Salutary effects of androstenediol on cardiac function and splanchnic perfusion after trauma-hemorrhage


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Submitted 31 March 2004; accepted in final form 23 April 2004

Shimizu, Tomoharu, Mashkoor A. Choudhry, Laszlo Szalay, Loring W. Rue III, Kirby I. Bland, and Irshad H. Chaudry. Salutary effects of androstenediol on cardiac function and splanchnic perfusion after trauma-hemorrhage. Am J Physiol Regul Integr Comp Physiol 287: R386–R390, 2004. First published April 29, 2004; 10.1152/ajpregu.00214.2004.—Recent studies have shown that dehydroepiandrosterone (DHEA) administration after trauma-hemorrhage (T-H) improves cardiovascular function and decreases cytokine production in male animals. Although androstenediol, one of the metabolites of DHEA, is reported to have estrogen-like activity, it remains unknown whether androstenediol per se has any salutary effects on cytokines and cardiovascular function after T-H. To examine this effect, male Sprague-Dawley rats underwent laparotomy and were bled to and maintained at a mean arterial blood pressure of 35–40 mmHg for ∼90 min. The animals were resuscitated with four times the volume of maximal bleedout volume in the form of Ringer lactate. Androstenediol (1 mg/kg body wt iv) or vehicle was administered at the end of resuscitation. Twenty-four hours after resuscitation, cardiac function and organ blood flow were measured by using 85Sr-microspheres. Circulating levels of nitrate/nitrite and IL-6 were also determined. Cardiovascular function and organ blood flow were significantly depressed after T-H. However, these parameters were restored by androstenediol treatment. The elevated plasma IL-6 levels after T-H were also lowered by androstenediol treatment. In contrast, plasma levels of nitrate/nitrite were the highest in the androstenediol-treated T-H animals. Because androstenediol administration after T-H decreases cytokine production and improves cardiovascular function, this agent appears to be a novel and useful adjunct for restoring the depressed cardiovascular function and for cytokine production in males after adverse circulatory conditions.

Hemorrhagic shock; nitric oxide; nitric oxide synthase; adiol; 5-androstene-3β,17β-diol

Hemorrhagic shock results in a rapid decrease in cardiac output and organ blood flow. Furthermore, studies have shown that intestinal perfusion remains depressed even after the recovery of cardiac output by fluid resuscitation (29). This splanchnic hypoperfusion after hemorrhagic shock also activates the inflammatory cascade. The depressed cardiovascular function and excess production of inflammatory mediators play an important role in the development of multiple organ failure after hemorrhagic shock (20).

Previous work from our laboratory has shown that left ventricular performance, cardiac output, and organ blood flow in the liver, small intestine, and kidney decreased significantly after trauma-hemorrhage (3, 4, 25), and plasma levels of IL-6 were elevated under those conditions in male animals (1, 25). However, clinical and laboratory studies have shown that gender differences exist in the organ and immune function after hemorrhagic shock (13, 23, 31). In this regard, our previous studies have shown that male sex steroids have deleterious effects and female sex steroids produce beneficial effects on cardiovascular functions after trauma-hemorrhage (5, 16, 31).

In particular, studies have shown that administration of a single dose of estrogen after trauma and hemorrhagic shock improved cardiovascular and hepatocellular functions (18, 25). In addition, dehydroepiandrosterone (DHEA) is the most abundant steroid hormone in plasma and is an intermediate in the pathway for the synthesis of testosterone and estrogen. DHEA treatment after trauma-hemorrhage has been reported to improve organ functions and normalize cytokine production after circulatory stress (9, 15).

Androstenediol (adiol or 5-androstene-3β,17β-diol) is one of the metabolites of DHEA. Androstenediol has been reported to have greater protective effects than DHEA against lethal bacterial infections and endotoxin shock (7). Furthermore, androstenediol has also been reported to produce protective effects after ionizing radiation in mice (21, 34). These studies have shown a significant improvement in survival of mice treated with androstenediol after whole body ionizing irradiation with gamma rays. The estrogen-like activities of androstenediol are observed at physiological concentration in breast cancer cells. Androstenediol causes an increase in estrogen receptor-dependent β-galactosidase activity in yeast (26). Furthermore, Schmidt et al. (30) reported the conversion of DHEA to downstream steroid hormones in macrophages. These investigators suggested that the conversion of DHEA leads to an increase of downstream effecter hormones in macrophages that may play an important role in local immunomodulation (30).

