Protective Effects of DHEA and AED against Viral, Bacterial and Parasitic Infections

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ABSTRACT

The use of agents that can boost host immunity to combat infections may be of considerable benefit in the treatment of animals' infectious outbreaks and complement the available modes of various treatments. This report deals with the role of beta androstenes as agents that up-regulate host immune response to a level that enables the host to resist lethal infection by viruses, bacteria, and parasites. The agents reviewed consist of a specific subgroup of androstene steroids that increase the levels of the TH1 cytokines such as, IL-2, IL-3, and IFNγ. Similarly to hydrocortisone, they suppress inflammation, but do not suppress immunity and function in the maintenance of the TH1/TH2 balance and immune homeostasis. We report that DHEA, and AED up-regulate immune resistance and protect the host from lethal infection by RNA and DNA viruses, Gram positive and Gram negative bacteria, parasitic infections, and stress mediated immune suppression. These agents provide a unique new avenue for the control, mitigation, and prevention of animal diseases by viral, bacterial and parasitic infections. Moreover, immune up-regulation may have a significant role in limiting antibiotic resistant and stress mediated infection.

Key words: Dehydroepiandrosterone (DHEA), Androstenediol (AED), Viral, Bacterial, Parasitic, Infections

INTRODUCTION

The principal defenses of the body against infections are derived from the immune system consequently, the availability of new agents that function to up-regulate host immunity and increase host resistance against infections and other deleterious conditions would be highly advantageous. The use of agents that can boost host immunity to combat infections may be of considerable benefit in the treatment of animals' infectious outbreaks and complement the available modes of treatments. Indeed, viral, bacterial and parasitic infections exert a dual role; first by undermining animals' health and secondarily, as a source of human infection. The latter can be a direct infection or indirect exposure to toxins transmitted in the food chain. Zoonotic and food borne diseases are of national and international importance and close monitoring is of paramount importance to reduce outbreaks (1, 2). A recent example of this interaction between animals and humans is illustrated by Graham *et al.* 2008 with avian influenza, where the poultry production methods are a significant factor in the spread of pandemic avian influenza (3).

This report details the role of beta androstenes as agents that up-regulate the host immune response to a level that enables the host to resist lethal infection by viruses, bacteria, and parasites (4-14). These agents consist of a specific subgroup of steroid that also mediates a rapid recovery of hematopoietic precursor cells after destruction by whole

body lethal radiation injury and increases survival following hemorrhagic trauma and shock (13-14). *In vivo*, the androstenes increase the levels of the TH1 cytokines such as, IL-2, IL-3, and IFNγ. Similarly to hydrocortisone, they suppress inflammation but do not suppress immunity; androstenes function in the maintenance of the TH1/TH2 balance and immune homeostasis. Selective examples and possible applications and use of these immune regulatory agents for the treatment, mitigation and control of animal infections are provided.

Experimental findings

In vivo experiments demonstrated that a single subcutaneous (SC) injection of Dehydroepiandrosterone (Δ 5 androstene 3β, 17 one, DHEA) protected female mice from a lethal challenge with human herpes type 2 or male mice from a lethal challenge with hu-
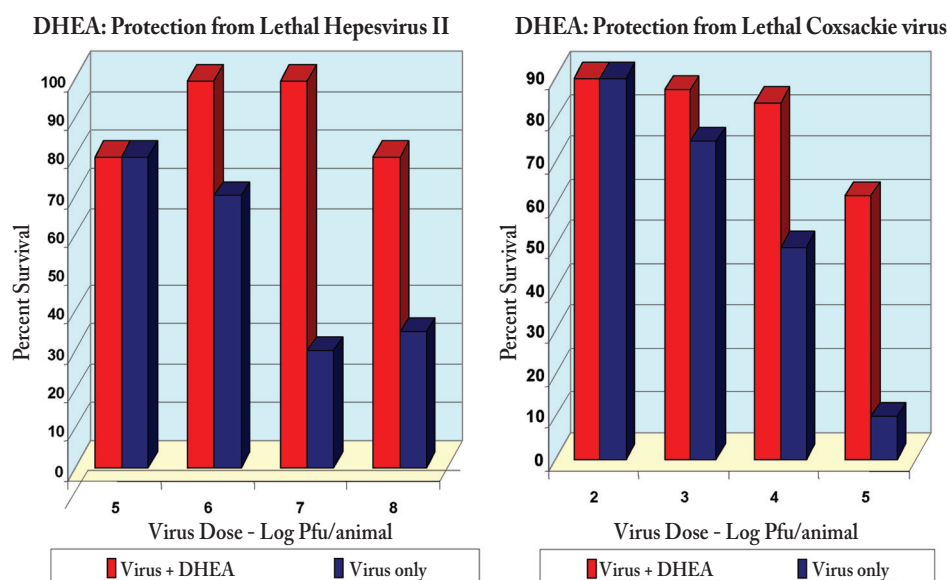

