PAPER

Dehydroepiandrosterone

An Inexpensive Steroid Hormone That Decreases the Mortality Due to Sepsis Following Trauma-Induced Hemorrhage

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Background: Recent studies suggest that male sex steroids play a role in producing immunodepression following trauma-hemorrhage. This notion is supported by studies showing that castration of male mice before trauma-hemorrhage or the administration of the androgen receptor blocker flutamide following trauma-hemorrhage in noncastrated animals prevents immunodepression and improves the survival rate of animals subjected to subsequent sepsis. However, it remains unknown whether the most abundant steroid hormone, dehydroepiandrosterone (DHEA), protects or depresses immune functions following trauma-hemorrhage. In this regard, DHEA has been reported to have estrogenic and androgenic properties, depending on the hormonal milieu.

Objective: To determine whether administration of DHEA after trauma-hemorrhage has any salutary or deleterious effects on immune responses, and whether it improves the survival of animals subjected to subsequent sepsis.

Design: Male C3H/HeN mice underwent laparotomy (ie, trauma-induced) and hemorrhagic shock (blood pressure, 35 ± 5 mm Hg for 90 minutes) followed by fluid resuscitation, or sham operation. The animals then received 100 mg of DHEA (4 mg/kg) or propylene glycol (hereafter referred to as vehicle). At 24 hours after trauma-hemorrhage and resuscitation, the animals were killed and blood, spleens, and peritoneal macrophages were harvested. Splenocyte proliferation and interleukin (IL) 2 release and splenic and peritoneal macrophage IL-1 and IL-6 release were determined. In a separate set of experiments, sepsis was induced by cecal ligation and puncture at 48 hours after trauma-hemorrhage and resuscitation. For those studies, the animals received vehicle, a single 100-µg dose of DHEA, or 100 µg/d DHEA for 3 days following hemorrhage and resuscitation. Survival was monitored for 10 days after the induction of sepsis.

Results: Administration of DHEA restored the depressed splenocyte and macrophage functions at 24 hours after trauma-hemorrhage. Moreover, daily administration of DHEA for 3 days significantly increased the survival of animals subjected to subsequent sepsis ($P = .01$).

Conclusion: The finding that DHEA markedly improves the depressed immune functions and survival of animals subjected to subsequent sepsis suggests that short-term treatment with DHEA after trauma-hemorrhage is a safe and novel approach for preventing immunodepression and for decreasing the mortality rate due to subsequent sepsis.

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SEVERAL STUDIES indicate that cell-mediated immune responses are markedly depressed in male patients following trauma and hemorrhagic shock, and that these changes persist for as long as 10 days after resuscitation.[1,2] Moreover, these depressed immune responses have been associated with an increased susceptibility to and mortality due to subsequent sepsis.[3,4] Recent studies have suggested that male sex steroid hormones play a significant role in producing immunodepression following trauma-induced hemorrhage (hereafter referred to as trauma-hemorrhage).[4-6] Support for this assertion comes from studies that showed that depletion of testosterone by castration of male mice before trauma-hemorrhage prevents the depression of splenocyte immune functions.[5] Alternatively, administration of a testosterone receptor antagonist, eg, flutamide, in normal male animals following trauma-hemorrhage restores the depressed immune responses and increases the survival rate of animals subjected to subsequent sepsis. In contrast to male mice, female mice in the proestrus state of the estrus cycle demonstrate enhanced immune responses following trauma-hemorrhage.[7] Thus, it appears that elevated levels of female sex hormones, ie, prolactin and estrogen, in the proestrus

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MATERIALS AND METHODS

ANIMALS

Inbred male C3H/HeN mice (Charles River Laboratories, Wilmington, Mass), 7 weeks of age (body weight, 24-27 g) were used. All procedures were performed in accordance with the guidelines set forth in the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health, Bethesda, Md. This project was approved by the Institutional Animal Care and Use Committee of Rhode Island Hospital and Brown University, Providence.

EXPERIMENTAL GROUPS

Male mice were randomized into 4 groups (6-8 mice per group). Mice in groups 3 and 4 were subjected to trauma-hemorrhage, whereas the mice in groups 1 and 2 underwent the sham procedure. Groups 1 and 3 received 0.1 mL subcutaneous propylene glycol injection (hereafter referred to as vehicle) following experimental treatment, whereas groups 2 and 4 received subcutaneous injection of DHEA, 100 µg per mouse (4 mg/kg). This dose of DHEA has been reported to produce an immunoenhancing effect on the depressed immune functions in mice following thermal injury.[12] In additional studies, following trauma-hemorrhage and resuscitation, mice were randomized into 3 groups (15 mice per group) and were subjected to subsequent sepsis. Group 1 received vehicle after resuscitation and again on the first and second postoperative days. Group 2 received a single subcutaneous injection of DHEA, 100 µg, immediately after resuscitation. Group 3 was treated with DHEA after resuscitation and again on the first and second postoperative days. In all experimental groups, polymicrobial sepsis was induced by cecal ligation and puncture (CLP) 48 hours after trauma-hemorrhage and resuscitation. The survival rate was monitored for up to 10 days after the induction of sepsis.

