and tap water. They were maintained in a room with controlled temperature (24°C) under a 12 h light/dark cycle. Mice were immunized intraperitoneally (i.p.) with 0.05 mL of live TC-83 VEE virus suspension, containing 1.7 x 10^6 PFU/mL, in 0.4% BABS. Virus stock from IVIC was replicated in VERO cell culture and contained $10^{6.78}$ LD_{50}.

DHEA (Sigma Chemical Co., St. Louis, MO, U.S.A.) was dissolved in 50% ethanol. Mice were injected with DHEA (10 mg/Kg), in 0.3 mL, subcutaneously (s.c.), 4 hours before vaccination. Control mice were injected with the diluent.

Anti-VEE IgM antibody titers were determined by an enzyme linked immunosorbent assay (ELISA) 7, 14 and 21 days after vaccination with TC-83 virus (11).

Live VEE virus (10 LD_{50}) was injected in mice treated with DHEA, 21 days after immunization, and 2 to 5 days later they were sacrificed to determine viral titers in serum and brain (12). Serial dilutions of serum or brain homogenates, from VEE controls and infected DHEA treated animals, were added to confluent monolayers in 24 well culture plates that were incubated at 37°C for 2 h for viral adsorption. The standard overlay (Eagle's medium 2x plus 0.5% agarose) was added. Plates were incubated at 37°C in 5% C02 for 48 h, until the cytopathic effect was evident. Plaques were counted after staining monolayers with 0.2% crystal violet.

Data are expressed as mean ± SEM and analyzed by means of the Student's t test or the analysis of variance, followed by Dunn's multiple comparison test, where appropriate. Differences were considered statistically significant when $p < 0.05$.

RESULTS

In mice treated with a single dose of DHEA, a significant increase ($p<0.01$) of antibody titers (Control = 1:1.680 vs. DHEA treated group = 1:4.320) was detected at week 2 after vaccination with TC-83 virus (Fig. 1).

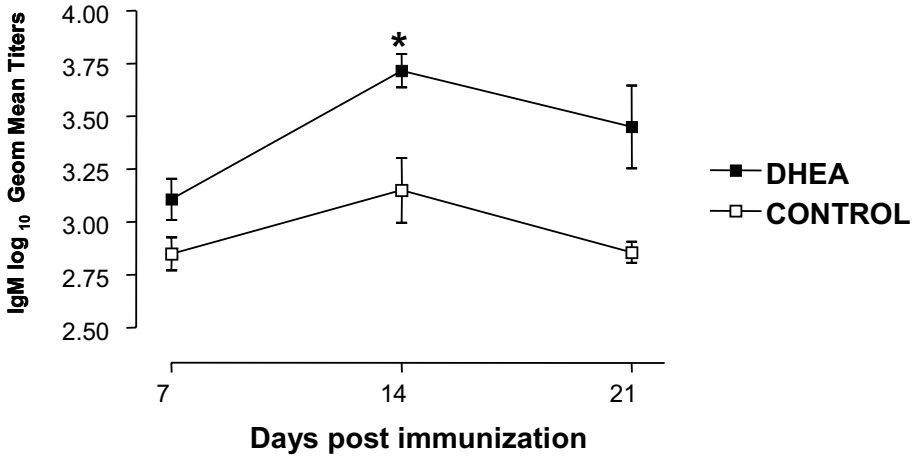

Fig. 1. Serum antibody response to TC-83 VEE with DHEA treatment (10 mg/kg). Data represent mean ± SEM; * $p < 0.05$ when compared to control group.

In mice immunized with TC-83 virus and infected with live VEE virus DHEA treatment decreased significantly ($p < 0.01$) blood virus levels at day 2 post infection (p.i.) (DHEA treated group = 4.33 vs. Control group = 4.62), although the values were similar on day 3 p.i. (5.58 *vs* 5.35). Brain virus levels were also reduced significantly ($p < 0.0001$) by DHEA on day 4 (6.71 *vs* 7.31) p.i. but the results were similar on day 5 p.i. (7.28 *vs* 7.31) (Table I).

DISCUSSION

The immunomodulating activity of DHEA has been previously demonstrated in viral infections (7,8). The present study also shows that the administration of this hormone, to mice vaccinated with VEE TC-83, enhances the humoral immune response. The increase in IgM titers due to DHEA could improve the efficiency of immunization and provide an effective protection against live

TABLE I
EFFECT OF DEHYDROEPIANDROSTERONE ON VIRAL TITERS IN MICE IMMUNIZED WITH TC-83 VIRUS AND INFECTED WITH LIVE VEE VIRUS

Treatment	Blood		Brain	
	Log_{10} PFU/mL		Log_{10} PFU/g	
	Day 2	Day 3	Day 4	Day 5
VEE Control	4.617 ± 0.029	5.347 ± 0.068	7.128 ± 0.048	7.314 ± 0.083
VEE + DHEA	4.333 ± 0.058*	5.577 ± 0.052	6.713 ± 0.003*	7.280 ± 0.005

Live VEE virus (10 LD_{50}) was injected to mice treated with DHEA, 21 days after TC-83 virus immunization. Then, 3-5 mice were sacrificed daily for viral detection. Values are expressed as mean ± SEM. * $p < 0.05$ compared to control.

