Androstenediol inhibits the trauma-hemorrhage-induced increase in caspase-3 by downregulating the inducible nitric oxide synthase pathway

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Kiang JG, Peckham RM, Duke LE, Shimizu T, Chaudry IH, Tsokos GC. Androstenediol inhibits the trauma-hemorrhage-induced increase in caspase-3 by downregulating the inducible nitric oxide synthase pathway. J Appl Physiol 102: 933–941, 2007. First published November 16, 2006; doi:10.1152/japplphysiol.00919.2006.—Soft tissue trauma and hemorrhage (T-H) diminishes various aspects of liver function, while it increases hepatic nitrate/nitrite, inducible nitric oxide synthase (iNOS), and endothelin-1 levels. Treatment with androstenediol (AED) inhibits the T-H-induced alterations of the above parameters. We sought to identify the molecular events underlying the beneficial effect of AED. Exposure of rats to T-H significantly increased the caspase-3 activity and protein, whereas treatment with AED significantly limited these increases. AED treatment also suppressed the T-H-induced increase in iNOS by effectively altering the levels of key transcription factors involved in the regulation of iNOS expression. Immunoprecipitation and immunoblotting analyses indicated that T-H increased apoptosome formation, and AED treatment significantly decreased it. Modulating the iNOS protein by transfecting cells with iNOS gene or small interfering RNA further confirmed the correlation between iNOS and caspase-3. Our data indicate that AED limits caspase-3 expression by suppressing the expression of transcription factors involved in the production of iNOS, resulting in decreased apoptosome. AED can potentially be a useful adjuvant for limiting liver apoptosis following T-H shock.

SEVERE HEMORRHAGIC SHOCK, EVEN with resuscitation, results in depressed liver blood flow (51) that appears to contribute to liver injury and an inflammatory response. It is evident that maintenance of liver circulation after hemorrhagic shock is critical in limiting liver injury (49, 57). The maintenance of liver circulation relies partly on endothelin-1 and nitric oxide (NO) (2, 27, 37, 43, 58). However, an increased inducible NO synthase (iNOS) in hemorrhaged liver has been reported (8, 11, 23, 43, 47, 48). Treatment of animals with iNOS inhibitors and experiments in iNOS gene knockout mice have shown that iNOS derived NO participates in liver injury and in the inflammatory cascade produced by hemorrhage (15, 20, 30, 31).

Androstenediol (AED) is one of the metabolites of the most abundant steroid hormone in plasma dehydroepiandrosterone and is an intermediate in the pathway for the synthesis of testosterone and estrogen. AED has been reported to have protective effects against ionizing radiation-induced injury in mice (28, 55), lethal bacterial infections, and endotoxic shock (35). AED has also been shown to preserve cardiovascular function (42) and hepatic function (44) after trauma plus hemorrhage (T-H), to reduce injury from behavioral stress (14), to promote wound repair (14), and to attenuate inflammation caused by viral infection (5). AED treatment also inhibits hemorrhage-induced decreases in portal blood flow, bile production, and serum albumin levels, and increases portal pressure, hepatic nitrate/nitrite, iNOS, and endothelin-1 levels (43). Although it has been suggested that the decrease in endothelin-1 and the induction of endothelial NO synthase improve hepatic perfusion, downregulation of iNOS decreases liver damage. The underlying mechanism of protective effects of AED has not been elucidated, however.

It is known that many caspases are involved in the intrinsic pathway of apoptosis in which caspase-3 plays a key role in triggering the occurrence of apoptosis (17, 18, 24). Caspase-3 is an aspartate-specific cysteiny1 protease. It is evident that hemorrhage increases caspase-3 cellular activity (22, 23) and decreases cellular ATP levels in various tissues (6, 7, 20, 23, 38, 50). Agents that increase inducible heat shock protein 70 kDa and inhibit iNOS have been shown to ameliorate the hemorrhage-induced cell injury (19–21). In this study, we...
examined the mechanisms whereby AED confers liver protection in animals subjected to soft tissue trauma followed by hemorrhage (T-H). We report that AED limits the T-H-induced increases in liver caspase-3 and apoptosome formation by limiting the expression of iNOS at the transcriptional level. AED can potentially be a useful adjuvant for inhibiting liver apoptosis following T-H.

MATERIALS AND METHODS

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996 edition. The animal protocol was reviewed and approved by Institutional Animal Care and Use Committee at University of Alabama.