TRAUMA-HEMORRHAGE PROCEDURE

Mice were lightly anesthetized with methoxyflurane (Metofane, Pitman-Moore, Mundelein, Ill) and restrained in a supine position, and a 2.5-cm midline laparotomy (ie, trauma-induced) was performed. This was then closed aseptically in 2 layers using commercially available sutures (6-0 Ethilon, Ethicon, Inc, Somerville, NJ). Following this, both femoral arteries were aseptically cannulated with polyethylene 10 tubing (Clay-Adams, Parsippany, NJ) using a minimal dissection technique. Blood pressure was constantly monitored by attaching 1 of the catheters to a blood pressure analyzer (Digi-Med, Louisville, Ky). On awakening, the animals were bled rapidly through the other catheter to a mean arterial blood pressure of 35 ± 5 mm Hg (prehemorrhage blood pressure, 95 ± 5mm Hg), which was maintained for 90 minutes. At the endof that period, the animals were resuscitated with lactated Ringer solution (4 times the shed blood volume for 30 minutes) to provide adequate fluid resuscitation. Lidocaine hydrochloride was applied to the groin incision sites, the catheters were removed, the vessels were ligated, and the groin incisions were closed. Animals undergoing sham operation underwent the same groin dissection, which included ligation of both femoral arteries; however, neither hemorrhage nor fluid resuscitation was performed. No mortality was observed in this trauma-hemorrhage model.

CECAL LIGATION AND PUNCTURE

Polymicrobial sepsis was induced at 48 hours after hemorrhage and resuscitation using the model of CLP described by Baker et al.[13] Briefly, mice were lightly anesthetized with methoxyflurane, and a 2.0-cm midline incision was made. The cecum was isolated, a 6-0 silk ligature was placed around it, and the cecum was ligated just below the ileocecal valve. The cecum was then punctured twice with a 22-gauge needle, a small amount of bowel contents was extruded through the puncture holes, and the cecum was returned to the peritoneal cavity. After application of lidocaine (Xylocaine) on the incision site, the abdominal incision was closed in 2 layers (using 6-0 Ethilon sutures). Isotonic sodium chloride solution (20 mL/kg body weight) was administered subcutaneously at that time. Previous studies have demonstrated that blood cultures taken from rats or mice following CLP are positive for gram-positive (eg, *Streptococcus bovis*) and gram-negative (eg, *Bacteroides fragilis, Escherichia coli, Klebsiella* species, and *Proteus mirabilis*) bacteria as early as 1 hour after CLP.[13]

BLOOD-, TISSUE-, AND CELL-HARVESTING PROCEDURES

The animals were killed with methoxyflurane overdose 24 hours after the completion of the experiment to obtain the spleen, peritoneal macrophages, and whole blood samples. The mice were killed at the same time of the day to avoid fluctuations due to circadian rhythm.

Whole blood was obtained using cardiac puncture and placed in microcentrifuge tubes (Microtainer; Becton Dickinson and Co, Rutherford, NJ). The tubes were then centrifuged at 16 000*g* for 15 minutes at 4°C. Plasma was separated, placed in pyrogen-free microcentrifuge tubes, immediately frozen, and stored (–80°C) until assay.

DETERMINATION OF PLASMA DHEA LEVELS

Plasma DHEA levels were determined using a commercially available radioimmunoassay (Diagnostics Systems Laboratory, Webster, Tex) as described by the manufacturer.

PREPARATION OF SPLENOCYTE CULTURE

The spleens were removed aseptically and placed in separate Petri dishes containing ice-cold phosphate-buffered saline so-

state contribute to sexual dimorphism in the immune response following trauma-hemorrhage.[8]

The steroid hormone dehydroepiandrosterone (DHEA) is the most abundant steroid hormone in plasma and is an intermediate in the pathway for the synthesis of testosterone and estrogen. However, depending on the hormonal milieu, androgenic and estrogenic effects of DHEA have been reported.[9] Moreover, DHEA has been shown to have immunoenhancing effects on splenocytes harvested from normal animals.[10] This effect of DHEA on immune functions might be an indirect effect due to antagonizing some of the immunosuppressive effects of glucocorticoids [10] or conversion of DHEA to estrogen. Alternatively, DHEA might mediate direct im-

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lution (PBS). Splenocytes were isolated as previously described,[1] and the ability of the splenocyte cultures to produce lymphokines in response to a mitogenic challenge was assessed by incubation for 48 hours (at 37°C, 5% carbon dioxide [CO_2], and 90% humidity) in the presence of 2.5-µg/mL concanavalin A (Pharmacia–LKB Biotech Inc, Piscataway, NJ). After incubation, the cell suspension was centrifuged at 300*g* for 15 minutes, and the supernatants were harvested and stored at –80 °C until assay for interleukin (IL) 2 and IL-3 production. In addition, the splenocyte suspensions were incubated in a 96-well microtiter plate (Corning Glass Co, Corning, NY) in 100-µL aliquots. The cells' ability to proliferate in response to mitogenic stimulation with 0 (negative control) or 2.5-µg/mL concanavalin A was determined by incubation for 48 hours at 37°C in a 5% CO_2 atmosphere with 90% humidity. The extent of proliferation was measured using hydrogen-3–thymidine incorporation technique, previously described by Stephan et al.[3]