Previous studies indicate that vascular endothelial cell dysfunction occurs early after trauma-hemorrhage and may contribute to further alterations in tissue perfusion and cellular function (33). Angele et al. (2) demonstrated that L-arginine (i.e., the substrate for constitutive nitric oxide synthase, cNOS) restored the depressed cardiac output and organ blood flow and decreased plasma levels of IL-6 (2). Recently, androstenediol has been reported to increase nitric oxide synthase (NOS) activity in the vagina of ovariectomized rabbits (32).

Although androstenediol has been reported to produce the above-mentioned salutary effects, it remains unknown whether this metabolite or its parent compound is responsible for...
producing the salutary effects after trauma-hemorrhage. We hypothesized that androstenediol itself is a useful adjunct for improving the depressed cardiovascular function and cytokine production after trauma-hemorrhage. To test this hypothesis, we examined whether administration of androstenediol has any salutary effects on cardiovascular function and cytokine production in males after trauma-hemorrhage. In addition, plasma levels of nitrate/nitrite were determined to evaluate systemic nitric oxide production.

MATERIALS AND METHODS

Animals. Adult male (275–325 g) Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were used in this study. All experiments were performed in adherence to the National Institutes of Health Guidelines for the Use of Experimental Animals and approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

Experimental procedures. A nonheparinized model of trauma-hemorrhage in the rat, as previously described, was used in this study (31). Briefly, male Sprague-Dawley rats (275–325 g) were fasted overnight before the experiment but allowed water ad libitum. The rats were anesthetized by isoflurane (Attune, Mirad, Bethlehem, PA) inhalation before the induction of soft tissue trauma (i.e., 5-cm midline laparotomy). The abdomen was then closed in layers, and catheters were placed in both femoral arteries and the right femoral vein [polyethylene (PE-50) tubing; Becton-Dickinson, Sparks, MD]. The animals were then restrained in a supine position, and the wounds were bathed with 1% lidocaine (Elkins-Sinn, Cherry Hill, NJ) throughout the surgical procedure to minimize postoperative pain.

The rats were then allowed to awaken, after which they were bled to a mean arterial pressure (MAP) of 35–40 mmHg within 10 min. The time at which the animals could no longer maintain an MAP of 35–40 mmHg was defined as the time at which the animals could no longer maintain an MAP of 35–40 mmHg without infusing some fluid. The MAP was maintained until 40% of the shed blood was returned in the form of Ringer lactate. After resuscitation, the catheters were removed, the vessels were ligated, and skin incisions were closed with sutures. Sham-operated animals underwent the same groin dissection, which included the ligation of the femoral artery, and catheters were placed in the femoral vein to administer agents; however, neither trauma-hemorrhage nor resuscitation was carried out. The animals were returned to their cages and were allowed food and water ad libitum until death. The animals were killed at 24 h after the end of resuscitation.

In the treatment group, 1 mg/kg body wt androstenediol (Steraloids, Newport, RI) was administered intravenously at the end of the resuscitation. In the vehicle-treated group (control group) rats received the same volume of vehicle (Intralipid, 1 ml/kg body wt, Sigma, St. Louis, MO).

Measurement of plasma levels of nitrate/nitrite. Blood samples were obtained and placed in microcentrifuge tubes at 24 h after the end of resuscitation or sham operation, and plasma was separated by centrifugation, immediately frozen, and stored at −80°C until assayed. The production of systemic nitric oxide was evaluated by measuring plasma nitrate/nitrite levels using a commercially available colorimetric assay kit (Cayman Chemical, Ann Arbor, MI).

Measurement of plasma levels of IL-6. Plasma levels of IL-6 were determined using ELISA kits (Pharmingen, San Diego, CA) according to the manufacturer’s instructions.

Measurement of heart performance, cardiac output, and organ blood flow. In an additional set of animals, heart performance was evaluated at 24 h after resuscitation or sham operation, as previously described (4). Briefly, rats were anesthetized with pentobarbital sodium (~30 mg/kg body wt), and a PE-50 catheter was inserted into the left ventricle via right carotid artery. The maximal rates of left ventricular pressure increase (+dP/dmax) and decrease (−dP/dmax) were determined with a heart performance analyzer.

