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ARGINASE BLOCKADE PROTECTS AGAINST HEPATIC DAMAGE IN WARM ISCHEMIA-REPERFUSION

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Abstract

Background—Liver ischemia reperfusion (I/R) injury is associated with profound arginine depletion due to arginase release from injured hepatocytes. Nitric oxide (NO), shown to have protective effects in I/R, is produced by nitric oxide synthase (NOS) from the substrate arginine. The purpose of this study was to determine if nor-NOHA, a novel arginase inhibitor, would be able to increase circulating arginine levels and decrease hepatic damage following warm I/R.

Methods—C57BL/6 mice underwent partial liver warm I/R and were treated intraperitoneally with either nor-NOHA (100mg/kg) or saline. Serum and tissue samples were collected to measure liver enzyme levels, amino acids, and inflammatory mediators. The agent nor-NOHA (100 mg/kg) was administered 15 minutes before ischemia and immediately after reperfusion. Serum amino acid analysis was performed using HPLC.

Results—Arginase activity after hepatic I/R peaked at 3–6 h after reperfusion and resulted in a 10-fold drop in circulating arginine levels. Treatment with nor-NOHA inhibited arginase activity and reversed the arginine depletion after I/R while simultaneously increasing serum nitric oxide. In addition, circulating citrulline, a product of NOS activity, was increased in nor-NOHA-treated animals compared to controls. Inhibition of arginase also resulted in protection from hepatic I/R-induced damage in association with markedly lower hepatic TNF, IL-6, and inducible NOS mRNA levels compared to controls.

Conclusion—Arginase blockade represents a potentially novel strategy to combat liver injury under conditions of arginine deficiency. This protection may be mediated through the arginine-NO pathway.

Keywords

inflammation; nitric oxide; arginine; liver; arginase; ischemia reperfusion

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Introduction

Ischemia reperfusion (I/R) injury is a pathophysiologic process whereby hypoxic organ damage is accentuated following return of blood flow and oxygen delivery to the compromised tissue. Transient episodes of hepatic ischemia occur during solid organ transplantation, trauma, hypovolemic shock, and elective liver resection, when inflow occlusion or total vascular exclusion is used to minimize blood loss. The pathophysiology of liver I/R injury includes both direct cellular damage as the result of the ischemic insult as well as delayed dysfunction and damage resulting from activation of inflammatory pathways [1-4]. There is evidence that the L-arginine-nitric oxide pathway plays an important role in mediating this injury [5-7]. In liver preservation injury and various warm ischemia reperfusion models, nitric oxide has been shown to have effects ranging from alterations in perfusion through vasodilatory effects, antiinflammatory properties through inhibition of neutrophil activation, and various antiapoptotic properties. Given that L-arginine is the sole substrate for the family of nitric oxide synthases [8;9], modulating its availability may be an important therapeutic strategy by which to promote nitric oxide production.

Arginase-I, found primarily in the cytosol of liver parenchymal cells, catabolizes the hydrolysis of L-arginine to produce L-ornithine and urea. With any traumatic event, including liver I/R injury, arginase is released into the bloodstream and acts to deplete arginine while increasing ornithine production [10;11]. While supplementation with large amounts of L-arginine in I/R models improves liver injury, the arginine depletion from the release of arginase is not completely reversed [12;13]. Given the massive quantities of supplemental arginine required to provide even a modest benefit in decreasing liver injury, alternate strategies of increasing substrate availability for nitric oxide synthase were explored. Therefore, we hypothesized that an arginase inhibitor would increase arginine availability and improve liver injury by inhibiting the activity of circulating arginase in animals undergoing liver I/R.

The naturally occurring arginase inhibitor is N-omega-hydroxy-L-arginine (NOHA). It is produced as an intermediary step when L-arginine is metabolized by the NOS enzymes and is also a direct substrate for NOS [14;15]. Recently, a more potent synthetic analogue of NOHA was identified, N-omega-hydroxy-nor-L-arginine (nor-NOHA) [15-17]. This compound increases L-arginine availability by inhibiting arginase activity, but does not directly increase NO or citrulline production as it is not a substrate for NOS [15]. We chose to utilize this compound because of its low IC₅₀ and due to the fact that it does not directly interact as either a substrate or an inhibitor of NOS activity when compared to NOHA [15;18]. Previously we have shown in a cold I/R syngeneic rat liver transplant model that arginase inhibition reduces graft injury [4;19]. While many of the mechanisms of injury are shared between cold and warm I/R, the partial warm I/R model adopted for this study is more widely applicable to a multitude of clinical scenarios such as hypovolemic shock, elective liver resections, and trauma. Additionally, there are instances in which protective agents in cold I/R are not reproduced in models of warm hepatic I/R. In this study, we demonstrate that inhibition of the major catabolic enzyme of arginine restores circulating levels of arginine, increases substrate availability for NOS and decreases hepatic damage following warm I/R.

