Effect of plasma processing and storage on microparticle abundance, nitric oxide scavenging, and vasoactivity

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BACKGROUND: We set out to define the impact of collection, processing, and storage on plasma product microparticle (MP) abundance, potential for nitric oxide (NO) scavenging, and vasoactivity.

STUDY DESIGN AND METHODS: Three currently US licensed products were tested: liquid plasma (LP), fresh frozen plasma (FFP), and solvent detergent plasma (SDP), along with a product under development, spray-dried solvent detergent plasma (SD-SDP) with/without beads. Vasoactivity was assessed in vitro using rabbit aortic vascular rings; MP abundance was determined by flow cytometry; and NO scavenging capacity/rate was determined using a biochemical NO consumption assay. All samples were analyzed unprocessed and following centrifugation at two speeds (2,500× *g* to remove platelets, and 25,000× *g* to remove microparticles).

RESULTS: Significant differences in vasoactivity were observed, with SD-SDP minus beads demonstrating the greatest constriction and FFP the lowest constriction response. All products exhibited the same total NO scavenging capacity; however, significant differences were observed in the maximal rate of scavenging, with SD-SDP minus beads and FFP reacting fastest and SDP the slowest. Across all products, platelet and microparticle depletion had no effect on vasoactivity or NO scavenging (total or rate). Microparticles (RBC derived) were found only in FFP and LP, with relative abundance (LP > FFP). Additionally, storage had no effect on total or RBC-derived MP abundance, NO scavenging, or vasoactivity.

CONCLUSION: Although vasoactivity differed between plasma products, we did not find similar differences in either total or RBC-derived MP abundance or NO scavenging capacity/rate.

Plasma transfusion is a relatively common patient therapy; in the United States alone, 3.9 million plasma units were transfused in 2011.[1] Although widespread, much remains to be learned about the effectiveness, efficacy, and safety of the various plasma products transfused. In the United States, three distinct plasma products are commonly transfused: liquid plasma (LP), fresh frozen plasma (FFP), and solvent detergent plasma (SDP). Product selection normally depends on the clinical requirements of the patient; for example, to minimize allergic/immune reaction an SDP product might be chosen, while LP may be preferred when certain clotting factors (that would be lost to freezing) are required. Each plasma product has different collection, processing (Table 1), and storage characteristics/reconstitution requirements (Table 2). These collection, processing, and storage/reconstitution processes not only likely impact the content of coagulation and antithrombotic factors but also likely affect the vasoactivity of each product.

ABBREVIATIONS: FFP = fresh frozen plasma; LP = liquid plasma; MP = microparticle; MPP = microparticle-poor plasma; NO = nitric oxide; PE = phenylephrine; PPP = platelet-poor plasma; SDP = solvent detergent plasma; SD-SDP = spray-dried solvent detergent plasma.

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TABLE 1. Plasma transfusion product collection and processing techniques

Product	Donor	Processing
Liquid plasma (LP)	Single	Plasma obtained from whole blood separation or plasmapheresis. Plasma separation occurs 5 days before whole blood expiration.
Fresh frozen plasma (FFP)	Single	Plasma obtained from whole blood separation or plasmapheresis. Plasma frozen within 6 hours if blood collected in acid-citrate-dextrose or 8 hours if collected in citrate-phosphate-dextrose.
Solvent detergent plasma (SDP)	Pooled (up to 1000 donors)	Plasma mixed with solvent and detergent and incubated at 30°C for 4 hours. Sample subjected to extraction with oil and chromatography to remove the additions.
Spray-dried solvent detergent plasma (SD-SDP)	Pooled (1000–1500 donors)	Plasma mixed with solvent and detergent and incubated at 30°C for 4 hours. Sample subjected to extraction with oil and chromatography to remove the additions. Spray-drying accomplishment by atomization of LP to droplets and brief exposure to hot (up to 150°C) gas, followed by rapid evaporative cooling.

TABLE 2. Plasma transfusion product storage, reconstitution, and reconstituted storage limits

Product	Frozen	Storage temperature	Storage limit	Thawing	Reconstituted storage
Liquid plasma (LP)	Never	1–6°C	30 days	NA	NA
Fresh frozen plasma (FFP)	Frozen at not more than −18°C (0 °F)	−18°C (0 °F) or lower	12 months	20–30 minutes at 30–37°C (gentle agitation)	4 hours at RT 5 days at 1–6°C
Solvent detergent plasma (SDP)	Frozen	−18°C (0 °F) or lower	4 years	20–30 minutes at 30–37°C (gentle agitation)	24 hours at 4°C
Spray-dried solvent detergent plasma (SD-SDP)	Frozen	−18°C (0 °F) or lower	Undetermined	20–30 minutes at 30–37°C (gentle agitation)	24 hours at 4°C

NA = not applicable; RT = room temperature.

For some time, it has been known that fresh plasma scavenges significant amounts of nitric oxide (NO).[2] More recently, microparticle (MPs), especially those derived from RBCs,[3–5] but also those from other cell types,[6] have been shown to be vasoactive in vivo and to trap NO.[3,4,7] Despite these findings, the vascular reactivity of plasma transfusion products has not been systematically evaluated. We sought to determine the in vitro vasoactivity of plasma transfusion products; specifically, LP, FFP, SDP, and spray-dried solvent detergent plasma (SD-SDP). We also characterized MP abundance in each product in addition to the specific NO scavenging capacity and the (physiologically relevant) NO scavenging rate, to assess any contribution played by these factors. Finally, we also assessed the effect of plasma transfusion product storage duration on these factors.