Experimental protocols. A nonheparinized rat model of soft tissue T-H was used in this study (49). Male Sprague-Dawley rats [body weight (BW): 275–325 g; Charles River Laboratories, Wilmington, MA] were fasted overnight before the experiment, but allowed water ad libitum. The rats were anesthetized using isoflurane (Attane Minrad, Bethlehem, PA) inhalation. A 5-cm midline laparotomy was then performed to induce soft tissue trauma. Following this, the abdomen was closed in layers, and both femoral arteries and the right femoral vein were cannulated with PE-50 tubing (Becton-Dickinson, Sparks, MD). The animals were then restrained in a supine position, and the area of incision was bathed with 1% lidocaine (Elkins-Sinn, Cherry Hill, NJ).

Fig. 2. AED decreases T-H-induced increases in active and inactive forms of caspase-3. Using immunoblotting analysis, we measured caspase-3 levels, phosphorylation of Akt, and actin abundance in lysates of liver of sham-operated (n = 4), T-H-treated (n = 4), AED-treated (n = 4), and T-H + AED-treated (n = 4) rats. Their specific bands of proteins were quantitated densitometrically and normalized against actin. A: representative Western blots are presented. B: AED decreased T-H-induced increase in inactive caspase-3 protein. C: AED decreased T-H-induced increase in active caspase-3 protein. *P < 0.05 vs. sham-operated group, AED group, and T-H + AED group; **P < 0.05 vs. sham-operated group and T-H group, determined by χ² test. STD, standard.

Fig. 3. AED inhibits T-H-induced increases in inducible nitric oxide synthase (iNOS) and Kruppel-like factor (KLF)-6 and decrease in KLF-4. Using immunoblotting analysis, proteins of iNOS, KLF-4, KLF-6, and actin were assessed in lysates of liver of sham-operated (n = 4), T-H-treated (n = 4), AED-treated (n = 4), and T-H + AED-treated (n = 4) rats. Their specific bands of proteins were quantitated densitometrically and normalized with actin protein. A: representative Western blot is presented. B: AED increased KLF-4. *P < 0.05 vs. sham, T-H, and T-H + AED; **P < 0.05 vs. sham, T-H, and AED, determined by χ² test. C: AED decreased iNOS. D: AED decreased KLF-6. *P < 0.05 vs. sham group, AED group, and T-H + AED group; determined by χ² test.
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Hill, NJ) to minimize postoperative pain. The rats were then allowed to awaken and were rapidly bled to a mean arterial pressure (MAP) of 35–40 mmHg within 10 min. The time at which the animals could no longer maintain a MAP of 35–40 mmHg without infusing some fluid was defined as maximum bleed-out volume. The rats were maintained at this MAP until 40% of the shed blood was returned in the form of Ringer lactate (LR). The animals were then resuscitated with four times the volume of shed blood with LR over 60 min. Following 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 arteries and cannulation of the femoral vein, but the animals were neither subjected to T-H nor resuscitated. The animals were returned to their cages and were allowed food and water ad libitum and were killed 24 h after the end of resuscitation. All rats subjected to this protocol survived until euthanization.

In the treatment group, AED (1 mg/kg BW; Steraloids, Newport, RI) was administered intravenously immediately at the end of the resuscitation. In the vehicle-treated group (control group), rats received the same volume of vehicle (Intralipid, 1 ml/kg BW; Sigma, St. Louis, MO). A small portion of liver was removed from the treated rats and frozen immediately at −70°C until used for immunoblotting and biochemical assays.

Tissues were first minced and then sonicated for 15 s and then centrifuged at 10,000 g for 10 min. The supernatant was saved for determining the total amount of protein in each lysate sample and performing immunoblotting analysis. The cellular caspase-3 activity was measured. Proteins and moieties were assessed by immunoblotting.

Cell culture. Human intestinal epithelial T84 cells, Hep60 cells, FHs74 Int cells, and CRL-1550 cells (American Type Cell Culture, Rockville, MD) were grown in DMEM, hybrid-care medium, and RPMI-1640, respectively, in a humidified incubator with a 5% CO2 atmosphere. Each medium was supplemented with 2 mM glutamine, 4.5 g/l glucose, and 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin, pH 7.4 (Quality Biological, Gaithersburg, MD). Cells were fed every 3–4 days.

