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Losartan, an angiotensin II receptor blocker, fails to attenuate pulmonary dysfunction in ovine burn and smoke model

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by

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Thesis

Presented to the Faculty of the Graduate School of

The University of Texas Medical Branch

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science

The University of Texas Medical Branch

August, 2008

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Publication No._____

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The University of Texas Medical Branch, 2008

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Elevated levels of angiotensin II (AngII) in the lung have been shown in response to burn and smoke inhalation injury. AngII has been associated with increased nitric oxide levels, leading to nuclear oxidative damage in Type II lung epithelial cells. Losartan is an AngII, AT_{1A} specific receptor blocking. Using an ovine model of acute respiratory distress, we hypothesized that blocking AngII receptors with losartan would improve the clinical conditions seen in response to the injury. Chronically prepared adult ewes received a 40% total body surface area third degree flame burn followed by 48 breaths of cotton smoke. The sheep were randomly divided into two groups: control (injury, no treatment, n=7) and treatment (injury + losartan, n= 7). Losartan was given intravenously as a bolus (50 mg) at 1hour post-injury. All animals received 4ml/kg/%burn of Ringer's lactate and were mechanically ventilated. Control animals showed severe signs of acute lung injury evidenced by deterioration of pulmonary gas exchange (PaO_2/FiO_2, pulmonary shunt fraction), increased lung water content, and increased pulmonary transvascular fluid flux, and vascular permeability index. Although there was a trend for the reduction of lung lymph flow and permeability index, none of these alterations could be shown to be statistically affected by this dosage of losartan in this 24-hour ovine model of acute lung injury.

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HYPOTHESIS

Millions of thermal injuries are reported each year in the United States, commonly these burn injuries simultaneously occur with smoke inhalation injury as well. Such injuries often result in the development of multiple organ failure due to the body's innate reaction to redirect blood to only the brain and heart thus depleting other important organs. Reperfusion of these systemic organs causes release of materials that injure the lungs. Oftentimes, patients develop acute respiratory distress syndrome, which is a severe inflammation of the lung parenchyma that can be life threatening if untreated. Studies have shown that angiotensin II levels are elevated in response to this injury and therefore may be a major contributor to the inflammation. Studies have shown that angiotensin II has proinflammatory abilities that are exerted on leukocytes, endothelial cells, and vascular smooth muscle cells[1]. Based on these current reports, we wanted to determine if treatment with losartan, an angiotensin II receptor blocker, attenuates the clinical symptoms of ARDS that occur in response to burn and smoke inhalation injury. It is hypothesized that losartan will block angiotensin II binding to its receptor and thus prevent inflammatory mediation and ultimately attenuate the clinical conditions seen in this ovine ARDS model. This hypothesis will be tested in sheep that have been operatively prepared for chronic study. The combination injury will be accomplished producing a 40% total body surface area 3rd degree burn and insufflation the lungs with 48 breaths of cotton smoke.

Specific Aim **Determine if losartan, an angiotensin II receptor blocker, attenuates clinical symptoms seen in response to burn and smoke inhalation injury in an ovine model.**

Using an ovine model, adult female ewes were subjected to 40% total body surface area 3rd degree flame burn as well as 48 breaths of cotton smoke while under deep anesthesia. They were awakened, fluid resuscitated, placed on a ventilator, and had their cardiopulmonary variables monitored and blood samples taken at set time points for the 24 hours following injury. The treatment group received 50mg bolus of losartan i.v. 1 hr post injury. Hemodynamics and other variables were measured an assessed for differences between control and treatment groups.

BACKGROUND

Thermal Injury An estimated 2.4 million burn injuries are reported each year in the United States. Of those reported injuries, about 650,000 receive professional care and 75,000 victims of these injuries are hospitalized. Approximately 20,000 burn patients have thermal injuries covering at least 25% of their total body surface area (TBSA). Thirty years ago, an estimated 9,000 fatalities occurred each year due to burn injuries in the U.S. Today, the number of fatalities has decreased greatly to an estimated 4,500.[2,3]

Recent studies have shown that many life-threatening complications may occur as a result of the inflammatory response incurred following the initial thermal injury. Patients may suffer from multiple organ failure (MOF) or sepsis following such an injury. Studies have shown that MOF may be a result of the body's reaction to the injury to redirect major blood flow to sustain viability of the brain and heart, thus depleting other major organs such as the intestines of the necessary blood supply. Such a traumatic injury can commonly trigger the development of Acute Respiratory Distress Syndrome (ARDS), an oftentimes fatal, inflammatory response of the lungs as reperfusion of the systemic organs causes the release of damaging materials into the lungs.[2]

Acute Respiratory Distress Syndrome ARDS is described as a lung disease caused by the inflammation of the lung parenchyma due to various direct and indirect issues (Table 1). This condition was first described in 1967 by Ashbaugh *et al*. as a syndrome characterized by stiff lungs, hypoxemia, and bilateral infiltrates on a chest X-ray[4]. In

1988, the Lung Injury Severity Score was developed and was commonly used in a critical care setting. By 1994, the American-European Consensus Conference Committee established a standard definition and clinical criteria of ARDS to be used universally.

Causes of ARDS

DIRECT LUNG INJURY	INDIRECT LUNG INJURY
Common causes	**Common causes**
Pneumonia	Sepsis
Aspiration of gastric contents	Severe trauma with shock and multiple transfusions
Less common causes	**Less common causes**
Pulmonary contusion	Cardiopulmonary bypass
Fat emboli	Drug overdose
Near-drowning	Acute pancreatitis
Inhalational injury	Transfusions of blood products
Reperfusion pulmonary edema after lung transplantation or pulmonary embolectomy	

Table 1. Listing of various causes of ARDS [5].

ARDS clinical criteria are described as follows: acute onset, bilateral infiltrates on chest X-ray, pulmonary artery wedge pressure <18mmHg, and PaO_2/FiO_2 <200mmHg[5]. Pulmonary gas exchange is commonly assessed by the ratio of arterial partial oxygen pressure (PaO_2) as a fraction of inspired oxygen pressure (FiO_2). Acute Lung Injury (ALI) is a less severe form of ARDS characterized by PaO_2/FiO_2 <300mmHg that can progress further into ARDS.