METHODS

Plasma sourcing and manufacture

Five different plasma products were tested. Ten units of each were analyzed. FFP and LP were purchased from the Blood Centers of the Pacific. Both products were obtained from whole blood processing. Briefly, whole blood was centrifuged (5000 *g*, 10 min, 4°C), and plasma was expressed out and separated from the RBCs. FFP was immediately frozen, and LP was stored in the refrigerator; neither were leukocyte reduced after collection. Entegrion, Inc. provided the SDP and SD-SDP (the SDP used to make SD-SDP was manufactured by Kedrion). The 10 SD-SDP units were split into two sets of five, one set containing beads and the other not. The product with beads was an experimental product designed to provide a "milling" effect on the powder during rehydration, assisting in breaking up any aggregates that formed in the rehydration process as a result of the hydrophobic nature of the dehydrated SD-SDP. The bead characteristics are proprietary; however, size prevents them from leaving the plasma bag (and would ultimately prevent infusion into a patient).

Blood typing

All FFP units were type AB; SDP units were from one Type A lot; SD-SDP units were from one Type AB lot; and nine LP units were Type O and one was Type B.

Plasma handling and storage

LP was shipped from San Francisco (Blood Centers of the Pacific) to St. Louis (at 4–6°C); thus, the earliest it could be tested was Day 3. Upon arrival, it was stored at 2–6°C for 28 days (and tested on Days 3, 14, and 28). FFP and SDP were thawed at 37°C and stored at 2–6°C for 5 days (and tested immediately after thaw/reconstitution and on Days 3 and 5). SD-SDP was reconstituted according to the manufacturer's instructions and stored at 2–6°C for 24 hours (and tested immediately after reconstitution and on Day 1) (Table 3).

Plasma preparations

Plasma refers to samples removed directly from the storage bag without further processing. Platelet-poor plasma (PPP) was prepared by centrifugation of the unadulterated plasma

TABLE 3. Outline of the plasma sample analysis schedule as a function of storage duration

Plasma product	Immediate (after thaw or reconstitution)	Day 1	Day 3	Day 5	Day 14	Day 28
LP			X		X	X
FFP	X		X	X		
SDP	X		X	X		
SD-SDP	X	X				

FFP = fresh frozen plasma; LP = liquid plasma; SDP = solvent detergent plasma; SD-SDP = spray-dried solvent detergent plasma.

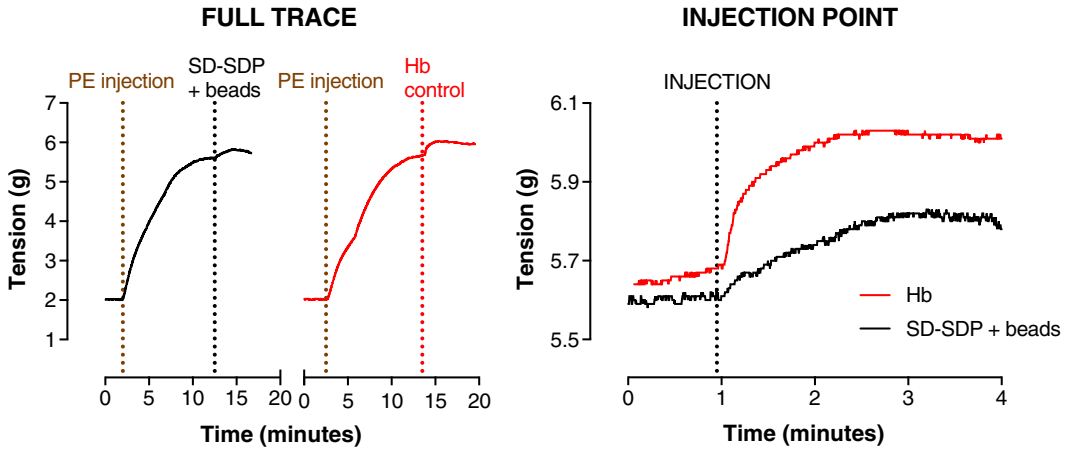

Fig. 1. Representative traces (SD-SDP without beads) from a single vascular ring demonstrating the experimental process to determine in vitro vasoactivity. Full traces (left) show rings preconstricted with PE (brown dotted lines indicate injection points). Black trace shows the injection of SD-SDP without beads and the resulting additional constriction response. Red trace shows the corresponding control injection of 1.5 nmoles of Hb and the resulting additional constriction response. Injection points are lined up and overlaid (right) for easier visual comparison of further constriction responses.

(2500× *g*, 15 min).[8,9] Microparticle-poor plasma (MPP) was prepared by centrifuging PPP plasma (25,000× *g*, 1 hr).[8,9] Experiments were performed to confirm platelet MP removal following this centrifugation protocol (Fig. S2, available as supporting information in the online version of this paper).

Vascular ring array

A previously validated in vitro vascular ring assay[10–12] was employed to determine vasoactivity of the various plasma products. Male New Zealand white rabbits (1.8–2 kg) were euthanized by intravenous injection of sodium pentobarbital. Aortas were harvested, and all nonvascular tissue was carefully dissected away. Vascular rings were prepared by cutting the aorta into 5-mm segments. Rings were mounted on a Radnotti vascular ring array (Harvard Apparatus), between two stainless steel hooks, one secured at the bottom of each 10-mL water-jacketed (37°C) chamber, the other connected to a force transducer (AD Instruments) via a silk thread. Resulting change in isometric tension was recorded by transducers linked to a data acquisition system (Powerlab 8Sp/octal bridge, AD Instruments) connected to a computer running data analysis software (LabChart 7, AD Instruments), as described.[13]

Vessel responsiveness was assessed following maintenance of steady-state 2-g resting tension for 30 minutes. Vessel preconditioning at 95% O_2, 5% CO_2, involved incubation with the alpha-adrenergic receptor agonist phenylephrine (PE) 10^{-6} mol/L and relaxation induced by acetylcholine 10^{-5} mol/L, to initiate a NO-based vasodilation response, indicating the presence of an intact endothelium. Endothelium-intact rings were identified as those demonstrating an acetylcholine relaxation response during preconditioning greater than 60% of the maximal induced tension (by PE). Any rings not producing this amount of relaxation were excluded from analysis.

