Androstenediol administration after trauma-hemorrhage attenuates inflammatory response, reduces organ damage, and improves survival following sepsis

László Szalay,* Tomoharu Shimizu,* Takao Suzuki, Ya-Ching Hsieh, Mashkoor A. Choudhry, Martin G. Schwacha, Kirby I. Bland, and Irshad H. Chaudry

Center for Surgical Research and Department of Surgery, University of Alabama at Birmingham, Alabama

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Despite numerous advances in intensive care medicine, sepsis following sepsis inflammatory response, reduces organ damage, and improves survival following sepsis. Am J Physiol Gastrointest Liver Physiol 291: G260–G266, 2006. First published March 30, 2006; doi:10.1152/ajpgi.00390.2005.—Although androstenediol (adiol or 5-androstene-3β,17β-diol), a metabolite of dehydroepiandrosterone (DHEA), has protective effects following trauma-hemorrhage (T-H), it remains unknown whether administration of adiol has any salutary effects on the inflammatory response and outcome following a combined insult of T-H and sepsis. Male rats underwent T-H shock [mean arterial pressure (MAP) 40 mmHg for 90 min] followed by resuscitation. Adiol (1 mg/kg body wt) or vehicle was administered at the end of resuscitation. Sepsis was induced by cecal ligation and puncture (CLP) at 20 h after T-H or sham operation. Five hours after CLP, plasma and tissue samples were analyzed for cytokines (IL-6 and IL-10), MPO, neutrophil chemotactic factor (CINC-3), and liver injury (alanine aminotransferase and lactate dehydrogenase). In another group of rats, the gangrenous cecum was removed at 10 h after CLP, the cavity was irrigated with warm saline and closed in layers, and mortality was recorded over 10 days. T-H followed by CLP produced a significant elevation in plasma IL-6 and IL-10 levels, enhanced neutrophil cell activation, and resulted in liver injury. Adiol administration prevented the increase in cytokine production, neutrophil cell activation, and attenuated liver injury. Moreover, rats subjected to the combined insult, receiving vehicle or adiol, had a 50% and 6% mortality, respectively. Since adiol administration suppresses proinflammatory cytokines, reduces liver damage, and decreases mortality after the combined insult of T-H and sepsis, this agent appears to be a novel adjunct to fluid resuscitation for decreasing T-H-induced septic complications and mortality.

liver enzymes; proinflammatory cytokines; neutrophils activation; myeloperoxidase

Despite numerous advances in intensive care medicine, sepsis and organ dysfunction remains the major cause of death in trauma patients as well as in patients following major surgery (6, 15, 37). A significant complicating factor following severe hemorrhage is the development of sepsis (5, 11, 23, 27). A growing body of data supports the notion that the prior hemorrhagic shock can significantly modulate the inflammatory response to a subsequent septic challenge. This is consistent with the “two-hit” model in which a major injury, such as hemorrhagic shock or trauma, acts as the “first hit” and sets up the host to manifest a suppressed or abnormal response to a secondary insult, such as the increased translocation of intestinal microbial or other such factors (8, 9, 13, 16).

The depressed organ perfusion and excessive production of proinflammatory mediators may play an important role in the development of multiple organ dysfunctions following hemorrhagic shock and sepsis (15, 21, 31). It has also been reported that systemic IL-6 levels increased after trauma-hemorrhage (T-H), and a sustained elevation in plasma IL-6 levels correlated with the evolving organ dysfunction (2, 9, 17, 38). Clinical data also confirmed that the increased IL-6 production is tied to a poor outcome (24).

Studies have demonstrated that the IL-6-mediated elevation in nitric oxide production is likely one of the detrimental effects induced by this cytokine. It has been reported that nitric oxide is a determinant of IL-6-mediated negative inotropic effects on the heart (10). Yu et al. (39) also demonstrated that IL-6 decreases cardiac contractility and enhances the synthesis of the inducible nitric oxide synthase (iNOS), resulting in an excess of nitric oxide production. In support of these findings, our studies have demonstrated that there was a close correlation between organ dysfunction and the elevation in plasma IL-6 levels (1–4, 13, 14, 17, 19, 38). Our recent study also indicated that the depressed cardiac function following T-H is associated with an increased in situ IL-6 production by the cardiomyocytes (38). The causal role of IL-6 in organ dysfunction has been further established by studies that showed that administration of anti-IL-6 antibodies improved organ function following T-H (34).

