Jay S. Epstein, MD
Jaroslav G. Vostal, MD, PhD
e-mail: jaroslav.vostal@fda.hhs.gov
Office of Blood Research and Review
Center for Biologics Evaluation and Review, FDA
Rockville, MD

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Nitric oxide levels increase during platelet concentrate production from buffy coats, but not during storage

There is some controversy regarding the potential of platelets (PLTs) to actively synthesize nitric oxide (NO) by nitric oxide synthase (NOS). As pointed out by Park and colleagues[1] in **TRANSFUSION**, NO is an important short-lived signaling molecule that activates soluble guanylyl cyclase thereby increasing levels of cyclic guanylyl monophosphate (cGMP) in many cells as well as chemically modifying biomolecules through nitrosylation and nitrosothiol formation. In PLTs cGMP antagonizes activation by decreasing intracellular Ca^{2+} levels, inhibiting cAMP breakdown, and activating cGMP-dependent kinases, which attenuate activation cascades. The results described by Park and colleagues are in line with our experience of nitrite/nitrate (NO^{2-}/NO^{3-}) measurements in PLT concentrates (PCs) prepared from pooled buffy coats.

For this study, PCs were stored at 22 ± 2°C in gas-permeable PL-2410 bags (Fenwal, Lake Zurich, IL) allowing molecular oxygen diffusion. In this milieu NO is oxidized to NO^{2-}/NO^{3-}, which are determined by the Griess assay.[2] In brief, cell-free samples are incubated with 0.2 U/mL nitrate reductase, 125 µmol/L nicotinamide dinucleotide phosphate, and 5 µM flavine-adenine dinucleotide for 90 minutes and then supplemented with *N*-ethylmaleimide (15 mmol/L) followed by 50% (vol/vol) absolute ethanol (all reagents from Sigma-Aldrich, St Louis, MO). The chromogenic Griess reagent *N*-1-naphthylethylenediamine is then added to a final 0.05% (wt/vol) in 3% (vol/vol) phosphoric acid and incubated for 30 minutes. Samples are subsequently cleared from protein by trichloroacetic acid precipitation. Paired blanks are included and a reference curve is generated by a serial dilution of $NaNO_2$ in ultrapure water.

Figure 1 shows that there is no significant change in NO^{2-}/NO^{3-} levels as a function of in vitro storage time ($p > 0.05$, $n = 9$), reflecting no major release of NO nor sequestering of NO^{2-}/NO^{3-} during storage. Also leakage from the container plastic (polyolefin in our case) is not observed. Together, this supports the data from Park and colleagues and confirms that PCs are not a main NO source.[1,3] However, we did find a significant difference before and after PC preparation from buffy coats (Fig. 2). Hereto, aliquots of plasma were taken before buffy coat preparation and pooled in equal volumes separately in the laboratory. Next, PCs were prepared following standard

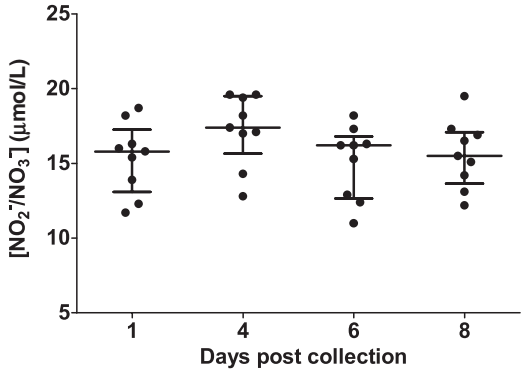

Fig. 1. NO^{2-}/NO^{3-} does not change during storage. Cell-free samples were analyzed for NO^{2-}/NO^{3-} content by standard chromogenic Griess assay (n = 9). Data are shown in function of storage time as days after collection with Day 0 as the actual day of collection. No differences were significant by one-way ANOVA with Tukey's posttest. All data sets were parametric (Prism, GraphPad, San Diego, CA).

