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AIDS 2016, **30**:2389–2394

A randomized, placebo-controlled pilot trial of *N*-acetylcysteine on oxidative stress and endothelial function in HIV-infected older adults receiving antiretroviral treatment

HIV-infected individuals are at higher risk for cardiovascular disease (CVD) compared with the general population [1]. This increased risk is exponentially greater in older HIV-infected persons [1]. Given that the survival rate of the HIV-infected population is nearing that of the general population with the widespread use of highly effective antiretroviral treatment (ART), there is growing concern that the risk of CVD will be magnified several-fold as this aging group continues to increase in prevalence. Thus, therapies to address this risk for CVD in older patients are needed.

Oxidative stress denotes inappropriately increased intracellular generation of reactive oxygen species (ROS) which in turn damages cellular lipid membranes and organelles with subsequent cellular dysfunction and death. Chronic oxidative stress has been associated with the aging process through accumulated cellular damage and senescence and thus is a strong candidate for the mechanism by which HIV may lead to premature aging [2].

It remains unknown if pharmacologically reducing ROS will reduce oxidative stress and improve CVD risk in HIV-infected patients already receiving ART.

Glutathione is the primary intracellular antioxidant responsible for controlling oxidative stress [3] and appears to be depleted in those with HIV infection [4]. *N*-acetylcysteine (NAC) is a sulfur hydroxyl compound that replenishes intracellular cysteine that is required for glutathione regeneration. NAC has been used safely in several small trials of HIV-infected patients not yet on ART [5–7] and in most, but not all, replenished glutathione stores and reduced oxidative stress measures. But trials are needed to determine if NAC can similarly reduce oxidative stress in ART-treated, virologically suppressed patients.

We performed a prospective, randomized, double-blind, three-arm, parallel-group, placebo-controlled, 8-week pilot trial in HIV-infected patients at least 50 years old and receiving virologically suppressive ART (ClinicalTrials.gov NCT01962961) to evaluate the potential efficacy and safety of PharmaNAC, a commercially available product considered to be a supplement and not requiring Food and Drug Administration approval (http://www.pharmanac.com/; BioAdvantex Pharma Inc., Mississauga, Ontario, Canada). PharmaNAC contains 900 mg of NAC as an effervescent tablet without the sulfur odor associated with other forms of NAC. Participants were equally randomized to one of the following three study arms: PharmaNAC 900 mg twice daily, PharmaNAC 1800 mg twice daily, or matching placebos. Randomization with varying block sizes was implemented at the entry visit with stratification for current smoking.

The primary objectives of this study were to compare the 8-week changes in levels of circulating malondialdehyde (MDA) and F2-isoprostane, both measures of oxidative stress, and flow-mediated dilation (FMD) of the brachial artery as a physiologic measure of endothelial function. The red blood cell oxidative stress markers reduced glutathione (GSH), oxidized GSH (GSSG), and GSH : GSSG ratios were measured on the first 15 participants enrolled into the trial (seven, six, and two in the PharmaNAC 900 mg bid arm, the PharmaNAC 1800 mg bid arm, and the placebo arm, respectively).

HIV-infected patients of age at least 50 years and receiving ART for at least 6 months and resulting in an HIV-1 RNA level less than 75 copies/ml at study screening were eligible. Primary exclusion criteria included diagnosed vascular disease (including congestive heart failure), history of portal hypertension or hepatic cirrhosis, diagnosis of asthma or COPD, previous receipt of stavudine or didanosine for more than 7 days, current receipt of daily vitamins C or E, and alcohol use more than the equivalent of 8 oz of wine daily for 7 days prior to screening. This study was approved by the Indiana University Institutional Review Board; all participants provided written informed consent.

For all analyses, an intention-to-treat approach was used. Statistical significance was considered if two-sided *P* values were less than 5%. As a pilot study, no formal sample size justification was performed, and enrollment was based primarily on availability of study resources. Multivariable linear regression models were constructed in those who had paired data available to evaluate changes in the oxidative stress markers (MDA, F2-isoprostanes, GSH, GSSG, and GSH : GSSG) and FMD; these models included study arm assignment and baseline values of each marker (as there were nonsignificant, but appreciable, imbalances of these markers at baseline) to determine their effects on changes at week 8.