Figure 1a and 1b: 100% survival is evident in DHEA treated female mice infected with lethal intracranial injection of 10^7 plaque forming units (PFU) of Herpes type II, while untreated infected animals had only a 30% survival, p<0.03.

Similarly, a single S.C. injection of 25 mg DHEA/ 25 gr mouse increased survival to 60% following a challenge with an infection dose that killed 90% of untreated animals.

DHEA was injected S.C. at 25mg/mouse in 0.2 ml dimethyl sulfoxide-ethanol (1:1), 4 h prior to intracranial or within 4 h after IP infection. Data represent survival up to a minimum of

21 days after infection, modified from Loria et al. (4).

Herpes simplex virus type 2 was delivered in 0.1 ml PBS by intracranial injection and Coxsackievirus B4 I.P injection. 192 animals were used in both experiments.

Table 1: Comparison of protective effect of DHEA and AED against Coxsackievirus B4 infection

	PERCENT ANIMAL SURVIVAL [1]	
Log virus dose PFU/animal	4	6
Virus Only	0	0
Virus + DHEA	83	0
Virus +AED	100	100

Doses: AED 8 mg, DHEA 25 mg per 25 gr mouse, respectively. No death occurred in the control group injected with vehicle. Total number of animals in the experiment = 144.

The AED group results are statistically different from virus alone, P< 0.0001.

At a dose of 10^6 PFU per animal, AED is significantly different from DHEA, P < 0.02.

[1]Modified from Loria and Padgett (5).

man enterovirus-coxsackievirus B4 (CVB4). As illustrated in Figure 1a and 1b, 100% survival is evident in DHEA treated female mice infected with a lethal intracranial injection of 10^7 plaque forming units (PFU) of Herpes type II, while untreated infected animals had only a 30% survival. Similarly, a single SC injection of 25 mg DHEA/25 gr mouse increased survival to 60% following a challenge with an infection dose that killed 90% of untreated animals. This protective effect of DHEA against intraperitoneal CVB4 or intracranial herpes virus infections was statistically significant, P ≤ 0.03. *In vitro*, DHEA did not have an effect on the growth rate or replication of bacteria or virus at any of the concentrations tested *in vitro* (4). To be effective, these steroids require a functioning immune system: this was evident since the genetically immune deficient mutants (the hairless HRS/J hr/hr) could not be protected when treated with these agents (4).

Androstenediol (AED, Δ 5 androstene 3β, 17β diol) is a derivative of DHEA which results from conversion of the 17
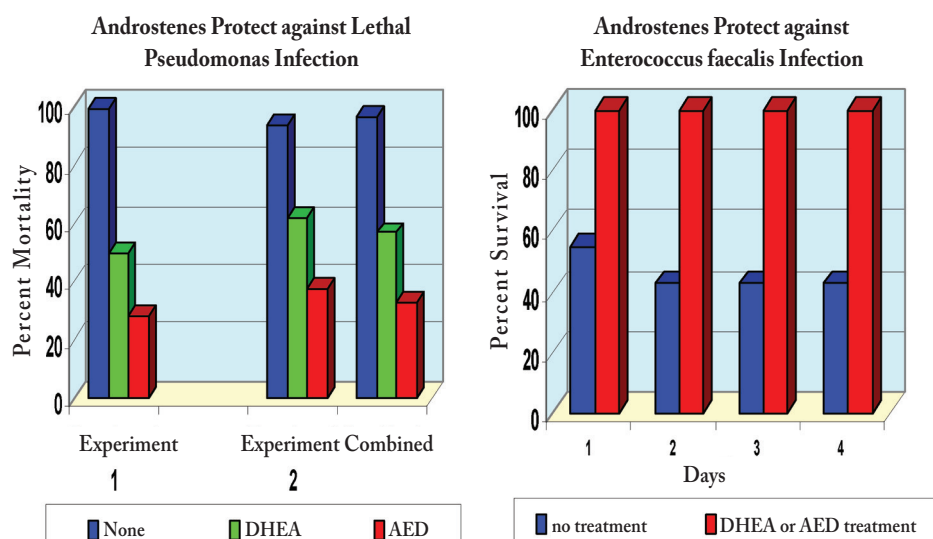
Figure 2a and 2b: DHEA treatment at a dose of 20 mg/animal, 2 h before *P. aeruginosa* injection protected 50% and 38.5% of the animals, respectively. A combination of the results of both experiments shows that 43% of the animals treated with DHEA were protected from a lethal *P. aeruginosa* infection. Six-month-old CD-1 female mice were infected with 2×10^7 cfu of *P. aeruginosa*; DHEA 20 mg s.c. 2 h before bacterial challenge. p<0.01 compared with control group. (total n=30) Similarly, 2 mg of AED resulted in 71.5% and 62.5% protection in two separate experiments. A combination of the results of both experiments shows that AED protected 67% of animals infected with a lethal dose of *P. aeruginosa*.