Criteria for Acute Respiratory Distress Syndrome

Definition	Criteria
American-European Consensus	1. Acute onset 2. PaO_2/FiO_2 ≤200 (regardless of PEEP level) 3. Bilateral infiltrates seen on frontal chest radiograph 4. PAWP ≤18mmHg (when measured) or no clinical evidence of left atrial hypertension
Lung Injury Severity Score	1. Chest radiograph score (number of quadrants with alveolar consolidation from 0-4) 2. Hypoxemia score (PaO_2/FiO_2 ≥300, 225-299, 175-224, 100-174, <100) 3. PEEP score (≥5, 6-8, 9-11, 12-14, ≥15cmH_2O) 4. Respiratory system compliance score (≥80, 60-79,

Table 2. Criteria for ARDS as defined by American-European Consensus and Lung Injury Severity Score [4].

This disease leads to severely impaired gas exchange and simultaneous release of inflammatory cytokines released from local epithelial and endothelial cells, thus causing inflammation, hypoxemia, and commonly resulting in MOF[6]. Additionally, neutrophils and T-lymphocytes migrate into the inflamed region of the lung parenchyma. This inflammatory response causes endothelial dysfunction, fluid extravasation from the microcirculation, impaired drainage of fluid from the lungs, and a reduction in surfactant production; thus inflammatory exudates enter the alveoli causing the formation of edema[5,7]. The edema formation increases the thickness of the space between the alveoli

and capillary and therefore, increasing the distance of diffusion of oxygen. This can severely impede pulmonary gas exchange and lead to hypoxia.

Angiotensin II Angiotensin II (AngII) is a potent vasoconstrictor endogenously released to help maintain a normal blood pressure in hypotensive conditions. Angiotensin was first discovered by two independent groups, headed by Braun-Menendez from Buenos Aires and Page from Indianapolis in 1939. It was characterized as the polypeptide responsible for the pressor effect of renal hypertension[8]. It is now known that AngII is produced via multiple cleavage actions of precursors.

Angiotensinogen is an α_2-globulin that is synthesized by the liver in small amounts and is released into the systemic circulation. Concomitantly, renin, a protein that is produced, stored, and released by the granular cells of the juxtaglomerular apparatus (JGA) of the kidney, is released and responsible for the proteolytic cleavage of angiotensinogen. Renin may be released by stimulation from the sympathetic nervous system causing the release of catecholamines responsible for the stimulation of juxtaglomerular cells via β-adrenergic receptors. Renin is also released as a result of decreased renal arterioloar perfusion pressure (sensed by baroreceptors in the wall of the afferent arteriole), or decreased sodium delivery to the distal tubules of the kidney. Angiotensinogen is cleaved into the decapeptide angiotensin I, which undergoes a second proteolytic cleavage by angiotensin converting enzyme (ACE), a dipeptidyl carboxypeptidase. ACE then cleaves two amino acids from AngI forming the octapeptide AngII. Additionally, ACE cleaves dipeptides from other substrates including

bradykinin, enkephalins, and substance P. ACE is located on the luminal surface of vascular endothelia throughout the entire body; moreover, it is present in abundant amounts in the lungs because they are rich in endothelial cells. AngI has also been shown to be cleaved by the serine protease, chymase, most commonly in the cardiac ventricles.[9,10]

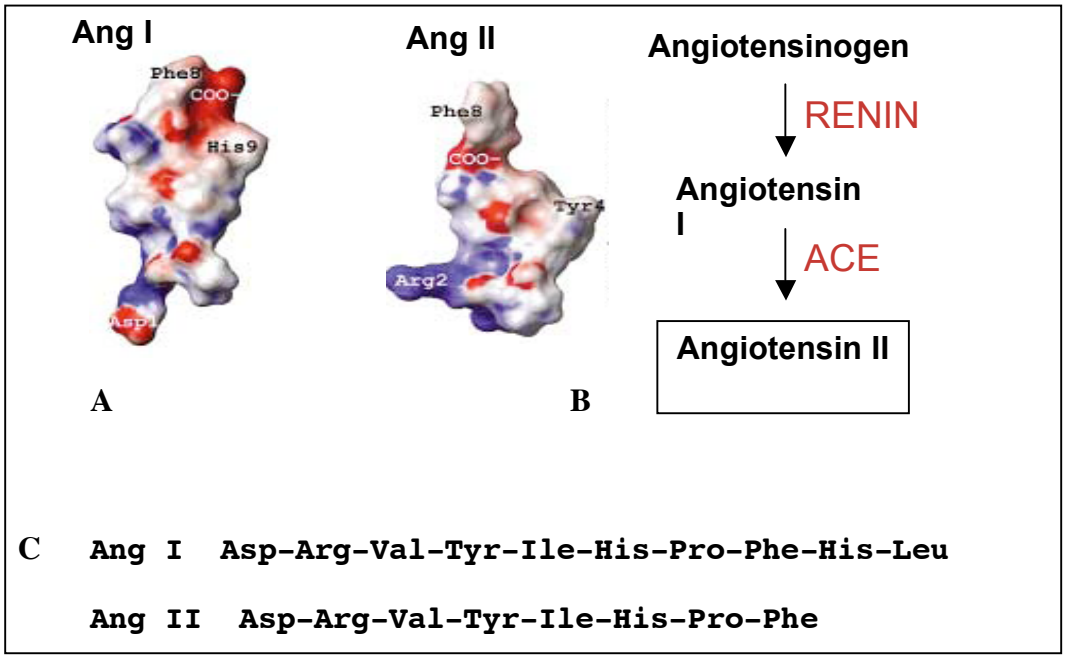

Figure 1. A) Protein structure of Ang I and Ang II [11]. B) Schematic of AngII production C) Amino acid structure of AngI (decapeptide) and AngII (octapeptide).

