Bradykinin production during donor plasmapheresis procedures

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Background and Objectives Extracorporeal circuits made of artificial substances may induce blood cells and humoral activation. Negatively charged surfaces may activate Factor XII and the prekallikrein-kinin cascade, resulting in bradykinin (BK) production. BK has been considered to be involved in severe hypotensive reactions occurring during therapeutic apheresis in patients taking angiotensin-converting enzyme (ACE) inhibitors or in those receiving platelet transfusion. In this study we investigated BK production during donor plasmapheresis procedures.

Patients and Methods Eighteen volunteer donors entered the study protocol. Nine of them were taking ACE inhibitors. Their blood pressure (BP) was monitored both pre- and post-apheresis, and BK determination was carried out using a competitive enzyme immunoassay (EIA), in plasma samples collected both during and at completion of the procedure. In addition, a limited number of thawed plasma units were checked for BK.

Results No side-effects were observed during the procedures. However, donors taking ACE inhibitors showed a higher variation of their systolic BP compared to those who were not taking ACE inhibitors, while diastolic BP percentage variations did not differ significantly between the two groups. The BK concentration was considerably higher in donors taking ACE inhibitors: 183 ± 26 versus 82 ± 6 ng/ml (P < 0.001) after the first collection cycle and 142 ± 20 versus 65 ± 11 ng/ml (P < 0.001) in the final samples. BK was also detected, at a lower concentration (15 ng/ml), in one out of four thawed plasma units obtained from donors taking ACE inhibitors and at 1 ng/ml in one out of two thawed plasma units from the control group.

Conclusion Donors taking ACE inhibitors and undergoing plasmapheresis showed higher levels of BK compared to the control group. Furthermore, the detection of BK in plasma units after a freeze–thaw procedure might explain the sudden hypotensive reaction occurring during therapeutic plasma exchange when plasmapheresis units are adopted as substitution fluids. Further investigations are needed to assess the real clinical importance of the presence of BK in plasma units.

Key words: Biocompatibility, bradykinin, donor plasmapheresis.

Introduction

Blood contact with artificial materials used in extracorporeal circuits for the removal of metabolites or toxins, collection of blood components or intraoperative blood salvage, may induce blood cell and humoral activation [1–3]. The issue of biocompatibility of artificial substances has therefore been questioned in recent years by several reports dealing with the onset of severe and sometimes life-threatening side-effects in patients undergoing extracorporeal plasma treatment [4–6], intraoperative blood salvage [7], therapeutic apheresis procedures [8,9] or even receiving bedside–filtered platelet units [10]. With regard to this latter point, it has been demonstrated that materials made of polyvinyl chloride (PVC) coated with a
negatively charged surface, may trigger activation of coagulation Factor XII (Hageman factor), with the subsequent production of kallikrein and further cleavage of high-molecular-weight kininogen (HMWK), resulting in the generation of bradykinin (BK) [11]. BK is a nonapeptide with strong vasodilatory properties. It induces histamine release and constriction of pulmonary airways [12]. Side-effects such as facial flushing, severe hypotension, abdominal pain, tachycardia, dyspnoea or even respiratory arrest, have been reported in patients given blood components through negatively (or, in a few cases, positively) charged leukodepletion filters [13–16], either receiving plasma protein fraction (PPF) during cardiopulmonary bypass (CPB), or during therapeutic apheresis (TA) procedures [7–9]. These unfavourable events were more frequent in patients given concomitant antihypertensive therapy with angiotensin-converting enzyme (ACE) inhibitors. BK has a plasma half-life of 15–30 seconds, and is rapidly degraded by endogenous kininases [17]. Given that ACE acts as a kininase II, ACE inhibitors might reduce endogenous BK catabolism by blocking ACE activity [18].

It has been recommended that in patients scheduled to undergo elective therapeutic apheresis procedures, ACE-inhibitor therapy should be withdrawn [9]. Nonetheless, to our knowledge no information is available about BK production and the occurrence of BK-related side-effects in donors undergoing collection of blood components using cell separators. We therefore investigated this issue in a small group of volunteers undergoing plasmapheresis.

Patients and methods

Donors

Eighteen volunteer donors fulfilling the Italian regulations for blood donation and apheresis [19] entered the study after signing an informed consent form. According to our national regulations and institution guidelines, monotherapy with antihypertensive drugs does not exclude volunteers from making a blood donation after a thorough medical examination. Therefore, donors were divided in two groups, each consisting of nine subjects: the ACE+ group comprised donors not taking ACE inhibitors, while the ACE− group consisted of individuals receiving monotherapy with ACE inhibitors for a median period of 36 months (range 18–60 months). Of the ACE− group, six donors were taking enalapril, and the three remaining donors were, respectively, taking perindopril, captopril and lisinopril. Subjects with a history of allergy or those on any other medication were excluded from the study, as were first-time donors or those donors < 25 years of age, given that a greater incidence of vaso-vagal reactions has been observed in younger donors [20]. Blood pressure (BP) was recorded by a trained nurse unaware of the status of the donor group, before and 5 min after completing the apheresis procedure. Side-effects occurring during the procedure were recorded.