The protective effects of DHEA and AED against a lethal E. faecalis infection. Mice were inoculated i.p. with 1 LD50 dose of the organism. Treatment with a single dose of either AED (8 mg/ animal) or DHEA (25 mg) 2 h before bacterial challenge afforded complete protection, whereas 57% of control animals died p<0.05, n=36.

keto group to a hydroxyl group at the 17 position. However, this minor chemical change resulted in remarkable increase in biological activity. The results presented in Table 1 show that one third lower dose of AED was more effective against 100 times greater virus dose challenge than DHEA.

DHEA and AED as protecting agent in Bacterial Infections.

Consequently, we examined the ability of DHEA and its derivative, AED, to up-regulate the host immune response to a challenge by other lethal infections with either the Gram negative or Gram positive bacteria. Figure 2a illustrates the protective effects of DHEA and AED against a dose of 2×10^7 colony forming units (CFU) of *Pseudomonas aeruginosa* that causes 100% mortality in CD-1 mice (8). Experiments 1 and 2 showed that DHEA treatment at a dose of 20 mg/animal, 2 h before *P. aeruginosa* injection protected 50% and 38.5% of the animals, respectively. A combination of the results of both experiments showed that 43% of the animals

treated with DHEA were protected from a lethal *P. aeruginosa* infection.

Similarly, 2 mg of AED resulted in 71.5% and 62.5% protection in two separate experiments. A combination of the results of both experiments showed that AED protected 67% of animals infected with a lethal dose of *P. aeruginosa*. The results showed that *in vivo* treatment with either DHEA or AED significantly (p ≤ 0.05) increased the survival of mice infected with a 100% lethal dose of *Pseudomonas aeruginosa*. Here too, AED was more effective than DHEA against *P. aeruginosa* infection since one tenth the needed DHEA dose was effective in achieving twice the level of protection. Similar results were shown when animals were infected with an LD50 of the Gram positive *Enterococcus faecalis*, Figure 2b. A single dose of either AED (8 mg/animal) or DHEA (25 mg/animal) 2 h before bacterial challenge protected all the animals, whereas 57% of untreated animals died (p < 0.05). Thus, these data showed that both DHEA an AED up-regulate host immunity, resulting in a protective effect against *E. faecalis* infection.

Effect of Immunosteroids DHEA and AED on Lipopolysaccharide Toxicity

During the course of Gram-negative infections, bacterial cell wall products, such as lipopolysaccharide (LPS) endotoxin are released, and induce intense pathophysiologic alterations (15,16). LPS alone is not the cause of the pathology, but rather the host response, which may be described as an "overshoot" of the immune system. One of the major responses to LPS *in vivo* is the rapid production and secretion of cytokines, the soluble mediators of inflammation,
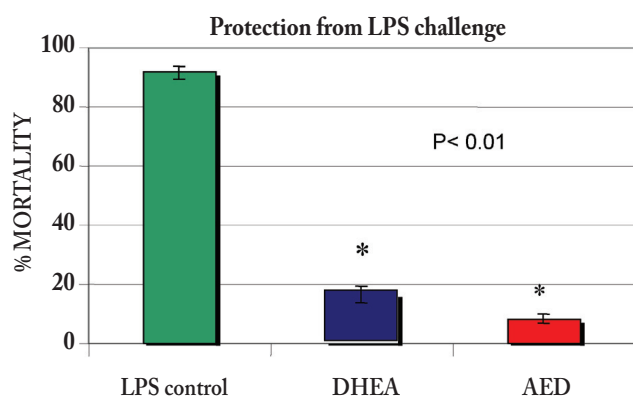
Figure 3: Protective effects of DHEA and AED against LPS endotoxic shock. CD-1 female mice were each challenged with 800 ug of LPS. The steroid, AED (0.4 mg/mouse) or DHEA (2 mg/mouse), was injected subcutaneously 1h before LPS challenge. Each group included 14 animals with 42 animals per experiment. A total of 84 animals were tested.

