Testosterone receptor blockade after trauma-hemorrhage improves cardiac and hepatic functions in males

DIERK E. REMMERS, PING WANG, WILLIAM G. CIOFFI, KIRBY I. BLAND, AND IRSHAD H. CHAUDRY
Center for Surgical Research and Department of Surgery, Brown University School of Medicine and Rhode Island Hospital, Providence, Rhode Island 02903

Remmers, Dierk E., Ping Wang, William G. Cioffi, Kirby I. Bland, and Irshad H. Chaudry. Testosterone receptor blockade after trauma-hemorrhage improves cardiac and hepatic functions in males. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2919–H2925, 1997.—Although studies have shown that testosterone receptor blockade with flutamide after hemorrhage restores the depressed immune function, it remains unknown whether administration of flutamide following trauma and hemorrhage and resuscitation has any salutary effects on the depressed cardiovascular and hepatocellular functions. To study this, male rats underwent a laparotomy (representing trauma) and were then bled and maintained at a mean arterial pressure (MAP) of 40 mmHg until the animals could not maintain this pressure. Ringer lactate was given to maintain a MAP of 40 mmHg until 40% of the maximal shed blood volume was returned in the form of Ringer lactate. The rats were then resuscitated with four times the shed blood volume in the form of Ringer lactate over 60 min. Flutamide (25 mg/kg) or an equal volume of the vehicle propanediol was injected subcutaneously 15 min before the end of resuscitation. Various in vivo heart performance parameters (e.g., maximal rate of the pressure increase or decrease), cardiac output, and hepatocellular function (i.e., the maximum velocity and the overall efficiency of indocyanine green clearance) were determined at 20 h after resuscitation. Additionally, hepatic microvascular blood flow (HMBF) was determined using a laser Doppler flowmeter. The results indicate that left ventricular performance, cardiac output, HMBF, and hepatocellular function decreased significantly at 20 h after the completion of trauma, hemorrhage, and resuscitation. Administration of the testosterone receptor blocker flutamide, however, significantly improved cardiac performance, HMBF, and hepatocellular function. Thus flutamide appears to be a novel and useful adjunct for improving cardiovascular and hepatocellular functions in males following trauma and hemorrhagic shock.

Early treatment of trauma victims with hemorrhagic shock, who survive the initial insult, includes control of ongoing bleeding and rapid restoration of intravascular volume to improve tissue perfusion. However, recent studies have shown that acute fluid resuscitation alone following trauma and hemorrhage (hereafter referred to as trauma-hemorrhage) does not restore or maintain hepatocellular function (21), microvascular blood flow (25), or cardiac output (24) in male animals. Furthermore, studies have indicated that nonspecific and specific immune functions are depressed following trauma-hemorrhage, which could explain the enhanced susceptibility to sepsis after such conditions (6). In this respect it has been demonstrated that females showed an enhanced immune response after trauma-hemorrhage as opposed to a decreased immune response in male mice (28). Furthermore, gonadectomy before the induction of trauma-hemorrhage in male mice prevents the occurrence of the immune depression (29), suggesting that male sex hormones may play an important role in the regulation of posttraumatic immunodepression. Additional studies have shown that testosterone receptor blocker blockade with flutamide, a nonsteroidal receptor antagonist, after hemorrhage in male mice restores the depressed immune function (4, 27). It has been demonstrated that testosterone is involved in other physiological events such as enhancing vasoconstriction (1, 13), which may play a role in producing organ dysfunction following trauma-hemorrhage. This effect can be inhibited by the administration of flutamide (1). Because testosterone receptor blockade following trauma-hemorrhage improves the depressed immune function, we hypothesized that administration of flutamide after trauma-hemorrhage and resuscitation will improve the depressed heart and liver functions under those conditions. Therefore, the aim of the present study was to determine whether testosterone receptor blockade with flutamide improves the depressed cardiovascular and hepatocellular functions after trauma and hemorrhagic shock in male animals.