CELL-LINE MAINTENANCE

The IL-2–dependent CTLL-2 cells were obtained from the American Type Culture Collection and maintained as previously described.[1]

ASSESSMENT OF LYMPHOKINE RELEASE

The capacity of the mixed splenocyte culture to produce IL-2 was assessed by determining the amount of IL-2 in the collected culture supernatant. Serial dilutions of the supernatants were added to CTLL-2 cells (1×10^5 cells/mL) and incubated for 48 hours at 37°C and 5% CO_2. At the end of this period, 0.037 MBq hydrogen-3–thymidine (specific activity, 24.8×10^{10} Bq/mmol; New England Nuclear, Wilmington, Del) was added to each well, and the cultures were incubated for an additional 16 hours. The cells were then harvested onto glass-fiber mats, and the β-decay was detected using liquid scintillation radiography.[1]

PREPARATION OF PERITONEAL AND SPLENIC MACROPHAGE CULTURE

Resident peritoneal macrophages were obtained from mice, and monolayers were established as previously described.[1,14] The spleens were removed aseptically and placed in separate Petri dishes containing cold (4°C) PBS. The spleens were dissociated by grinding and then suspended and used to establish a macrophage culture.[1] The macrophage monolayers were stimulated with 10 µg/mL of lipopolysaccharide per milliliter of Click medium containing 10% fetal calf serum for 48 hours (at 37°C, 5% CO_2, and 90% humidity) to assess the ability of the cells to release IL-1β and IL-6. At the end of the incubation period, the culture supernatants were removed, divided into aliquots, and stored at –80°C until assay for IL-1β and IL-6.

ASSESSMENT OF IL-1

Levels of IL-1β in the macrophage supernatants were determined using Sandwich–enzyme-linked immunosorbent assay as previously described.[15] In brief, 96-well plates (Nunc-Immuno Maxisorp; Inter-Med/Nunc VWR-Scientific, Batavia, Ill) were coated overnight (4°C) with 2.0 µg/mL monoclonal hamster anti–mouse IL-1β (Genzyme Diagnostics, Cambridge, Mass). Following a washing of the samples, the standard (1000 pg/mL recombinant mouse IL-1β, Genzyme Diagnostics) was added and then incubated overnight (4°C). After repeated washings, the plates were incubated at 37°C for 1 hour with a biotinylated polyclonal rabbit anti–mouse IL-1β (Genzyme Diagnostics) at a concentration of 0.8 µg/mL. The plates were washed and then incubated with horseradish peroxidase for 15 minutes at 37°C. Following washing, 100 µL of tetramethylbenzidine (Sigma Chemical Company, St Louis, Mo) was added to initiate color development. The optical density at 655 nm for each well was then determined on a microplate reader (EL-311; Bio-Tek Instruments Inc, Winooski, Vt). The concentration of IL-1β present in the samples was determined by interpolation against the standard curve.

ASSESSMENT OF IL-6 RELEASE

Activity of IL-6 in culture supernatant was determined by the degree of proliferation of the murine B-cell hybridoma cell-line 7TD1, which only grows in the presence of IL-6.[14] The 7TD1 (gift from Jacques Van Snick, MD) was maintained as previously described.[16] Serial dilutions of macrophage supernatants were added to 4×10^4 7TD1 cells/mL, and the cells were incubated for 72 hours at 37°C in 5% CO_2. For the last 4 hours of incubation, 20 µL of a 3-(4,5-dimethylthiazol-2-l)-2,5-diphenyltetrazolium-bromide solution (MTT; 5 mg/mL in RPMI 1640, Sigma Chemical Company) was added to each well (only viable cells incorporate MTT). The assay was stopped by aspiration of 100 µL supernatant from each well, with subsequent replacement by 150 µL 10% sodium dodecyl sulfate solution in PBS (lauryl sulfate, Sigma Chemical Company) to dissolve the dark blue formazan crystals. Using an automated microplate reader (EL-311, Bio-Tek Instruments Inc), the light absorbance was measured at 595 nm.

STATISTICAL ANALYSIS

Results are presented as mean ± SEM. One-way analysis of variance followed by the Student-Newman-Keuls test as a post hoc test for multiple comparisons were used to determine the significance of the differences between experimental means. Comparisons of plasma DHEA levels were analyzed using the Mann-Whitney rank sum test. Survival was compared using a *z* test. $P<.05$ was considered statistically significant.

munoenhancing effects via intracellular DHEA receptors, which have been found in T cells.[11]

Although immunoenhancing effects of DHEA have been reported in cells harvested from normal animals, it remains unknown whether DHEA produces any beneficial or deleterious effects on the depressed cell-mediated immune responses following trauma-hemorrhage, and whether DHEA increases the survival rate of animals subjected to hemorrhage and subsequent sepsis. Our aim, therefore, was to determine the effect of DHEA administration on the depressed splenocyte and macrophage function following trauma-hemorrhage and the effect of DHEA on the survival of animals subjected to subsequent sepsis.