Cardiac output and organ blood flow in the small intestine, liver, spleen, kidney, pancreas, adrenal grand, brain, heart, and lung were determined by using a radioactive microsphere technique as previously described (4). Briefly, 85Sr-labeled microspheres (~500,000 cpm; DuPont NEN, Boston, MA) were injected manually into the left ventricle. The reference blood sample was withdrawn from the femoral arterial catheter for 60 s at a rate of 0.7 ml/min. Isotonic sodium chloride solution was infused at the same rate to replace the volume of blood lost. The animals were killed, and abdominal organs were then harvested and weighed. The radioactivity in the organs and reference blood sample was counted with an automatic gamma counter (1470 Wizard; Wallac, Gaithersburg, MD). Cardiac output and organ blood flow were calculated according to the following two equations: 1) cardiac output = [(RBF × CT)/Cr] × 100/BW, and 2) organ blood flow = [(RBF × Ct)/Cr] × 100, where RBF is the reference blood sample withdrawal rate (0.7 ml/min), CT is counts per minute of total injected dose, Cr is counts per minute in the reference blood sample, BW is body weight (g), and Ct is counts per minute per gram of tissue.

Statistical analysis. Data are presented as means ± SE. Statistical differences between groups were determined by one-way ANOVA followed by Fisher’s least significant difference as a post hoc test. The differences were considered significant if P < 0.05.

RESULTS

Alterations in hemodynamic parameters. There was no significant difference in the hemodynamic parameters between androstenediol-treated and nontreated sham-operated animals. As shown in Fig. 1, cardiac output significantly decreased in vehicle-treated rats after trauma-hemorrhage. Androstenediol treatment after trauma-hemorrhage increased cardiac output, and the values were similar to those observed in sham-operated animals (Fig. 1A). Furthermore, the positive and negative dP/dmax shown in Fig. 1, B and C, were also significantly depressed at 24 h after trauma-hemorrhage. Treatment of rats with androstenediol prevented the decrease in positive dP/dmax and negative dP/dmax; however, negative dP/dmax was still significantly lower than in sham-operated animals (Fig. 1, B and C).

Mean blood pressure also decreased significantly in rats after trauma-hemorrhage (Fig. 2A). Treatment of rats with androstenediol after trauma-hemorrhage did not significantly improve mean blood pressure compared with vehicle-treated trauma-hemorrhaged animals. Furthermore, no significant difference in heart rate was observed among the different groups of animals (Fig. 2B).

Alteration in organ blood flow. There was no difference in organ blood flow between androstenediol- and vehicle-treated sham-operated groups (Table 1). Trauma-hemorrhage markedly decreased blood flow in the small intestine, liver, spleen, kidney, pancreas, and adrenal gland of the vehicle-treated rats. Androstenediol treatment after trauma-hemorrhage prevented the decrease in blood flow in the small intestine and liver. Furthermore, although androstenediol treatment after trauma-hemorrhage increased blood flow in the spleen, kidney, and pancreas, it was not restored to normal in these organs (Table 1). Androstenediol treatment did not improve blood flow in the adrenal gland. However, blood flow in the brain increased significantly in both the vehicle- and androstenediol-treated rats after trauma-hemorrhage. There was no significant differ-
Plasma levels of nitrate/nitrite. Plasma levels of nitrate/nitrite increased significantly at 24 h after trauma-hemorrhage. Androstenediol treatment further increased nitrate/nitrite levels in rats after trauma-hemorrhage (Fig. 3), but it did not influence the nitrate/nitrite levels in the sham-operated rats.

Plasma IL-6 levels. Plasma IL-6 levels were significantly elevated after trauma-hemorrhage compared with sham-operated rats in the vehicle treatment group. However, androstenediol treatment markedly decreased the levels of IL-6 after trauma-hemorrhage (Fig. 4).

DISCUSSION

The present study showed that administration of androstenediol after trauma-hemorrhage improved left ventricular performance, cardiac output, and organ blood in the small intestine, liver, spleen, and kidney. This improvement of cardiovascular function was associated with increase of plasma nitrate/nitrite levels. Furthermore, elevated circulating levels of IL-6 after trauma-hemorrhage were significantly reduced by androstenediol treatment.

