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A Randomized, Placebo-Controlled Pilot Trial of N-Acetylcysteine on Oxidative Stress and Endothelial Function in HIV-infected Older Adults Receiving ART:

NAC in HIV

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HIV-infected individuals are at higher risk for cardiovascular disease (CVD) compared to 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

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SUPPLEMENTAL DIGITAL CONTENT
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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, which 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 FDA approval (http://www.pharmanac.com/; BioAdvantex Pharma Inc.). PharmaNAC contains 900mg 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 900mg twice daily, PharmaNAC 1800mg 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 GSH (reduced glutathione), GSSG (oxidized glutathione), and GSH:GSSG ratios were measured on the first 15 participants enrolled into the trial (7, 6, and 2 in the PharmaNAC 900bid arm, the PharmaNAC 1800mg bid arm, and the placebo arm, respectively).

HIV-infected patients of age ≥ 50 years and receiving ART for at least six months and resulting in an HIV-1 RNA level <75 copies/mL at study screening were eligible. Primary exclusion criteria 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 (ITT) 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, GSH:GSSG) and FMD; these models included study arm assignment and baseline values of each marker (as there were non-significant, but appreciable, imbalances of these markers at baseline) to determine their effects on changes at Week 8.

Twenty-six patients 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, 9 were randomized to PharmaNAC 900 twice daily, 8 were randomized to PharmaNAC 1800mg twice daily, and 7 were randomized to placebo. The baseline characteristics of the study arms are shown in Supplemental Table 1. One participant in the PharmaNAC 1800mg 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 to 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 to placebo at Week 8. Of note, additional adjustments for race and sex in these models did not appreciably affect these results.

In this randomized, placebo-controlled, pilot trial, we found that use of PharmaNAC at either 1800mg twice daily or 900mg 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 reduced glutathione levels in red blood cells increased substantially while the levels of red blood cell oxidized glutathione levels decreased substantially, thereby leading to overall non-significantly increased ratios of GSH:GSSG with both doses of PharmaNAC compared to 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 to placebo. And in turn, FMD levels increased in both PhamaNAC arms compared to 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Week 8 changes in FMD and oxidative stress markers after adjusting for baseline values.

Outcome	Effect	Point estimate	P-value
F2-isoprostanes, pg/mL	1800mg bid vs. placebo	−6.87	0.51
	900mg bid vs. placebo	−7.87	0.44
	Baseline value	***−0.82***	***<.0001***
Malondialdehyde,μM	1800mg bid vs. placebo	0.017	0.98
	900mg bid vs. placebo	0.21	0.75
	Baseline value	0.17	0.65
Flow-mediated dilation, %	1800mg bid vs. placebo	0.75	0.44
	900mg bid vs. placebo	0.83	0.38
	Baseline value	***−0.56***	***0.0015***
GSH (reduced glutathione),μM	1800mg bid vs. placebo	177.60	0.67
	900mg bid vs. placebo	409.75	0.38
	Baseline value	−0.11	0.85
GSSG (oxidized glutathione),μM	1800mg bid vs. placebo	−22.49	0.62
	900mg bid vs. placebo	−11.69	0.80
	Baseline value	***−0.76***	***0.049***
GSH:GSSG	1800mg bid vs. placebo	7.90	0.71
	900mg bid vs. placebo	23.40	0.23
	Baseline value	−0.53	0.36