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RESULTS

EFFECT OF EXOGENOUS ADMINISTRATION OF DHEA

Administration of a single dose of DHEA, 100 µg, increased circulating plasma DHEA levels by 125% (1.12 ± 0.17 and 1.14 ± 0.19 ng/mL for animals undergoing sham operation and trauma-hemorrhage, respectively) compared with vehicle-treated animals (0.49 ± 0.07 and 0.55 ± 0.08 ng/mL for animals undergoing sham operation and trauma-hemorrhage, respectively) ($P = .03$, Mann-Whitney rank sum test). Following trauma-hemorrhage, circulating plasma DHEA levels were similar to those of the corresponding vehicle- and DHEA-treated animals undergoing sham operation.

EFFECT OF DHEA ADMINISTRATION ON SPLENOCYTE FUNCTION FOLLOWING TRAUMA-HEMORRHAGE

The splenocyte proliferative capacity was similar in vehicle- and DHEA-treated animals undergoing sham operation (**Figure 1**, A). Following trauma-hemorrhage, a significant depression of splenocyte proliferation was found in vehicle-treated animals (–74.6% vs vehicle-treated animals undergoing sham operation; $P = .01$). In contrast, splenocyte proliferative capacity in DHEA-treated animals was found to be similar to that seen in animals undergoing sham operation.

There were no significant differences in the levels of IL-2 release by splenocytes from the vehicle- or DHEA-treated animals undergoing sham operation (Figure 1, B). Trauma-hemorrhage resulted in a significant depression of splenocyte IL-2 release in vehicle-treated animals (–51.4% vs vehicle-treated animals undergoing sham operation; $P = .04$). Administration of DHEA following trauma and hemorrhagic shock restored the depressed IL-2 release to levels slightly, but not significantly, higher than those in animals undergoing sham operation.

EFFECT OF DHEA ADMINISTRATION ON MONOKINE RELEASE BY SPLENIC AND PERITONEAL MACROPHAGES

No significant difference in the release of splenic macrophage IL-1β was evident between the vehicle- and DHEA-treated animals undergoing sham operation (**Figure 2**, A). However, animals subjected to trauma-hemorrhage and receiving vehicle showed significantly decreased IL-1β release (–56.7% vs vehicle-treated animals undergoing sham operation; $P = .04$). In contrast, DHEA-treated animals subjected to trauma-hemorrhage had normal IL-1β release.

Splenic macrophage release of IL-6 was also comparable in both groups undergoing sham operation. Vehicle-treated mice subjected to trauma-hemorrhage showed significantly decreased splenic macrophage IL-6 release (–47.9% vs vehicle-treated animals undergoing sham operation; $P = .04$). However, administration of DHEA significantly increased the depressed IL-6 release following trauma-hemorrhage (+102.8% vs vehicle-treated mice subjected to trauma-hemorrhage; $P = .04$).

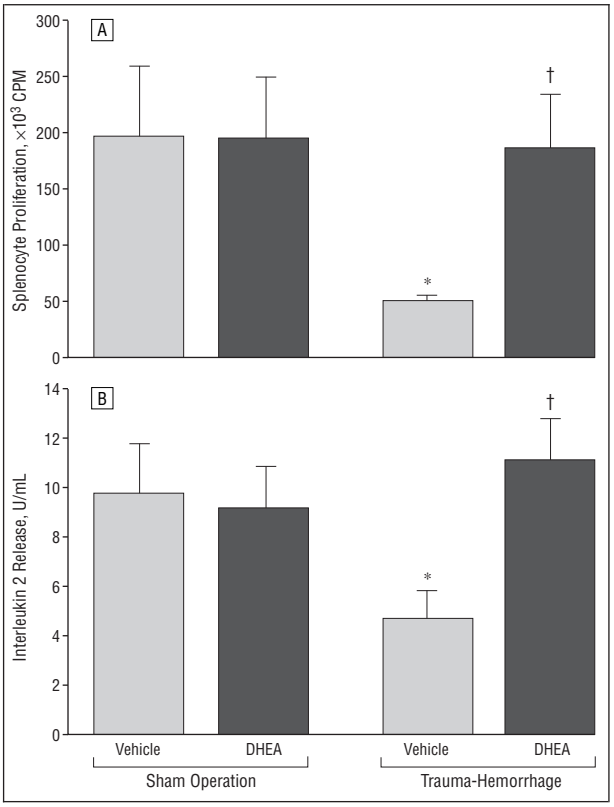

Figure 1. *Splenocytes harvested at 24 hours after trauma-induced hemorrhage (trauma-hemorrhage) or sham operation from male C3H/HeN mice treated with propylene glycol (vehicle) or 100 µg of dehydroepiandrosterone (DHEA) (7 mice per group). A, The splenocyte proliferation capacity in the presence of 2.5-µg/mL concanavalin A was measured by hydrogen-3–thymidine incorporation. B, Release of interleukin (IL) 2 by splenocytes stimulated with 2.5-µg/mL concanavalin A was determined by a specific bioassay (CTLL-2). Analysis of variance was used to determine significant differences. Asterisk indicates* P<.05 *vs vehicle-treated animals undergoing sham operation; dagger,* P<.05 *vs vehicle-treated animals subjected to trauma-hemorrhage.*

