ADAMTS–13 activity and von Willebrand factor levels in methylene blue photo-inactivated plasma processed by either the Springe method or an ‘in house’ system

J. del Río-Garma, 1 A. Pereira, 2 J. L. Arroyo, 3 J. Mateo, 4 A. Alvarez-Larrán, 5 C. Martínez, 3 J. Muncunill, 6 L. Barbolla, 3 on behalf of the Spanish Group of Apheresis (Grupo Español de Aféresis GEA)

1 Complexo Hospitalario, Ourense, Spain
2 Service of Hemotherapy and Hemostasis, Hospital Clinic Provincial, Villarroel, Barcelona, Spain
3 Centro de Hemoterapia y Hemodonación de Castilla y León, Valladolid, Spain
4 Hospital Sant Pau, Barcelona, Spain
5 Hospital del Mar, Barcelona, Spain
6 Fundació Banc de Sang i Teixits de les Illes Balears, Palma de Mallorca, Spain

Background and Objectives Methylene blue photo-inactivated plasma (MBPIP) has been reported to be less effective than fresh-frozen plasma (FFP) in the treatment of thrombotic thrombocytopenic purpura, which suggests a reduced content of the von Willebrand factor metalloprotease ADAMTS-13 in MBPIP.

Materials and Methods ADAMTS-13 activity and von Willebrand factor antigen (vWF:Ag) levels were measured in plasma before and after photo-oxidation by either the Springe method or a commercial ‘in house’ system as well as in cryoprecipitate-poor plasma (CPP) and FFP (20 units each).

Results Levels of ADAMTS-13 activity in MBPIP processed by the Springe method or the commercial ‘in house’ system were comparable to one another and did not significantly differ from levels found in FFP [median (range): 114% (57–139%), 99% (74–123%), and 106% (70–130%), respectively]. ADAMTS-13 activity was significantly reduced in CPP [median (range): 87% (70–107%) as compared with FFP (P < 0.05). Levels of vWF:Ag decreased after photo-oxidation by both methods.

Conclusion In vitro ADAMTS-13 activity was conserved in MBPIP processed by the two photo-oxidation methods analysed and did not significantly differ from levels found in FFP.

Key words: ADAMTS-13, blood component transfusion, plasma exchange, thrombotic thrombocytopenic purpura.

Introduction Plasma exchange with infusion of fresh-frozen plasma (FFP) is the standard of care for thrombotic thrombocytopenic purpura (TTP). Plasma exchange is effective in TTP, presumably because it replaces the von Willebrand factor (vWF) cleaving protease ADAMTS-13 that is deficient in many patients with TTP and also because it removes the causative inhibitory antibodies [1].

Several plasma products have been reported to be as effective as FFP in the treatment of TTP, including cryoprecipitate-poor plasma (CPP) [2] and plasma submitted to virus inactivation with solvent-detergent (SD) [3] or psoralens and ultraviolet (UV) light (i.e. Intercept® plasma, Cerus, Concord,
CA, USA) [4]. In accordance with these clinical observations, ADAMTS-13 activity was found to be preserved in all these kinds of therapeutic plasma [5–8].

With regard to methylene blue-photoactivated plasma (MBPIP), reports from Spain have shown that it is less effective than FFP in the treatment of TTP [9,10]. This clinical observation suggested that MBPIP might bear a reduced content of ADAMTS-13 activity, a hypothesis that was not confirmed by three studies reporting normal levels of ADAMTS-13 in MBPIP [5,8,11]. However, it should be noted that the total number of plasma units analysed in these previous studies was relatively small and that only the ‘in house’ photo-inactivation system developed by Macopharma was tested. Since ADAMTS-13 activity is highly variable in healthy people, a larger number of photo-inactivated plasma units must be analysed before drawing a definitive conclusion. In addition, as many patients included in the above-cited Spanish reports received MBPIP processed by the Springe method, an evaluation of ADAMTS-13 activity in this kind of plasma is warranted before rejecting the hypothesis of a reduced content of ADAMTS-13 in MBPIP.