For the experimental portion, rings were preconstricted with PE 10^{-6} mol/L (a dose previously identified to achieve approximately 50% to 60% maximal constriction in rabbit aortic rings). Once plateau constriction had been achieved, plasma product (200 µL) was injected into each bath and the subsequent maximal constriction was recorded (Fig. 1). Following washout from the plasma product injection and return to initial baseline 2-g tension, each ring was preconstricted once again (PE 10^{-6} mol/L), after which cell-free hemoglobin (Hb) (1.5 nmoles) was injected (100 µL of 15 µM/L Hb solution), and the subsequent maximal constriction response recorded (Fig. 1). Percentage of maximal constriction from the plasma injection was divided by the percentage of maximal constriction induced by 1.5 nmoles of cell-free Hb and multiplied by

100. Cell-free Hb (i.e., Hb in suspension) was obtained following lysis of washed RBCs diluted in phosphate-buffered saline (PBS; freeze/thaw), lysate centrifugation (25,000× *g* 10 min, 4˚C) to remove membrane, and passage through a G25 Sephadex sizing column (equilibrated in PBS). Normalization to the Hb constriction (which represents the physiologic effect of a consistent degree of NO scavenging) accounted for any variance in individual ring segment responsiveness to a known vasoconstricting stimulus. For each experiment, eight aortic ring preparations were run in parallel. In the experimental portion, following PE preconstruction, three rings were injected with plasma, three with PPP, and two with MPP. Following washout and a further preconstriction with PE, every ring was injected with Hb.

Flow cytometry

Simultaneous to the NO scavenging and vascular reactivity studies (reported herein), complete MP phenotype analysis was also performed on the exact same plasma products. This phenotype analysis has been previously published[14] and the methods fully described.[14] Herein, we have reanalyzed this original MP data in a new manner to reflect the purpose of this paper.

Methodologically, in brief, flow cytometry (Accuri C6, Becton Dickinson) was performed on PPP samples stored at −80°C for batch analysis. Frozen PPP aliquots were thawed for 10 minutes at 37°C and analyzed within 2 hours. Each plasma product was incubated (10 μL, 15 min, protected from light) with lineage-specific monoclonal antibodies to identify RBCs (CD235a-APC), platelets (CD41a-PerCP-Cy5.5), and leukocytes (CD45-PE-Cy7). Following incubation, samples were diluted with stain buffer up to a total volume of 1 mL. Sample gating was set using the unstained sample. APC-, PerCP Cy5.5–, and PE-Cy7–conjugated isotype control mouse IgG were negative controls. Threshold setting and data acquisition was performed according to software manufacturer recommendations (Accuri C6, Becton Dickinson).

Microparticle phenotyping was performed using phosphatidylserine-positive (annexin V-positive) MPs. MPs with cellular origins from cell types thought to influence vascular reactivity were selected for analysis (i.e., platelets, RBCs, and leukocytes). The following antibodies were used: annexin V-FITC, CD41a- PerCP Cy5.5, CD62P-APC, CD235a-APC, CD47-PerCP-Cy5.5, CD45-PE-Cy7, CD66b-PerCP-Cy5.5, CD3-PE, CD14-APC, CD154-APC, CD105-PE, CD144-PerCP-Cy5.5, CD62E-APC, CD106-APC, CD142-PE, and CD35-PE. Absolute-count tubes (TruCOUNT, Becton Dickinson) quantified absolute MP counts within different plasma products. Small bead calibration kits (Bangs Laboratories) were used to measure microparticles within the constant region of 0.2 μm to 1 μm. Positive control acquisition was based on full stains minus one, and the compensation was set against antibody capture beads with single and tandem fluorochromes. PPP from each plasma product (10 μL) was incubated with lineage-specific monoclonal antibodies (2 μL) ± annexin V (2 μL) in the presence of $CaCl_2$ (2.5 mM for 30 min at room temperature, protected from light). After incubation, samples were diluted with stain buffer or annexin V–binding buffer up to a total volume of 300 μL. Data acquisition was performed as suggested by the software manufacturer (Accuri C6, Becton Dickinson).[14] Total MP numbers include all annexin V–positive MPs falling within the submicron gate (i.e., both phenotyped and nonphenotyped).

Nitric oxide scavenging assay

A previously well validated NO scavenging assay[2] was employed to determine the maximal rate and total capacity for NO scavenging of the various plasma transfusion products (Fig. S1A). A custom-made glass reaction cell[15] (Fig. S1B), was filled with PBS (3.5 mL, pH 7.4, 37°C) (IKA Works Inc.). Spermine NONOate (in 0.01 N NaOH) was injected into the reaction cell, generating NO in a first-order process (half-life 39 min, 37°C, yielding 2 moles of NO per mole of parent compound).[16,17] Evolved NO, carried by sparging helium, was passed through an inline condenser (removing water vapor) to a NO chemiluminescence analyzer (TEA 810, Ellutia) generating an electrochemical NO baseline signal (approx. 1500–1700 mV). Following maintenance of a stable signal (approx. 5 min), 60-μL samples of plasma (as either plasma, PPP, or MFP diluted 1/10 in PBS) were injected into the NONOate solution, resulting in transient loss of NO signal (i.e., inverse peaks), allowing quantification of NO scavenging capacity by plasma samples. Following each injection, the reaction chamber was flushed, refilled with PBS and NONOate, preventing excessive foaming from plasma proteins.