Gender dimorphism was observed in the cardiac function and tissue perfusion after trauma-hemorrhage. Previous studies from our laboratory have shown that proestrus female rats, which have high circulating levels of estrogen and progesterone, maintain cardiac output and higher splanchnic perfusion compared with male rats after trauma-hemorrhage (3). Our previous studies have also shown that administration of a single dose of estrogen after trauma-hemorrhage improved

Table 1. Organ blood flow after trauma-hemorrhage

<table>
<thead>
<tr>
<th></th>
<th>Sham Operated</th>
<th>T-H</th>
<th>+ Vehicle</th>
<th>Androstenediol</th>
<th>Vehicle</th>
<th>Androstenediol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small intestine</td>
<td>145±12</td>
<td>132±12</td>
<td>104±9*</td>
<td>151±52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>130±5.3</td>
<td>136±7.9</td>
<td>103±6.4*</td>
<td>167±12†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>118±19</td>
<td>83±8</td>
<td>34±6.2*</td>
<td>67±12†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>523±39</td>
<td>548±33</td>
<td>178±24*</td>
<td>327±44†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>166±29</td>
<td>143±22</td>
<td>48.6±6.7†</td>
<td>64.7±7.6†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>417±55</td>
<td>472±21</td>
<td>296±54†</td>
<td>296±33†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>45.1±5.6</td>
<td>54.1±3.4</td>
<td>67.4±4.6†</td>
<td>74.1±5.7†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>500±85</td>
<td>515±44</td>
<td>580±39</td>
<td>612±55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>54.4±10</td>
<td>44.6±20</td>
<td>44.8±4.9</td>
<td>43.8±17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SE of 6–7 animals per group. Organ blood flow measurements (ml/min kg body wt⁻¹) were performed at 24 h after sham operation or trauma-hemorrhage (T-H). For further details, see MATERIALS AND METHODS. *P < 0.05 vs. other groups; †P < 0.05 vs. sham operated.

ence in blood flow in the heart and lung among the different group of animals (Table 1).

Plasma levels of nitrate/nitrite. Plasma levels of nitrate/nitrite increased significantly at 24 h after trauma-hemorrhage. Androstenediol treatment further increased nitrate/nitrite levels in rats after trauma-hemorrhage (Fig. 3), but it did not influence the nitrate/nitrite levels in the sham-operated rats.

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Gender dimorphism was observed in the cardiac function and tissue perfusion after trauma-hemorrhage. Previous studies from our laboratory have shown that proestrus female rats, which have high circulating levels of estrogen and progesterone, maintain cardiac output and higher splanchnic perfusion compared with male rats after trauma-hemorrhage (3). Our previous studies have also shown that administration of a single dose of estrogen after trauma-hemorrhage improved
cardiovascular and hepatocellular functions (18, 25). Alternatively, testosterone receptor blockade after trauma-hemorrhage by flutamide in normal male rats restored the depressed cardiac function, blood flow, oxygen delivery, and consumption in all organs tested (5). Additionally, Jarrar et al. (15) demonstrated that DHEA administration after trauma-hemorrhage restored the depressed cardiac and hepatocellular functions in male rats. DHEA is the most abundant steroid hormone in plasma and is an intermediate in the pathway for the synthesis of testosterone and estrogen. The salutary effects of DHEA were not observed if the estrogen receptor antagonist ICI-182780 was administered with DHEA. However, plasma levels of 17β-estradiol and testosterone were not significantly altered in animals receiving DHEA (15). Thus the above studies collectively suggest that gender dimorphism does exist in the cardiac function and tissue perfusion after trauma-hemorrhage.

In this study, we examined the effect of androstenediol on cardiac functions after trauma-hemorrhage because androstenediol is one of the metabolites of DHEA. This agent has been reported to have protective effects against lethal bacterial infections and endotoxin shock. The results presented in this manuscript clearly suggest that treatment of rats with single dose of androstenediol (1 mg/kg iv) after trauma-hemorrhage significantly improved cardiac function, prevented the elevation in IL-6, and further increased nitrate/nitrite levels under those conditions. Whether androstenediol increases endothelial NOS (eNOS) and whether this increase is responsible for the elevated nitrate/nitrite levels remains to be determined. It should be pointed out, however, that the dose of androstenediol that was used in this study did not restore blood flow in some organs. Whether androstenediol at higher doses or repeat treatment would restore blood flow to those organs remains unknown. We did not use a higher dose of androstenediol or repeat treatment because a number of potential risk factors associated with higher dose and long-term androstenediol use have been reported (34). Long-term oral supplementation with androstenediol appears to adversely affect blood lipids (i.e., decrease of high-density lipoprotein concentration) and increase the risk of pancreatic cancer or prostate cancer (8). Nonetheless, our findings suggest that administration of a single dose (1 mg/kg iv) of androstenediol as an adjunct to resuscitation after trauma-hemorrhage is effective for restoring and maintaining cardiovascular function and cytokine production after trauma-hemorrhage.