Materials and Methods

Experimental procedures. We used the previously described nonheparinized model of trauma-hemorrhage in the rat (21, 24, 25) with minor modifications. Briefly, male Sprague-Dawley rats (275–325 g) were fasted overnight before the experiment but allowed water ad libitum. The rats were anesthetized by methoxyflurane inhalation before the induction of trauma (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 wounds were bathed with 1% lidocaine (Elkins-Sinn, Cherry Hill, NJ) throughout the surgical procedure to reduce postoperative pain. Rats were then bled and maintained at a mean arterial pressure (MAP) of 40 mmHg until the animals could not...
maintain a MAP of 40 mmHg unless extra fluid in the form of Ringer lactate was given. This time was defined as maximum bleed out, and the amount of withdrawn blood was noted. After this, the rats were maintained at MAP of 40 mmHg until 40% of the maximum bleed out volume was returned in the form of Ringer lactate. The animals were then resuscitated (pump 22, Harvard Apparatus, South Natick, MA) with four times the volume of the withdrawn blood over 60 min (−45 ml/rat) with Ringer lactate. It should be mentioned that the shed blood was not used for resuscitation. Fifteen minutes before the end of the resuscitation period the rats received 25 mg/kg body wt flutamide (Scherer, Kenilworth, NJ) subcutaneously or an equal volume (−0.5 ml) of the nontoxic vehicle propanediol. The catheters were then removed, the vessels ligated, and the skin incisions closed with sutures.

After the rats were returned to their cages, they were allowed food and water ad libitum. At 20 h after the completion of fluid resuscitation, the animals were anesthetized with methoxyflurane and then catheterized via the right jugular vein. Under continued general anesthesia with pentobarbital sodium (25–30 mg/kg body wt), cardiac output, hepatocellular function, and hepatic microvascular blood flow (HMBF) were measured in each animal.

All animal experiments were performed according to the guidelines of the “Animal Welfare Act” and the Guide for the Care and Use of Laboratory Animals (NIH Publication No. (NIH) 85–23). This project was approved by the Institutional Animal Care and Use Committee of Rhode Island Hospital.

Measurement of cardiac output. A PE-50 catheter was placed into the right carotid artery to measure MAP and heart rate. The arterial catheter was then replaced by a 2.4-Fr fiberoptic catheter that was connected to an in vivo hemoreflectometer (Hospex Fiberoptics, Chestnut Hill, MA). The fiberoptic catheter was positioned with the tip at the origin of the carotid artery from the aortic arch as confirmed by characteristic changes of the optical density measurements recorded by the hemoreflectometer. In previous experiments, we were able to verify the exact position of the fiberoptic catheter at autopsy. These experiments confirmed that the characteristic changes of the optical density measurements indicate the entrance of the fiberoptic catheter into the aortic arch times the volume of the withdrawn blood. Indocyanine green solution (ICG, Cardio Green, Becton Dickinson) was injected via the catheter in the jugular vein (1 mg/ml aqueous solvent as a 50-µl bolus). Twenty ICG concentrations per second were recorded for 30 s with the aid of a data acquisition program (Asyst+, Asyst Software, Rochester, NY). The area under the ICG dilution curve was determined according to a previous publication (24) to calculate cardiac output. Cardiac output was then divided by the body weight to determine cardiac index. Cardiac index was divided by heart rate to calculate stroke volume. MAP was divided by cardiac index to calculate total peripheral resistance (TPR). The central venous pressure was not measured in the present experiment because we did not find major alterations of this parameter following trauma-hemorrhage and resuscitation in the rat (24). The calculated value of the TPR should be considered as an estimated one.

Measurement of hepatocellular function. Hepatocellular function was measured by the in vivo ICG clearance technique. ICG was administered by bolus injection (50 µl) of 1, 2, and 5 mg/ml ICG in aqueous solvent. The arterial concentration of ICG was recorded each second for 5 min. After this, the initial velocity of ICG clearance for each dose was calculated after performing a nonlinear regression of the ICG clearance curves according to an e-raised second-order polynomial function (10). The initial velocities of ICG clearance were then plotted against the ICG doses according to the methods of Lineweaver-Burk (9). This results in a straight line, allowing the determination of the maximum velocity (Vmax) of ICG clearance and the Michaelis-Menten constant (Km). In this active hepatoceleular membrane transport system, Vmax represents the functional hepatocyte ICG receptors, whereas Km represents the efficiency of the active transport process (21).

Measurement of in vivo heart performance. After the determination of cardiac output and hepatocellular function, the fiberoptic catheter in the right carotid artery was replaced with PE-50 tubing, which was manually stretched to reduce the outer diameter by ~50%. Under pressure control this catheter was carefully advanced into the left ventricle. The position of the catheter was confirmed by recording the characteristic left ventricular pressure curve. Data were analyzed from an in vivo heart performance analyzer (MicroMed, Louisville, KY) as described in our previous publication (16). Various left ventricular performance parameters such as the maximal rate of the pressure increase (+dP/dtmax) and decrease (−dP/dtmin), as well as end-diastolic pressure in the left ventricle were documented with a data acquisition system (DMSI 200–8; Micro-Med).