Total NO scavenged was determined by measuring the area under curve (AUC) of the inverse peaks. Of note, this response occurs over minutes and indicates the total capacity of the sample to capture NO. Consequently, we also developed a novel methodology to determine the more physiologically relevant readout of the maximal NO scavenging rate (since plasma and endothelium never reach steady state, by virtue of blood flow kinetics). This response, which occurs within approximately 10 to 15 seconds of sample injection, more closely represents the in vivo dynamics of NO scavenging in the vascular tree. Maximal rate of NO scavenging was determined following smoothing of the raw data (Savitsky-Golay; 30-point, polynomial degree 2). Following smoothing, a first derivative plot of 5-second intervals was fitted to the data to find the 5-second interval with the maximal slope (i.e., corresponding to the maximal rate of NO scavenging). These data were then plotted for each sample (Fig. 2).

Statistics

Results are presented as mean ± standard error of the mean. Column statistics were performed to determine data distribution normality. Where appropriate, groups were compared by paired or unpaired t test (Student's or Wilcoxon rank sum) or repeated-measures analysis of variance (Bonferroni's post hoc test) (PRISM, GraphPad Inc.).

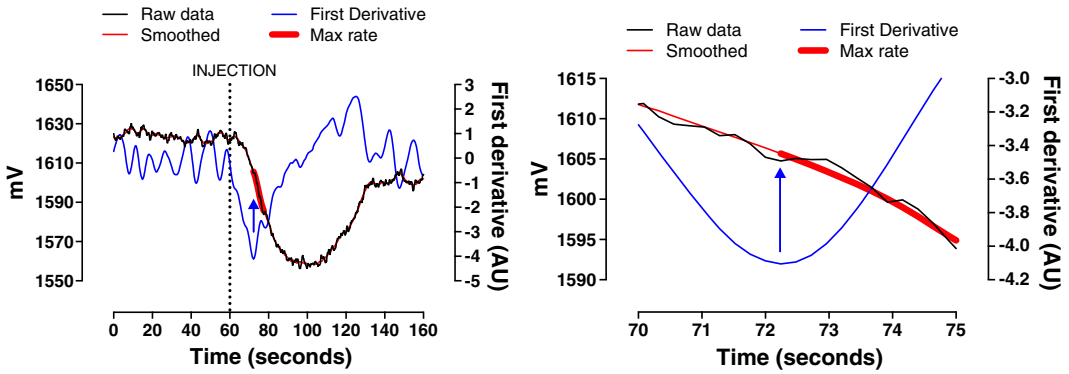

Fig. 2. Representative trace from LP injection demonstrating the methodology used to determine the maximal rate of NO scavenging, including signal smoothing, plotting of the first derivative signal and identification of the 5-second interval of maximal NO scavenging. Right is zoomed in to demonstrate the exact identification of the start point of maximal NO scavenging using the first derivative.

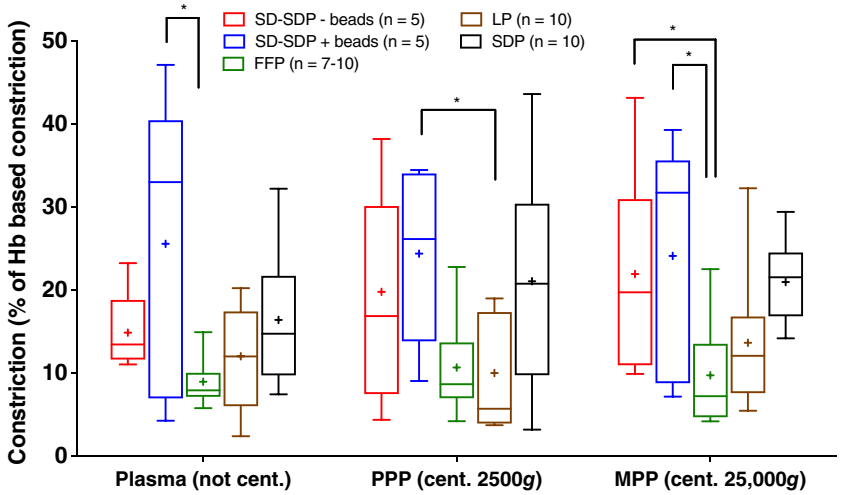

Fig. 3. Vasoactivity of the various plasma transfusion products from initial measurement (i.e., FFP following thaw, SDP and SD-SDP ± beads following reconstitution, and LP Day 3). Samples measured directly from the storage bag (plasma not centrifuged), or following centrifugation at 2500× g for 15 minutes (PPP); or following centrifugation of PPP at 25,000× *g* for 1 hour (MPP). SD-SDP without beads demonstrated the greatest constriction response in the ring bioassay, with FFP the least. The same trends were observed across all sample measurements (i.e., plasma, PPP, and MPP samples).