iNOS gene construct. iNOS gene was used in this study. cDNA of human iNOS was obtained from Dr. N. Tony Eissa (Baylor College of Medicine, Houston, TX). A 3,362-bp coding sequence with Hind III and Xho I restriction sites at each terminals of human iNOS gene was subcloned from this full length of human iNOS cDNA. With the use of PCR application, the forward and reverse primers were 5′-CT AAG GCT ATG GCC GGC TGT CCT GGG AAA TTT CTG TTC-3′ and 5′-GAC TCG AGC TCA GAG CGC TGA CAT CTC CAG GCT-3′, respectively. After digestion and purification of the PCR amplification product, the expression cDNA sequence was used for insertion into the vector. The vector used in this study was pcDNA3 (Invitrogen, San Diego, CA). The expression cDNA of iNOS gene was inserted between the Hind III and Xho I sites of pcDNA 3.1 vector. The expression construct then was sequenced to confirm its correct sequence and open reading frame (20).

Transient gene transfection. Cultured cells (1.5 × 10⁵) were grown in six-well plates. Cells in each well added with 4-μg iNOS expression plasmid in 0.5-ml antibiotic-free growth medium using Lipofectamine 2000 transfection kit (Invitrogen). The equal amount of cells was also mixed with blank pcDNA 3.1 vector as a control group. Then 1.5-ml DMEM was added to each well, and the cells were placed back into the incubator for 24 h to allow the transient transfection.

iNOS small interfering RNA transfection. To decrease iNOS protein, RNA interference technology was used. Two designed pairs of oligoduplexes targeted against iNOS were purchased from Qiagen (Valencia, CA). The target sequences of those oligoduplexes are NOS-S sense strand, 5′-ACACAAGGAGACCUACCAGCTT-3′, and NOS-AS antisense strand, 5′-GCUGUAGGUGUCCGUUGUTT-3′, respectively. A nonspecific oligoduplex (nonsilencing control, target-
once with 500-μl PBS wash buffer. The pellet was resuspended in the 50 μl of electrophoresis sample buffer without 2-mercaptoethanol, boiled for 5 min, then centrifuged for 30 s to remove the agarose beads. The supernatant was incubated with 5% 2-mercaptoethanol at 37°C for 1 h. Twenty-five microliters of sample were loaded onto precast 10% Tris-glycine polyacrylamide gels for Western blots.

**Western blots.** Samples were resolved on SDS-polyacrylamide slab gels (precast 10% gel, Invitrogen). Protein was blotted onto a nitrocellulose membrane (type NC, 0.45 μm, Schleicher and Schuell) using a Novex blotting apparatus and the manufacturer’s protocol. The nitrocellulose membrane was blocked by incubation for 90 min at room temperature in PBS containing 5% nonfat dried milk. The blot was then incubated for 60 min at room temperature with the selected antibody against actin, Kruppel-like factor (KLF)-4, KLF-6, NFκB-p65, NFκB-p50, iNOS, p53, Bcl-2 (Santa Cruz Biotechnology), inactive caspase-3, active caspase-3, caspase-9 (Epitomics, Belmont, CA), and cytochrome c (Upstate, Lake Placid, NY), at 1 μg/ml in PBS-5% BSA. The blot was washed three times (10 min each) in Tris-buffered saline-0.1% Tween 20 before incubating for 60 min at room temperature with a ×1,000 dilution of species-specific IgG peroxidase conjugate (Santa Cruz Biotechnology) in PBS-1% gelatin. The blot was washed six times (5 min each) in Tris-buffered saline-0.1% Tween 20 before detection of the peroxidase activity using the Enhanced Chemiluminescence kit (Amersham Life Science Products, Arlington Heights, IL).

**Measurements of caspase-3 activity.** Caspase-3 activity was determined using the CASCAP-3 Cellular Activity Assay Kit PLUS (Biomol, Plymouth Meeting, PA). Change in absorbance was measured at 405 nm with a SpectraMax plate reader and SOFTmax Pro 3.1.1 software (Molecular Devices, San Diego, CA). Data were normalized to total protein, and caspase-3 activity was expressed as picomoles pNitroaniline per minute per microgram protein.

**Statistical analysis.** All data are expressed as means ± SE. One-way ANOVA, χ² test, and Student’s t-test were used for comparison of groups with 5% as a significant level.