* Statistically significant in duplicate experiments at p<0.01 by ANOVA (8).

such as tumor-necrosis-factor (TNFα) (17,18) and IL-1 (19,20). Toxicity can be reduced by administration of potent immunosuppressive glucocorticoids (21) which inhibit the production of TNFα and other cytokines if given prior to LPS challenge (22,23). We have previously shown that administration of LPS or the administration of sera from LPS-treated mice induced penetration into the CNS of attenuated non-neuroinvasive viruses (24). While, Danenberg *et al*. (1992) reported that administration of LPS induced the secretion of TNFα and corticosterone (25) Ben- Nathan *et al*. (1999) showed that this effect of LPS can be prevented by the use of DHEA (8).

TNF is considered to be a major proximal mediator of septic shock, a claim substantiated by the finding that passive immunization against TNFα protects mice from the lethal effects of LPS (18). TNFα is not the sole mediator of LPS-induced phenomena (19), but rather acts in conjunction with other cytokines, augmenting their activity (23, 26). As reported, by Zuckerman *et al*. (1992) and Lehmann *et al*. (1987) endotoxic shock is mediated not only by TNFα but also by other cytokines involved in septic shock, such as IL-1 and IL-6 (26, 27) and showed that TNF injection alone can cause lethal toxicity similar to LPS treatment. Based on these studies, we reported that the protective effects of DHEA or AED was accomplished in part by lowering TNF levels, as

illustrated in Figure 3. DHEA reduced LPS induced mortality by about 70%, and half the dose of AED by 80%. Such treatment should also mitigate the cascade effects in endoxins septic shock which includes the elevations of cytokines IL-1 and IL-6 (28).

Based on the available data, we concluded that DHEA and AED mediate host protection by up-regulation of host immunity and host resistance, and not by direct antiviral or antibacterial effects. A summary of the range of protection by the androstenes is illustrated in Table 2. It is of importance to emphasize that because of their action in boosting host resistance, DHEA or AED may potentiate the actions of certain antibiotics, leading to a reduced use and have the potential to protect the host, infected with antibiotic resistant organisms.

DHEA Effect on Parasitic Infections

Experimental data has show that DHEA and DHEA sulfate (DHEA-S), its soluble form in the circulation, are effective in the treatment of many parasitic infections; several examples are provided below. Experimental Chagas' disease in the Wistar rat treated with DHEA resulted in modulation of the immune response during the acute and chronic phases of disease. Results show that SC administration of 40 mg/kg DHEA was associated with *ex-vivo* elevation of IL-12 and nitrous oxide (NO) levels during the acute phase and an increase in spleen cell proliferation during the chronic phase of the disease (41). Brazao *et al*. (2010) combined treatment of DHEA and zinc in animals infected with *Trypanosoma cruzi* resulted in an increase in macrophage count and the level of IFNγ and NO (41).

DHEA-S treatment was also effective in reducing the mortality rate of animals infected with *T. cruzi* Bolivia strain. DHEA-S treatment was superior to treatment with benznidazole alone or to the combined treatment of DHEA-S+ benznidazole. DHEA-S administration to *T. cruzi* infected rats also enhanced the levels of peritoneal macrophages IFNγ, IL-2 and NO production (42).