RESULTS

Vascular reactivity

Initial (baseline) measurement

Vasoactivity was significantly different between plasma products at their initial (baseline) measurement (Fig. 3); FFP measured immediately after thawing, SDP and SD-SDP ± beads measured immediately following reconstitution, and LP measured on Day 3 (following delivery from San Francisco; see Table 3). SD-SDP + beads demonstrated significantly greater vasoactivity compared to FFP in the uncentrifuged plasma analysis (25.55 ± 8.0 vs. 8.94 ± 1.1; $p < 0.05$). Similarly, in the PPP analysis, SD-SDP + beads demonstrated significantly greater vasoactivity than LP (24.36 ± 4.8 vs 9.99 ± 2.3; $p < 0.05$), and in the MPP analysis, both SD-SDP without beads and SD-SDP with beads demonstrated significantly greater vasoactivity than FFP (21.91 ± 3.8 vs. 24.09 ± 6.4 vs. 9.72 ± 2.1, respectively; $p < 0.05$). Across all analyses, FFP consistently demonstrated the lowest aortic constriction responses and SD-SDP with beads the highest.

Measurement neat and following removal of platelets (PPP) and microparticles (MPP)

Centrifugation of the plasma samples had no effect on vasoactivity, with no significant difference observed between plasma, PPP, and MPP samples from the same transfusion product (Fig. 3).

Storage

Storage duration had no effect on the vasoactivity of any of the plasma products. No significant differences were observed

between initial measurement and up to 1 day of storage for the SD-SDP ± bead samples, 5 days for the FFP and SDP samples, and 28 days for LP (Table SS1, available as supporting information in the online version of this paper).

Nitric oxide (NO) scavenging

Initial (baseline) measurement

All the plasma transfusion products scavenged NO to the same extent (i.e., had the same total NO scavenging capacity), as determined by area under the curve analysis of the scavenging assay signals (Fig. 4A). Of note, however, significant differences were observed between plasma products in the maximal rate of NO scavenging (Fig. 4B). SD-SDP without beads and FFP demonstrated significantly greater maximal rates of NO scavenging than SDP in the uncentrifuged plasma analysis (5.73 ± 0.22 vs. 5.48 ± 0.19 vs. 4.26 ± 0.22; $p < 0.01$ and $p < 0.05$, respectively). In the PPP analysis SD-SDP without beads once again demonstrated a significantly greater maximal rate of

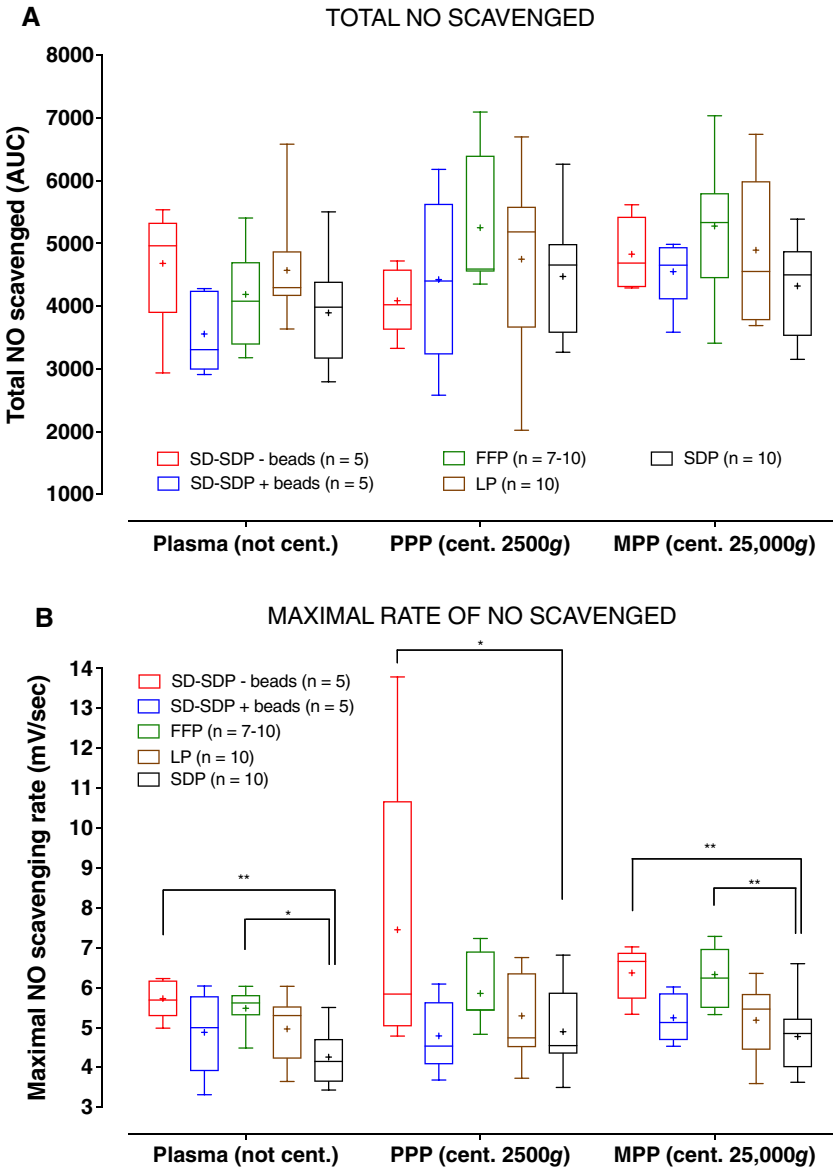

Fig. 4. NO scavenging capacity (total NO scavenged) (A) and maximal rate of nitric oxide scavenged (B) of the various plasma transfusion products from initial measurement (i.e., FFP following thaw, SDP and SD-SDP ± beads following reconstitution, and LP Day 3). Samples measured directly from the storage bag (plasma not centrifuged) or following centrifugation at 2500× *g* for 15 minutes (PPP) or following centrifugation of PPP at 25,000× *g* for 1 hour (MPP). No difference was observed between any of the products in terms of NO scavenging capacity (total NO scavenged). However, significant differences were observed in the maximal NO scavenging rate between plasma products, with the same trend across the plasma, PPP, and MPP samples. SD-SDP without beads and FFP demonstrated the highest NO scavenging rates and SDP the lowest.