The present study was aimed at ascertaining whether MBPIPs processed by the Springe method or the ‘in house’ system developed by Macopharma retain a normal level of ADAMTS-13 activity as compared with FFP and cryoprecipitate-poor plasma.

Materials and methods
ADAMTS-13 activity and von Willebrand factor antigen (vWF:Ag) levels were measured in 20 plasma units of MBPIP processed by the Springe method at BIOMAT (Grupo Grifols, Barcelona, Spain), 20 units of MBPIP processed ‘in house’ by the Macopharma system, 20 units of FFP, and 20 units of CPP. All the 80 plasma units were prepared from group A whole blood donations collected at the regional blood bank (Centro de Hemoterapia y Hemodonación) of Castilla y León (Valdolid, Spain).

Plasma preparation and inactivation
Whole blood was collected into quadruple bags with 63 ml of CPD (Grifols, Barcelona, Spain) and cooled on plates of butane-diol for a maximum of 16 h. Whole blood was then centrifuged at 3300 g for 12 min at 22 °C (Haerex Cryofuge 6000i; Kleinostheim, Germany) and processed to red blood cells, platelets and plasma (Compomat G4 system, Fresenius-Hemocare, Bad Homburg, Germany). All plasma units were processed and frozen within 24 h after collection by means of a Jewet JRF (Thermo Scientific, Walthman, MA, USA) quick freezing system and stored at −35°C or less.

In-house MBPIP was produced by the Macopharma Macotronic MB system (Mouveaux, France) as previously described [12]. In brief, plasma units were white blood cell reduced (PLAS 4, Macopharma), mixed with one tablet of anhydrous methylene blue (85 mg) in a BSV700XS bag, homogenized, illuminated (180 J/cm²), quickly frozen and stored at −35°C. Plasma intended to virus inactivation by the Springe method was kept frozen at −35°C and sent to BIOMAT, where it was thawed at 37°C, mixed with 4–6 ml of methylene blue solution (adjusted for the plasma volume in each bag), illuminated with visible light at a minimum of 45 000 lux for 60 min and frozen again at −35°C [13]. The procedure was performed according to Good Manufacturing Practice rules and under computer control. Frozen units were thereafter returned to the regional blood bank. For the preparation of CPP, FFP was thawed at 4°C for 14–24 h and centrifuged at 5000 g for 10 min at 4°C (Haerex Cryofuge 6000i). The resulting cryoprecipitate was removed and the supernatant fraction was frozen again and kept at −35°C or below until use.

Measurement of ADAMTS-13 activity and vWF:Ag levels
Pre-processing samples were taken from the original plasma bag just after fractionation and before any further manipulation. Post-processing samples consisted in segments removed from the plasma bag after the plasma was photo-inactivated and frozen. Samples from CPP and FFP consisted of segments removed from the bag just after freezing. At each step, care was taken to thoroughly mix the tube contents with plasma in the bag before segmenting the tube. All samples were stored at −80°C until use and thawed at 37°C just before being assayed.

ADAMTS-13 activity was measured by fluorescence resonance energy transfer (FRET) technology (ADAMTS-13 Activity Assay, ATS-13, GTI Diagnostics, Waunakee, WI, USA), with a Fluoroskan Ascent fluorometer (Thermo Scientific, Helsinki, Finland) [14]. VWF:Ag was measured by means of an in-house enzyme-linked immunosorbent assay using antibodies from Dako (Lostrup, Denmark). Results were the average of duplicate experiments.