**RESULTS**

**T-H induces dysfunction in liver.** Our laboratory has found that hemorrhage alone does not lower liver biochemical parameters, such as cellular ATP and caspase-3 enzymatic activity (20, 22). Addition of trauma to hemorrhage induces a significant ATP loss by 88 ± 2% and a marked elevation of caspase-3 by threefold (Fig. 1). Therefore, T-H becomes a useful model for studying liver under severe assaults (43). In Chaudry’s laboratory, T-H has also been shown to increase portal pressure, alanine transferase, and NO production; AED treatment effectively limits these changes (43). Both groups of rats treated with T-H alone and with T-H plus AED all survived 24 h after resuscitation. Liver tissues were analyzed to investigate the effect of T-H on caspase-3 and its relationship to iNOS.

**AED decreases T-H-induced increases in cellular caspase-3 enzymatic activity.** Hypoxia has been shown to alter cellular caspase-3 enzymatic activity in human cultured cells (25), rat ileum and lung (23), and mouse jejunum, lung, heart, kidney, and brain (21), and it is considered to be a reliable biomarker of apoptosis. We decided to determine whether T-H altered caspase-3 enzymatic activity in rats subjected to T-H. T-H increased cellular caspase-3 enzymatic activity in liver tissue by 332 ± 21% (P < 0.05 vs. sham controls). When rats subjected to T-H were treated with AED, the caspase-3 enzymatic activity returned to basal levels in the liver. AED alone did not alter the basal enzymatic activity of caspase-3 protein (Fig. 1). Therefore, treatment with AED eliminated the T-H-induced increase in cellular caspase-3 enzymatic activity.

**AED treatment attenuates T-H-induced increases in caspase-3 expression in the absence of Akt phosphorylation.** To determine whether the T-H-induced increase in cellular caspase-3 activity was due to the increased caspase-3 protein, we measured caspase-3 levels in liver tissue lysates of animals subjected to T-H and sham-operated animals. Furthermore, to determine whether the T-H-induced increase in caspase-3 activity involved preferential increases in the inactive or active form of caspase-3, we blotched electrophoretically separated liver lysates with antibodies against the 35-kDa (inactive) and 17-kDa (active) forms of caspase-3. Both the inactive and active forms of caspase-3 were increased in the livers of T-H-treated animals (Fig. 2). The levels of inactive and active
forms of caspase-3 were increased by 216 ± 5 and 246 ± 4%, respectively, in the livers of T-H-treated animals compared with sham-handled animals (P < 0.05 vs. sham controls). Treatment with AED alone reduced caspase-3 levels by only 50 ± 12 and 25 ± 3%, respectively (P < 0.05 vs. sham controls). Similarly, treatment with T-H and AED also diminished caspase-3 levels by 50 ± 2 and 34 ± 18%, respectively (P < 0.05 vs. sham controls; Fig. 2, B and C).

Because increased caspase-3 activity has been attributed to the phosphatidylinositol 3-kinase/Akt pathway (17), we determined Akt phosphorylation at Ser473 and Thr308 by immunoblotting. We failed to detect Akt phosphorylation at serine or threonine residues, which indicates that a pathway other than phosphatidylinositol 3-kinase/Akt is responsible for the increase in caspase-3 (Fig. 2A).

**AED inhibits the T-H-induced increases in iNOS.** It has been established that hemorrhage upregulates the expression of iNOS in mouse jejunum, lung, heart, kidney, and brain (21), and rat ileum and lung (23). Because inhibition of iNOS results in decreases in caspase-3 activity in human intestinal cells and Jurkat T cells (25), we asked whether AED could reduce the T-H-induced altered expression of iNOS. Indeed, T-H increased iNOS in the liver (Fig. 3, lane 2), and AED treatment significantly inhibited this increase (Fig. 3, lane 4). This observation suggests that the effect of AED on the caspase-3 activity may be related to the effect of AED on the expression of iNOS.

It has been known that KLF-4 and KLF-6 are transcriptional factors that control the expression of the iNOS gene. Specifically, KLF-6 upregulates, whereas KLF-4 has been shown to downregulate, iNOS gene expression at the transcriptional level (21, 23, 54). In the rat liver, T-H increased KLF-6 and decreased KLF-4. Treatment with AED decreased KLF-6 and increased KLF-4 (Fig. 3). It appears, therefore, that the effect of AED on iNOS is mediated through effects on transcription factors known to control the expression of iNOS.