NO scavenging than SDP (7.45 ± 1.65 vs. 4.9 ± 0.31; $p < 0.05$). In the MPP analysis, once again both SD-SDP without beads and FFP demonstrated significantly greater maximal rates of NO scavenging than SDP (6.37 ± 0.3 vs. 6.06 ± 0.24 vs. 4.78 ± 0.28; $p < 0.01$, respectively). Across all analyses SD-SDP without beads and FFP consistently demonstrated the greatest maximal rate of NO scavenging, and SDP the lowest.

Measurement neat and following removal of platelets (PPP) and microparticles (MPP)

Centrifugation of the plasma samples had no effect on NO scavenging (total or maximal rate). No difference was observed in terms of NO scavenging between plasma, PPP, and MPP samples from the same transfusion product (Fig. 4A and B).

Storage

Storage had no effect on NO scavenging (total or maximal rate) of any of the plasma products. No significant differences were observed between initial measurement and up to 1 day of storage for the SD-SDP ± bead samples, 5 days for the FFP and SDP samples, and 28 days for LP (Tables S2 and S3, available as supporting information in the online version of this paper).

Microparticles

In a previously published report,[14] we fully characterized the MP cellular origins (including RBC-, platelet-, WBC-, endothelium-, granulocyte/neutrophil-, monocyte-, T cell–, and tissue factor–derived MPs) of the exact same plasma products analyzed herein for NO scavenging and vasoactivity. In a reanalysis of this original MP data, we separated the SD-SDP group into two subgroups (with and without beads), that were not differentiated in our previous publication, due to their differing NO scavenging and vasoactivity responses, despite the fact that MP numbers in these two preparations did not differ significantly (which is why they were reported as one group in our original publication[14]). Of note, MPs derived from RBC, platelet, and WBC cellular origins were by far the most abundant, while those from other cell types were negligible.

Initial (baseline) measurement

Neither of the SD-SDP products contained significant numbers of total MPs or RBC (CD235a+)-derived MPs; however, MPs were clearly identifiable in the SDP, FFP, and LP plasma products, with relative abundance LP > FFP > SDP. The modest numbers of MPs in all products probably reflect the reason why following centrifugation there was no change in measured vasoactivity or NO scavenging capacity/rate, that is, comparing plasma to PPP and MPP.

Storage

Storage had no effect on total or RBC (CD235a+)-derived MP abundance. No significant differences were observed between initial measurement and up to 1 day of storage for the SD-SDP ± bead samples, 5 days for the FFP and SDP samples, and 28 days for LP (Tables S4 and S5, available as supporting information in the online version of this paper).

Correlations

We did not find any directional change in total or RBC (CD235a+)-derived MP abundance that could account for the differences in vasoactivity or NO scavenging observed between plasma products. Similarly, differences in NO scavenging did not correlate with product vasoactivity (Fig. 6).

DISCUSSION

We systematically evaluated plasma product vasoactivity and found that 1) all preparations demonstrated significant vasoactivity, within a physiologically relevant range; 2) SD-SDP with beads demonstrated the most vasoactivity; 3) all preparations demonstrated significant NO scavenging, within a physiologically

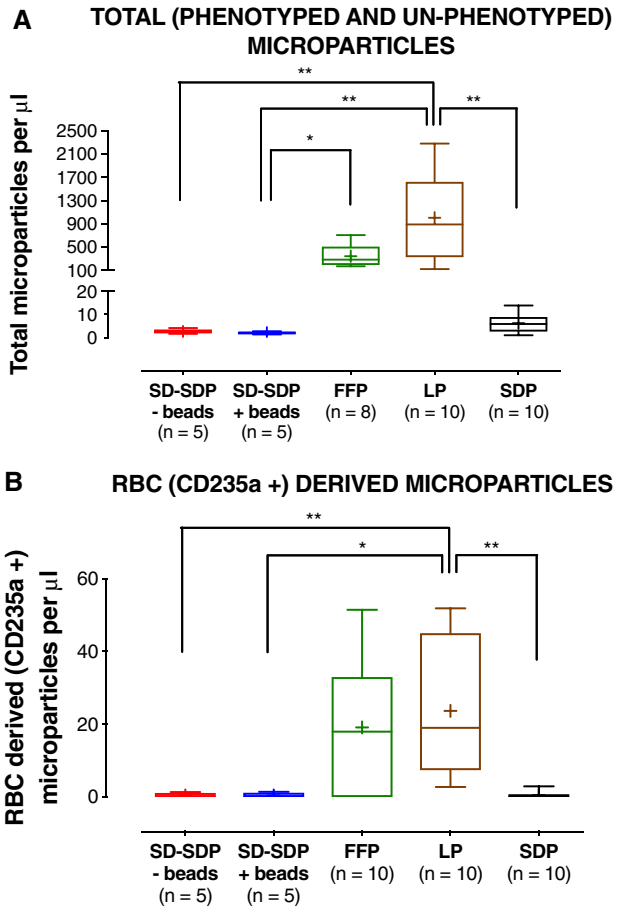

Fig. 5. Total microparticle load (A) and RBC (CD235a+)-derived MP load (B) of the various plasma transfusion products from initial measurement (i.e., FFP following thaw, SDP and SD-SDP ± beads following reconstitution, and LP Day 3). Only FFP, LP, and SDP demonstrated measurable levels of total MPs, with LP > FFP > SDP. Only FFP and LP demonstrated measurable levels of RBC-derived MPs.

relevant range; 4) SD-SDP demonstrated the highest rate of NO scavenging (preparations did not otherwise differ); 5) no parameters were influenced by storage duration; and 6) MPs were found only in FFP and LP. Notably, contrary to expectations, we found no relationship among MP abundance, NO scavenging, and vasoactivity.