Cryptosporidiosis is a life threatening parasitic disease in the immune compromised host and DHEA treatment was reported to be effective. Ten golden Syrian Hamsters were treated with DHEA for 7 days prior to infection with 1×10^6 *C. parvum* oocysts. DHEA was shown to be an effective prophylactic agent in this model (38). This experiment was reproduced in mice with similar findings showing

Table 2: The Range of Protection by Androstenes

Agent	Class	Family	Strain
Viruses	RNA	Picornavirus	Coxsackie virus B4 (Loria *et al.*, 1988)
		Flavivirus	Semliki Forest Virus (Ben-Nathan *et al.*, 1991)
		Alphavirus	West Nile Virus (Ben-Nathan *et al.*, 1991) Japanese Encephalitis virus (Chang 2005) Venezuelan Equine Encephalomyelitis virus (Negrette *et al.*, 2001)
		Myxovirus	Influenza (Padgett *et al.*, 1997)
		Retrovirus	Mammary tumor virus (Schwartz 1979) Murine Leukemia (Raghi-Niknan *et al.*, 1997)
	DNA	Herpesvirus	Herpes Type 2 (Loria *et al.*, 1988) Herpes Type 1 (Daigle and Carr 1998)
Bacteria		Gram Positive	*Enterococcus faecalis* (Loria *et al.*, 1988)
		Gram Negative	*Pseudomonas aeruginosa* (Ben-Nathan *et al.*, 1999) *Klebsiella pneumonia* (Whitnall *et al.*, 2000)
Parasites		Trypanosoma cruzi	Y strain (Dos Santos *et al.*, 2005)
		Malaria	*Plasmodium falciparum* (Leenstra *et al.*, 2003)
		Coccidia-Isospora	*Cryptosporidium pavum* (Rasmussen *et al.*, 1995)
Non infectious Agent			Lipopolysaccharide (Danenberg, *et al.*, 1992) (Ben-Nathan *et al.*, 1999) 7,12 dimethyl benz (A) anthracene and urethane induced tumors (Schwartz 1981, Li *et al.*, 1994)

a significant reduction in intestinal and stool oocyts counts. DHEA was more effective if administered prior to infection.

In departure from other findings, Vargas-Villavicencio *et al.* 2008 administered DHEA at dose of 200 μg/25g BALB/c female or male mice one week prior to infection and every other day for the duration of 8 weeks, resulting in a 50% reduction of parasite load as compared to untreated, infected animals. The protective effect was independent of the host immune response since DHEA did not affect the levels of IL-1, IFNγ, IL-4 or IL-10 mRNA. *In vitro*, evidence showed a dose dependent effect of DHEA treatment on the reduction of motility and viability of *T. crassiceps*. These findings may indicate a metabolic effect of lower hormone doses on parasitic infection independent of the immune up-regulation evident in other infections (43).

Protective Efficacy of DHEA Against Viral Encephalitis

Arboviruses are transmitted by insect vectors, i.e., mosquitos, ticks, insects and by mechanical means (44). Many different arboviruses circulate among wild animals, and cause diseases to humans and to agriculturally important domestic animals. An excellent review with extensive details is provided by Kuno, G. and Chang, GJ. (2005) (44).

Arboviruses pose a constant threat of major outbreaks by existing strains and the emergence of new epidemics. As an example, West Nile virus (WNV) is one of the arboviruses which dramatically expanded its geographic distribution and now has a global distribution associated with encephalitis (45,46). It is a mosquito-transmitted flavivirus, first isolated from a febrile adult woman in the West Nile District of Uganda in 1937 (47). WNV is a single stranded plus RNA virus, and a member of the Japanese encephalitis antigenic complex of the genus Flavivirus, family Flaviviridae (48, 49). Until 1999, West Nile Virus was found in Africa, the Middle East, parts of Asia, Southern Europe and Australia. It then suddenly emerged in New York, rapidly spreading throughout the United States and has since caused considerable acute mortality and morbidity (50). The clinical man-

Table 3: The Protective effect of DHEA on Mice Infected with West Nile Virus (WNV).

Day of DHEA Treatment	Mortality D/T	Percent survival
-1	3/10	70*
0	5/10	50*
1	5/10	50*
2	6/10	40
3	8/10	20
Control	9/10	10

Mice were injected once SC with 1 gr/kg of DHEA on day -1, 0, 1, 2, or 3 days after virus infection.
WNV: 100 plaque forming units (PFU) /mouse was injected I.P.
* p<0.05 compared to control untreated group.
D/T = Dead/total

Table 4: DHEA Protection against Encephalitis Virus Infections (in percentage)

	West Nile Virus Flavivirus	Sindbis Virus Alfavirus	Simliki Forest Virus Alfavirus
Untreated Infected Control	100	71	90
DHEA treatment	50	21	30