Measurement of HMBF. HMBF was measured on the liver surface by laser Doppler flowmetry (22). Briefly, the abdomen was reopened and a flat flow probe connected to a laser Doppler blood perfusion monitor (Laserflo, model BPM 403A, TSI, St. Paul, MN) was placed on the liver surface. HMBF represents the microvascular red blood cell flux in ~1 mm² surface organ tissue with a unit of milliliter per minute per 100 g tissue as indicated by the manufacturer. Although this is a widely used technique for the determination of alterations in organ surface perfusion, the flow unit should be considered as an arbitrary one. Statistics. Results are presented as means ± SE. The data were analyzed with one-way analysis of variance followed by the Fisher’s least-significant difference test as a post hoc test for multiple comparisons. The differences are considered significant at a P value <0.05. There were 8 animals in the sham plus vehicle group, 7 animals in the sham plus flutamide group, 11 animals in the hemorrhage plus vehicle group, and 10 animals in the hemorrhage plus flutamide group.

RESULTS

The mean time to reach maximum bleed out was 45 ± 1 min. The average shed blood volume was 11 ± 0.1 ml/rat. The average time until the end of hemorrhage was 91 ± 2 min. There was no significant difference in these parameters between the hemorrhaged animals that subsequently received flutamide or vehicle. Effects of flutamide on MAP and TPR. MAP decreased significantly at 20 h after the completion of hemorrhage and resuscitation in both hemorrhaged groups compared with sham-operated animals. Administration of flutamide during fluid resuscitation, however, significantly increased MAP compared with the vehicle-treated group (Fig. 1A). TPR was also significantly decreased in both hemorrhaged groups compared with shams. There was no significant difference between the flutamide- and vehicle-treated animals 20 h following trauma-hemorrhage and resuscitation (Fig. 1B). Effects of flutamide on cardiac index and stroke volume. As shown in Fig. 2A, cardiac index was significantly decreased in the hemorrhaged animals compared with shams. Administration of flutamide, however, improved cardiac index to a level that was not
significantly different from sham values. Similarly, stroke volume decreased in the hemorrhaged- and vehicle-treated animals. Flutamide treatment increased stroke volume, and the values were not significantly different from shams (Fig. 2B).

Effects of flutamide on heart performance. The maximum rate of pressure increase ($+\frac{dP}{dt_{\max}}$) in the left ventricle was significantly decreased following trauma-hemorrhage (Fig. 3A). However, flutamide treatment increased $+\frac{dP}{dt_{\max}}$ after trauma-hemorrhage and resuscitation, and there was no statistical difference between hemorrhaged- and flutamide-treated animals and sham-operated rats (Fig. 3A). The maximum rate of pressure decrease in the left ventricle ($-\frac{dP}{dt_{\max}}$) was also significantly decreased after hemorrhage compared with sham controls. Although flutamide administration significantly increased $-\frac{dP}{dt_{\max}}$ values compared with vehicle animals following trauma-hemorrhage and resuscitation, these values were lower than sham controls (Fig. 3B). Heart rate and the end-diastolic pressure were not different between the various groups (Table 1).

Effects of flutamide on HMBF and hepatocellular function. HMBF was significantly decreased following trauma-hemorrhage and resuscitation compared with shams. Flutamide treatment increased HMBF but the values were significantly lower than shams (Fig. 4). Hepatocellular function, i.e., $V_{\text{max}}$ (Fig. 5A) and $K_m$ (Fig. 5B), was significantly depressed following trauma-hemorrhage and resuscitation compared with sham controls. Flutamide significantly improved hepatocellu-
Comparison between sham-operated animals with vehicle and flutamide treatment. There was no significant difference between sham-operated animals treated either with the vehicle or flutamide in any of the above-mentioned parameters (Table 2).