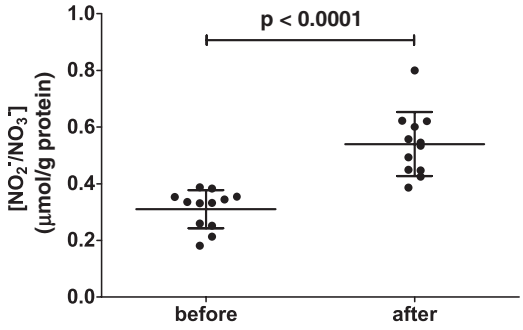

Fig. 2. NO^{2-}/NO^{3-} is increased during PC preparation from buffy coats. Cell-free paired samples before and after PC preparation were analyzed for NO^{2-}/NO^{3-} content by a standard chromogenic Griess assay (n = 12). Total protein concentration was determined in all samples using the bicinchoninic acid assay. The NO^{2-}/NO^{3-} content is expressed per gram of protein to correct for dilutions made during SSP+ supplementation. Horizontal and error bars represent means and standard deviation, respectively. A paired t test was used to determine statistical difference. Data sets were parametric.

component preparation procedures and an aliquot of these was analyzed as a paired sample. Because the buffy coat method involves a dilution step with PLT additive solution (SSP+, MacoPharma, Milan, Italy), amounts of NO^{2-}/NO^{3-} are expressed per gram of protein as a means of standardization. Total protein was determined in all samples concurrently by bicinchoninic acid assay (Thermo Fisher Scientific, Rockford, IL). Before PC preparation, samples contained 0.31 ± 0.07 µmol/g (mean ± SD; n = 12) compared to 0.54 ± 0.11 µmol/g after the procedure ($p < 0.0001$; paired t test; Fig. 2). Based on the dilution with SSP+ (containing 1.46 ± 0.84 µmol/L NO^{2-}/NO^{3-}, n = 4), a mean value of 0.35 ± 0.07 µmol/g protein is theoretically expected, which is significantly less than experimentally determined.

Expression of functional NOS in PLTs is controversial, questioning factual enzymatic NO synthesis in this cell type.[3] Our data and those from Park and colleagues now indicate that there is no measurable NO production in PC during storage. This observation seems unexpected since paracrine NO signaling during PLT storage is intuitively reasonable, for example, to counteract cell activation as part of the PLT storage lesion. Maybe control of oxidative damage explains limited NO release, since NO is quickly scavenged by superoxide anion generating a series of potentially harmful radicals. Therefore, PLTs in storage conditions may need to compromise unwanted oxidative damage with unwanted cell activation, or maybe NOS is just simply absent or not functional in thrombocytes.

While Park and colleagues assessed apheresis PCs, we analyzed PCs prepared from pooled buffy coats and demonstrate that the procedure itself increases NO^{2-}/NO^{3-}. White blood cells and red blood cells (RBCs) reside in buffy coats and both are able to synthesize NO but RBC-NOS does so at a rate comparable to endothelial cells and in levels that alter PLT behavior.[4] Furthermore, RBCs function as a major repository for NO_2^-, which might be released through either hemolysis or diffusion when reduced to NO by deoxyhemoglobin.[5] Whether NO is actively or passively released during PC production is unclear because our method does not distinguish between both. Moreover, the exact role of NO^{2-}/NO^{3-} increase during PC production is equally unknown, but it may be that NO signaling at least aids in preventing overt PLT activation during pooling, dilution, and subsequent centrifugation.

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CONFLICT OF INTEREST

All authors do not have any conflicts of interest.

Hendrik B. Feys, PhD
e-mail: hendrik.feys@rodekruis.be
Philippe Vandekerckhove MD, PhD
Veerle Compernolle, MD, PhD
Transfusion Research Center
Belgian Red Cross Flanders
Brugge, Belgium

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