DHEA serial i.p. injection of 10mg/kg on days -1 and 0 before and days 2, 4, and 6 after virus inoculation. 18-20 animals for each group. Adapted from Ben-Nathan *et al.*, 1991, Arch. Virol. 120:263-271

ifestations of WNV in humans range from asymptomatic seroconversion to fatal meningoencephalitis, with symptoms including cognitive dysfunction, muscle weakness and flaccid paralysis (51- 54). Compromised immunity, age and genetic factors (55, 56) are correlated with greater risk for neurological disease. There is no effective human WNV vaccine to protect populations at risk. Currently, the only effective manner to provide immediate resistance to WNV is by the passive administration of WNV-specific antibodies (57-60). An animal vaccine is currently in use (61, 62). However, we used the murine model of WNV to determine the protective efficacy of DHEA against lethal viral encephalitis. The murine model is a good experimental model for such studies, because WNV causes a systemic infection in mice and the virus invades the central nervous system (CNS), resulting in death within 1-2 weeks (63, 64).

Ben-Nathan *et al.* (1991) and (1992) tested the *in vivo* activity of DHEA by intraperitoneal injection with the drug suspended in either dimethyl-sulfoxide (DMSO), paraffin oil or soybean oil for subcutaneous injection. Serial injection of DHEA at doses from 5 to 20 mg/kg on days -1 and 0 before and on days 2, 4, and 6 after infection with WNV doses of 10, 100, 500 or 1000 PFU/mouse, resulted in protection against WNV. DHEA treatment protected 50%-70% of the mice as compared to 0-30% in control non-treated infected mice. DHEA treatment not only reduced death rate but postponed the onset of disease and

death by 2-3 days in animals that succumbed (29, 65). A single subcutaneous injection of DHEA (20 mg/kg) before or after virus inoculation (500 PFU/mouse) protected 70% of the mice against lethal WNV infection (Table 3). The drug was more effective against WNV when injected one day prior to infection and 50% when injected one day post infection.

DHEA treatment reduced WNV level in the spleen by 2 log PFU and by 2-3 log PFU in the brain of infected mice, as compared to non-treated mice. It delayed the onset of the disease and increased the ability of the host to control virus replication and neuroinvasiveness through various immune mechanisms (29). Administration of DHEA caused an increase in thymus and spleen weight in control mice as well as in WNV infected mice. If animals were immune-suppressed and virus infected, DHEA treatment caused a greater increase in thymus and spleen tissues weight than in DHEA treated control uninfected mice (65). Similar results were reported against infection with Venezuelan Equine Encephalomyelitis virus (VEE) in mice following vaccination with the TC-83 VEE virus (31). In this case, a single DHEA dose of 10 mg/kg, 4 hours before vaccination increased antibody titers against TC-83 VEE virus at 14 days after immunization. When vaccinated animals were challenged with live VEE virus 21 days after immunization and treated with DHEA, both viremia and brain virus levels were reduced. This suggests that DHEA treatment could enhance the efficiency of immunization against VEE virus in mice (31).

Table 5: Corticosterone Increases Viremia in mice Infected with Sindbis Virus (SVN)

Corticosterone Treatment Group	Blood virus level - log PFU/ml
SVN	3.2 ± 0.14
SVN + 1250ng i.v	3.9 ± 0.12
SVN + 2500ng i.v	4.5 ± 0.1*
SVN + 5000ng i.v	5.2 ± 0.31*

Corticosterone was injected intravenously one day after virus inoculation.
SVN was injected intraperitoneally
* p<0.01 as compared to untreated group.
  (Ben-Nathan, D *et al*. 1996, Arch. Virol. 141:1221-1229, 1996)

Figure 4: The effect of DHEA on mice mortality infected with WN-25 virus (2x10^5 PFU/mouse) and exposed to stress (cold or isolation) or injected with dexamethasone. DHEA was suspended in RSSP and injected i.p.(10mg/kg) on day 1 before, Day 0, and days 2, 4, 6 and 8 after inoculation and exposure. Cold stress (1±0.5°C) was introduced from day of inoculation until 8 d post inoculation. Dexamethasone was injected i.m. (2mg/kg) 2h before and 24h after virus inoculation. Number of mice in each group with or without DHEA are: no stress 20; cold stress 18; isolation stress 16; dexamethasone treatment, 18.
* p<0.01 DHEA treated vs. non-treated group
  (Ben-Nathan et al 1992).