Due to the fact that AngII production directly relies on renin release, stimulation and inhibition of renin release become the main components in regulating the amount of AngII produced. Stimulation of renin release was described earlier, and inhibition, or turning off, of renin release is mostly controlled by negative feedback. Restoration of renal perfusion pressure and increased sodium and fluid reabsorption are homeostatic

negative feedback measures that are mostly responsible for decreased renin release[12]. Other studies show that AngII also has a feedback effect by increasing juxtaglomerular cell calcium ion levels[13]. Downstream signal transduction of AngII promotes intracellular calcium release, which in turn promotes vasoconstriction; and AngII can also inhibit the cyclic AMP system. Increased calcium ion concentrations and decreased cAMP in JG cells are responsible for the suppression of renin release[12]. In contrast, decreased calcium concentration and increased cAMP concentrations are responsible for direct stimulation[12].

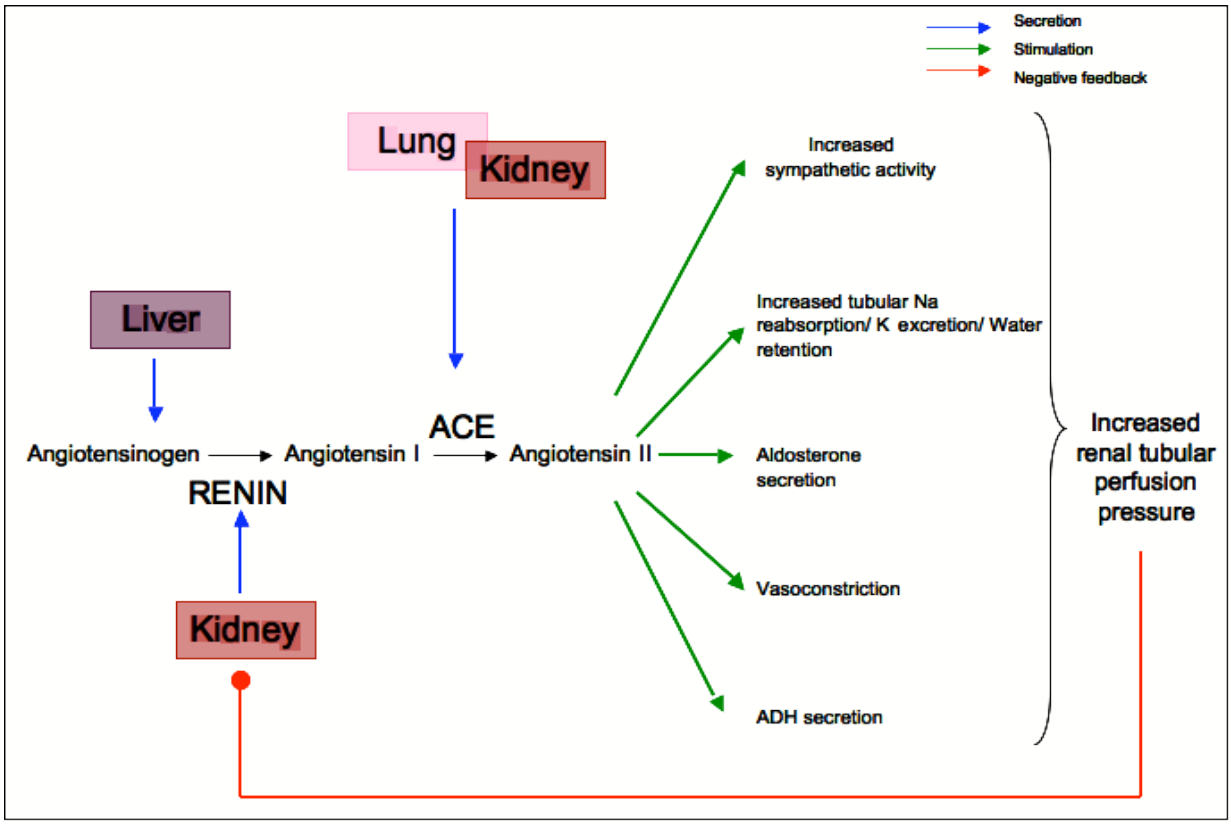

Figure 2. Schematic of AngII production and its actions.

Angiotensin Receptor The angiotensin receptors are G-protein coupled receptors responsible for the signal transduction of the renin-angiotensin effector hormone[10]. This class of receptors can be divided into four subtypes: AT_1, AT_2, AT_3, and AT_4. More information stands to be elucidated about AT_2, AT_3 and AT_4, but AT_1 has been quite well characterized.

AT_1 receptor mediates various biological pathways upon activation by AngII, including: vasoconstriction, aldosterone synthesis and secretion, cardiac hypertrophy, decreased renal blood flow, renal renin inhibition, renal tubular sodium reuptake, and increased sympathetic nervous system activity[14]. Signal transduction via the AT_1 receptor takes place initially via phospholipase C (PLC) activation. PLC enzymatically cleaves inositol triphosphate (IP_3) from a membrane phospholipids. IP_3 stimulates the release of calcium ions from non-mitochondrial intracellular site, such as the sarcoplasmic reticulum. It also stimulates production of diacylglycerol (DAG). Increased intracellular calcium ion concentration is thus responsible for inducing vasoconstriction while DAG activates protein kinase C (PKC) which stimulates aldosterone synthesis.[12]

