

Award Number:

W81XWH-09-1-0484

TITLE:

Mitochondrial Permeability Transition in Pathogenesis of Hemorrhagic Injury: Targeted Therapy with Minocycline

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REPORT DATE:

July 2010

TYPE OF REPORT:

Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

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# REPORT DOCUMENTATION PAGE

*Form Approved*  
*OMB No. 0704-0188*

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<b>1. REPORT DATE (DD-MM-YYYY)</b> 01-06-2010		<b>2. REPORT TYPE</b> Annual Report		<b>3. DATES COVERED (From - To)</b> 1 JUL 2009 - 30 JUN 2010	
<b>4. TITLE AND SUBTITLE</b>  Mitochondrial Permeability Transition in Pathogenesis of Hemorrhagic Injury: Targeted Therapy with Minocycline				<b>5a. CONTRACT NUMBER</b>	
				<b>5b. GRANT NUMBER</b> W81XWH-09-1-0484	
				<b>5c. PROGRAM ELEMENT NUMBER</b>	
<b>6. AUTHOR(S)</b>  John J. Lemasters				<b>5d. PROJECT NUMBER</b>	
				<b>5e. TASK NUMBER</b>	
				<b>5f. WORK UNIT NUMBER</b>	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  Department of Education, South Carolina  Charleston, SC 29425-0001				<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> U.S. Army Medical Research and Materiel Command Fort Detrick, MD 21702-5012				<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>	
				<b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b>  Approved for public release; distribution unlimited	
<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b>  Patients that initially survive hemorrhage and resuscitation may develop a systemic inflammatory response syndrome (SIRS) that leads to injury and dysfunction of vital organs (multiple organ dysfunction syndrome, MODS), particularly to the liver and kidney. SIRS and MODS may involve mitochondrial dysfunction. Minocycline and doxycycline are tetracycline derivatives that are cytoprotective to liver, brain and other organs in various models of hypoxic, ischemic and oxidative stress, which may act by preserving mitochondrial function. We determined whether minocycline and doxycycline protect liver and kidney in a mouse model of hemorrhage and resuscitation. Minocycline and doxycycline each decreased liver enzymes and creatinine in the blood after hemorrhage/resuscitation compared to vehicle. Minocycline and doxycycline also significantly decreased liver necrosis and liver and kidney apoptosis. In conclusion, minocycline and doxycycline when administered only after resuscitation decrease liver and kidney injury after hemorrhage/resuscitation. These safe and widely used agents might be useful clinically to prevent SIRS and MODS after hemorrhagic shock.					
<b>15. SUBJECT TERMS</b> Doxycycline, hemorrhage, kidney, liver, minocycline, mitochondria, permeability transition, resuscitation					
<b>16. SECURITY CLASSIFICATION OF:</b>			<b>17. LIMITATION OF ABSTRACT</b>  UU	<b>18. NUMBER OF PAGES</b>  4	<b>19a. NAME OF RESPONSIBLE PERSON</b> USAMRMC
<b>a. REPORT</b> U	<b>b. ABSTRACT</b> U	<b>c. THIS PAGE</b> U			<b>19b. TELEPHONE NUMBER (include area code)</b>

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### **Introduction**

Despite improvements in evacuation, 35% of deaths from far forward battlefield injuries occur prior to arrival to a field hospital. Even after successful hemorrhagic resuscitation, late deaths from multiple organ failure occur in up to 50% of victims of severe multiple trauma and hemorrhage. Early intervention is therefore needed and must be applied in a far forward setting to help stabilize injured combatants and to lessen the likelihood of unsuccessful resuscitation and late development of the systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS).

We hypothesize that mitochondrial dysfunction is central to the pathobiology leading to these deaths and that onset of the mitochondrial permeability transition is the fundamental pathophysiological event leading to mitochondrial failure and its dire bioenergetic consequences. Accordingly, we will make direct measurements by intravital multiphoton microscopy to determine whether onset of the mitochondrial permeability transition and resultant mitochondrial dysfunction does occur in the livers and kidneys of mice subjected to hemorrhage and resuscitation. Furthermore, we will assess the ability of minocycline, an agent that prevents the mitochondrial permeability transition by blocking mitochondrial calcium uptake, to decrease liver and kidney injury and improve overall survival after hemorrhage and resuscitation. Recently, we have discovered that doxycycline, another tetracycline derivative, is even more strongly cytoprotective in models of hypoxic and ischemic injury. Accordingly, we have added an examination of minocycline to our originally planned experiments.