Experimental Procedures

Materials

N^G-omega-hydroxy-nor-L-arginine was purchased from Bachem Bioscience, Inc. King of Prussia, PA.

Animals

Male C57BL/6 mice (8-12 weeks old) were purchased from the Jackson Laboratory (Bar Harbor, ME). All animals were maintained in a laminar-flow, specific pathogen-free atmosphere at the University of Pittsburgh. Animal protocols were approved by the Animal Care and Use Committee of the University of Pittsburgh and the experiments were performed in adherence to the National Institutes of Health Guidelines for the use of Laboratory Animals.

Liver Ischemia

A non-lethal model of segmental (70%) hepatic warm ischemia was used. This I/R protocol was initiated with the abdominal wall being clipped of hair and cleansed with betadine. Under sodium pentobarbital (40 mg/kg, IP) and methoxyflurane (inhalation) anesthesia, a midline laparotomy was performed. With the use of an operating microscope, the liver hilum was dissected free of surrounding tissue. All structures in the portal triad (hepatic artery, portal vein, and bile duct) to the left and median liver lobes were occluded with a microvascular clamp (Fine Science Tools, San Francisco, CA) for 60 minutes, and reperfusion was initiated by removal of the clamp. This method of segmental hepatic ischemia prevents mesenteric venous congestion by permitting portal decompression through the right and caudate lobes. The abdomen was covered with a sterile plastic wrap to minimize evaporative loss. Throughout the ischemic interval, evidence of ischemia was confirmed by visualizing the pale blanching of the ischemic lobes. The clamp was then removed and gross evidence of reperfusion based on immediate color change was assured before closing the abdomen with continuous 4-0 polypropylene suture. Either the absence of ischemic color changes or the lack of response to reperfusion was a criterion for immediate sacrifice and exclusion from further analysis. Temperature was monitored by rectal temperature probe and was maintained at 37°C by means of a warming pad and heat lamp. At the end of the observation period following reperfusion, the mice were anesthetized with inhaled methoxyflurane and were sacrificed by exsanguination. Sham animals underwent anesthesia, laparotomy, and exposure of the portal triad without hepatic ischemia. Animals were sacrificed at predetermined time points after reperfusion for serum and liver samples.

Experimental design

Nor-NOHA (100 mg/kg) was given by intraperitoneal injection to animals 15 minutes prior to ischemia and immediately after reperfusion. Our selection of dosing regimen was based on previous testing of nor-NOHA in normal animals where doses up to 200 mg/kg IV $\times$ 2 resulted in no changes AST, ALT, or creatinine. Doses up to 400 mg/kg IV $\times$ 2 resulted in minimal increases in AST and ALT with no increase in creatinine [19]. Therefore the dose used in the current study (100 mg/kg I.V. $\times$ 2) was well below toxic doses.

Sodium Dodecyl Sulfate/Polyacrylamide Agarose Gel Electrophoresis

Cytosolic liver proteins were prepared as described [20] and quantitated with bicinchonic acid protein assay reagent (Pierce Chemical, Rockford, IL). Western blot analysis for arginase I protein was performed using 1 μ l of serum protein on 13% SDS-PAGE as described [20]. Primary polyclonal chicken antibody to rat arginase I (1:50,000; a generous gift from the Sidney M. Morris Jr., PhD, Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine) and secondary peroxidase-conjugated rabbit anti-chicken IgY (1:5,000; Jackson ImmunoResearch Laboratories, West Grove, PA) were used for the arginase I Western blot. Membranes were developed with the Super Signal West Pico chemiluminescent kit (Pierce, Rockford, IL) and exposed to film.