The protective efficacy of DHEA was also demonstrated against other lethal viral infections of the central nervous system (CNS). In addition to WNV described above, tests against the neurovirulent and neuroinvasive strain of Sindbis virus (SVNI) and Semliki Forest virus (SFV) both belonging to the alphavirus family were done. DHEA administration at a dose of 10 mg/kg on days -1 and 0 before and days 2, 4, and 6 after virus inoculation reduced the mortality by 50% and 60% in WNV, SVNI and SFV, respectively as compared to control-untreated infected mice (Table 4). It is evident that DHEA may have a significant protective effect against infection by many different Encephalitic viruses.

DHEA effects on stress induced immunosuppression and viral encephalitis

Glucocorticoids have been used extensively to inhibit inflammation, specifically by interfering with activation of cell mediated function of lymphocytes and macrophages (66- 69). In a series of experiments, it was found that when mice infected with WNV are stressed, it will result in higher mortality. Treatment with DHEA prevents mortality in all models of stress in mice infected with WNV or with attenuated arboviruses (29,65,70).

DHEA prevented encephalitis induced by attenuated arboviruses in stressed mice or following dexamethasone and corticosterone injection (64). Exposure of WN-25 (a variant of West Nile virus) or SVN (neuroadapted Sindbis virus) inoculated mice to stress (cold or isolation) treatment, induced
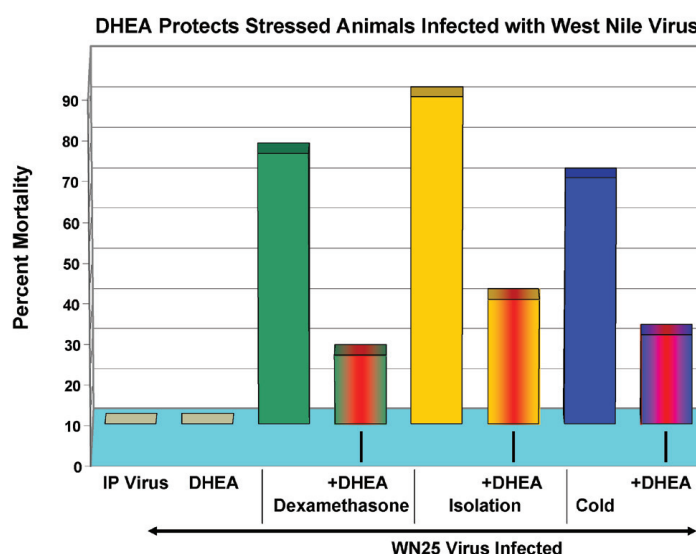
viral encephalitis and mortality while in non-stressed, inoculated mice, no mortality was observed. Administration of dexamethasone or corticosterone induced mortality of 67% and 50% respectively, compared with no death in control inoculated mice. DHEA treatment reduced mortality of the stressed, inoculated mice by 45-50% and in the dexamethazone-treated group by 50%. Moreover, DHEA enhanced the humoral immune response, prevented involution of lymphoid organs in stressed or dexamethasone treated mice, and reduced the secretion of corticosterone induced by cold stress (Figure 4).

Previously, Ben-Nathan *et al*. (1996) reported that exposure of virus inoculated mice to cold stress or corticosterone injection resulted in significant elevation of viremia and marked increase in mortality as compared to untreated control (64). The effects caused by cold stress and the administration of corticosterone on viral levels in the blood are shown in Table 5.

Potential Application to Veterinary Infections

As shown above, both DHEA and AED are very effective in boosting host immunity and preventing morbidity and mortality caused by a Picornavirus - coxsackievirus B4 as well as other RNA viruses. Indeed, animals are infected by many different RNA viruses: among them Foot-and-Mouth disease virus which is also a member of the Picornavirus family.

The disease is highly infectious and devastating in farm animals, causing blisters in the mouth and feet of cattle, swine, sheep, goats, deer, and other cloven-hoofed animals. It causes death in young animals. It is important to realize that during the 2001 epidemic in the United Kingdom resulted in the slaughter of more than 6.5 million animals. Humans may be mechanical carriers but are not infected by this virus (71). This Foot and Mouth disease virus should not be confused with hand-foot and mouth disease in humans which is caused by a Coxsackie A virus, also an enterovirus and a member of the Picornavirus family.