The precise mechanism responsible for producing the beneficial effects of androstenediol on cardiac function and organ blood flow is not known. Nonetheless, several studies have shown that hemorrhagic shock induces a cascade of proinflammatory cytokines that are associated with immunosuppression (12), hemodynamic depression, and organ dysfunction (6). In this regard, studies by Mizushima et al. (25) have shown that administration of estrogen after trauma-hemorrhage reduced circulating levels of IL-6. Knoferl et al. (17) also reported that administration of DHEA after trauma-hemorrhage significantly reduced the elevated circulating IL-6 levels, suggesting an attenuation of the inflammatory response under those conditions. Furthermore, Kuebler et al. (19) have demonstrated that progesterone treatment after trauma-hemorrhage also significantly reduces plasma IL-6 levels (19). Consistent with these findings, the present findings also indicate that the elevated circulating levels of IL-6 after trauma-hemorrhage were significantly reduced by androstenediol treatment. Because elevated levels of IL-6 have been correlated with cardiac dysfunction in many injury conditions (14, 22) as well as after trauma-hemorrhage (17, 19, 25), it is likely that attenuation of IL-6 production could be a factor responsible for androstenediol salutary effect in preventing cardiac dysfunction after trauma-hemorrhage.

In addition to IL-6, we also found an increase in nitrate/nitrite production after trauma-hemorrhage, and treatment with androstenediol further elevated systemic nitrate/nitrite levels. Eckhoff et al. (11) have shown that estrogen administration significantly reduced hepatic injury after ischemia-reperfusion to the liver, and this effect was associated with increased serum nitric oxide metabolites. Our result showed further induction of nitrate/nitrite in the androstenediol-treated rats. Although we have not determined whether the increase in nitrate/nitrite levels is due to inducible NOS or cNOS, it is likely that the increase in NOS, especially eNOS activity in androstenediol-treated animals, may contribute to the improvement of cardiac output and splanchnic perfusion after trauma-hemorrhage.

Several studies have shown salutary effects of androstenediol treatment on immune functions after injury such as endotoxin shock, sepsis, and radiation injury (7, 21, 34). Ben-Nathan et al. (7) reported that androstenediol had greater protective effects on survival rate than DHEA against lethal bacterial infections and endotoxin shock. In contrast, Padgett and Loria (28) have shown that androstenediol had little influence on the secretion of LPS-induced proinflammatory cytokine (IL-6, TNF-α, and IL-1) from macrophages in vitro. Whether the difference in the results of Ben-Nathan et al. (7) and Padgett and Loria (28) is due to in vivo vs. in vitro use of DHEA remains unclear.

Although the precise mechanism responsible for the beneficial effects of androstenediol on cytokine production remains unknown, it is possible that androstenediol mediates its action via the estrogen receptor(s). In this regard, several studies have shown that estrogens have protective effects on the cardiovascular system via the rapid nongenomic and long-term genomic mechanisms (10, 24). Furthermore, studies have shown that administration of a single dose of estrogen after trauma-hemorrhage improved cardiovascular and hepatocellular functions and splanchnic perfusion (18, 25). Recent study from our laboratory (35) has also shown that estrogen attenuated the production of IL-6 by Kupffer cells from both sham-operated

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**Fig. 4.** Effects of androstenediol on plasma IL-6 levels at 24 h after sham operation or trauma-hemorrhage. Data are means ± SE (n = 6 animals/group). *P < 0.05 vs. other groups.
and trauma-hemorrhage animals in a dose-dependent manner. Because Kupffer cells have been reported to be the major source of IL-6 production after trauma-hemorrhage (27), it is likely that similar to estrogen, androstenediol treatment down-regulates the production of IL-6 from Kupffer cells. However, further studies are needed to determine such a paradigm.

In summary, our study indicates that androstenediol administration after trauma-hemorrhage improves cardiac function and organ blood flow and decreases cytokine production. This improvement of cardiovascular function may be associated with attenuation in IL-6 levels and an increase in plasma nitrite/nitrate levels. Because androstenediol produced the above-mentioned salutary effect, these results lead us to conclude that androstenediol appears to be a useful adjunct for restoring the depressed cardiovascular functions and attenuating cytokine production after trauma-hemorrhage.

ACKNOWLEDGMENTS

We thank Z. F. Ba for superb technical assistance during these studies.

GRANTS

This investigation was supported by National Institutes of Health Grant R37-GM-39519.

REFERENCES