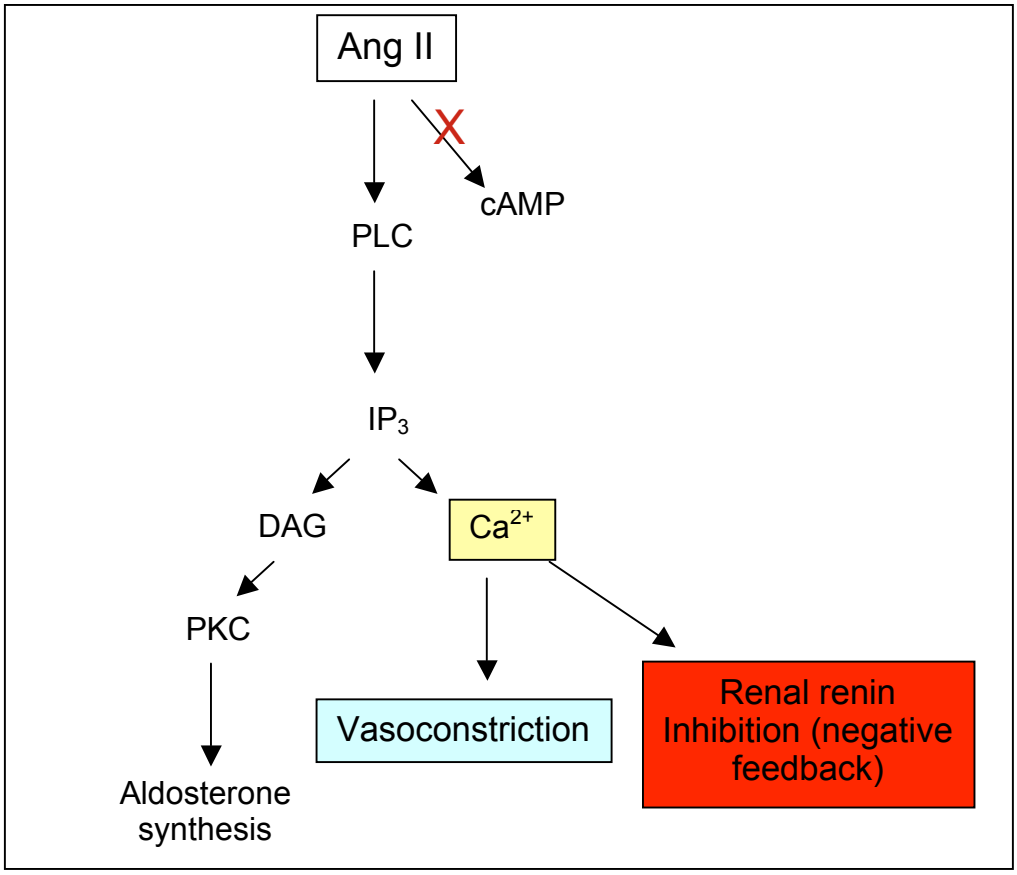

Figure 3. Schematic of Angiotensin II signaling cascade and downstream events.

Aside from the biological effects listed above, AngII is also known to induce severe inflammation. Several inflammatory mediators are stimulated by AngII, thus eliciting an acute inflammatory response.

Inflammatory mediators stimulated by Angiotensin II

NF-κB	Nuclear factor-κB
IκB	Inhibitor of κB
NADPH oxidase	Reduced nicotinamide-adenine dinucleotide phosphate dehydrogenase
TNF-α	Tumor necrosis factor-α
IL-6	Interleukin-6
ROS	Reactive oxygen species
$O_2^{\bullet}$	Superoxide
PAI-1	Plasminogen activator inhibitor-1
AP-1	Activator protein-1
MMP	Matrix metalloproteinase

Table 3. List of inflammatory mediators that can be stimulated by angiotensin II [1].

AngII can be further cleaved into the metabolites AngIII and IV, which are also agonists of the AT_1 receptor. They however, have lower affinities for the receptor as compared to AngII and are thus less potent vasocontrictors[15]. AngIII has been shown to exert specific growth effects while AngIV possesses unique receptors distributed widely amongst tissues[15]. Further research is still required on these metabolites to elucidate their roles in the renin-angiotensin system.

Losartan Losartan, an antihypertensive pharmaceutical, is a non-competitive, selective angiotensin II Type 1 receptor blocker that was designed in 1986 by DuPont. It undergoes first-pass metabolism by cytochrome P450 where is it converted to an active carboxylic metabolite, which is 10 to 40 times more potent than losartan.

In its salt form, it is chemically described as 2-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]imidazole-5-methanol monopotassium salt.[16]

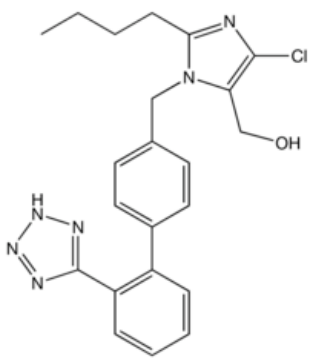

Figure 4. Chemical structure of losartan.

There are now 7 variable angiotensin receptor antagonists available: losartan, candesartan, eprosartan, irbesartan, olmesartan, telmisartan, and valsartan[17]. Losartan has been shown to antagonize the majority of all physiological effects of AngII[16]. It is commonly chosen over an ACE inhibitor due to the possibility of alternative pathways of AngII formation, including that of chymase.

SIGNIFICANCE

It has been established that AngII has the ability to induce an inflammatory response via several possible pathways in leukocytes, endothelial cells, and vascular smooth muscle cells. Studies using a murine model have effectively shown that AngII levels are elevated in response to ARDS[18]. The murine model in these studies used an acid aspiration protocol to induce ARDS formation as well as sepsis-induced acute lung injury using caecal ligation and perforation. Additionally, the study showed that injection of mice with a catalytically active recombinant form of ACE2, a homologue of ACE that functions as a negative regulator of the renin-angiotensin system, attenuated ARDS symptoms[19]. Another study by the same group showed that mice with a knockout for ACE2 had markedly worse lung function than the wild type as defined by increased lung elastance, pulmonary edema, and leukocyte accumulation.

Several studies with angiotensin receptor blockers (ARBs) have shown a suppression of proinflammatory cytokines in patients with hypertension[20]. For example, plasma levels of various cytokines such as MCP-1, TNF-α, PAI-1, and IL-6 were reduced in response to treatment of human hypertension with ARBs. All of these cytokines are possible inflammatory mediators induced by AngII.

Determining if losartan is effective in attenuating ARDS symptoms would prove beneficial in progressing therapeutic measures for burn patients. Previous studies have used less direct methods of inducing ARDS in that the injury is not necessarily representative of ARDS development under thermal injury circumstances. Using an

ovine model that directly receives burn and smoke inhalation injury along with losartan treatment allows a closer representation of the effectiveness of this study.

METHODS

This study was approved by the Animal Care and Use Committee of the University of Texas Medical Branch. All the animals were handled according to the guidelines established by the American Physiology Society and National Institutes of Health.