Almeida *et a l*.,2008, reported that depressed DHEA levels increased sickness response in lame dairy cows, which again emphasizes the need to monitor these hormone levels (72). The experimental data outlined above strongly suggest that DHEA and AED may be effective agents in enhancing immunity and host resistance to limit Foot-and-Mouth disease virus outbreaks.

Bovine Virus Diarrheal Virus (BVDV) is an enveloped, single-stranded RNA virus, a member of the Pestivirus genus belonging to the Flaviviridae family. Symptoms of infection in addition to diarrhea include respiratory and bleeding disorders. It spreads easily and some animals become carriers for life. The main effect of vaccination to BVDV has been to limit transmission but has not been effective in preventing disease (73).

The results reported above and the data in Table 4 illustrates the protective effects of DHEA against viruses of the Flavivirus family.

Bluetongue (BT) virus, an orbivirus of the Reoviridae family, includes 24 known serotypes, is transmitted to ruminants via certain species of biting midges (*Culicoides spp.*) and causes thrombo-hemorrhagic fevers mainly in sheep and occasionally also in cattle and deer, and can infect all ruminant species. The large number of known antigenic strains makes vaccination a tenuous approach (74). Tests should be recommended to determine whether DHEA and AED could be
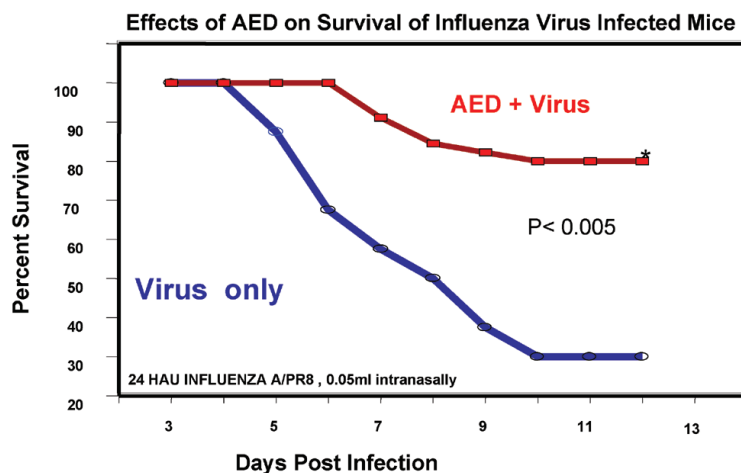
Figure 5: AED treatment improved survival of influenza-infected mice. Male C57BL/6J mice were treated with 8 mg/ mouse AED, squares - red line n= 45 or control vehicle open circles - blue line n=40 4 h prior to infection with 24 HAU influenza A/PR8 virus. p<0.005. Modified from (6).*

effective in enhancing host immune response or mitigating infection following vaccination.

Influenza viruses belong to the Orthomyxoviridae family, are RNA viruses that affect birds and mammals. Influenza viruses may cause an asymptomatic infection in wild aquatic birds which function as a reservoir for the infection of domestic poultry and swine and may be highly pathogenic in other species. Avian and swine influenza infection may lead to selection of new influenza strains which infect humans and give rise to pandemics (75).

Influenza A infection of dogs and cats from horses has been reported. Some of these infections can be fatal to pets. Recently, influenza H3 and H5 antigenic strains derived from natural clinical infections in carnivores lead to selection of new antigenic strains affecting dogs and cats (76).

Our previous results show that AED is highly effective in boosting host resistance to influenza infection as illustrated in Figure 5 with 80% survival rate. Similarly, Padgett *et al*. 1997 reported that AED and AED sulfate significantly increase resistance to influenza infection and increase vaccine efficacy (77, 78). Clearly the data show that AED may a valuable agent in the control of influenza infection.

CONCLUSIONS

The introduction of hydrocortisone and other steroids into therapy was a watershed event in medicine. Nevertheless,

the untoward effects associated with corticosteroid therapy are well documented. The present group of androstenes, particularly dehydroepiandrosterone and beta androstenediol counteract stress mediated immune suppression and are potent immune enhancing agents which also counteract the immune suppressive effects of cortisone.

These agents provide a unique new avenue for the control, mitigation, and prevention of diseases by viral, bacterial, and parasitic infections. Moreover, immune up-regulation, may have a significant role in limiting antibiotic resistant infections. These agents have low toxicity, are stable without refrigeration, and can be easily marketed and distributed.

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