Similar to previous reports from fresh plasma,[2] we observed significant NO scavenging by the plasma transfusion products. Nevertheless, somewhat unexpectedly, considering the vastly different processing of the various transfusion products, all demonstrated very similar total NO scavenging (Fig. 4A). This was even more unexpected given the different MP loads of the various plasma products. Even those samples containing a higher total (phenotyped and nonphenotyped MPs) and RBC (CD235a+)-derived MP abundance (i.e., LP; Fig. 5) did not demonstrate any change in vasoactivity or NO scavenging following MP depletion.

There are several possible explanations for our findings, which should be confirmed in complementary preparations (isolated–perfused lung, cannulated resistance arterioles, pressure myography, etc.). We did not measure free Hb abundance, which may have accounted for observed NO scavenging and would not be altered by MP depletion. While cell-free Hb would appear the most obvious sink for NO bioactivity in plasma (that would not be removed via centrifugation), other possible sinks could also include 1) heme-based enzymes (i.e., ceruloplasmin), 2) nonenzymatic oxidants (i.e., oxygen- or nitrogen-based radicals), or 3) antioxidants (i.e., low-mass thiols). Unfortunately, we did not undertake the complex partitioning experiments that would have been necessary to attribute this plasma bioactivity to any specific agent(s). In addition, the aortic ring protocol followed (observing additional vasoconstriction following phenylephrine preconstriction), while standard, does not offer the same dynamic range as would observing