Animal Model

Surgical Preparation Fourteen adult Merino ewes (25-35kg) were allowed adjustment time to the laboratory conditions. Following anesthesia under halothane, an incision was made in the femoral triangle followed by insertion of a polyvinyl chloride catheter (16-gauge, 24-inches, Becton Dickinson Infusion Therapy Systems, Sandy, UT) into the right femoral artery up to the descending aorta for continuous measurement of systemic arterial blood pressure and intermittent arterial sampling of blood. A Swan-Ganz pulmonary artery, thermodilution, flow-directional, flotation catheter (model 131F7, Edwards Lifesciences, Irvine, CA) was inserted into the pulmonary artery via a percutaneous stick in the right external jugular vein for measurement of mean pulmonary arterial pressure as well as for intermittent mixed venous blood sampling. A right thoracotomy was performed between the 6^{th} and 7^{th} ribs using surgically aseptic conditions. The caudal mediastinal lymph node was identified followed by cannulation of the efferent vessel using Silastic tubing (602-015, Dow Corning, Midland, MI). The incision was closed and a second incision was made between the 10^{th} and ll^{th} ribs for ligation of the caudal end of the lymph node and for cauterization of the diaphragm in order to reduce systemic

lymph drainage into the node. A chest tube was left in place for drainage and the incision was closed. A left thoracotomy, in the fifth intercostal space, was then performed in order to insert a Silastic catheter into the left atrium. The sheep were awakened and allowed free access to food and water.[21, 22]

Recovery During a 5 day recovery period following surgery, animals were connected to pressure transducers (PXMK 1590, Edwards Lifesciences, Irvine CA) in order to monitor mean arterial and left atrial pressures (V24C, Philips, Bothell, WA). All readings were taken when the sheep were standing in metabolic cages and zero calibration for the transducers was taken at the level of the olecranon joint, which is considered to be at the level of the right atrium. An i.v.infusion of Ringer's lactate solution (2ml/hr/kg IV) was also started following surgery. Cardiac output was determined using the thermodilution technique[23]; the mean recordings of three 10ml volumes of 5% dextrose solution at 0°C were used as indicators.[21, 22]

ARDS Model All animals received a tracheostomy under halothane anesthesia. Each animal then received 40% total body surface area third-degree flame burn while under halothane anesthesia. Body surface area (BSA) was determined by the formula, BSA= (body weight, kg)$^{2/3}$ * 0.084m^2/kg. Between burning of each side of the animal, the sheep were insufflated with 4 sets of 12 breaths of cotton smoke <40°C using a modified bee smoker[22]. An arterial blood sample was drawn and analyzed for carboxyhemoglobin using a CO-oximeter (682 CO-oximeter, Instrumentation Laboratory, Lexington, MA)

after each set of delivered smoke as an indicator of inhalational injury. The baseline time point was set at the end of smoke insufflation and the animals were monitored for the following 24 hours.

Ventilation All sheep were placed on a ventilator with positive end-expiratory pressure of 5cm H_20 and tidal volume maintained at 15ml/kg. The sheep were ventilated with an FiO_2 of 100% oxygen for the first three hours post-injury for increased clearance of carboxyhemoglobin. FiO_2 was subsequently adjusted to maintain PaO_2 >80mmHg. Initial respiratory rate was set at 20 breaths/min and subsequently adjusted to maintain a $PaCO_2$ between 25 and 35mmHg.

Fluid Resuscitation All sheep received fluid resuscitation via i.v. infusion with Ringer's solution following the Parkland formula, 4ml/kg/% burned body surface area.

Experimental Groups Fourteen sheep were randomly divided into two groups: control and treatment. Animals in the control group received the burn and smoke injury. Animals in the treatment group received burn and smoke injury followed by 50mg bolus of Losartan i.v., 1hr post-injury. We originally performed a dose response experiment injecting 1μg/kg, 2μg/kg, 4μg/kg, 8μg/kg, and 25mg and saw no changes (data not shown). The physician recommended dosage is 25-100mg/day[16]. Based on these two principles, we settled on a 50mg/24hr dosage. Both groups received the same fluid resuscitation and mechanical ventilation.

Measured Variables

Cardiopulmonary hemodynamics The following variables were continuously monitored throughout the 24-hour experimental time period: cardiac output, heart rate, mean arterial pressure, left atrial pressure, pulmonary arterial pressure, pulmonary artery wedge pressure, and central venous pressure. Blood gases were taken of both arterial and mixed venous blood samples at different time points (blood has analyzer, 1032 IL, Instrumental Laboratory, Lexington, MA). PaO_2/FiO_2 ratio was measured to evaluate pulmonary gas exchange. Lung lymph flow rate, lung lymph protein, and plasma protein were measured to calculate the vascular permeability index, which is used as an indicator of pulmonary transvascular fluid flux. Vascular permeability index is calculated as (lymph protein/plasma protein)*lung lymph flow rate.

Molecular Biology

Angiotensin Measurement Angiotensin II plasma levels were extracted and measured using a radioimmunoassay kit from Alpco Diagnostics (Salem, NH). The kit is based on the principle that AngII present in the samples competes with radioactive labeled AngII for the AngII antibody. A solid-phase second antibody binds to antibody-bound AngII. Thus, the more AngII present in the sample, the less radiolabeled AngII is present as it has been outcompeted for binding the primary antibody. Plasma concentration was initially chosen for measuring AngII because it allowed for measurement over a time

course using one animal instead of only at baseline and 24hrs. Measuring over a time course helps provide more information about when changes in concentration occur.

Statistics

A two-way ANOVA was run on all data followed by a Bonferroni adjustment to assess significance between the control group and the treatment group. Significance occurs if $P<.05$.

RESULTS

Animal Model During the 24hr period following the burn/smoke injury, the animals of both groups were monitored; their hemodynamics were recorded at set time points and blood gas samples were run accordingly to help monitor gas exchange. Left ventricular stroke work index (LVSWI) /left atrial pressure (LAP) relationship was decreased in both groups indicating a decrease in cardiac function. LVSWI was calculated with the data from the measured hemodynamics using the following equation: Stroke Volume Index * (Mean Arterial Pressure – Left atrial pressure) * 0.0136. Stroke Volume Index can be calculated by: (Cardiac Output/Heart Rate)/Body Surface Area. There was no statistical difference of LVSWI-LAP between the groups.