DISCUSSION

Fluid resuscitation remains the cornerstone of the treatment of severely traumatized patients with hemorrhagic shock. However, acute fluid resuscitation alone after trauma-hemorrhage in rats does not restore or maintain organ functions (21, 24, 25). Furthermore, it has been demonstrated that nonspecific and specific immune functions are depressed after trauma-hemorrhage despite fluid resuscitation (6). The depression in immunological and physiological responses are apparent immediately after trauma-hemorrhage and persists for a prolonged period of time (6, 15). Sexual dimorphism, however, has been reported with respect to immune functions. For instance, it has been reported that female mice in the proestrus state maintain splenic immune functions and show an increased survival rate after sepsis compared with male mice (30). In addition, females show an enhanced immune response following trauma-hemorrhage as opposed to a depressed immune response in male mice (28). Furthermore, studies indicate that testosterone is at least in part involved in the immune depression in males following trauma-hemorrhage because castrated male mice showed a maintained immune function under such conditions compared with a markedly depressed function in noncastrated animals (29). Recently, it has been shown that testosterone receptor blockade with flutamide in male mice following trauma-hemorrhage and resuscitation restores the depressed immune response (4, 27). These results, therefore, collectively suggest that the immune depression following trauma-hemorrhage is a testosterone and/or testosterone receptor-associated event. We are currently examining the effects of gonad-al
ectomy in male rats on the in vivo heart performance up to 4 h following trauma-hemorrhage. The preliminary results suggest that gonadectomy in male rats before trauma-hemorrhage restores the depressed in vivo heart performance that was observed in noncastrated males following trauma-hemorrhage (unpublished observation). Other investigators have demonstrated that testosterone is involved in physiological mechanisms such as the regulation of the vascular response and the coagulation system (11, 13), which may play a significant role in the pathophysiology of trauma-hemorrhage (25, 26). Thus we hypothesized that testosterone receptor blockade not only improves the depressed immunological response but also the physiological response (i.e., cardiovascular and hepatocellular functions) following trauma-hemorrhage and resuscitation. The aim of the present study, therefore, was to determine whether testosterone receptor blockade with flutamide following trauma-hemorrhage and resuscitation improves cardiac output, left ventricular performance, hepatic perfusion, and hepatocellular function in male rats.

The data presented in this study indicate that the left ventricular performance was significantly depressed at 20 h after trauma-hemorrhage and resuscitation in vehicle-treated animals, as demonstrated by a marked decrease in \( +\frac{dP}{dt_{\text{max}}} \) and \( -\frac{dP}{dt_{\text{max}}} \), cardiac output, and stroke volume. These findings confirm our previous results, which showed that the cardiac function was decreased at 7.5 h and remained depressed at 20 h after trauma-hemorrhage (15, 16, 24). In flutamide-treated animals, however, we found a restored \( +\frac{dP}{dt_{\text{max}}} \) and an improved \( -\frac{dP}{dt_{\text{max}}} \) after trauma-hemorrhage and resuscitation. The improvement in the left ventricular function was reflected by the restored cardiac index and stroke volume in the flutamide-treated animals. Furthermore, MAP was significantly elevated in these animals. HMBF and the hepatocellular function were also significantly increased in the hemorrhaged and flutamide-treated animals. Taken together, our data clearly demonstrate that testosterone receptor blockade in males following trauma-hemorrhage and resuscitation improves the depressed cardiovascular and hepatocellular functions. It should be pointed out that we did not observe any adverse or beneficial effects of testosterone receptor blockade with flutamide in nonhemorrhaged animals. This would suggest that testosterone effects on cardiovascular and hepatocellular functions following trauma-hemorrhage and resuscitation are beneficial. Therefore, testosterone receptor blockade may modify the pathophysiological responses to trauma and hemorrhagic shock.

Previous studies have shown that plasma testosterone levels do not change significantly after trauma-hemorrhage and resuscitation (29). In view of this, it appears that inhibition of the effectiveness of physiological testosterone levels is helpful to the host following hemorrhagic shock. The dose of flutamide used in this study (25 mg/kg body wt sc) was selected because the same dose was found to be effective in previous studies dealing with the effect of trauma-hemorrhage on the depressed immune response (4, 27).