Androstenediol (adiol or 5-androstene-3β,17β-diol), a metabolite of dehydroepiandrosterone (DHEA), was shown to have greater protective capacity than DHEA on lethal bacterial infections and endotoxin shock (4). Additionally, it is reported that the conversion of DHEA leads to an increase in downstream effector hormones in macrophages, which may play an important role in local immunomodulation (25). Administration of adiol following T-H has also been shown to be effective in reducing plasma IL-6 production and improving cardiovascular and hepatic function (29, 30).

Although adiol has been reported to produce the above-mentioned salutary effects following T-H or endotoxin shock, it remains unknown whether its salutary effects are also evident in a two-hit model of T-H and sepsis. Therefore, the present
study was undertaken to determine whether administration of adiol has any salutary effects on inflammatory mediators and on the survival of animals following T-H and sepsis. We hypothesized that adiol would have salutary effects on organ function and cytokine production even after a compound insult such as T-H and sepsis.

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 groups. Following cannulation of the femoral arteries and a femoral vein, the animals were divided randomly into two groups, one group undergoing a T-H protocol and the second group in which only a sham operation was performed. These animals were then further divided into two subgroups as follows: at the end of resuscitation (or at the matching time point after sham operation), one subgroup was treated with androstenediol (adiol, 1 mg/kg body wt; Steraloids, Newport, RI), and the other group received the same volume of vehicle (vehicle, Intralipid, 1 ml/kg body wt; Sigma, St. Louis, MO). Twenty hours after the T-H or sham operation, the animals were anesthetized again, and cecal ligation and puncture was performed in both T-H and sham animals as described previously (5).

T-H procedure. A nonheparinized model of T-H, as previously described, was used in this study (17). Briefly, male Sprague-Dawley rats were fasted overnight before the experiment but allowed water ad libitum. Animals were anesthetized using isoflurane (Attane; Minrad, Louis, MO). Twenty hours after the T-H or sham operation, the animals were anesthetized again, and cecal ligation and puncture was performed in both T-H and sham animals as described previously (5).

RESULTS

Cytokine production and iNOS expression. Five hours after CLP, animals of the T-H + vehicle + CLP group had significantly higher plasma IL-6 and IL-10 levels compared with CLP + vehicle (Figs. 1A and 2A). In addition, the IL-6 and IL-10 mRNA expressions were also elevated following a...
combined insult of T-H and CLP in the liver, heart, and lung in the vehicle group (Figs. 1, B–D, and 2, B–D). The adiol-treated T-H group subjected to CLP showed attenuated plasma IL-6 and IL-10 levels similar to shams. In addition, mRNA transcripts of the cytokines in this group also remained at a significantly lower level in the heart and the lung. In the liver, the IL-6 transcription was also reduced significantly, but the reduction in the IL-10 mRNA content did not reach statistical difference.

In the group of animals not undergoing T-H prior to CLP, adiol did not induce significant alterations in plasma IL-6 or IL-10 levels. In the lung, IL-6 mRNA expression was significantly reduced in the adiol-treated T-H group subjected to sepsis compared with CLP group receiving vehicle. The expression of IL-6 and IL-10 mRNA in the liver, heart, and lung were not affected by adiol treatment.

Similar to the cytokine release, iNOS expression in liver, heart, and lung was also significantly upregulated following T-H and subsequent CLP. Adiol administration following T-H significantly prevented the increase of iNOS mRNA expression in the liver, heart, and lung (Fig. 3, A–C).

Neutrophil accumulation in the lung and the heart. MPO activity, an indicator of neutrophil accumulation, was determined in the lung and heart samples. Five hours after CLP, animals in the T-H group receiving vehicle following T-H had significantly higher heart and lung MPO levels compared with CLP animals receiving vehicle and not undergoing prior T-H (Fig. 4, A and B). In addition, adiol treatment significantly reduced MPO levels in both lung and heart following a combined insult of T-H and CLP. Adiol did not influence MPO activity significantly in the CLP group not subjected to prior T-H.

Neutrophil CINC-3 in the liver, lung, and heart. There was a significant increase in CINC-3 levels in the liver and the heart...
following T-H and CLP (Fig. 5, A and B). Similarly, lung CINC-3 levels also displayed a trend for enhanced production, but the increase in lung CINC-3 levels following a combined insult of T-H and CLP did not reach statistical significance (Fig. 5C). Treatment of rats with adiol prevented the increase in CINC-3.