### **Body**

**Minocycline Decreases Liver Injury after Hemorrhagic Shock and Resuscitation in Mice.** Patients that initially survive hemorrhage and resuscitation may develop a systemic inflammatory response syndrome (SIRS) that leads to injury and dysfunction of vital organs (multiple organ dysfunction syndrome, MODS). SIRS and MODS may involve mitochondrial dysfunction. Under pentobarbital anesthesia, C57BL6 mice were hemorrhaged to 30 mm Hg for 3 h and then resuscitated with shed blood plus half the volume of lactated Ringer's solution containing minocycline (10 mg/kg body weight), tetracycline (10 mg/kg body weight) or vehicle. Serum alanine aminotransferase (ALT), necrosis, apoptosis and oxidative stress were assessed 6 h after resuscitation. Mitochondrial polarization were assessed by intravital microscopy. After H/R with vehicle or tetracycline, ALT increased to 4538 U/L and 3999 U/L, respectively. Minocycline treatment decreased ALT to 1763 U/L ( $p<0.01$ ). Necrosis and TUNEL also decreased from 24.5 % and 17.7 cells/field, respectively, after vehicle to 8.3% and 8.7 cells/field after minocycline. Tetracycline failed to decrease necrosis (23.3%) but decreased apoptosis to 9 cells/field ( $p<0.05$ ). Additionally, minocycline (67%) and tetracycline (77%) decreased caspase-3 activity in liver homogenates ( $p<0.05$ ). Lipid peroxidation was decreased after resuscitation with minocycline about 70% but not after resuscitation with tetracycline ( $p<0.05$ ). Intravital microscopy showed that minocycline preserved mitochondrial polarization after H/R ( $p<0.05$ ). Minocycline decreases liver injury after hemorrhage and resuscitation by preventing MPT-dependent mitochondrial dysfunction. Minocycline might be useful clinically after hemorrhage shock and resuscitation to prevent SIRS and MODS.

**Doxycycline as Well as Minocycline Decrease Liver and Kidney Injury After Hemorrhagic Shock and Resuscitation in Mice.** Patients that initially survive hemorrhage and resuscitation may develop a

systemic inflammatory response syndrome (SIRS) that leads to injury and dysfunction of vital organs (multiple organ dysfunction syndrome, MODS), particularly to the liver and kidney. SIRS and MODS may involve mitochondrial dysfunction. Minocycline and doxycycline are tetracycline derivatives that are cytoprotective to liver, brain and other organs in various models of hypoxic, ischemic and oxidative stress and which may act by preserving mitochondrial function. Here, our **Aim** was to determine whether minocycline and doxycycline protect liver and kidney in a mouse model of hemorrhage and resuscitation. **Methods:** Under pentobarbital anesthesia, C57BL6 mice were hemorrhaged to 30 mm Hg for 3 h and then resuscitated with shed blood plus half the volume of lactated Ringer's solution containing minocycline (10 mg/kg body weight), doxycycline (5 mg/kg body weight) or vehicle. Serum alanine aminotransferase (ALT), serum creatinine and urea, and caspase-3 activity were assessed 6 h after resuscitation. Liver and kidney histology and immunochemistry were also assessed at 6 h after the resuscitation. **Results:** After resuscitation with vehicle, ALT increased to 1988 U/L. Minocycline and doxycycline decreased ALT to 857 U/L and 789 U/L, respectively ( $p < 0.001$ ). After resuscitation with vehicle, blood creatinine increased to 273  $\mu$ M. Minocycline and doxycycline treatment decreased blood creatinine to 109  $\mu$ M and 92  $\mu$ M, respectively ( $p < 0.05$ ). Minocycline and doxycycline treatment also significantly decreased liver necrosis and liver and kidney apoptosis. Caspase-3 activity in liver homogenates was decreased by 52% ( $p < 0.01$ ). **Conclusion:** Minocycline and doxycycline even when administered only after resuscitation decrease liver and kidney injury after hemorrhage/resuscitation. These safe and widely used agents might be useful clinically to prevent SIRS and MODS after hemorrhagic shock and other systemic stresses.

### **Key Research Accomplishments**

We have shown that minocycline and doxycycline even when administered only after resuscitation each decrease liver and kidney injury after hemorrhage/resuscitation. These safe and widely used agents might be useful clinically to prevent SIRS and MODS after hemorrhagic shock in civilian and military medicine.

### **Reportable Outcomes**

A manuscript is in preparation entitled "Minocycline Decreases Liver Injury after Hemorrhagic Shock and Resuscitation in Mice" for the journal *Shock*. An abstract entitled "Minocycline and Doxycycline Decrease Liver and Kidney Injury After Hemorrhagic Shock and Resuscitation in Mice" by Andaleb Kholmukhamedov, Christoph Czerny, Jiangting Hu, Justin Schwartz, Venkat Ramshesh and John J. Lemasters has been accepted for presentation at the Annual Meeting of the American Association for the Study of Liver Diseases in Boston, MA, October 29-November 2, 2010.

### **Conclusion**

Minocycline and doxycycline are safe and widely used agents might be useful clinically to prevent SIRS and MODS after hemorrhagic shock in civilian and military medicine.

### **References**

None.

### **Appendices**

None