Testosterone receptors can be found in almost every cell population, including immune competent cells, myocytes, and hepatocytes (18). Because flutamide selectively blocks the intracellular testosterone receptor, the processes responsible for the improved cardiovascular and hepatocellular functions found in the present study may originate from this mechanism. Although the precise mechanism responsible for the salutary effects of flutamide on organ functions is not known, several possibilities should be considered. These include the following. First, testosterone receptor blockade initiates intracellular changes that protect hepatocytes from damage following low-flow conditions. These alterations could be initiated by inhibition of specific intracellular effects of testosterone and/or enhancing possible nonspecific effects of testosterone, which have been described for various steroid hormones (7). Furthermore, it is possible that testosterone receptor blockade enhances specific cellular effects of other sex hormones, such as estradiol, which are known to produce beneficial effects after trauma-hemorrhage (2, 3). Second, recent studies have indicated that testosterone increases vascular smooth muscle thromboxane A2 receptors in the aorta (13). It has also been shown that testosterone treatment enhances the vasconstrictor response to thromboxane A2 in the coronary circulation (11, 13), which can be blocked by administration of flutamide (11). Additionally, it has been shown that testosterone treatment inhibits the synthesis of prostacyclin by rat aortic smooth cells in culture (14). Thus it is possible that flutamide administration inhibits the vasoconstrictive actions of thromboxane A2, which may lead to better organ perfusion. Third, administration of tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)) is known to decrease the left ventricular contractility (19) and hepatocellular function (20). Because TNF-\( \alpha \) is significantly elevated following trauma-hemorrhage and resuscitation (5, 12), it is also possible that testosterone receptor blockade decreases cytokine release, which would be associated with better organ functions (15). Finally, it is known that testosterone and/or estradiol modify the stress response of the hypothalamic-pituitary-adrenal axis (8, 17). In previous experiments we found a tendency toward higher plasma levels of corticosterone in gonadectomized (29) or flutamide-treated mice (4, 27) compared with untreated animals after trauma-hemorrhage and resuscitation. In those experiments, plasma corticosterone was taken 24 and 72 h after the initiation of the experiment. However, it could be possible that testosterone receptor blockade alters the adrenal response to trauma-hemorrhage and resuscitation during the first few hours, resulting in improved cardiovascular and hepatocellular functions in the flutamide-treated animals as observed in the present study. Nevertheless, further studies are required to evaluate these possibilities and to pinpoint the precise mechanism of the salutary effects of flutamide.
It could be argued that the improved cardiac function is responsible for the improved HMBF and for the improved hepatocellular function in this study. However, HMBF was improved by ~15%, whereas $V_{\text{max}}$ and $K_m$ were improved by ~50% in flutamide-treated animals compared with vehicle-treated rats. Thus it is likely that flutamide administration improves hepatocellular function via a blood flow independent effect. Nevertheless, additional experiments are required to determine the precise mechanisms leading to an improved hepatocellular function following trauma-hemorrhage and flutamide administration.

It can also be argued that the measurement of cardiac performance in vivo as opposed to in vitro represents a limitation of our study. Although this might be viewed as a shortcoming, we feel that measuring cardiac contractility in vivo has allowed us to gain a better understanding of how the heart responds to ongoing changes (i.e., crystalloids resuscitation) during various stages of hemorrhagic shock (16). However, studies measuring cardiac contractility in vitro are needed to assess further how flutamide treatment following trauma-hemorrhage and resuscitation affects various left ventricular performance parameters under constant preload, afterload, and heart rate.

We measured cardiac output with the ICG dilution technique along with the use of this technique for determination of the hepatocellular function as described in our previous publications (21). The ICG clearance has been found to be a specific and extremely sensitive early indicator of hepatocellular dysfunction after various adverse circulatory conditions and allows in vivo determination of hepatocellular function without blood sampling or any toxic side effects (9, 21). The determination of $V_{\text{max}}$ and $K_m$ with three different doses as described previously represents the hepatocellular function independent of the hepatic blood flow (9, 10). HMBF was measured with laser Doppler flowmetry. This method has been shown to correlate well with other techniques, such as the radiolabeled microsphere method, despite some limitations such as the limited depth of the measurement in the tissue (22). It should be mentioned that the rats were not heparinized throughout the entire study period because previous experiments from our laboratory and others have shown that preheparinization improves microvascular blood flow, cardiac output, renal and hepatic function, as well as vascular endothelial function following trauma-hemorrhage (23). It should also be noted that the shed blood was not used for resuscitation. This was done to keep the model simple and also to study the potential beneficial effects of various pharmacological agents.

In summary, our data indicate that testosterone receptor blockade after trauma-hemorrhage and resuscitation significantly improves MAP, cardiac output, various left ventricular performance parameters such as $+\Delta p/\Delta t_{\text{max}}$, $-\Delta p/\Delta t_{\text{max}}$, HMBF, and hepatocellular function in males. We therefore conclude that testosterone receptor blockade may be a novel and useful adjunct to improve the cardiovascular and hepatocellular functions in male trauma patients following hemorrhagic shock. However, further experiments are required to determine the precise mechanisms responsible for the beneficial effects of testosterone receptor blockade on cardiovascular and hepatocellular responses following trauma-hemorrhage and resuscitation.

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REFERENCES