SYBR Green Real Time RT-PCR

Total RNA was extracted from whole liver using the TRIzol reagent (Life Technologies, Inc., Grand Island, NY) according to the manufacturer's instruction. mRNA for TNF α , IL-6, iNOS, eNOS, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was quantified in duplicate by SYBR Green twostep, real-time RT-PCR. After removal of potentially contaminating DNA with DNase I (Life Technologies), one μ g of total RNA from each sample was used for reverse transcription with an oligo dT (Life Technologies) and a Superscript II (Life Technologies) to generate first-strand cDNA. PCR reaction mixture was prepared using SYBR Green PCR Master Mix (PE Applied Biosystems, Foster City, CA) using the primers as previously described [21;22]. Thermal cycling conditions were 10 min at 95°C followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min on an ABI PRISM 7000 Sequence Detection System (PE Applied Biosystems). Each gene expression was normalized with GAPDH mRNA content.

Nitric Oxide Assay

Nitrate and nitrite are the stable degradation products of nitric oxide in vivo and provide a method to quantify nitric oxide levels in biological samples. Measurement of these products was done using the Colorimetric Non-enzymatic Assay for Nitric Oxide kit (Oxford Biomedical; Oxford, Michigan). Briefly, 50 μ L of serum was deproteinized using ZnSO $_4$ and subsequently incubated with cadmium to convert the nitrate to nitrite. The quantitation of total nitrite using the Griess Reagent is then accurate for total nitric oxide in the samples. The assay was performed according to protocol and data analyzed as suggested by manufacturer.

Arginase Activity Assay

L-arginine is hydrolyzed to ornithine and urea. Based on this principle, a colorimetric assay in which ornithine gives a color product with ninhydrin. One unit of arginase activity is defined as the amount of Mn $^{2+}$ activated enzyme that produces 1 μ mol of ornithine /min at 37°C [23; 24].

Histopathology

Formalin fixed liver samples were embedded in paraffin and cut to 6 μ m thick sections. Tissues were stained with hematoxylin and eosin and slides were assessed for inflammation and tissue damage.

Liver function tests

To assess hepatic function and cellular injury following hepatic ischemia, serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) levels were measured using the Opera Clinical Chemistry System (Bayer Co., Tarry Town, NY).

Amino Acid Analysis

To assess serum amino acid levels following rat liver transplantation, 100 μ L of serum samples were deproteinized with 100 μ L of 1.5M HClO $_4$ followed by neutralization with 50 μ L of 2M K $_2$ CO $_3$. Arginine, ornithine, citrulline, and polyamine levels were measured using HPLC analysis as previously described [25]. To assess tissue arginine levels following liver reperfusion, 250 mg of tissue was homogenized in 1ml of 1.5M HClO $_4$ and neutralized with 0.5 ml of 2M K $_2$ CO $_3$. The extracts were used for amino acid analysis by HPLC.

Statistical analysis

Results of serum liver injury tests are expressed as the mean $\pm$ standard error of the mean (SEM). Group comparisons were performed using the student's t-test or single-factor ANOVA. Differences were considered significant at $p < 0.05$.

Results

Arginase activity peaks 3 hours following hepatic I/R and depletes serum arginine levels

After any traumatic event with hepatocyte injury, arginase is released into the bloodstream. In our model of warm hepatic I/R, circulating arginase protein is seen as early as 30 minutes following reperfusion. This release peaks at approximately 3 hours and begins to decline 6 hours following reperfusion. Arginase is virtually undetectable by western blot analysis in serum from animals undergoing I/R 12 to 24 hours following reperfusion (Fig. 1A). This increase in circulating arginase protein is also accompanied by an increase in arginase activity (Fig. 1B). Furthermore, the increase in serum arginase following I/R is paralleled by depletion in circulating L-arginine as measured by HPLC analysis (Fig. 2). Control animals not undergoing I/R have much higher levels of serum arginine at baseline (Fig. 2). These results demonstrate that after hepatic I/R, arginase is released into the serum and is associated with a significant reduction in circulating arginine.

Nor-NOHA treatment decreases circulating Arg-I in animals undergoing hepatic I/R

In order to suppress the effects of massive arginase release following I/R, a pharmacologic arginase inhibitor, nor-NOHA, was used. Mice undergoing hepatic I/R were treated with nor-NOHA (100mg/kg) 15 minutes prior to ischemia and immediately following reperfusion. Serum arginase protein is decreased in animals treated with nor-NOHA at 3 and 6 hours after reperfusion compared to control saline animals (Fig. 3A). These results suggest that arginase inhibition with a pharmacologic agent reduces the amount of circulating arginase following warm hepatic I/R by reducing hepatocellular injury and necrosis.