Twenty-six patients were screened for inclusion into this trial; two of these failed screening due to disqualifying laboratory values with the remaining 24 participants enrolled and randomized between December 2013 and May 2014. Of these 24, nine were randomized to PharmaNAC 900 mg twice daily, eight were randomized to PharmaNAC 1800 mg twice daily, and seven were randomized to placebo. The baseline characteristics of the study arms are shown in Supplemental Table 1, http://links.lww.com/QAD/A963. One participant in the PharmaNAC 1800 mg bid arm stopped study participation due to the detection of anal cancer not thought to be due to study participation. Another participant was discontinued due to the development of a severe rash that was later determined to be due to scabies and not thought to be due to study participation. The numbers of adverse events were similar amongst the three study arms without any appreciable differences in specific symptoms, laboratory toxicities, or diagnoses.

Results of models incorporating baseline values of the endpoints of interest on the changes from entry to week 8 are shown in Table 1. We found that baseline levels of F2-isoprostanes, FMD, and GSSG were significantly associated with their respective changes at week 8. We also found that treatment with PharmaNAC at each dosage compared with placebo led to non-significant, but appreciable, decreases in F2-isoprostane levels, increases in GSH, reductions in GSSG, and increases in GSH : GSSG at week 8. However, there were no appreciable differences in the changes in MDA with PharmaNAC at either dosage compared with placebo at Week 8. Of note, additional adjustments for race and sex in these models did not appreciably affect these results.

Table 1. Week 8 changes in flow-mediated dilation and oxidative stress markers after adjusting for baseline values.

Outcome	Effect	Point estimate	*P* value
F2-isoprostanes (pg/ml)	1800 mg bid vs. placebo	−6.87	0.51
	900 mg bid vs. placebo	−7.87	0.44
	Baseline value	*−0.82*	*<0.0001*
MDA (μM)	1800 mg bid vs. placebo	0.017	0.98
	900 mg bid vs. placebo	0.21	0.75
	Baseline value	0.17	0.65
FMD (%)	1800 mg bid vs. placebo	0.75	0.44
	900 mg bid vs. placebo	0.83	0.38
	Baseline value	*−0.56*	*0.0015*
GSH (μM)	1800 mg bid vs. placebo	177.60	0.67
	900 mg bid vs. placebo	409.75	0.38
	Baseline value	−0.11	0.85
GSSG (μM)	1800 mg bid vs. placebo	−22.49	0.62
	900 mg bid vs. placebo	−11.69	0.80
	Baseline value	*−0.76*	*0.049*
GSH : GSSG	1800 mg bid vs. placebo	7.90	0.71
	900 mg bid vs. placebo	23.40	0.23
	Baseline value	−0.53	0.36

FMD, flow-mediated dilation; GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, malondialdehyde.

In this randomized, placebo-controlled, pilot trial, we found that use of PharmaNAC at either 1800 mg twice daily or 900 mg twice daily for 8 weeks was generally well tolerated in HIV-infected adults at least 50 years old and who were receiving virologically suppressive ART. We found that that the levels of GSH in red blood cells increased substantially, whereas the levels of red blood cell GSSG decreased substantially, thereby leading to overall nonsignificantly increased ratios of GSH : GSSG with both doses of PharmaNAC compared with placebo. This suggests that this preparation of NAC may enhance the ability of cells to neutralize higher levels of ROS. In fact, we did find that F2-isoprostane levels, but not MDA levels, decreased with both PharmaNAC dosages compared with placebo. And, in turn, FMD levels increased in both PhamaNAC arms compared with placebo, suggesting an improvement in endothelial function with NAC. In this initial pilot study, the small sample sizes likely precluded finding statistically significant differences between either of the two doses of PharmaNAC and placebo for the primary endpoints of oxidative stress and endothelial function. As we believe that these changes are clinically relevant, a more definitive, longer term trial with adequate power appears to be justified.

Acknowledgements

We thank the study participants for their generous participation. We also thank Ms Paula Johnson, NP for study coordination, Ms Julnica Scott for performing the vascular ultrasonography studies, and Ms Lauren Kennedy for data entry.