Donor plasmapheresis procedure

Plasma collection was performed using an Autopheresis C device (Baxter, Deerfield, IL). This cell separator, which has an extracorporeal blood volume up to 230 ml, allows for a virtually cell-free plasma collection by using a combined centrifugation–filtration technique: donor blood is drawn with a 16-gauge needle; it passes through a PVC disposable set and filtration occurs by means of a magnetic-guided rotating filter with a 0.65-micron micropore nylon membrane [21,22]. After completion of each collection/filtration cycle (lasting 5–9 min), plasma-poor blood is eventually reinfused to the donor. The procedure usually takes 30–45 min for completion, with four to eight collection/reinfusion cycles. Anticoagulation is carried out by using ACD-A at a ratio of 1:12–1:16. All donors were given saline (200–300 ml) before disconnection.

Laboratory tests

The blood chemistries of each donor were checked before the procedure and in samples drawn from the plasma collection bag as quality controls. In the latter, sampling was performed at the end of the procedure by a three-way stopcock situated in the collection line. Complete blood counts were performed by means of an automated instrument (Argos; Roche, Milan, Italy) in basal samples and in final samples from the plasma collection bag, C1 and C2, carried out using a Beckman HPS device (Beckman Inc., Fullerton, CA). Plasma protein electrophoresis was performed by using a colorimetric assay (Itachi Italy) in basal samples and in final samples from the plasma collection bag. C1 and C2, using an automated instrument (Cosmofed 3200; Ciampolini, Florence, Italy) and determination of plasma protein was performed by using a colorimetric assay (Bachi Inc., Osaka, Japan).

BK assay

Plasma sampling was performed as described above, at the end of the first collection cycle and at the end of the procedure, by using a refrigerated syringe after careful resuspension and mixing of the plasma collection bag. After sampling, plasma was aliquoted into 2-ml amounts and stored in 5-ml cryovials (Nalgene, Rochester, NY) at ~80 °C until analysis. Each vial contained a cocktail of protease inhibitors consisting of disodium-EDTA (Carlo Erba Spa, Milan, Italy) and aprotinin (Sigma Inc., St Louis, MO), at a final concentration of 5 μg and 3 μl, respectively. Disodium-EDTA inhibits kininase I, while aprotinin is a generic protease inhibitor. BK determination was carried out using a competitive enzyme immunoassay (EIA) (Markit-M-Bradykinin; Dainippon, Osaka, Japan). All analyses, as well as standard dilutions (from
shown in Table 1 with procedure parameters. The two groups values and analyses performed on the collected plasma, are

Results

Statistics

were used as controls during analysis of each batch.

plasmapheresis without the addition of protease inhibitors, standard whole-blood donation, as well as samples from described above. Normal plasma, i.e. cell-free samples from a Europe, Leuven, Belgium) and samples were processed as of storage at –45° ACE, angiotensin-converting enzyme; ACD-A, acid citrate dextrose-formula A; F, female; M, male.

NS, not statistically significant.

Data are shown as mean ± SD.

5000 pg/well to 19·5 pg/well were carried out in duplicate. After plotting the absorbance of the standard dilutions on a semilogarithmic sheet, the BK concentration/well of each sample was read against the standard curve. In the event of a high BK concentration, it was necessary to dilute the sample and to repeat the analysis. The sensitivity of the test, i.e. the lower limit of detection, was 0·173 ng/ml (7·2 pg/well).

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All samples were analysed in duplicate, and the final value given for each sample represents the mean of the two absorbances. BK was checked in four plasma bags from the ACE+ group and in two from the ACE– group after 2–4 months of storage at –45 °C. Sampling was performed immediately after thawing through a sampling-site coupler (Terumo Europe, Leuven, Belgium) and samples were processed as described above. Normal plasma, i.e. cell-free samples from a standard whole-blood donation, as well as samples from plasmapheresis without the addition of protease inhibitors, were used as controls during analysis of each batch.

Statistics

Statistical analysis was performed using computer-specific software (GraphPad Prism, San Diego, CA). Data are shown as mean ± SD, unless otherwise stated. Non-parametric analysis (Mann–Whitney U-test) was used for abnormally distributed data. P-values of < 0·05 were considered as significant.

Results

The characteristics of the donors, predonation biochemical values and analyses performed on the collected plasma, are shown in Table 1 with procedure parameters. The two groups did not differ with regard to predonation blood count; and all plasma units collected were cell free, i.e. no white blood cells (WBC), red blood cells (RBC) or platelets were detected by means of the automatic cell counter (data not shown). Individuals of the ACE+ group were older (52 ± 9 versus 39 ± 10 years of age, P < 0·001) and had a higher average body weight (80 ± 15 versus 65 ± 8 kg, P < 0·023) than individuals of the ACE– group. Procedural parameters, i.e. duration, processed blood volume and collected plasma volume, were similar in the two groups and no procedural-related side-effects were observed in either group. However, significant differences between pre- and postapheresis systolic blood pressure (SBP) values in both groups were observed: 123 – 142 versus 16 ± 4 mmHg, P < 0·05 in the ACE+ group and 144 ± 14 versus 126 ± 16 mmHg, P < 0·05 in the ACE– group, even though ACE+ individuals showed wider percentage variations of SBP (13 ± 10 versus 4 ± 4%, P < 0·05). For diastolic blood pressure (DBP), only ACE+ subjects showed significant differences pre- and post-apheresis, while the values for ACE– individuals approached, but did not reach, statistical significance (data not shown). Similarly, DBP percentage variations did not differ when comparing ACE+ and ACE– groups (9 ± 3 versus 14 ± 7%, respectively, P = NS).