**AED inhibits T-H-induced increase in NFκB.** The promoter region of iNOS gene has 10 NFκB binding sites. Hemorrhage increases NFκB binding to the iNOS gene, thereby leading to an increased iNOS gene expression (40). We found that T-H increased both p65 and p50 units of NFκB in the liver tissues (Fig. 4A, lane 2). Treatment with AED vastly decreased both proteins (Fig. 4A, lane 4). Like KLF-4 and KLF-6, it appears that the effect of AED on iNOS gene expression is also mediated by preceding effects on NFκB (Fig. 4), another transcription factor known to control iNOS gene expression (56).

**T-H increases apoptosis formation.** Caspase-3 enzymatic activity has been shown to be activated by complexes of cytochrome c and caspase-9, termed the “apoptosome” (17, 18). Therefore, we used immunoprecipitation and immunoblotting analysis to detect and measure the apoptosome. The complexes were detected in liver lysates immunoprecipitated with anti-caspase-9 antibody first and then immunooblotted with anti-cytochrome c antibody. Lysates from T-H liver displayed an increase in cytochrome c protein (Fig. 5A). To further confirm the presence of the complex formation between caspase-9 and cytochrome c, we immunoprecipitated the lysate with anti-cytochrome c antibody and immunooblotted with anti-caspase-9 antibody. Lysates from T-H liver also displayed an increase in caspase-9 (Fig. 5B), suggesting that T-H results in an increased apoptosome formation, thereby leading to an increased caspase-3 enzymatic activity.

**AED inhibits T-H-induced increase in apoptosome formation.** Treatment with AED at the end of the resuscitation period inhibited the increase in apoptosome formation in liver of T-H-treated rats (Fig. 5). Liver lysates immunoprecipitated with anti-caspase-9 antibody and immunoblotted with anti-cytochrome c antibody exhibited a significantly low level of cytochrome c protein (Fig. 5A). Similarly, lysates immunoprecipitated with anti-cytochrome c antibody and immunoblotted with anti-caspase-9 antibody also exhibited a low level of caspase-9 protein (Fig. 5B).

Fig. 6. AED fails to block T-H-induced decreases in p53 and Bcl-2. Using immunoblotting analysis, proteins of p53, Bcl-2, and actin were assessed in lysates of liver of sham-operated (n = 4), T-H-treated (n = 4), AED-treated (n = 4), and T-H+AED-treated (n = 4) rats. Their specific bands of proteins were quantitated densitometrically and normalized with actin protein. A: representative Western blot is presented. B: AED did not block the T-H-induced decrease in p53. C: AED did not block the T-H-induced decrease in Bcl-2. *P < 0.05 vs. sham group and AED group; determined by χ² test. **P < 0.05 vs. sham-operated group, T-H-treated group, and T-H+AED-treated group, determined by χ² test.
AED fails to inhibit T-H-induced decreases in p53 and Bcl-2. The p53 is a proapoptosis protein, and Bcl-2 is an antiapoptosis protein. Hemorrhage alone for 1 h has been shown to increase mRNA of p53 and Bcl-2 in rat lung and Bcl-2 in ileum (23). We wanted to determine whether p53 and Bcl-2 were altered 24 h after T-H treatment. Figure 6 shows that T-H significantly decreased p53 and Bcl-2, whereas the AED treatment failed to correct the observed decreases, suggesting that the AED inhibitory action is rather specific to the iNOS pathway.

iNOS protein regulates the level of caspase-3 activity. To verify the correlation between the protein level of iNOS and the caspase-3 activity, cells were transiently transfected with iNOS gene. Western blotting shows increased iNOS protein after transfection for 24 h (Fig. 7A, lane 1). No iNOS protein was detected in control cells (Fig. 7A, lane 2) or vector-transfected cells (Fig. 7A, lane 2). The caspase-3 activity in iNOS gene-transfected cells increased by twofold (P < 0.05 vs. control and vector transfected).

To further confirm this correlation, RNA interference technology was adopted. Cells were treated with iNOS siRNA 24 h before hypoxia. The hypoxic cells in the absence of iNOS siRNA displayed increases in iNOS (Fig. 7B, lane 2) and caspase-3 enzymatic activity (Fig. 7B). Treatment with iNOS siRNA before hypoxia prevented these increases (Fig. 7B), suggesting that iNOS regulates caspase-3.

DISCUSSION

Hemorrhagic shock invariably causes delayed pathology, including multiple organ dysfunction (3, 19). Pathology results from distinct molecular processes, and it is believed that reversal or prevention of the molecular abnormalities should result in tissue and organ protection. Accordingly, in this study, we sought to investigate the molecular events that underwrite the known ability of AED (a metabolite of the most abundant steroid hormone in plasma, dehydroepiandrosterone) to protect liver damage in animals subjected to T-H.