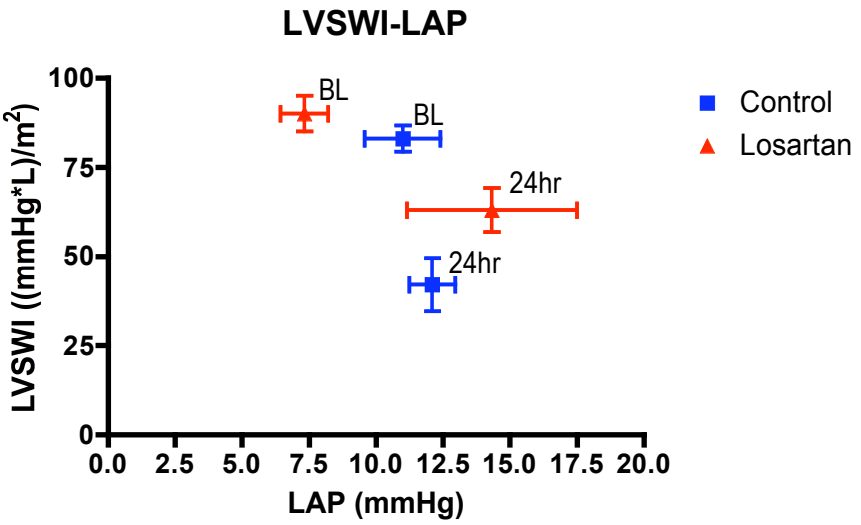

Figure 5. Left ventricular stroke work index-left atrial pressure relationship decreases in both study groups indicating decreased cardiac function.

Pulmonary gas exchange decreased in both groups and is represented as the ratio PaO_2/FiO_2. In our study both groups had a decrease in gas exchange ability with a PaO_2/FiO_2 ratio <300mmHg, indicating criteria for Acute Lung Injury. Additional representation of poor lung function is formation of edema, as fluid formation makes oxygen diffusion more difficult across the endothelium. Edema is measured by a wet/dry ratio of lung tissue harvested at the end of the study. Neither of these measurements contains statistical difference between groups.

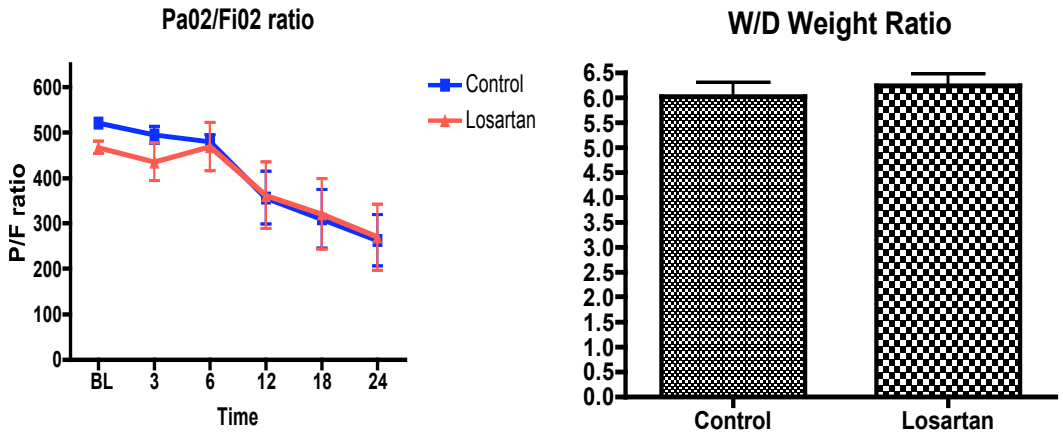

Figure 6. PaO_2/FiO_2 falls below 300mmHg in both study groups indicating decreased gas exchange ability. W/D weight ratio is increased in both groups indicating edema formation as inflammatory response to injury.

Fluid balance is another major factor in this type of injury where there tends to be an increase in vascular leakage. Normally in a healthy lung, fluid and protein leak mostly through small gaps between capillary endothelial cells. However, the injury to the

endothelium causes an increased vascular permeability resulting in transvascular fluid flux[7]. Lung lymph flow increased in both animal groups and while there was a trend for the losartan to maintain a lower lymph flow, there was no statistical difference between groups except at 12hrs. Lymph flow increases as it establishes a new equilibrium due to edema formation and acts as an indicator of inflammation. Additionally, the vascular permeability index showed an increase in transvascular fluid flux. Again, the trend was for losartan to maintain a lower index, but it was not significant between study groups. Lastly, retention of fluid is also an indicator of inflammation and occurs due to increased vascular permeability. Both groups had an increase in fluid retention over the 24 hr period.