The release of IL-1β by peritoneal macrophages (Figure 2, C) was comparable in both groups undergoing sham operation. Vehicle-treated mice had significantly depressed peritoneal macrophage IL-1β release following trauma-hemorrhage (–52.9% vs vehicle-treated mice undergoing sham operation; $P = .006$). Peritoneal macrophage release of IL-1β in DHEA-treated mice, however, was found to be comparable to IL-1β release by macrophages from animals undergoing sham operation.

SURVIVAL FOLLOWING TRAUMA-HEMORRHAGE AND SUBSEQUENT SEPSIS

Within all groups, the highest mortality rate was observed during the first 4 days following the onset of sepsis (**Figure 3**). After the second day following CLP, only 7 of 15 of the vehicle-treated animals were alive compared with 11 of 13 in the group of animals receiving a single 100-µg dose of DHEA. Moreover, animals

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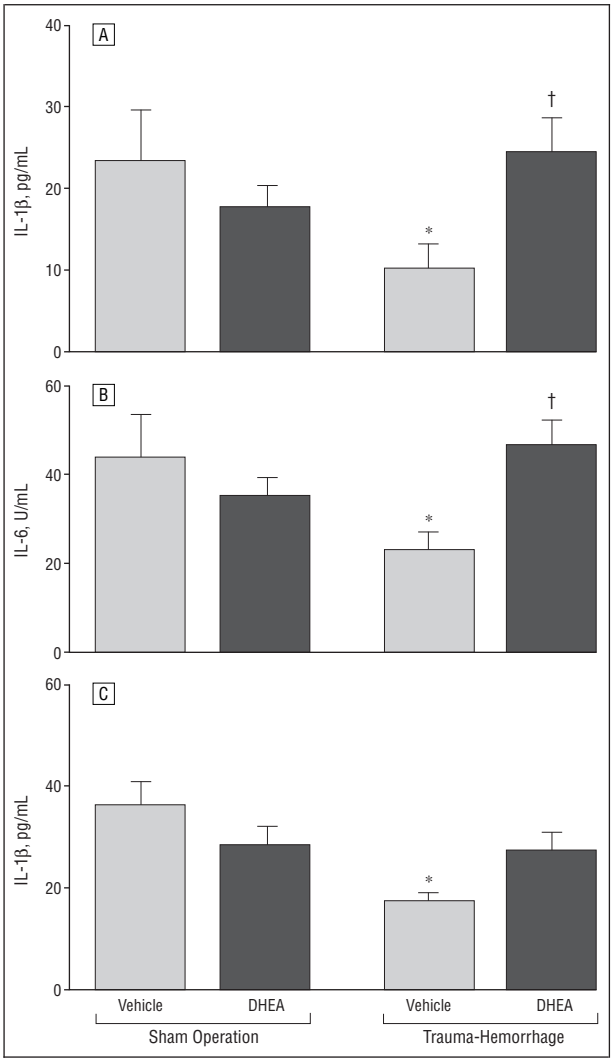

Figure 2. *Release of interleukin (IL) 1β (A) and IL-6 (B) by splenic macrophages and release of IL-1β by peritoneal macrophages (C) from male C3H/HeN mice (treated with propylene glycol [vehicle] or 100 μg of dehydroepiandrosterone [DHEA]) (7 mice per group) at 24 hours after trauma-induced hemorrhage (trauma-hemorrhage) or sham operation in the presence of 10-μg/mL lipopolysaccharide. Levels of IL-1β were detected using enzyme-linked immunosorbent assay; levels of IL-6, by a specific bioassay (7TD1). Analysis of variance was used to determine significant differences. Asterisk indicates* P<.05 *vs vehicle-treated animals undergoing sham operation; dagger,* P<.05 *vs vehicle-treated animals subjected to trauma-hemorrhage.*

receiving DHEA 3 times after trauma-hemorrhage had a significantly higher survival rate compared with vehicle-treated animals (92% [12/13] of the animals were alive on day 2 after CLP; $P<.05$). The beneficial effects of DHEA administration persisted during the entire observation period, as animals receiving a single dose of DHEA exhibited a higher survival rate compared with vehicle-treated animals, although this difference did not reach statistical significance (38% [5/13] vs 20% [3/15] in vehicle-treated mice on day 10 after the induction of sepsis; $P=.52$). The survival rate of animals receiving 3 doses of DHEA was significantly higher compared with that of animals receiving vehicle (77% [10/13] vs 20% [3/15] in vehicle-treated mice on day 10 after the induction of sepsis; $P=.01$).