To further investigate the mechanism by which nor-NOHA treatment decreases hepatic injury during I/R, serum levels of circulating nitric oxide were measured. The assay employed measures both nitrate and nitrite, the two stable end products of nitric oxide production, by conversion of nitrate to nitrite with spectrophotometric quantification of total nitrite. The serum from animals treated with nor-NOHA contained significantly increased levels of nitric oxide end products compared to saline control animals (Fig. 3B). This suggests that the protective effect of nor-NOHA involves increasing the availability of arginine for nitric oxide synthesis.

Nor-NOHA decreases hepatic upregulation of iNOS but not eNOS during warm I/R

To determine the effect of warm I/R on nitric oxide producing enzymes, the expression of iNOS and eNOS in hepatic tissue was determined by real-time PCR. This analysis revealed low basal levels of iNOS mRNA expression in hepatocytes in sham operated animals. This was significantly increased at 6 hrs of reperfusion (Fig. 4A). Treatment with nor-NOHA significantly decreased the iNOS induction as compared to saline control treated animals following I/R. Interestingly, eNOS mRNA expression in hepatic tissue was also significantly increased following I/R (Fig. 4B). In contrast to iNOS, nor-NOHA treatment did not decrease the expression of eNOS mRNA as compared to saline control treated animals.

Nor-NOHA treatment reverses arginine depletion and increases citrulline following warm I/R

To determine if the diminished serum arginase protein expression after treatment with nor-NOHA restored circulating arginine levels, the effect of arginase inhibition on arginine levels was evaluated following hepatic I/R. Serum amino acid analysis by HPLC showed that serum arginine is increased in nor-NOHA treated animals at both the 3 and 6 hr time points but did not significantly change serum arginine levels in sham operated animals (Fig. 5A). Serum citrulline, a product of nitric oxide synthase metabolism of arginine, was also analyzed. Low baseline levels were detected in the serum of sham operated animals which were significantly

increased following I/R (Fig. 5B). Additionally, at both 3 and 6 hr time points, serum citrulline was also significantly increased in the circulation of animals treated with nor-NOHA compared to animals treated with saline (Fig. 5B). These results suggest that increasing the amount of available arginine by inhibition of arginase leads to increased substrate utilization by nitric oxide synthase and production of citrulline.

Arginase inhibition is protective in hepatic I/R

To determine whether arginase inhibition was associated with decreased markers of hepatocellular injury, serum ALT was measured. Pharmacologic inhibition of arginase in animals undergoing hepatic I/R resulted in a significant decrease in serum ALT at both the 3 and 6 hr time points (Fig. 6A). Liver histology confirmed the ALT estimation of liver damage. Severe sinusoidal congestion and hepatocellular necrosis was present in liver tissue from control mice whereas minimal damage was noted in samples from nor-NOHA treated mice (Fig. 6B).

Hepatic tissue inflammatory mediators are decreased with nor-NOHA treatment

Inflammatory cytokines, such as TNF α and IL-6, have been shown to play key roles in the pathophysiology of hepatic I/R injury (12, 13). We measured hepatic levels of these cytokines after I/R using real time quantitative PCR. Hepatic expression of IL-6 and TNF α mRNA was significantly decreased in animals treated with nor-NOHA compared to control animals (Fig. 7).

Discussion

The damage to the liver caused by I/R continues to be an important limiting factor in many clinical settings such as liver surgery, transplantation, and low flow states. Large amounts of arginase, sufficient to result in marked arginine depletion, are released from hepatocytes following hepatic I/R injury. Since the L-arginine/NOS pathway has been recognized to play critical roles during inflammation, the purpose of this study was to determine if arginase blockade could effectively attenuate liver injury in a model of warm hepatic I/R. The major and novel findings of this investigation are: 1) serum arginase protein levels and enzyme activity are increased after warm hepatic I/R, and result in rapid circulating arginine depletion; 2) arginase inhibition with nor-NOHA is associated with significantly increased nitric oxide production as well as circulating arginine and 3) restoring serum arginine levels improved liver histology and biomarkers of hepatocellular inflammation and necrosis.