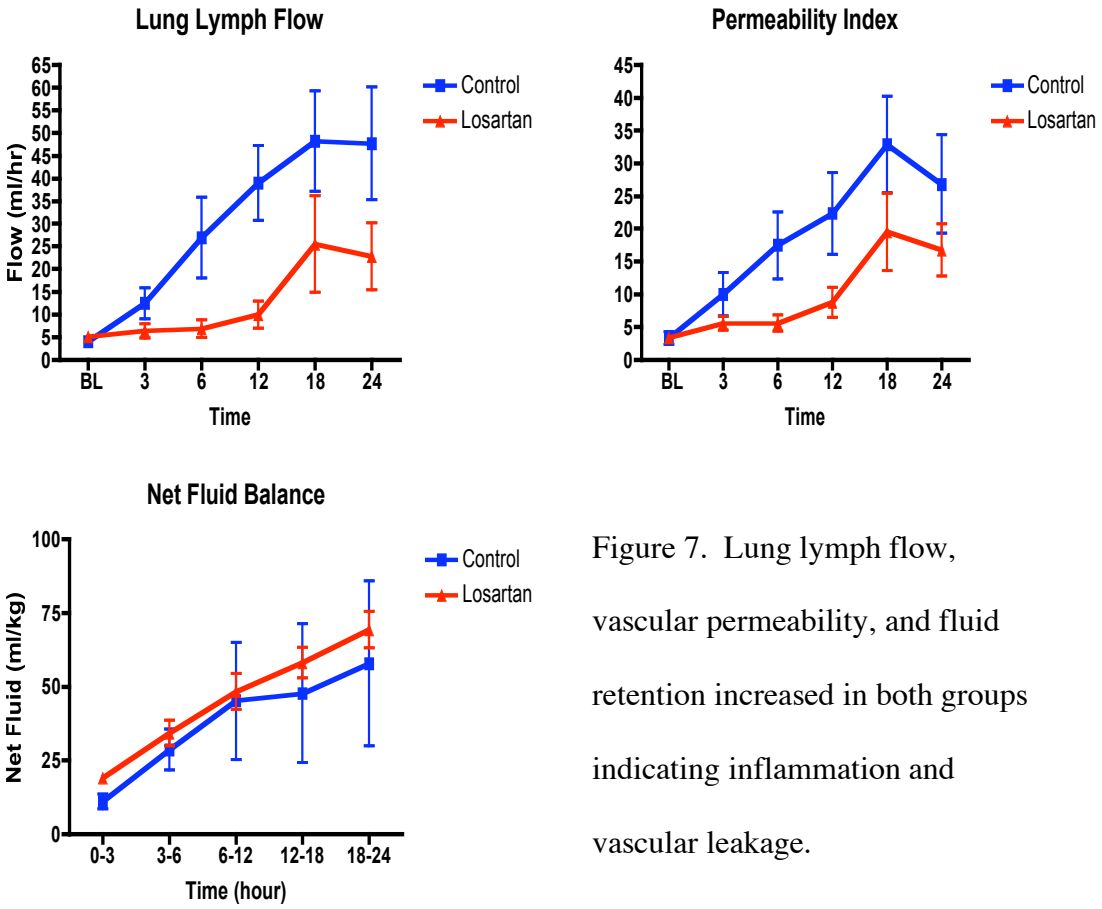

Figure 7. Lung lymph flow, vascular permeability, and fluid retention increased in both groups indicating inflammation and vascular leakage.

Angiotensin Measurement Although previous studies show elevated AngII levels in mice, we wanted to confirm this in our ovine model. In this study, we saw no significant changes throughout the 24 hr period or differences between groups.

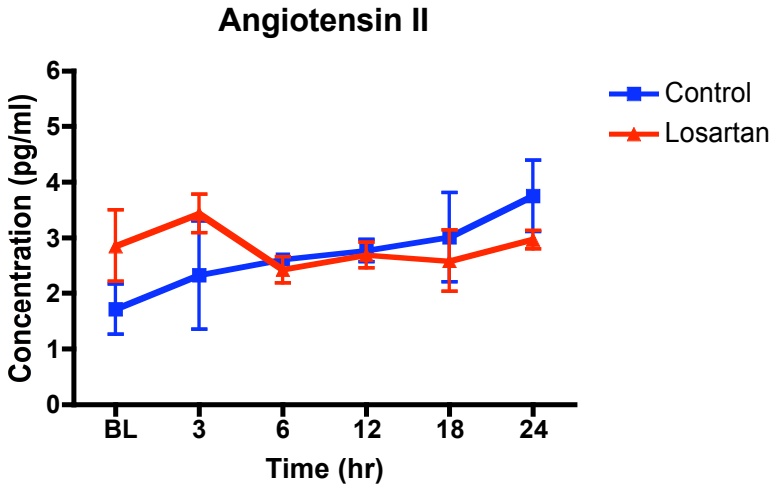

Figure 8. Plasma AngII levels measured by radioimmunoassay in ovine burn/smoke model showed no significant difference between study groups.

DISCUSSION

While this study has shown representation of the ovine burn and smoke inhalation ARDS model, it seems that the chosen losartan dose does not attenuate the clinical symptoms. The study groups, both control and treatment, showed typical signs of ARDS formation with no significant difference between them. There was decreased gas exchange ability as marked by the decline in PaO_2/FiO_2. The left ventricular stroke work index was decreased indicating a decline in cardiac functional ability. There was a marked increase in inflammation as indicated by the increase in pulmonary edema as shown by the wet to dry weight ratio of lung tissue, the increase in lung lymph flow, vascular permeability, and fluid retention.

Interestingly, angiotensin II levels did not significantly change in response to the burn/smoke injury nor was there a significant difference between the control and treatment groups. The lack of change in plasma levels of AngII within each group could be attributed to various circumstances. The first reason being that two major steps in the protocol of the radioimmunoassay kit could have allowed for loss of AngII. During the extraction phase, the ovine plasma did not filter through the extraction tubes with as much ease as expected even though the samples had be spun prior to filtration, the filters had a tendency to clot and thus prevent full extraction of all AngII from the plasma. Also, during the final step, the residue containing AngII had to be resuspended in scintillation buffer, which is highly viscous and difficult to retain the entire sample. These two steps could have contributed to a possible loss of AngII from each sample.

If in fact, the recorded amounts of ovine plasma AngII are accurate, the lack of change seen in plasma levels of AngII could have an alternative explanation. A murine study looking at levels of AngI and AngII in both plasma and renal tissue in response to ACE inhibitors showed that the AngII concentration in the tissue of vehicle treated rats was substantially higher in the renal tissue than the plasma[25]. Furthermore, a murine bleomycin-induced acute lung injury model reported a pulmonary AngII concentration fivefold higher than that of systemic AngII[26]. Perhaps in our study, plasma AngII levels are not as affected as the tissue levels, in particular the lung tissue. Several studies support the existence of local renin-angiotensin systems and that the majority of AngII production is in fact coming from peripheral tissue instead of the systemic circulation[27]. This concept suggests that kidney-derived renin and liver-derived angiotensinogen are made available to the tissue by the systemic circulation and AngII is thus produced by local tissue converting enzyme[27]. This could be consistent with our data given that lung tissue is known to contain large amounts of angiotensin converting enzyme. Further studies would require analysis of lung tissue AngII levels and perhaps systemic renin and angiotensinogen levels.

As stated earlier, there was no significant difference between study groups of plasma AngII levels. We had expected to see a greater level of AngII in the losartan treated group indicating that the receptors were being blocked and leading to a greater concentration of plasma AngII. However, our results were not as anticipated and could be explained by the inability to fully block AT1 receptors, and thus the need for a larger bolus dosage of losartan. We had based our chosen 50mg dose on physician

recommendation, which was between 25 and 100mg daily. Additionally, our dose response study (as previously described) was not showing any effects. Perhaps a future study would entail treating the sheep with an extremely large dosage of losartan as was done in a murine ventilator-induced lung injury study where the animals were treated with 30mg/kg losartan[28]. This study successfully showed losartan to attenuate clinical symptoms of this lung injury.