With regard to BK determination, we observed significant differences between the ACE+ group and the ACE– group, both at the end of the first collection cycle (183 ± 26 versus 82 ± 6 ng/ml, P < 0·0001) and at the end of the procedure: 142 ± 20 versus 65 ± 11 ng/ml, P < 0·0001 (Fig. 1). The intra-assay coefficient of variation (CV) ranged from 7·8% (mean 82, SD 6) to 14·2% (mean 183, SD 26). Plasma samples collected and stored without protease inhibitors were negative for BK. Interestingly, BK was detected in one out of four
thawed plasma units from the ACE⁺ group at a 15-ng/ml concentration and in one out of two plasma units from the ACE⁻ group at a concentration of 1 ng/ml.

**Discussion**

It was recently demonstrated that negatively (and sometimes positively) charged materials may induce activation of the prekallikrein-BK cascade through activation of coagulation Factor XII (Hageman) during blood component transfusion or apheresis procedures [9,11]. This results in severe and sometimes life-threatening situations, mainly in patients given ACE inhibitors as hypertension treatment, owing to blockade of the physiological activity of ACE as a kininase II [4–7]. We therefore investigated possible BK production during donor plasmapheresis procedures performed in a small group of volunteer donors, some of whom were taking ACE inhibitors. All plasmapheresis procedures were well tolerated with no side-effects and the plasma collected was of high quality, confirming the effectiveness of the procedures, as previously shown [21–24]. Nevertheless, a significant reduction in SBP was observed, particularly in the ACE⁺ group, while DBP varied significantly only in the ACE⁻ group. Taking into account the very high level of BK production observed mainly in the ACE⁺ group, it is surprising that the BP was of such a limited range. A possible explanation is the very high protein filtration rate permitted by the centrifugation/filtration technique employed by the autopheresis device. We therefore imagine that the great majority of BK generated was driven into the collected plasma, and only a limited amount of BK was infused back to the donors. At this point, it is worth remembering that 0.8–1.2 μg/kg body weight (b.w.) of BK can induce a BP drop of 20 mmHg, and that this effect could be increased 20–50-fold in subjects taking ACE inhibitors [12]. The concentration of BK found in our series was considerably higher than that observed in different settings.

In fact, BK levels of up to 0.470 ng/ml have been found in the venous blood of patients undergoing low density lipoprotein (LDL)-apheresis [6], and BK levels of up to 2.4 ng/ml have been found during haemodialysis procedures performed with an AN69 membrane [25]. Furthermore, BK has been shown to increase to up to 25 ng/ml during platelet concentrate filtration performed with negatively charged leukodepletion filters [13] and to 6.8 ng/ml during autologous blood filtration, with the occurrence of severe hypotension. In this latter case, the recipient was taking ACE-inhibitor therapy [16].

Such wide ranges of BK concentrations reported in different settings might be influenced by: different materials and different electric charges; physical phenomena such as shear forces, pump squeezing and contact with air; and by blood flow and centrifugation speed [2]. As to this last point, we must underline that our experimental conditions were quite different from those reported in other settings (except haemodialysis): blood flow during donor plasmapheresis ranges from 70 to 100 ml/min [21], while blood flow during blood component filtration is ~ 20–30 ml/min [13] and during therapeutic apheresis usually ranges from 30 to 70 ml/min [3,9]. In some of the previously reported studies, BK determination has been performed in venous blood samples, while in the present study sampling was performed at the stage immediately after filtration, thus reducing the clearance effects of endogenous kininases. Our data show that even in donors taking ACE inhibitors, a residual kininase activity persists; in fact, BK levels at the end of the collection were very much lower than those observed at the end of the first collection cycle, with significant differences between groups and, possibly, among donors [11]. Considering this latter point, we observed residual BK in one out of four thawed plasma units from the ACE⁺ group (15 ng/ml) and in one out of two plasma units from the ACE⁻ group (1 ng/ml). The disappearance of BK in the other thawed units from the ACE⁺ group could easily be explained by a residual kininase activity, given that ACE accounts for 76%
of total endogenous kininase activity [26], while the presence of traces of BK in the units from the ACE group might be explained by the great interindividual variation in kininase activity [11,13]. We emphasize that our investigation, performed only in a limited donor series and using a single apheresis device, should be confirmed with a larger number of donors (and plasma units) and on different cell separators adopting different techniques, using centrifugation only or filtration through hollow polypropylene fibres. Further research is needed to evaluate the real impact of residual BK in apheresis plasma units, if these are used as replacement fluid during plasma-exchange procedures [26].

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