Our data suggests that nor-NOHA's action may be through the L-arginine/NO pathway. The protective effects of enhancing arginine availability may be mediated partly by improved substrate availability for NOS. Systemic arginine depletion due to arginase release after I/R may lead to decreased NO production and affect hepatic blood flow. Prior work has shown that mice deficient in endothelial NOS (eNOS) have worsened liver damage following I/R compared to wild type mice [6]. The protective effects of eNOS derived NO appears to be partly due to alterations in total hepatic blood flow. This study demonstrates increases in expression of both iNOS and eNOS mRNA in hepatic tissue following I/R. The increase in iNOS mRNA expression, but not eNOS mRNA, is attenuated with nor-NOHA treatment, likely representing decreased liver injury and decreased production of cytokines known to induce iNOS expression (such as TNF α as in Fig. 6). Further investigation into the mechanism of protection by nor-NOHA revealed significantly increased levels of nitric oxide metabolites in the serum of nor-NOHA treated animals following I/R, reflecting increased nitric oxide production in these animals. Therefore, it appears that limiting arginine availability for eNOS following massive arginase release affects hepatic blood flow and subsequent hepatocellular

damage. Further, the protective effects from nor-NOHA treatment appear to involve increasing arginine availability for NOS and increased nitric oxide production.

Monitoring systemic blood pressure during nor-NOHA administration will be important given the role of arginine in NO dependent relaxation in vascular smooth muscle cells [26]. Indeed, we found that citrulline, a product of NOS metabolism of arginine, is increased in mice treated with nor-NOHA. However, our study does not elucidate which isoform of NOS is utilizing the increased available arginine due to nor-NOHA. Measuring serum levels of nitrite and other more direct markers of nitric oxide production would be useful in determining whether the increased arginine following nor-NOHA protects via an NO-dependent mechanism. It is noteworthy that nor-NOHA treatment also restored the depleted levels of circulating arginine levels in a rodent model of liver transplantation, and improved liver graft injury [19]. Furthermore, some therapeutic interventions that may be beneficial in the cold I/R setting have not been proven to apply in warm hepatic I/R models. Inhaled carbon monoxide has been shown to be protective in liver transplant preservation injury [27] but not in a rodent model of warm hepatic I/R injury (personal observation). Furthermore, warm I/R occurs in a variety of clinical settings including elective liver resections, trauma, and hypovolemic shock. This suggests that arginase blockade may serve as a useful strategy during multiple forms of hepatic redox stress, and is not limited to warm I/R injury. One recent study utilizing human subjects undergoing warm I/R liver resections versus transplantation demonstrated that there were differences in arginase activity and arginine availability between the two clinical scenarios [28]. However, this paper did not specifically examine pharmacologic arginase inhibition in either model or its effect on arginine availability. These findings further highlight the complex metabolic pathways involved in liver injury and underscore the potential for arginase blockade as a possible therapeutic intervention.

The arginase/NOS competition for arginine catabolism in various cell populations can lead to beneficial or adverse consequences. This depends largely on which pathway is blocked or enhanced and in which cell population [29]. Inhibiting arginase degradation of arginine may lead to decreased downstream products such as polyamines and proline [30]. These products are important mediators of cellular proliferation and collagen synthesis, respectively [31]. Thus, there may be potential adverse effects on liver regeneration and wound healing in animals treated with an arginase inhibitor. However, proline can also be produced from glutamate and glutamine in the small intestine, and the relative importance of this synthetic pathway for endogenous provision of proline is currently unclear [32]. In certain populations of immune cells, intracellular arginase is upregulated by various stimuli such as lipopolysaccharide and inflammatory cytokines [33]. Local arginine depletion by myeloid suppressor cells has been shown to have effects on T-cell receptors and function [34]. Given the important role that the immune cells play during and after hepatic I/R injury, it would be interesting to examine the effects of arginine depletion on both Kupffer and dendritic cell function in the liver and the effects of restoring arginine availability with nor-NOHA. Modulation of the local immune population of cells also has important implications for the role that the arginase/NOS system may play in liver transplant tolerance.