Given the overall results of the data, it appears that losartan was ineffective in attenuating the clinical symptoms seen in response to a burn and smoke inhalation injury in an ovine model. Further studies are necessary not only to confirm elevated angiotensin II levels in response to this injury, but also to possibly eliminate angiotensin receptors blockers as a therapeutic treatment method for this injury. This would allow future research to focus on more effective treatment methods for ARDS.

References

1. Dandona P, Dhindsa S, Ghanim H, Chaudhuri A. Angiotensin II and inflammation: the effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockade. *J Human Hypertens* 2007; 21: 20-27.

2. National Institutes of Health. "Fact Sheet: Burns and Traumatic Injury". September 2006. National Institutes of Health. 4 April 2008. http://www.nih.gov/about/researchresultsforthepublic/BurnsandTraumaticInjury.pdf.

3. Brenner, RA. "Medical Care Guide: Burn Statistics". 2002. Burn Survivor. 4 April 2008. http://www.burnsurvivor.com/burn_statistics.html.

4. Dancey DR, Hayes J, Gomez M, Schouten D, Fish J, Peters W, Slutsky AS, Stewart TE. ARDS in patients with thermal injury. *Intensive Care Med* 1999; 25: 1231-1236.

5. Ware LB and Matthay MA. The Acute Respiratory Distress Syndrome. *N Eng J Med* 2000; 342: 1334-1349.

6. Pugin J, Verghese G, Widmer MC, Matthay MA. The alveolar space is the site of intense inflammatory and profibrotic reactions in the early phase of acute respiratory distress syndrome. *Crit Care Med* 1999; 27: 304-312.

7. Ware, LB and Matthay MA. Acute Pulmonary Edema. *N Eng J Med* 2005; 353: 2788-2796.

8. Frohlich ED. Sixtieth Anniversary of Angiotensin. *Hypertension* 2001; 38: 1245.

9. Greenspan FS and Gardner DG. "Chapter 10: Endocrine Hypertension". Basic & Clinical Endocrinology 6th ed. McGraw Hill: New York, NY 2001.

10. Boron WF and Boulpaep EL. "Chapter 39: Integration of Salt and Water Balance". Medical Physiology updated edition. Elsevier Saunders: Philadelphia, PA 2005.

11. Spyroulias GA, Nikolakopoulou P, Tzakos A, Gerothanassis IP, Magafa V, Manessi-Zoupa E, Cordopatis P. Comparison of the solution structures of angiotensin I & II. *Eur J Biochem* 2003; 270: 2163-2173.

12. Seldin DW and Giebisch G. The Kidney: Physiology & Pathophysiology 2nd ed, Vol. 2. Raven Press, New York 1992. pp. 1413-1422.

13. Shade RE, Davis JO, Johnson JA, Gotshall RW, Spielman WS. Mechanism of action of AngII and ADH on renin secretion. *Am J Physiol* 1973; 224: 926-943.

14. Baker, RD. Cardiovascular Physiology. Strand Street Press: Galveston, TX 2003.

15. Marshall RP. The Pulmonary Renin-Angiotensin System. *Curr Pharmaceutical Design* 2003; 9: 715-722.

16. Physicians Desk Reference 58th ed. Thompson PDR: Montvale, NJ 2004. pp. 1952-1956.

17. Ribiero AB and Gavras H. Angiotensin II Antagonists- Clinical Experience in the Treatment of Hypertension, Prevention of Cardiovascular Outcomes and Renal Protection in Diabetic Nephropathy and Proteinuria. *Arq Bras Endocrinal Metab* 2006; 50: 327-333.

18. Imai Y, Kuba K, Rao S, Huan Y et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 2005; 436: 112-116.

19. Oudit GY, Kassiri Z, Patel M, Chappell M, Butany J, Backx P, Tsushima R, Scholey J, Khokha R, Penninger J. Angiotensin II-mediated oxidative stress and inflammation mediate the age-dependent cardiomyopathy in ACE2 null mice. *Cardiovascular Res* 2007; 75: 29-39.

20. Dandona P, Kumar V, Aljada A, Ghanim H, Syed T, Hofmayer D, Mohanty P, Tripathy D, Garg R. Angiotensin II receptor blocker valsartan suppresses reactive oxygen species generation in leukocytes, nuclear factor-kappa B, in mononuclear cells of normal subjects: evidence of an anti-inflammatory action. *J Clin Endocrinol Metab* 2003; 88: 4496-4501.

21. Brazeal BA, Honeycutt D, Traber L, Toole J, Herndon D, Traber D. Pentafraction for superior resuscitation of the ovine thermal burn. *Crit Care Med* 1995; 23: 332-339.

22. Enkhbaatar P, Cox RA, Traber LD, Westphal M, Esechie A, Morita N, Prough D, Herndon D, Traber D. Aerosolized anticoagulants ameliorate acute lung injury in sheep after exposure to burn and smoke inhalation. *Crit Care Med* 2007; 35: 2805-2810.

23. Mathews L and Singh RK. Cardiac output monitoring. *Ann Card Anaesth* 2008; 11: 56-58.

24. Sugi K, Theissen JL, Traber LD, Herndon DN, Traber DL. Impact of carbon monoxide on cardiopulmonary dysfunction after smoke inhalation injury. *Circ Res* 1990; 66: 69-75.

25. Allan DR, McKnight JA, Kifor I, Coletti CM, Hollenberg NK. Converting enzyme inhibition and renal tissue angiotensin II in the rat. *Hypertension* 1994; 24: 516-522.

26. Marshall RP, Gohlke P, Chambers RC, Howell DC, Bottoms, SE, Unger T, McAnulty RJ, Laurent GJ. Angiotensin II and the fibroproliferative response to acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2004; 286: L156-L164.

27. Campbell DJ. Circulating and Tissue Angiotensin Systems. *J Clin Invest* 1987; 79: 1-6.

28. Yao S, Feng D, Wu Q, Li K, Wang L. Losartan Attenuates Ventilator-Induced Lung Injury. *J Surg Res* 2008; 145: 25-32.

Vita

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