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Increased Survival in Septic Mice Using Whole Body Periodic Acceleration (pGz)

Adams, Jose A.[(1)] ,Uryash, Arkady[(1)]. Lopez, Jose R.[(1)], Sackner, Marvin, A.[(2)]

[1]*Mount Sinai Medical Center Miami Beach FL, Div. Neonatology*
[2] *Sackner Wellness Products LLC*

Corresponding author:

Jose A. Adams, M.D.
Mount Sinai Medical Center
Div Neonatology
4300 Alton Road - Miami Beach, Florida 33140
Tony-Adams@msmc.com

Abstract

Sepsis constitutes a major cause of mortality and healthcare expenditure. Whole body periodic acceleration (pGz) is the repetitive motion of the body in the head to foot axis using a motion platform. pGz induces pulsatile shear stress to the vascular endothelium, which increases constitutive nitric oxide, prostacyclin, and adrenomedullin, all which stabilize endothelial cellular junctions and are cytoprotective. We hypothesized that treatment with pGz prior to sepsis, would reduce mortality in mice given a lethal dose of LPS. Mice were randomized to 3-day (1hr per day) treatment with pGz (pGz-LPS) or control (CONT-LPS). A lethal dose of LPS was given and behavioral scoring and survival monitored every 15 min for 48hrs. Tissue was harvested for nitric oxide synthase isoforms protein expression. In a separate group, a nitric oxide synthase inhibitor (L-NAME) was given during pGz or control. There were no survivors in the control group 30 hrs after LPS, in contrast pGz improved survival to 60% and maintained this survival beyond 48 hrs. L-NAME, markedly reduced survival. This study is the first to show that a non-invasive, non-pharmacological technology pGz has potential to significantly improve outcome from sepsis.

Introduction

Sepsis strikes more than one million people in the USA, and is estimated to kill 28-50%. Far more people die of sepsis than the combined number of deaths from prostate, breast cancer, and AIDS. Worldwide incidence of sepsis has been estimated to be close to 19 million per year, but true incidence is unknown. Additionally, sepsis is one of the most costly conditions treated in the USA amounting more than $20 billion in 2011 (Angus and van der Poll 2013, Singer, Deutschman et al. 2016). Sepsis is treated with antibiotics and managed with supportive therapies such as fluid replacement, ventilatory and, hemodynamic support and in some instances corticosteroids. Unfortunately, since the 1960's all targeted clinical pharmacologic trials have failed to significantly alter mortality from sepsis.

Sepsis disrupts endothelial barrier, reduces microcirculatory flow and produces an intense

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inflammatory and oxidative stress response which challenges targeted therapies. Lipopolysaccharide (LPS) injected in animals mimics sepsis allows study of endothelial barrier dysfunction and intense inflammatory and oxidative stress responses.

Increased endothelial nitric oxide (eNO), prostacyclin, sirtuitin 1 (SIRT1) and adrenomedullin, all stabilize endothelial cellular junctions and are anti-inflammatory, and antioxidants decrease reactive oxygen species (ROS) burden. The latter mediators are increased by pulsatile shear stress to the vascular endothelium.

Figure 1. Panel A. *Linear Motion platform (Scilogex SKL-480, Scilogex, Rocky Hill, CT) which can perform pGz in small animal models. Un-sedated animals are placed in the rodent holder. Sinusoidal head to foot (Z-plane) motion is imparted to produce pulsatile shear stress.* ***Panel B.*** *Exer-Rest® (NIMS, Miami Florida) motion platform used to impart whole body period acceleration in human subiects.*

Whole body periodic acceleration (pGz) is the sinusoidal motion of the body in a headward - footward direction (similar to a mother moving a baby carriage back and forth) pGz is non-invasive means produced by a motion platform to add pulses to the circulation (*Figure 1*) Such pulses increase shear stress to the vascular endothelium. pGz increases eNO in heart and isolated vessels, and increases production of prostacyclin, SIRT1 and adrenomedullin as well as upregulation of antioxidants. Additionally, pGz is cardioprotective when administered to animal models of focal or whole body ischemia reperfusion injury. (Adams, Bassuk et al. 2005, Uryash, Wu et al. 2009, Uryash, Bassuk et al. 2015)

We hypothesized that treatment with this non-invasive technology prior to sepsis, would reduce mortality in mice given a lethal dose of LPS.