Liver injury. Following the combined insult of T-H and CLP, plasma LDH (Fig. 6A) and ALT (Fig. 6B) levels were significantly higher than in the groups receiving CLP alone. Plasma LDH was reduced significantly in the adiol-treated T-H group subjected to CLP; however, it remained at a higher level than the levels in the adiol + CLP group. A small but insignificant reduction in ALT level was also observed in the former group. Furthermore, adiol did not influence plasma LDH and ALT levels in animals receiving CLP alone in the absence of T-H.

Survival rate. CLP alone resulted in no mortality within the 10-day survival observation period (Fig. 7). The animals in the T-H group subjected to CLP had a mortality rate of 50% within 3 days after CLP. Adiol treatment, however, improved the survival rate in the combined model of T-H + CLP, and the survival rate was similar to that observed in the CLP group that did not undergo prior T-H.

DISCUSSION

The present results indicate that adiol administration reduces cardiac IL-6 and iNOS gene expressions following the combined insult of T-H and sepsis. Since IL-6 plays a major role in the development of multiple organ failure following hemorrhage, it is possible that adiol treatment would improve the survival of animals subjected to T-H and sepsis, via reducing IL-6. The present results also indicate that the higher IL-6 levels were associated with higher mortality and that lower levels of IL-6 with adiol administration were associated with decreased mortality. Previous studies from our laboratory have shown that Kupffer cells are the major source of circulating IL-6 following T-H as well as sepsis (3, 22). In accordance with these data, Kupffer cells isolated from posthemorrhaged or postseptic animals also showed a markedly higher IL-6 production in vitro (16). Our preliminary study indicates that the Kupffer cells isolated from T-H rats subjected to subsequent sepsis also showed an increased IL-6 production, which was decreased to sham levels by administration of adiol following T-H (unpublished observations). Our recent study has shown that following T-H, IL-6 production by cardiomyocytes locally can also significantly produce cardiac depression under those conditions (38). The present study shows that IL-6 mRNA levels in the heart and plasma IL-6 levels also increased significantly in the group subjected to T-H and sepsis. Studies have also shown that high IL-6 levels decreased cardiac contractility, and this was associated with an enhanced synthesis of iNOS and increased NO production by the myocytes (39). In this regard, the significance of iNOS induction and the deleterious effects of excessive NO on contractile function has been well-demonstrated (28, 33). In addition, the contribution of iNOS in the induction of organ injury following hemorrhage or septic insult has also been suggested (5, 7, 18, 31, 32). Our recent findings revealed that adiol administration following T-H decreased plasma IL-6 levels and improved cardiac func-

Fig. 4. Effects of adiol treatment on neutrophil accumulation in the heart (A) and lung (B). Tissue myeloperoxidase activities were measured at 5 h after CLP; n = 6 animals/group. *P < 0.05 vs. corresponding CLP alone group. #P < 0.05 vs. corresponding Veh-treated group.

Fig. 5. Effect of adiol treatment on tissue cytokine-induced neutrophil chemoattractant (CINC-3) levels in the liver (A), heart (B), and lung (C) at 5 h after CLP; n = 5–6 animals/group. *P < 0.05 vs. corresponding CLP alone group.
tion (29). However, that study did not examine the effects of adiol following a combined insult of T-H and induction of sepsis, which was examined in this study. Previous studies from our laboratory have also shown a correlation between the adiol-mediated attenuation of iNOS expression and reduction in hepatic injury (30). Although the significance of iNOS induction still remains unclear, it is likely that the observed downregulation of iNOS gene expression in the liver, heart, and lung in this study contributes to the adiol-mediated salutary effects on organ functions following T-H and the induction of subsequent sepsis.

The results indicate that MPO activity in the heart and lung increased significantly in the combined model of T-H and sepsis, indicating neutrophil activation under those conditions. In contrast, the MPO activity was unaffected in the heart as well as in the lung at 5 h after the onset of sepsis. However, since we did not use normal rats for comparison, it could be argued that the MPO was indeed increased with sepsis alone. Although this may be so, the fact remains that adiol had no effect on heart or lung MPO in the model of sepsis alone, thereby suggesting that 5 h may be too early for sepsis in itself to produce neutrophil activation. Further support for the notion that 5 h after the onset of sepsis without prior T-H did not produce any significant adverse effects comes from the finding that liver LDH and ALT levels were also not increased at that interval without or with adiol administration. These results are in accordance with our previous results that showed that the liver enzymes were not increased at 5 h after CLP (35). Furthermore, CINC-3 was not affected significantly at 5 h after CLP, and although CINC-3 levels were detected in the liver and lung, the levels were not affected by adiol, again suggesting that these are normal levels of CINC-3 in the liver and lung. Altogether, the results as summarized in this manuscript clearly suggest that adiol prevents both the inflammatory response and organ dysfunction following a combined insult of T-H and CLP.

