

AD _____

Award Number: W81XWH-10-1-0508

TITLE: Efficacy of Gamma-glutamylcysteine (GGC) in Ischemia-reperfusion Injury

PRINCIPAL INVESTIGATOR: Stanley T. Omaye, Ph.D.

CONTRACTING ORGANIZATION: Nevada System of Higher Education
Reno, NV 89557-001

REPORT DATE: U&A 2012

TYPE OF REPORT: ~~Other~~

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

| | | | | | |
|--|-------------------------|-------------------------------|---|--------------------------------------|---|
| 1. REPORT DATE U&f à^! 2012 | | 2. REPORT TYPE Øø æ | 3. DATES COVERED 1 July 201€ – 30 Ù^! ç{ à^! 2012 | | |
| 4. TITLE AND SUBTITLE Efficacy of Gamma-glutamylcysteine (GGC) in Ischemia-reperfusion Injury | | | 5a. CONTRACT NUMBER | | |
| | | | 5b. GRANT NUMBER W81XWH-10-1-0508 | | |
| | | | 5c. PROGRAM ELEMENT NUMBER | | |
| 6. AUTHOR(S) Stanley T. Omaye | | | 5d. PROJECT NUMBER | | |
| | | | 5e. TASK NUMBER | | |
| E-Mail: [{ æ^O~ }!Èâ~ | | | 5f. WORK UNIT NUMBER | | |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Nevada System of Higher Education Reno, NV 89557-001 | | | 8. PERFORMING ORGANIZATION REPORT NUMBER | | |
| 9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012 | | | 10. SPONSOR/MONITOR'S ACRONYM(S) | | |
| | | | 11. SPONSOR/MONITOR'S REPORT NUMBER(S) | | |
| 12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited | | | | | |
| 13. SUPPLEMENTARY NOTES | | | | | |
| 14. ABSTRACT Öæ { æ^ çæ ^ & • çã ^ ÁÖÖÖDá !^&• ! [!Áã^] çã^ Á! Á! çæç } ^ ÁÖÜPDá æ Á @, } Á! Á çãã çãæã^ Á d^ • Á Á @ { æ Á ~ { àããÁ^ á } á [çãã^ Á! • ÁPWXOÖDÁÖÖÁ! ç & ç æ çæ • ç çãæã^ Á d^ • Á Á çãã } Á Á^ } ^ Á ç! ^ • ç } Á Á ç çã ç ç á^ • Á ç á^ } á^ } ç Á ç & ^ æ^ á^ á^ & çã } Á Á^ } á [* ^ } [• ÖUPÁ^] ç • á ÉÖÁ ç ç! ^ Á ç áã • Á! [! ç * Á ç Á^ & ç Á ÁÖÖÁ ç çã } ç æ^ á^ á^ [! çããÁÖÖDá ÁPWXOÖÁ Á Á^ } á Á çã ç Á ç ç! ^ Á! ç & ç á ç çæ ç ç çãæã^ Á d^ • Á ç çãã Á^ çæ ^ } ç çÖÖÖÁ ç } ^ Á Á @! ^ Á^ * * ^ • ç ç ÖÖÖÁ Á Á^ } Á Á^ } á ^ Á & [] [] } á ç ç Á ç! ç Á! ç & ç } Á! [Á çãæã^ Á d^ • Á Á } [ç^ Á [á^ çãã } Á Á^ } ^ Á ç! ^ • ç } Á Á ç çã ç ç á^ • Á^ : ^ { ^ ÉÖ í ç * Á ç Á É [] ç ç! ç áá^ Á ç ç ç á^ á^ Á } çã^ Á ÖÖÖÁ • ç * Á ç Á ç! ç á^ • ç çã [ç ç á^ [{ Á! á^ } ç Á^] ç Á^ ç çã^ } çã^ Á ç ç! ç! { ç ç & Á ç çã & [{ çæ *! ç ç Á^ ç ç á^ ÇÚSÖDÁ • ç * Á^ [! ç á^ ç & çã } Á! Á^ ç ç! ç * ÁÖÖÖÁ ç^ Á! ÉÖ ç çã * Á^ * * ^ • ç ç ÖÖÖÁ [• • Á^ Á^ { ç! ç ç ç^ á^ Á ç ç á^ ÁÖÖÖÁ & } & } çãã } Á^ } á^ á^ Á @ • ç [* çãã^ * ^ ÉÁ | | | | | |
| 15. SUBJECT TERMS Oxidative stress, antioxidants, glutathione, gamma-glutamylcysteine. | | | | | |
| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT UU | 18. NUMBER OF PAGES Áí | 19a. NAME OF RESPONSIBLE PERSON USAMRMC |
| a. REPORT U | b. ABSTRACT U | c. THIS PAGE U | | | 19b. TELEPHONE NUMBER (include area code) |

Table of Contents

| | <u>Page</u> |
|------------------------------|-------------|
| Introduction | 3 |
| Body | 3 |
| Key Research Accomplishments | 3 |
| Reportable Outcomes | 4 |
| Conclusion | 4 |
| References | |
| Appendices | 4 |

INTRODUCTION (from previous report):

Hemorrhagic shock is a leading cause of death in military combat and civilian trauma (Bellamy, 1984; Lieu, et al., 2004). Better understanding of the associated cellular biochemical changes that occur in ischemia-reperfusion (IR) injury can lead to efficacious therapies (Thomas et al., 2008). Therefore, we have been studying the feasibility of using gamma-glutamylcysteine (GGC) a dipeptide precursor for glutathione as a potential compound in modulating the oxidative stress associated with IR injury.