Materials and Methods

Periodic Acceleration

Adult mice (C57BL6, Jackson Laboratory, Bar Harbor, ME) (n=36) where randomized to receive pGz prior to LPS administration (pGz-LPS, n=18) or control LPS treated (CONT-LPS, n=18). pGz was performed by placing conscious mice into a mice holder (Kent Scientific, Connecticut, USA) and the holder placed on a motion platform (Figure 1) which moves mice at a frequency of 480 cycles/min, acceleration of ± 3.0mt^2/sec, for 1 hour daily for 3 consecutive days. CONT-LPS mice were also placed in the mouse holder but not on the motion platform. On the third day of pGz, LPS was administered intraperitoneally (IP). A separate group (n=10) served as Sham.

LPS model and Behavioral Scoring

Lipopolysaccharide (E.Coli O111B4, Sigma-Aldrich, St Louis, MO) a dose of 40mg/kg, lethal dose, was injected I.P. The animals where placed in their original housing, water and food provided for ad libitum consumption. A video recorder was used to record the entire period after LPS administration. A robust behavioral scoring system used to evaluate sepsis severity in mice was utilized. The Behavioral Scoring Criteria uses 8 categories with a 4-point scale per category (Shrum, Anantha et al. 2014). Lower scores are normal, and a maximum score of 32 is moribund. Behavioral scoring was performed in each mouse at 15 min intervals until death or survival and discontinued 48 hr after LPS.

To determine whether or not the effects of pGz are in part nitric oxide (NO) dependent, in a separate group of mice (n=18 per group) a nonspecific nitric oxide synthase inhibitor NG-nitro-L-arginine methyl ester (L-NAME, 100mg/kg/day) was administered to both CONT- LPS and pGz-LPS treated animals, for 7 days in drinking water prior to LPS administration.

Protein Analysis

In a separate group of animals (n=10 per group) analysis of nitric oxide synthases protein expression in myocardium was carried out using Western Blot. Animals were sacrificed at 90 and 360 min after LPS and hearts excised and processed to extract Nitric Oxide Synthases (NOS). Protein extraction was performed as previously described (Uryash, Bassuk et al. 2015). The primary antibodies used included eNOS, p-eNOS $^{(Ser1177),)}$, iNOS and nNOS (Santa Cruz Biotechnology, Inc. Santa Cruz, CA.) Individual Protein loading control used was Glyceraldehyde-3-phosphatedehydrogenase (GAPDH) Blots were visualized by Enhanced Chemifluorescence (ECF)

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(GE Healthcare Bio-Sciences Corporation, Piscataway, NJ) on Storm 860 Imaging System (GE Healthcare Bio-Sciences Corporation, Piscataway, NJ). Fluorescent signal intensities and protein levels were quantified using ImageQuant software (GE Healthcare Bio-Sciences Corporation, Piscataway, NJ). The study protocol was approved by Mount Sinai Medical Center Animal Use and Care Committee.

Results and Discussion

Behavioral scoring showed a significant difference early in the course of sepsis (8 hr after LPS) between CONT-LPS and pGz-LPS groups. These early differences were associated with significant survival in pGz-LPS group. There was no survival beyond 30 hr after LPS administration in LPS control mice. In contrast, 60% of pGz-LPS treated mice survived beyond 48 hr after LPS ($p < 0.0001$). The NO inhibitor L-NAME abolished the pGz response and hastened mortality in CONT-LPS animals. (*Figure 2*)

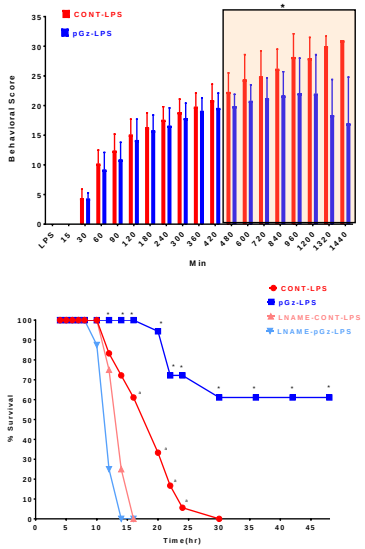

Figure 2. Panel A ***<u>Behavioral Scoring after LPS</u>*** *in LPS and pGz-LPS mice performed every 15 min for the first 1440 min. Shaded area denotes significant difference between LPS and pGz-LPS from 480 to 1440 min (X±SD)* ***Panel B. <u>Survival after LPS</u>.*** *In CONT-LPS, pGz-LPS, and LNAME-CONT-LPS and LNAME-pGz- LPS groups. There was no survival in CONT-LPS group 30 hr after LPS administration. pGz improved survival to 60% beyond 30 hr. L-NAME blunted the pGz response and hasten mortality in CONT-LPS. (*p< 0.0001 CONT-LPS vs pGz-LPS and LNAME, [a]p< 0.01 CONT-LPS vs LNAME*

NOS protein expression at 90 min after LPS showed a significant increase in iNOS and decrease in nNOS. pGz significantly increased eNOS, p-eNOS and nNOS and markedly decreased iNOS expression.