A number of studies have also shown that IL-10 is capable of inhibiting the production of macrophage-derived proinflammatory cytokines. Since ablation of Kupffer cells by gadolinium chloride also decreased circulating IL-10 levels following T-H, it indicates that Kupffer cells also represent the major cellular source of elevated circulating IL-10 levels under those conditions (26). In view of these results, it can be suggested that adiol-induced salutary effects are mediated, at least in part, by a decreased cytokine production by Kupffer cells. Although the precise mechanism responsible for the salutary effects of adiol on cytokine production remains unknown, it is possible that adiol mediates its salutary effects via the estrogen receptor(s). In this regard, studies have indicated that estrogen-like activity of adiol is observed at physiological concentration in breast cancer cells (20). Furthermore, adiol causes an increase in estrogen receptor-dependent β-galactosidase activity in yeast (20). Previous studies from our laboratory have shown that proestrus female mice, which have high circulating levels of estrogen and progesterone, have a significantly lower mortality following T-H and the induction of subsequent sepsis (9) or sepsis alone (40) than male counterparts. Moreover, the salutary effects of DHEA (a parent compound of adiol) on organ functions are abolished if the estrogen receptor antagonist ICI 182,780 is administered with DHEA (12). This suggests that in addition to adiol, DHEA also mediates its effects via the estrogen receptor(s) (12).

Another potential mechanism of the salutary effect of adiol could be the activation of peroxisomes. Waxman (36) has demonstrated that adiol can activate peroxisome proliferator gamma (PPAR-γ). Activated PPAR-γ could reduce organ injury in T-H shock and systemic inflammation in polymicrobial sepsis (1, 41). Our recent study indicated that adiol treatment following T-H activated PPAR-γ, and treatment with PPAR-γ antagonist prevented the salutary effects of adiol under those conditions (unpublished observations). Thus it is likely that adiol produces its salutary effects via PPAR-γ; however, more studies are needed to delineate the precise mechanism of the salutary effects of this compound following T-H and/or sepsis.

Previous studies have shown that CLP alone induces an inflammatory response at a time point later than 5 h, causes organ dysfunction, and potentiates the inflammatory response following T-H (9, 13, 16, 35). However, CLP-induced inflammatory response in the absence of T-H was not observed at an
early time point (i.e., 5 h after CLP). The primary reason to perform CLP in this study was not to determine the effect of CLP on the inflammatory response or organ function, rather its potential effect on the post-T-H inflammatory response and organ function. Since at 5 h there was no effect of CLP on inflammatory response, we selected this time point to be optimum to differentiate between the responses induced by CLP and T-H alone as well as the responses induced in the event that the two, CLP and T-H, are combined. Thus measurement at 5 h after CLP has allowed us to determine the effect of CLP on the T-H-induced inflammatory response and organ function and whether those potential effects are prevented in adiol-treated animals.

Our studies suggest that T-H followed by CLP produced a significant elevation in plasma IL-6 and IL-10 levels, enhanced neutrophil infiltration in lung and heart, and caused liver injury. These alterations in inflammatory mediators accompanied with an increase in mortality following T-H and sepsis. Adiol administration suppresses proinflammatory cytokines and decreases mortality rates following T-H and sepsis. The improved survival from sepsis by adiol treatment following T-H appears to be due to decreased organ damage and improved organ functions. We found that these improvements are accompanied with a reduction in IL-6 and/or iNOS production. Collectively, these findings suggest a relationship between increased inflammatory mediators and mortality following T-H and sepsis. Nevertheless, more studies are required to delineate the mechanism by which the decrease in inflammatory response results in an increase in antimicrobial defense. Furthermore, whether adiol modulation of global inflammatory response or any particular chemokine or cytokine is responsible for the salutary effects remains to be established. In summary, although the precise mechanism of the salutary effects of adiol requires further investigations, our studies suggest that adjuvant use of adiol following T-H appears to be a novel approach for reducing the mortality rates following T-H and the occurrence of subsequent sepsis.

GRANTS

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