VEE challenge. However, the reduction in viremia and brain viral titers in mice immunized with the TC-83 virus and then infected with the VEE virus was detected only on days 2 and 4, respectively. On the following day, the titers were not significantly different, indicating that the protection provided by DHEA was only partial. A higher dose or a different scheme of treatment with this hormone could be necessary to get a total protection against the infection with the VEE virus.

The protective effect of DHEA against lethal viral infections in young mice has previously been demonstrated (7,10). The enhancement in the immune response against VEE TC-83 virus, observed with DHEA treatment in this study, is in agreement with previously reported findings (8) in aged mice vaccinated against influenza virus or immunized with recombinant Hepatitis B Surface Antigen (9). Our data seem to indicate that DHEA treatment affects the host resistance against the virus via a peripheral immunostimulating effect.

VEE occurs as an endemic or epidemic disease in Central and South America, as well as in the southern United States. The currently used vaccines are not fully efficient (5,6). For these reasons, the increase of immune response to the vaccine TC-83 obtained with DHEA treatment is of importance and warrants further investigation on its possible use to improve human and equine immunization against VEE virus.

REFERENCES

1. CALISHER C.H., KARABATSOS N.: Arbovirus serogroups: definition and geographic distribution. In: Monath T.P. Ed. The arboviruses: epidemiology and ecology. Vol 1. Boca Ratón: C.R.C. Press 1988; pp. 19-57.
2. SOTO-ESCALONA A., FINOL L.T., RYDER S.: Estudio de un brote de encefalitis venezolana en el Distrito Páez, Estado Zulia en Octubre de 1968. Invest Clín 1969; 10:45-54.
3. NEGRETE A., MOSQUERA J.: Epidemia de encefalitis de 1959 en Maracaibo (San Francisco), Estado Zulia, Venezuela. Manifestaciones Clínicas y terapéutica antibiótica. Invest Clín 1974; 15:11-44.
4. WEAVER S.C., SALAS R., RICO-HESSE R., LUDWIG G.V., STEVEN-OBERSTE M., BOSHELL J.: Reemergence of epidemic Venezuelan equine encephalomyelitis in South America. The Lancet 1996; 348: 436-440.
5. ENGLER R.J. M., MANGIAFICO J.A., JAHRLING P., KSIAZEK T. G., PEDROTTI-KRUEGER M., PETERS C. J.: Venezuelan equine encephalitis-specific immunoglobulin responses: Live attenuated TC-83 versus inactivated C-84 vaccine. J Med Virol 1992; 38: 305-310.

6. BURKE D.S., RAMSBURG H.H., EDELMAN R.: Persistence in humans of antibody to subtypes of Venezuelan equine encephalomyelitis virus after immunization with attenuated TC-83 VEE virus vaccine. J Infect Dis 1977; 136: 354-359.
7. LORIA R.M., INGE T.H., COOK S.S., SZAKAL A. K., REGELSON W.: Protection against acute lethal viral infection with the native steroid Dehydroepiandrosterone (DHEA). J Med Virol 1988; 26: 301-314.
8. DANENBERG H.D., BENYEHUDA A., ZAKAY-RONES Z., FRIEDMAN G.: Dehydroepiandrosterone (DHEA) treatment reverses the impaired immune response of old mice to influenza vaccination and protects from influenza infection. Vaccine 1995; 13:1445-1448.
9. ARANEO B.A., WOODS M.L., DAYNES R.A.: Reversal of immunosenescence phenotype by Dehydroepiandrosterone: hormone treatment provides an adjuvant effect on the immunization of aged mice with recombinant Hepatitis B surface antigen. J Infect Dis 1993; 167: 830-840.
10. BEN-NATHAN D., LACHMI B., LUSTIG S., FEUERSTEIN G.: Protection by Dehydroepiandrosterone in mice infected with viral encephalitis. Arch Virol 1991; 120:263–271.
11. ROSATO R.R., MACASAET T. F., JAHRLING P.B.: Enzyme linked immunoabsorbent assay detection of immunoglobulins G and M to Venezuelan equine encephalomyelitis virus in vaccinated and natural infected humans. J Clin Microbiol 1989; 26: 421-425.
12. BERGOLD G.H., MAZZALI R.: Plaque formation by arboviruses. J Gen Virol 1968; 2: 273-284.