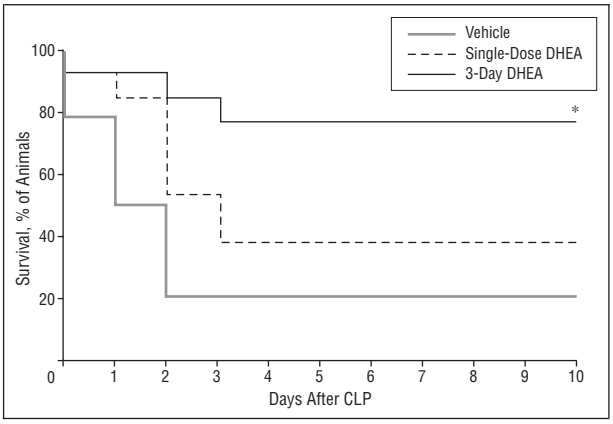

Figure 3. *Survival rate of male C3H/HeN mice (15 mice per group) for 10 days after trauma-induced hemorrhage (trauma-hemorrhage) followed by cecal ligation and puncture (CLP). Following trauma-hemorrhage, animals received propylene glycol (vehicle), a single dose (100 μg) of dehydroepiandrosterone (DHEA), or 100 μg/d of DHEA for 3 days. The* z *test was used to determine significant differences. Asterisk indicates* P<.05 *vs vehicle-treated mice.*

COMMENT

Several studies have shown that cell-mediated immunity is markedly depressed in male patients following trauma-hemorrhage, despite adequate fluid resuscitation.[17] This depression persists for up to 10 days after resuscitation and is associated with increased susceptibility to sepsis.[1,3] In addition, male sex steroid hormones appear to be involved in initiating the depression of immune responses.[5,14] This suggestion is supported by the observation that depletion of testosterone in male mice by castration 2 weeks before the onset of trauma-hemorrhage prevents the depression of immune responses.[5] Moreover, administration of a testosterone receptor antagonist, eg, flutamide in normal male animals following trauma-hemorrhage restores the depressed immune responses after trauma-hemorrhage and improves survival following subsequent sepsis.[4,18]

In contrast to male mice, female mice in the proestrus state demonstrate enhanced immune functions following trauma-hemorrhage.[7] Since the proestrus state of the estrus cycle is characterized by increased plasma levels of female sex hormones such as estrogen and prolactin, these hormones may be responsible for producing the immunoenhancing effects following trauma-hemorrhage. In this regard, administration of prolactin in male patients following trauma-hemorrhage has been shown to restore the depressed immune responses.[8] Although serum concentrations of DHEA are 20 times higher than those of any other steroid hormone, [9] little attention has been paid to the effects of DHEA on immune responses following trauma-hemorrhage. Furthermore, androgenlike and estrogenlike effects of DHEA have been reported, depending on the hormonal milieu.[9] The aim of our study, therefore, was to determine whether the inexpensive steroid hormone DHEA has any beneficial or deleterious effects on the depressed immune function following trauma-hemorrhage. Our results indicate that administration of DHEA following trauma-hemorrhage restores the depressed splenocyte as well as splenic and

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Statement of Clinical Relevance

A number of studies have shown that numerous immunological functions are markedly depressed following trauma-induced hemorrhage, contributing to an increased susceptibility to subsequent sepsis. The most abundant steroid hormone in the plasma, dehydroepiandrosterone (DHEA), has been reported to have immunostimulatory properties under normal conditions (cells). Our study indicates that administration of DHEA after hemorrhagic shock restores the depressed immune functions and improves survival from subsequent sepsis. Since short-term therapy with the steroid hormone DHEA has no adverse effects, administration of this inexpensive agent in male patients should be considered a novel and useful adjunct for the treatment of immune dysfunction encountered in trauma patients.

peritoneal macrophage function. We administered DHEA at a dose of 100 µg per animal, since this dose has been shown to have an immunoenhancing effect on splenocyte functions following thermal injury.[19] Furthermore, administration of DHEA on 3 consecutive days following trauma-hemorrhage significantly improved the survival rate of animals subjected to subsequent sepsis compared with vehicle-treated animals. Previous studies also have demonstrated that normalization of the depressed macrophage and splenocyte functions in flutamide-treated animals following hemorrhagic shock was associated with decreased lethality of subsequent sepsis.[4] Thus, the improved survival rate in DHEA-treated animals following trauma-hemorrhage and subsequent sepsis appears to be due to the restoration of the depressed immune function.

Additional support for the findings that DHEA has immunoenhancing properties comes from the studies of Araneo et al,[12] who demonstrated that DHEA restores the depressed splenocyte functions following thermal injury. Studies also have shown that in vitro treatment with DHEA of splenocytes from thermally injured mice restores the depressed lymphokine release.[12] Moreover, the decreased resistance of thermally injured mice to *Listeria monocytogenes* infection was prevented in DHEA-treated animals.[12] In another report, DHEA decreased the mortality rate in animals infected with viruses such as herpes simplex virus type 2, Coxsackievirus B4, and bacteria (eg, *Enterococcus faecalis* and *Pseudomonas aeruginosa*).[20] These results, therefore, collectively suggest immunoenhancing effects of DHEA following severe infection or traumatic injury.