Statistical methods
The investigation was designed as a non-inferiority study and powered to detect a therapeutically relevant reduction of ADAMTS-13 activity, arbitrarily settled at 20 or more percent points, with 85% probability and a 5% α risk. Comparisons between pre- and post-processing plasma samples were done by the Wilcoxon test for matched data. Comparisons across different kinds of plasma were done by the Mann–Whitney test. Correlation between ADAMTS-13 activity and vWF:Ag levels was investigated by the Spearman rank test. All calculations were performed using the statistical software Stata/SE 9 (www.stata.com).
Table 1  Statistical analysis of changes in ADAMTS-13 activity and von Willebrand factor antigen (vWF:Ag) levels after methylene blue photo-inactivation of plasma by the Macopharma or the Springe methods. Comparison with fresh-frozen plasma (FFP) and cryoprecipitate-poor plasma (CPP)*

<table>
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<tr>
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<th>ADAMTS-13 activity (% normal controls)</th>
<th>vWF:Ag levels (U/ml)</th>
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<tbody>
<tr>
<td>Macopharma</td>
<td></td>
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<tr>
<td>Pre</td>
<td>100 (81–132)</td>
<td>98 (58–165)</td>
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<tr>
<td>Post</td>
<td>99 (79–123)</td>
<td>70 (45–122)*</td>
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<td>Pre</td>
<td>104 (58–138)</td>
<td>108 (63–153)</td>
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<tr>
<td>Post</td>
<td>114 (57–139)*</td>
<td>66 (38–100)*</td>
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<tr>
<td>FFP</td>
<td>106 (70–130)</td>
<td>114 (81–138)</td>
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<tr>
<td>CPP</td>
<td>87 (70–107)</td>
<td>20 (10–39)</td>
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*Values stand for the median (range) of the 20 plasma units.
*Not significantly different from FFP.
*Based on 16 plasma units (4 were broken during processing).
*Significantly different from FFP at the 0·05 level.

Results

Levels of ADAMTS-13 activity and vWF:Ag in the original plasma and in samples taken after photo-inactivation are summarized in Table 1. As it can be seen, the original plasma units assigned to each inactivation method had comparable levels of ADAMTS-13 activity and vWF:Ag and they did not significantly differ from levels found in FFP. ADAMTS-13 activity did not significantly change between pre- and post-processing samples for any of both photo-oxidation methods. The percentage point variation in ADAMTS-13 activity after plasma photo-inactivation by either the Springe method or the ‘in house’ commercial system were comparable to one another [median (range): –0·5 (–16 to 26) and –8 (–32 to 38), respectively; P > 0·1], and post-inactivation levels did not significantly differ from those found in FFP. ADAMTS-13 activity was significantly lower in CPP than in FFP (P < 0·05).

As compared to the original plasma, levels of vWF:Ag were significantly decreased in the samples taken after virus inactivation by either of the two photo-oxidation methods (P < 0·01 in both cases). Post-inactivation vWF:Ag levels were similar in both photo-oxidation methods. Levels of vWF:Ag in CPP were significantly reduced as compared to FFP (P < 0·001).

There was no correlation between ADAMTS-13 activity and vWF:Ag levels in the 60 samples taken from donated plasma prior to any manipulation (Spearman’s R statistic = 0·17; P > 0·1).

Discussion

The present study shows that virus inactivation in plasma using the Springe method or the ‘in house’ system developed by Macopharma does not decrease ADAMTS-13 activity, as measured in vitro. They also show that ADAMTS-13 activity varies largely among healthy people, with levels ranging from 58 to 138% in pre-processing donor plasma.