We report herein that T-H causes significant increases in cellular caspase-3 activity and protein in the liver of rats subjected to T-H. Caspase-3 is an aspartate-specific cysteiny1 protease that plays a key role in cell apoptosis (17, 18). It has been shown that caspase-3 activity is increased by hypoxia (12, 25), ischemia (10, 36, 53), hemorrhage (9, 22, 26, 29, 32, 33, 60), and in other pathological conditions (1, 33, 34, 39, 46, 52). Interventions that lead to inhibition of caspase-3 activity (60), such as treadmill exercise (26), hypertonic saline resuscitation (33), or LR resuscitation (23), have been shown to reduce tissue apoptosis and brain damage in hemorrhaged animals. It is also evident that treatment of cells (25) or animals (21) with iNOS inhibitors results in decreased cellular caspase-3 activity. We found that AED treatment limits the expression of iNOS, a finding that implies that iNOS expression regulates the expression of caspase-3 through undetermined pathways. This conclusion is corroborated by previous findings (25) in human intestinal epithelial and T cells, which demonstrated that pharmaceutical manipulation of iNOS results in changes in caspase-3 expression. The fact that AED limits the increased expression caused by T-H of two iNOS transcriptional enhancers, KLF-6 and NFκB (p65 and p50 components), and prevents the decrease of the suppressor KLF-4, strongly suggests that AED controls the expression of iNOS by regulating the expression of known regulators of the iNOS gene transcription. NFκB activity has been reported increased also in septic (4) and hemorrhagic shock (40, 41). It is presumed that the affected transcription factor promoter regions define androgen response elements through which the transcription rate is

![Fig. 7. Correlation between the iNOS level and the caspase-3 activity. Human CRL1550 intestinal cells were transfected with iNOS gene or treated with iNOS small interfering RNA (siRNA). A: iNOS gene transfection increased iNOS protein and caspase-3 enzymatic activity (n = 3). *P < 0.05 vs. control (CON) and vector-treated groups, determined by one-way ANOVA. B: treatment with iNOS siRNA decreased iNOS and caspase-3 enzymatic activity. Cells were treated with nonsilencing oligo or iNOS siRNA for 24 h, then exposed to 10 mM NaCN for 1 h to allow hypoxia to occur, and recovered for 23 h to allow iNOS upregulation (n = 3). *P < 0.05 vs. CON group, siRNA-treated group, and siRNA + hypoxia (HX)-treated group, determined by χ2.

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AED treatment inhibits KLF-6 and NFκB, activating caspase-3 (18), leading to apoptosis. The apoptosome therefore activates caspase-3 (18), leading to apoptosis. AED treatment inhibits KLF-6 and NFκB and increases KLF-4. Subsequently, iNOS expression is reduced followed by reduction in caspase-3 activity and apoptosis. The observation of the decreased p53 and Bcl-2 in liver resulted from T-H is not in agreement with that reported in jejunum and lung of rats exposed to hemorrhage (23) and cultured human cells exposed to NaCN-induced hypoxia (25). The discrepancy can be due to the differences of insults, the time to collect tissues after the insults, and types of tissues studied. Since AED failed to correct the decreased p53 and Bcl-2 in liver, it appears that the inhibitory action of AED on the iNOS pathway is rather specific.

A schematic representation of the finding of this study is shown in Fig. 8. T-H increases KLF-6 and NFκB and inhibits KLF-4, resulting in an increased expression of iNOS. This increase in iNOS leads to increase NO production (35) that reacts with O2• to form ONOO•. Peroxynitrite leads to mitochondrial swelling and release of cytochrome c that subsequently is caged by caspase-9 to form the apoptosome. The apoptosome activates caspase-3 (18), leading to apoptosis. AED treatment inhibits KLF-6 and NFκB and increases KLF-4. Subsequently, iNOS expression is reduced followed by reduction of caspase-3 activity and apoptosis.

In summary, AED, a metabolite of the most abundant steroid hormone in plasma dehydroepiandrosterone, protects rat liver from apoptosis caused by T-H. The protective effect of AED is mediated by its ability to regulate the transcription factors of iNOS and subsequently to inhibit apoptosome formation and cellular caspase-3 activity. We conclude that the liver protective effect of AED can potentially be a useful adjuvant for inhibiting liver apoptosis after T-H shock.

DISCLAIMER

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