The underlying mechanisms by which DHEA mediates its salutary effect on immune cells following trauma-hemorrhage, however, remain unclear. It has been suggested that DHEA mediates its immunomodulating properties indirectly through its conversion to other steroid hormones. In this respect, depending on the hormonal milieu, DHEA has been shown to have an estrogenlike or androgenlike effect.[9] In male patients who typically have low estrogen and high androgen plasma levels, DHEA appears to have estrogenic properties, whereas, in premenopausal females in whom high estrogen and low androgen plasma levels are more common, androgenic effects have been reported with DHEA.[9] Since our studies demonstrate that DHEA administration following trauma-hemorrhage is immunoprotective, DHEA might act estrogenically in male patients. However, it remains to be determined whether DHEA, due to its conversion to androgens, has any deleterious effects on the immune responses following trauma-hemorrhage. Blauer et al[10] demonstrated that DHEA antagonizes the suppressive effects of dexamethasone on lymphocyte proliferation. In this regard, increased plasma glucocorticoid levels have been reported following trauma-hemorrhage.[8] However, the antiglucocorticoid properties do not entirely explain the effectiveness of DHEA in improving immune responses following trauma-hemorrhage, thermal injury, and infection.[21] This suggestion is based on the findings of Araneo et al,[21] who showed that, whereas DHEA prevented the depression of splenocyte functions after thermal injury, administration of the steroid receptor blocker mifepristone (RU486) only produced a partial improvement of splenocyte functions. Other investigators have suggested a direct effect of DHEA on immune cells. Meikle et al[11] demonstrated the presence of an intracellular DHEA receptor through which DHEA enhanced IL-2 and interferon gamma production in activated murine and human T cells in vitro. Our results showed that administration of DHEA did not enhance the immune responses in animals undergoing sham operation, which would indicate that DHEA is immunostimulatory only in an immunologically compromised host but not in healthy animals. Since plasma DHEA levels did not change following trauma-hemorrhage, our findings further suggest that the number of DHEA receptors or the receptor affinity for DHEA might be altered under such conditions. Thus, provision of the additional agonist, ie, DHEA, for the receptor is helpful for restoring the depressed immune responses following trauma-hemorrhage.

In summary, our findings indicate that administration of DHEA restores the depressed splenocyte function as well as splenic and peritoneal macrophage cytokine release following trauma-hemorrhage in male mice. Moreover, since survival rates were significantly higher in the DHEA-compared with vehicle-treated animals, it appears likely that DHEA improves the function of macrophages at other sites. Since DHEA restores the depressed immune function following trauma-hemorrhage in male mice and decreases the mortality due to subsequent sepsis, administration of this inexpensive steroid hormone should be considered a novel and useful approach for the treatment of immune dysfunction in male trauma patients.

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DISCUSSION

Michael S. Dahn, MD, Detroit, Mich: This is a carefully designed study regarding the immunostimulating properties of DHEA in a hemorrhagic shock–septic rodent model. DHEA is a remarkable agent. In addition to its immune-enhancing properties, as you have aptly demonstrated, numerous other effects have previously been identified, including inhibition of glucocorticoid output, augmentation of insulin-like growth factor I, relief of insulin resistance, inhibition of reperfusion associated oxidant damage, etc. I mention these effects, as some or all of them may be mechanistically relevant to your paper. You may want to comment on them.

With specific reference to your current findings, your report speaks for itself. It corroborates and enlarges a growing body of data indicating that this steroid hormone is a physiologic regulator of the immune process. As a result, my comments will be limited to a few observations, which I hope you will elaborate on.

Your cell culture studies uniformly demonstrated no cytokine production augmentation attributable to DHEA in the sham categories. This is in contrast to prior reports, which demonstrate immunostimulatory effects such as increased T lymphocyte IL-2 production, even in normal basal states. How do you account for these differences?

Also, following hemorrhage, you measured DHEA concentrations to be significantly increased by a factor of 2, 24 hours after a 100-µg dose. DHEA actually exists in 2 forms, an active form, which you measured with your immunoassay, and an inactive, sulfated form, which is present in much larger amounts and is partially protein bound. Can you comment on the role of this large reservoir of conjugated DHEA, which can be endogenously desulfated and made available for immune stimulation?

I took the liberty of contacting the manufacturer of the assay that you used for DHEA measurement, and I was advised that the antibody that is utilized in that kit exhibits a minor cross-reactivity with the sulfated form of DHEA. Is it possible that you are measuring changes in DHEA sulfate availability caused by effects other than DHEA administration?