BODY:

Specific goal – Determine GGC doses to inhibit cellular oxidative stress and inhibit cellular death.

1. GGC inhibition of oxidative stress in human endothelial cells.

The objective of this study was to investigate the efficacy of GGC on GSH synthesis and oxidative stress in human endothelial cells, as a model for cellular oxidative stress. We found that GGC plays a role in GSH synthesis as a substrate for the antioxidant GSH and in modulating expression of proteins related to antioxidant defense as an inducer or suppressor.

2. Co administration of GGC and conjugated linoleic acid (CLA) in human endothelial cells.

The objective of this study was compared effects of co-administration of GGC and CLA with GGC alone on oxidative stress. We confirmed that GGC can substitute as an antioxidant for GSH without increasing GSH levels. Co-administration of CLA with GGC had differential effects depending on the dose of CLA. We believe that due to its ease of permeability through cell membranes, GGC could be used as an intra and intercellular therapeutic agent in oxidative stress-related injuries and diseases.

3. (New, June 30 – Oct 31, 2012) The objective was to assess the potential ability of GGC to be absorbed. Use of everted gut sac isolated from rats for studying the transport of GGC (5, 10 and 15 micromol/Liter) across the small intestine. Everted gut sacs were isolated from SD rats weighing approximately 250 g (El-Gorab et al, 1975; Barth et al., 1998; Mahmoud, 2004). Approximately 20 to 30% of the GGC was recovered and was GGC concentration dependent following 30 minutes of incubation of the suspended guts.

KEY RESEARCH ACCOMPLISHMENTS:

Two studies using human endothelial cells have been completed, peer reviewed and published. The studies indicate that GGC has efficacy in oxidative stress and suggest the potential usefulness of this compound in injuries and diseases associated with oxidative stress.

A sensitive method using high performance liquid chromatography (HPLC) and fluorimetric detection has been developed.

Preliminary studies suggest that GGC crosses the rat intestinal wall and is concentration dependent.

REPORTABLE OUTCOMES:

Published Abstracts

Nakamura, Y. K., Dubick, M. A., and **Omaye, S. T.** Gamma-Glutamylcysteine (GGC) inhibition of oxidative stress in human endothelial cells. Emerging Topics Section, Society of Toxicology Annual Meeting, 2011, Washington, D.C.

Nakamura, Y. K., Dubick, M. A., and **Omaye, S. T.** Effects of co-administration of gamma-glutamylcysteine (GGC) and conjugated linoleic acid (CLA) on oxidative stress in human endothelial cells. American Chemical Society Annual meeting, Denver, Colorado, 2011.

Published Peer-Reviewed Manuscripts

Nakamura, Y.K., Dubick, M.A., and Omaye, S. T. Gamma-glutamylcysteine inhibits oxidative stress in human endothelial cells. *Life Sciences* 90: 116-121, 2012.

Nakamura, Y.K., Dubick, M.A., and Omaye, S. T. Modulation of oxidative stress by γ -glutamylcysteine (GGC) and conjugated linoleic acid (CLA) isomer mixture in human umbilical vein endothelial cells. *Food and Chemical Toxicol* 60: 1854-1859, 2012.

CONCLUSION:

Although further studies are warranted to develop a better understanding about the efficacy of GGC supplementation under various conditions, GGC has potential as a therapeutic compound in modulation of oxidative stress. GGC appears to be absorbed in the gut and available to systemic circulation. Future studies will be directed at to better understand the mechanisms of action and eventual application of GGC in oxidative stress associated with ischemia reperfusion and hemorrhagic shock.

REFERENCES:

Barthe, L., Woodley, J. F., Kenworthy, S. and Houin, G. An improved everted gut sac as a simple and accurate technique to measure paracellular transport across the small intestine. *Eur J Drug Metab Pharmacol* 23: 313-323, 1998.

Bellamy, R. F. 1984. The cause of death in conventional land warfare: implication for combat casualty care research. *Mil Med* 149: 55-62.

El-Gorab, M. I., Underwood, B. A., and Lerch, J. D. The roles of bile salts in the uptake of beta-carotene and retinol by rat everted gut sacs. *Biochimica et Biophysica Acta* 401: 2650277, 1975.

Liu, L-M, Hu, D-Y, Chen, H-S, and Hu, P-H. 2004. The effect of different volumes of fluid resuscitation on traumatic-hemorrhagic shock at high altitude in the unacclimated rat. *Shock*. 21: 93-96.

Mahmoud, M. r. Everted intestinal sacs as in vitro model for assessing absorptive of L-histidine under the effect of aspirine and gum acacia in male rats. *Egyptian J of Hospital Med* 16: 14-28, 2004

Thomas, w., Krah, J. F., Kauvar, D. S. and Baer, D. G. 2008. The combined influence of hemorrhage and tourniquet application on the recovery of muscle function in rat. *J Ortho Trauma* 22: 47-51.

APPENDICES:

1. Abstract for published manuscript in Life Sciences
2. Abstract for published manuscript in Food and Chemical Toxicology