The data are similar with respect to the effects of pGz on eNOS p-eNOs and nNOS expression at 360 min.

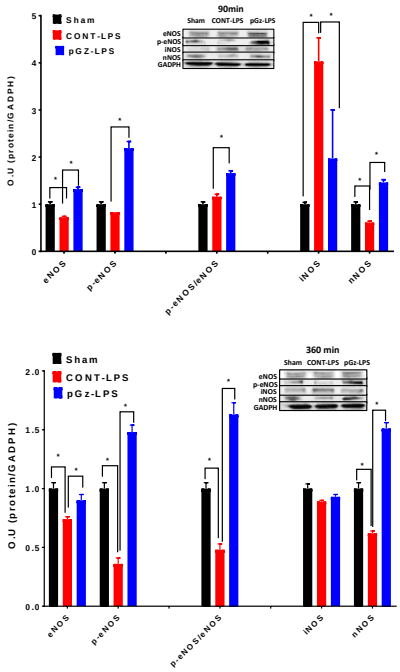

Figure 3. Panel A *NOS Protein Expression 90 min after LPS.* ***Panel B.*** *NOS Protein Expression 360 min after LPS (*p < 0.001 SHAM or CONT-LPS vs pGz-LPS). (X±SD*

iNOS was not significantly increased at 360 min in CONT-LPS or pGz-LPS treated animals. (*Figure 3)*

pGz is a noninvasive, non-pharmacological therapeutic intervention which improved survival in this lethal model of LPS. The severity of this model and high mortality rate with the dose of LPS utilized in this study, improvement in survival with pGz is quite significant. pGz via pulsatile shear stress elicits production of a host of mediators. eNOS is produced primarily in endothelial cells in very small quantities constitutively (micromolar), and is important as both a signaling molecule and mediator of anti-inflammatory signaling response. The observed increased in eNOS and phosphorylation (p-eNOS) would increase the cytoprotective and anti-inflammatory eNO (Mao, Chen et al. 2013, Zhao, Vanhoutte et al. 2015). In contrast, iNOS produced mainly by macropages and leukocytes in very large quantities (macromolar) produces marked hypotension and increase in pro-inflammatory cytokines. eNOS has also been shown to decreased iNOS production. pGz increased eNOS activity by its phosphorylation and blunted the significant rise in iNOS induced by LPS. nNOS has been shown to play an important role in myocardial function The observed decrease in nNOS by LPS has

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not been reported and may be significant in myocardial depression observed during LPS, particularly since nNOS has been shown to decrease TNF-α production in the heart (Zhang and Feng 2010). pGz restored nNOS levels at both 90 and 360 min after LPS.

Inhibition of NOS via LNAME, significantly reduced survival in both CONT-LPS and pGz-LPS treated animals. The latter suggest that NO is critical for survival in sepsis, and that in part, the salutary effects of pGz may be due to its increase NO. It is not surprising that NOS inhibition resulted in greater mortality, the latter was also observed in large clinical trials (Lopez, Lorente et al. 2004).

Figure 4. *Gentle Jogger ™ (Sackner Wellness, Miami Florida), A motorized device passively applies a tapping force to the bottoms of a user's feet through a pedal rocking mechanism, with the pedals tapping against a bumper to provide sufficient force to the bottom of the user's foot to introduce small pulses into the circulation. The device can be used in seated or supine posture.*

This study is the first to show that a non-invasive, non-pharmacological technology has the potential to improve outcomes from sepsis. In the clinical setting, pretreatment with pGz can realistically only be undertaken in hospitalized patients at risk for infection. It is estimated that 3-4% of all hospitalized patients will have a 'healthcare associated sepsis." Preliminary results with the same dose of LPS as in the current study revealed excellent survival *1 hr after LPS injection*. In the current study in mice, pGz was administered with a motorized platform. In humans, pGz can be given with a bed-like motorized platform but such a platform is costly and requires patient transfer. Recently, a simple noninvasive device that induces pulsatile shear stress has been developed as a wellness device, (Gentle Jogger® (Sackner Wellness Products LLC, Miami Florida) (Figure 4). It uses motorized pedals to repetitively tap the feet against a bumper to add pulses into circulation. It is portable, weighing 4.8 kg and can be applied in seated posture as well as within a bed attached to the footboard for hospitalized patients.

This device has potential for preconditioning in patients to mitigate the mortality of sepsis. Our study provides pre-clinical data for a clinical trial aimed at reducing mortality from sepsis.

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