In summary, the large amount of arginase that is released systemically and the ensuing arginine deficiency following warm I/R can be partially reversed using a selective pharmacologic inhibitor of this enzyme. Enhancing arginine availability and arginase blockade are associated with decreased hepatocellular injury. Thus, strategies aimed at arginase blockade following liver I/R may be important in a variety of clinical settings.

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Abbreviations

I/R	ischemia/reperfusion
NO	nitric oxide
NOS	nitric oxide synthase
nor-NOHA	n-omega-Hydroxy-nor-L-arginine
TNF-α	tumor necrosis factor-alpha

IL-6

interleukin 6

ALT

alanine aminotransferase

Figure 1. Serum arginase levels following reperfusion

Mice were subjected to 60 min ischemia and various times of reperfusion. (A) Western blot analysis of serum arginase protein expression was performed. Blot is representative of 3 separate experiments. (B) Time course of serum arginase activity following I/R was performed. The results are representative of three separate experiments.

Figure 2. Serum arginine following reperfusion

Mice were subjected to 60 min ischemia and various times of reperfusion. Serum amino acid concentration of arginine following reperfusion was measured by HPLC analysis. The results are representative of three separate experiments.

Figure 3. Serum arginase after nor-NOHA treatment

Mice treated with nor-NOHA or control saline were subjected to 60 min ischemia and three to six hours of reperfusion. (A) Western blot of serum arginase protein was performed. Control is serum from an animal that has not undergone I/R. (B) Serum levels of nitrate and nitrite were measured as the stable end products of nitric oxide metabolism. These markers of nitric oxide levels were significantly increased in nor-NOHA treated animals compared to saline control animals. Data represents mean $\pm$ SE, n = three to six mice per group. *P<0.05 vs. saline control treated mice.

Figure 4. Hepatic expression of iNOS and eNOS mRNA following I/R with arginase inhibition by nor-NOHA

Mice underwent either sham laparotomy or subjected to 60 min of ischemia and six hours of reperfusion were treated with either saline control or nor-NOHA. Hepatic expression of iNOS and eNOS were determined using real-time PCR. (A) I/R significantly increased iNOS mRNA expression in hepatic tissue. This effect was attenuated with nor-NOHA treatment. (B) Increased eNOS mRNA expression was demonstrated following I/R. Treatment with nor-NOHA did not effect eNOS mRNA expression. Data represents means $\pm$ SE, n = three to six mice per group. *P<0.05 versus saline control treated mice. #P<0.05 versus sham operated mice.

Figure 5. Effect of arginase inhibition on circulating amino acids, arginine and citrulline
Mice treated with nor-NOHA or control saline were subjected to 60 min ischemia and three to six hours of reperfusion. Sham mice also underwent laparotomy without I/R. Serum amino acids were measured by HPLC analysis. (A) Serum arginine is significantly increased in I/R operated animals treated with the arginase inhibitor, nor-NOHA. (B) Serum citrulline is low in sham operated animals and significantly increased following I/R. It is also significantly increased in animals treated with nor-NOHA. Data represents means $\pm$ SE, n = four to six mice per group. * $P < 0.05$ versus saline control treated mice. # $P < 0.05$ versus sham operated mice.

A.

B.

Figure 6. Effect of nor-NOHA treatment on serum transaminases and histology

(A) Mice treated with nor-NOHA or control saline were subjected to 60 min ischemia and three to six hours of reperfusion. Serum ALT levels were analyzed as a measure of hepatocellular injury. Data represents means $\pm$ SE, n = four to six mice per group. * $P < 0.05$ versus nor-NOHA-treated mice. (B) Hematoxylin-eosin-stained liver sections from animals treated with nor-NOHA or control saline undergoing 60 min ischemia and 6 hours following reperfusion (Original magnification $\times 100$). Decreased hepatic necrosis is seen in the nor-NOHA group compared to the saline-treated animals. Images are representative liver sections from six mice per group.

Figure 7. Hepatic IL-6 and TNF α with arginase inhibition

Real time RT-PCR was used to measure hepatic tissue cytokine content after I/R in mice treated with nor-NOHA or control saline. (A) Hepatic levels of IL-6 were significantly lower in nor-NOHA versus saline-treated animals. (B) Hepatic levels of TNF α were similarly reduced in nor-NOHA treated mice. Data represents means $\pm$ SE, n = four to six mice per group. * P<0.05 versus nor-NOHA- treated mice.