Author contributions: Study conception and design: S.K.G. and Z.L.; study performance: S.K.G., L.M.K., and Z.L.; data interpretation: S.K.G., L.M.K., M.A.C., and Z.L.; and drafting and critical revision of the manuscript: S.K.G., L.M.K., M.A.C., and Z.L.

Sources of support: This work was supported by the Indiana University Health – Indiana University School of Medicine Strategic Research Initiative. Additional support was provided by the Indiana Clinical and Translational Sciences Institute funded in part by Grant no. UL1TR001108 from the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical Translational Sciences Award. This investigation was conducted in a facility constructed with support from Research Facilities Improvement Program grant no. C06 RR020128-01 from the National Center for Research Resources, National Institutes of Health. BioAdvantex Pharma, Inc. provided *N*-acetylcysteine in the form of PharmaNAC and matching placebo as well as an unrestricted grant in support of the laboratory measures performed in this trial.

Dr Gupta has received unrestricted grant support from Gilead Sciences, Inc. and Bioadvantex Pharma, Inc.; consultancy fees from ICON/Oncolys and Bristol-Myers Squibb; and travel support to present research findings from Gilead Sciences, Inc. and Bristol-Myers Squibb.

Conflicts of interest

There are no conflicts of interest.

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Received: 27 July 2016; accepted: 29 July 2016.

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DOI:10.1097/QAD.0000000000001222

Augmentation of anti-simian immunodeficiency virus activity in CD8$^+$ cells by neutralizing but not nonneutralizing antibodies in the acute phase

An understanding of T-cell and neutralizing antibody (NAb)-protective correlates would contribute to the development of anti-HIV strategies [1]. Acute-phase NAb impairment in HIV and simian immunodeficiency virus (SIV) infection [1,2] suggests the importance of identifying anti-HIV mechanisms of antibodies.

Reports have described passive NAb efficacy in HIV/SIV sterile protection [3,4] and postinfection viremia reduction [5–12]. We have developed a model of passive NAb-based SIV control [7,13–15], in which Burmese rhesus macaques (*Macaca mulatta*) were intravenously administered with polyclonal neutralizing anti-SIV IgG (300 mg) at week 1 post-SIV_{mac239} challenge. This derives viral RNA accumulation in dendritic cells, elevated Gag-specific polyfunctional CD4$^+$ T-cell responses, in vitro CD8$^+$-cell virus-suppressive activity augmentation, and viremia control. When nonneutralizing SIV-binding IgG (non-NAbs) with significant ADCVI (antibody-dependent cellular viral inhibition) activity [14,16] was similarly infused, replication was uncontrolled [14]. In the present study, we examined whether the enhancement of virus-suppressive activity of CD8$^+$ cells is induced by non-NAbs.

We analyzed peripheral blood mononuclear cell (PBMC) frozen samples of 12 rhesus macaques in our previous studies [7,13–15,17,18], consisting of three groups administered with anti-SIV neutralizing IgG ($n = 4$), nonneutralizing IgG ($n = 4$), or control IgG ($n = 2$)/mock ($n = 2$), respectively. IgGs had been affinity-purified from NAb-inducing, non-NAb-inducing, or uninfected macaque pooled plasma (100% SIV_{mac239}-neutralizing titers were 1 : 16 in NAbs) [7,14,15]. Experiments had been conducted at the Tsukuba Primate Research Center, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN) after approval by the Committee on the Ethics of Animal Experiments of NIBIOHN under guidelines for animal experiments at NIBIOHN and National Institute of Infectious Diseases.

To compare NAb/non-NAb ADCVI activity [14], major histocompatibility complex-mismatched herlesvirus saimiri-immortalized HSC-F CD4$^+$ T cells [19] infected with SIV_{mac239} (multiplicity of infection 0.001) were cocultured with/without effector PBMCs from uninfected macaques [effector/target (E : T) ratio 4 : 1] under antibody presence. Day 7 supernatant virus was measured by p27 ELISA (Advanced Bioscience Laboratories, Rockville, Maryland, USA). Without PBMCs, NAbs exerted dose-dependent viral inhibition whereas non-NAbs showed no inhibition. With PBMC addition, NAbs and non-NAbs showed comparable ADCVI