Previous authors have already pointed out to the great variability in ADAMTS-13 levels among healthy individuals. For instance, in the study by Mannucci et al. [15], who used the collagen-binding assay, ADAMTS-13 activity ranged from 50% to more than 150%. A similar range of variation was reported by Kokame et al. [14] in normal controls assessed by the FRETS-VWF73 assay. Such a wide range of variability makes it necessary to analyse a large number of plasma units before establishing whether or not any manipulation of donated plasma has a significant effect on the levels of ADAMTS-13. Blood group influences ADAMTS-13 activity, with group O individuals having, on average, 10% more activity than people with ABO groups other than O [15]. This is the reason why we restricted our study to group A donors, in order not to introduce a known factor of ADAMTS-13 variability. Despite this precaution, it should be noted that ADAMTS-13 levels in the quartile of our blood donors with the highest metalloprotease activity nearly doubled the levels found in the lowest quartile, a feature whose clinical value at the time of selecting plasma donors for patients with TTP warrants further investigation. ADAMTS-13 activity temporarily decreases after physical stress, such as strenuous exercise [16], but this seems not likely to have influenced the levels we found in blood donors. On the other hand, it has been suggested that ADAMTS-13 activity is inversely correlated with vWF:Ag levels [15], an association that we were not able to corroborate in our blood donors.

Levels of ADAMTS-13 in transfused plasma have previously been found to be little affected by virus-inactivation methods based on SD [5,6] or psoralens and UV light (i.e. the Intercept® system) [8]. Furthermore, both SD plasma and Intercept® plasma have shown to be as effective as FFP when employed as replacement fluid in TTP patients treated by plasma-exchange [3,4]. With regard to MBPIP, three previous studies involving 6, 10 and 12 plasma units, respectively, all processed by the ‘in house’ system developed at Macopharma, did not find a significant reduction in ADAMTS-13 activity [5,8,11]. The present study expands these previous observations with a relevant number of plasma units and widens them by including MBPIP processed by the Springe method. The main procedural difference between the Springe method and the ‘in house’ system developed at Macopharma lies in the additional freezing-thawing cycle to which plasma is submitted in the Springe method. The freezing-thawing cycle
before the addition of methylene blue is intended to disrupt intact leucocytes, thereby exposing intracellular viruses to photo-oxidation (reviewed by Williamson et al. [17]). This step contributes to decrease the contents of some coagulation proteins [18], but our results show that it has no detrimental effect on the levels of ADAMTS-13 activity.

With regard to CPP, though a statistically significant decrease in ADAMTS-13 was found in this plasma component as compared to FFP, the clinical significance is doubtful, because CPP has demonstrated to be as effective as FFP in the treatment of TTP [2]. In our study, median ADAMTS-13 activity in CPP was 87% and activity lied above 70% in all the 20 CPP units that were tested, which is probably adequate to replace the deficient ADAMTS-13 found in TTP. We did not measured ADAMTS-13 in the cryoprecipitate corresponding to the evaluated CPP units, but our finding of a reduced metalloprotease activity in CPP is consistent with the observation by Scott et al. [7], who reported that ADAMTS-13 activity becomes concentrated in cryoprecipitate. Not surprisingly, vWF:Ag levels were markedly reduced in CPP.

Contrarily, to what was reported for SD plasma and Intercept® plasma, observational studies conducted in Spain have found that MBPIP is less effective than FFP in the treatment of TTP [9,10,19], despite previous authors [5,8,11] and the present study show that ADAMTS-13 activity is preserved in MBPIP. The reason why MBPIP would be less effective in TTP remains elusive. Virus inactivation of transfused plasma with methylene blue and light leads to photo-oxidative damage of several coagulation factor proteins [20], which impairs in vivo performance beyond what can be detected by the routine coagulation laboratory assays [21]. On the other hand, recent studies have shown that in vivo ADAMTS-13 activity is highly dependent on the intra-vascular flow conditions and the physiological interaction between platelets and vWF [22]. It is plausible therefore that virus inactivation in plasma with methylene blue and light leads to subtle changes in the ADAMTS-13 molecule that impair in vivo activity while passing undetected by the current in vitro assays. Alternatively, methylene blue and light could damage other, hitherto unknown plasma components contributing to TTP pathogenesis.

In conclusion, MBPIP prepared by either the Springe method or the ‘in house’ system developed at Macopharma retains normal levels of ADAMTS-13 activity as measured by the current laboratory assays.

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