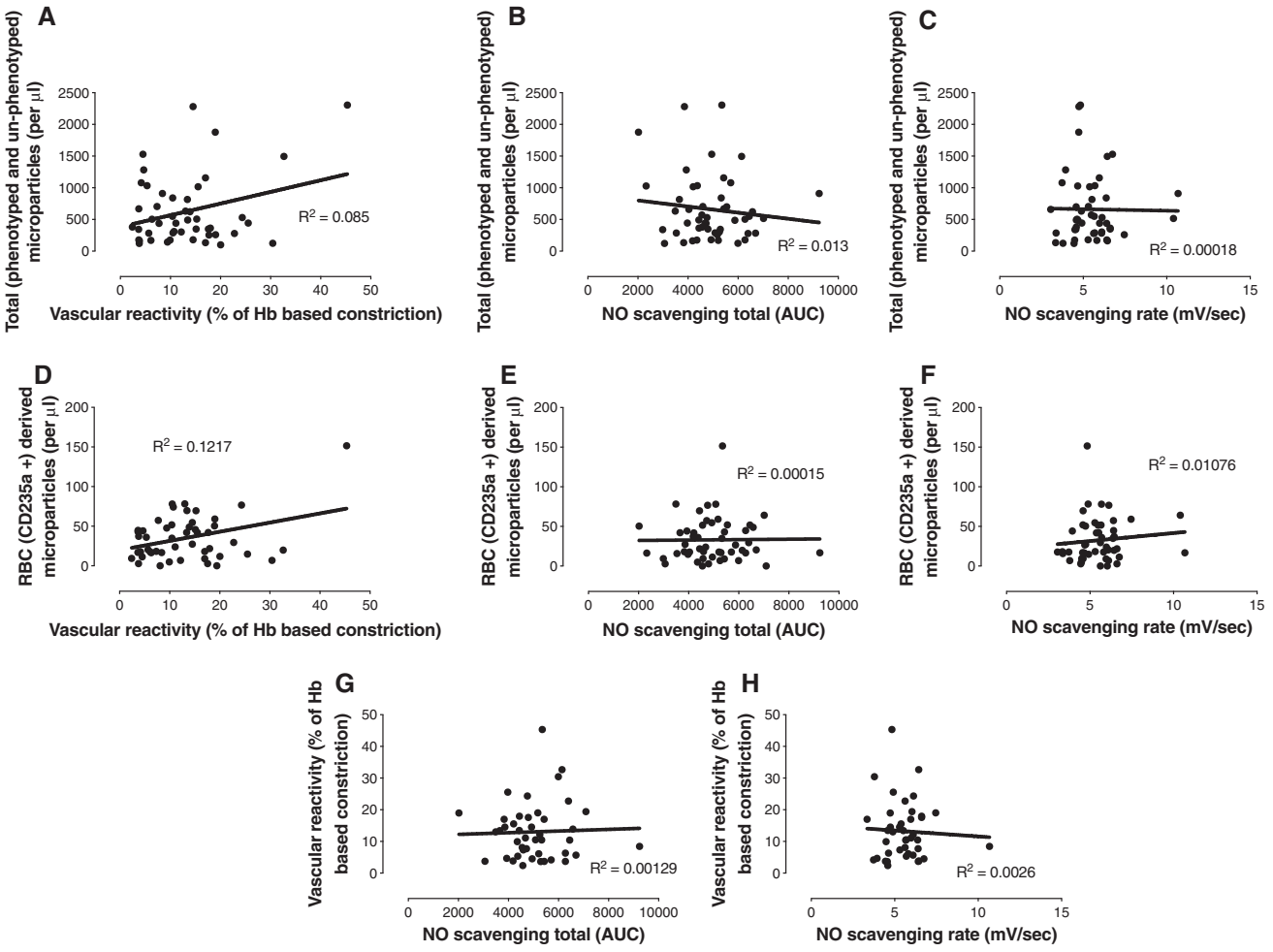

Fig. 6. Correlations between vascular reactivity, total and RBC (CD235a+)-derived MP abundance and NO scavenging capacity/rate. Correlations between total MP abundance and vasoactivity, and NO scavenging total and rate (A–C). Correlations between RBC-derived MP abundance and vasoactivity, and NO scavenging total and rate (D–F). Correlations between vascular reactivity and NO scavenging total and rate (G and H). No directional change was found in total or RBC-derived MP abundance that could account for the differences in vasoactivity or NO scavenging observed between plasma products. Similarly, differences in NO scavenging did not correlate with product vasoactivity.

plasma-based abrogation of an acetylcholine-based relaxation response[18]; we are currently validating this approach for this purpose. Certainly, however, it is possible that plasma vasoactivity acts through another (than NO) signaling system (adenosine, epoxides, etc.).

Although we did not observe statistically significant differences in vasoactivity or NO scavenging across the different plasma products, what is striking is the variability that was observed in these parameters within products and across similar products. Perhaps most remarkable is the difference in constriction response (Fig. 3) and maximal rate of NO scavenging (Fig. 4b) between SD-SDP with and without beads. One can only speculate that perhaps the beads themselves (that due to size restriction could not leave the bag) released or generated a component capable of demonstrating biologic vasoactivity and the ability to scavenge NO. Additionally, it is interesting to note that those products obtained from single donors (i.e., LP and FFP) appeared to demonstrate less vascular constriction variance (Fig. 3) than the pooled plasma products (in particular SD-SDP), which would be the opposite of what one might expect. However, one caveat might be that the pooled plasma products tested herein (SDP and SD-SDP) were obtained from a single lot, and greater variance might have been observed had we compared multiple lots.[14]

Our study was limited by the inability to test LP at Day 0 because of the shipping time. However, given the lack of change in any of the measured parameters as a function of storage duration, it is unlikely that this delay was of any note. Further potential limitation involves the use of the BD Accuri C6 flow cytometer, which has recently been suggested as unable to resolve particles less than 400 nm.[19] We performed calibration experiments on our BD Accuri C6 flow cytometer before sample analysis, using a small bead calibration kit, with beads measuring 0.2, 0.5, and 0.8 μm (Bangs Laboratories). These were used to fix the appropriate instrument settings to study MPs within the constant region between 0.2 and 1 μm.[14] Consequently, we believe our instrument was able to resolve MPs, down to 200 nm in size. Finally, we also caution that assaying plasma provides unique challenges, mainly related to excessive foaming in purged or gased systems. This factor was another reason why we chose to analyze maximal rate of NO scavenging, not only because of its greater relevance in terms of in vivo NO processing but also because the rapid response time (within seconds) occurred well before the formation of excessive foam, most likely offering much more accurate analysis.

CONFLICT OF INTEREST

SCR, FTM, RM, DDT, EHW, EF, KAT, and AD have disclosed no conflicts of interet. PCS is on the Scientific Advisory Board for Entegrion.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Fig. S1. (A) Experimental setup for the biochemical NO scavenging assay. (B) Details of the custom-made glass reaction cell showing gas inflow (blue) and outflow (red) from the chimney, the empty reaction cell, and the chimney and reaction cell fully assembled.

Fig. S2. (A) Analysis was performed on fresh plasma centrifuged to remove platelets (2,500× *g*, 15 min) and MPs (25,000× *g*, 60 min), as outlined in the methods (analysis performed on an Acea Biosciences, Novocyte3000). Submicron size was determined using a small bead calibration kit, with beads measuring 0.2, 0.5, and 0.8 μm (Bangs Laboratories); Green = noise from buffer alone; orange = 0.2-μm beads; blue = 0.5-μm beads—three populations of these blue beads represent singlet, doublet, and triplet signals resulting from beads sticking together; red = 0.8-μm beads. (B)Samples were stained with CD41 Pacific blue (405 nm) and annexin V (488 nm); gray = unstained; fuschia = stained. Panels on the left show the CD41+ populations in a representative sample of both MPP and PPP. The CD41+ population was then overlaid on the bead size–dictated scatter parameters (see right). The black dots are the CD41+ particles in the submicron range. PPP has far more dots than MPP, as one would expect. (C) Based on platelet marker and submicron size, we determined that MPP had significantly less microparticles than PPP ($p < 0.05$; paired t test).

TABLE S1. Effect of transfusion plasma storage on vasoactivity (% of hemoglobin-based constriction).

TABLE S2. Effect of transfusion plasma storage total NO scavenging (AUC). # $p < 0.01$ immediate versus Day 1. * $p < 0.05$ immediate versus Day 1.

TABLE S3. Effect of transfusion plasma storage on maximal NO scavenging rate (mV/sec). * $p < 0.05$ Day 3 versus Day 14. # $p < 0.05$ immediate versus Day 1.

TABLE S4. Effect of transfusion plasma storage on total microparticle abundance (MP/μl). * $p < 0.05$ immediate versus Day 1. # $p < 0.01$ immediate versus Day 1.

TABLE S5. Effect of transfusion plasma storage on RBC-derived (CD235a positive) MP abundance (microparticles/μl). * $p < 0.05$ immediate versus Day 3. # $p < 0.01$ immediate versus Day 5.