Most important, several reports using nonrodent models, including a recently reported study in *Shock* using an experimental design similar to yours but utilizing a porcine model, failed to demonstrate a physiologic or survival benefit associated with DHEA administration. Since most of the immune-enhancing effects of DHEA have been demonstrated in rats and mice, and these animals are substantially more dependent upon steroids than mammals, could it be that the stage is being set for the identification of an effect in rodents, which may be clinically insignificant in humans?

As a final note, these studies required pretreatment with DHEA prior to the septic stimulus. How would you envision using this steroid hormone clinically, and are there any negative consequences to the use of this agent? I look forward to hearing reports on human studies using this agent.

Dr Angele: With respect to the first question you raised concerning the lack of augmentation of cytokine production by DHEA in cells from sham-operated animals, you indicated that this was in contrast to previous reports, which demonstrated immunostimulatory effects, such as increased T-lymphocyte IL-2 production, even in the normal basal state. In this regard, I would like to mention that the previous studies dealt exclusively with the in vitro effects of DHEA on T cells from normal animals. Although in vitro DHEA administration has been shown to have immunostimulatory effects on splenocytes, in vivo DHEA administration at a dose comparable to that used in our studies has not been found to have any immunostimulatory effects in sham animals in other studies as well. Thus, the present findings are not in contrast to our previous findings. Dr Dahn also indicated that DHEA concentrations were significantly increased, by a factor of 2, 24 hours after administration of 100 µg of DHEA. I would like to clarify that, following trauma-hemorrhage without DHEA administration, there was no increase in DHEA concentrations, and that an increase in DHEA concentrations in sham as well as in post-hemorrhage animals was observed only if

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the animals were treated with DHEA. Dr Dahn also correctly pointed out that DHEA exists not only in active form, which is measured by immunoassay, but also in an inactive, sulfated form, which is partially protein bound. This point is well taken, and we will attempt to address that in future studies.

Dr Dahn further mentioned that large animal studies have failed to demonstrate a physiological or survival benefit following hemorrhagic shock associated with DHEA administration and, therefore, the question is whether DHEA works only in rodents and may not be applicable in the clinical arena. The study Dr Dahn cited utilized a porcine bib-lethal model of hemorrhage and endotoxemia, and thus one would not expect any beneficial effects on survival under those conditions. Furthermore, the study cited incorporated male and female animals. In this respect, DHEA has been shown to have estrogenic or androgenic effects depending on the different hormonal milieu in males and females. Therefore, the difference in results between the study cited and ours might be due to the fact that they used both female and male animals in their studies. Moreover, there are a number of studies that indicate that DHEA has an immunoenhancing effect in humans; for example, significantly higher plasma antibody titers.

Dr Dahn's final comment was with respect to how we would envision using the DHEA clinically and whether there might be any negative consequences of the use of DHEA. Since we have shown that administration of DHEA improved the survival of animals following trauma-hemorrhage and subsequent sepsis, and we did not really observe adverse effects in our animals nor have adverse effects been reported with such a short-term treatment of 3 days of DHEA, we could envision using DHEA in patients once they are brought to the emergency room in order to decrease susceptibility and maybe alter their morbidity to sepsis under those conditions.

Stewart C. Wang, MD, PhD, Ann Arbor, Mich: Excellent work, as always, from your laboratory. Dr Dahn mentioned that there are quite a few associations between DHEA and various effects in the body, and of particular importance in this particular situation may be its effects on immune cell cytokine production. Drs Araneo and Daynes, out of Salt Lake City, had earlier published that DHEA tends to shift local lymphokine responses toward more of a T_H1 type response. Have you looked at the more specific T-helper subset cytokines, such as interferon gamma, IL-10, IL-13, IL-12, specifically looking at T_H1-T_H2 shift?

A second question: It is well known that certain subspecies of mice have very specific T_H1-T_H2 responses, which may be very important here. Have you looked at this model in other strains of mice, particularly with regards to whether there is a shift in the T_H balance?

Dr Angele: We measured the release of T_H1 cytokines by T-lymphocytes such as IL-2 and gamma interferon in the present study. The results indicate the DHEA administration normalized the depressed T_H1 release following trauma-hemorrhage. As of yet, we have not determined the release of T_H2 cytokines, eg, IL-10, following trauma-hemorrhage and DHEA administration. We will address the effect of this drug on T_H2 cytokine release in future studies.

Dr Wang's second comment dealt with the administration of DHEA in alternate strains following trauma-hemorrhage. We have not yet utilized strains other than C3H/HeN mice, but your point is well taken and we will examine this in the future.

Steve A. Calvano, PhD, New Brunswick, NJ: If I understand your survival studies correctly, you are giving 60 µg/d per mouse, and I assume that is intraperitoneally. Is there any evidence that DHEA has a bacteriostatic or bactericidal effect, especially since you are delivering it directly to the peritoneum, where the infection is present?

Dr Angele: Since DHEA was administered subcutaneously in the present study, one would not expect a direct effect of DHEA on the bacteria in the peritoneal cavity. Thus, the increased survival rate of DHEA-treated animals following trauma-hemorrhage and subsequent sepsis does not appear to be due to a direct bacteriostatic or bactericidal effect of DHEA.

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