Leukocyte Depletion of Red Cell Components Prevents Exposure of Transfusion Recipients to Neutrophil Elastase

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Abstract

Background: Polymorphonuclear leukocytes contain a large number of enzymes and bactericidal proteins stored in granules. Neutrophil activation induces degranulation and immediate release of these bioactive substances, including human neutrophil elastase (HNE) also known as elastase-2 (ELA2), which may contaminate whole blood units and blood components. ***Materials and Methods***: The HNE concentration was determined in the supernatants of blood components with a commercial enzyme-linked immunosorbent assay (ELISA). The effect of leukocyte depletion and storage was evaluated by testing whole blood, buffy-coat-reduced, and leukocyte-depleted red cell units. Buffy-coat-derived platelets and plasma were also tested. ***Results***: HNE concentrations at day 1 were about 50 µg/l in all types of red cell components with the exception of leukocyte-depleted red cells (<0.26 µg/l). In leukocyte-depleted red cells, platelets and plasma, no significant increase was observed during storage. In whole-blood units and buffy-coat-reduced red cells, the HNE concentrations increased steadily and often exceeded 1,000 µg/l when the units expired. ***Conclusion***: Leukocyte depletion may limit the inadvertent infusion of bioactive substances derived from polymorphonuclear leukocytes, of which HNE is but one example. The accumulation of HNE in buffy-coat-reduced red cells may be greater than that of whole blood units. HNE accumulates during storage and its quantity may have pathophysiologic significance. Platelets and plasma derived from buffy coats contain some HNE, but leukocyte-depleted red cells virtually none. However, we consider the accumulation of HNE in these components not to be clinically important. The quantities, kinetics, and occurrence in various blood components of HNE contamination differ from those observed with cytokines.

Introduction

Many types of blood components are contaminated by bioactive substances derived from leukocytes and platelets. The kinetics of this and the blood components affected vary according to the source of the contamination [1-6]. For example, the contamination by cytokines from lymphocytes is clinically relevant [7, 8]. These cytokines accumulate during the storage of platelets prepared from platelet-rich plasma [9, 10], whereas platelets prepared from buffy coats may be virtually free of them [11]. In contrast to cytokines, which may be active at concentrations of nanograms per liter, enzymes from granulocytes, such as human neutrophil elastase (HNE), are effector proteins by themselves and often

occur in concentrations of milligrams per liter. During intraoperative blood salvage, such amounts of HNE and other granulocyte enzymes were released, and could be removed by washing before retransfusion [12, 13]. In whole blood units, HNE concentrations increased 30-fold during storage and reached 1,500 μg/l [14-16]. Baseline HNE concentrations were not influenced by filtration through a 40-μm polyester filter [16]. In whole blood filtered before storage, HNE was significantly lower at days 13 and 21 but did not differ at day 35, compared with unfiltered whole blood [16]. Similar HNE contamination occurs in red cells units [15, 17, 18]. Prestorage leukocyte depletion by filtration prevented HNE accumulation, whereas poststorage filtration with a third-generation polyester filter did not [17]. Granulocyte products other than HNE, such as platelet-activating factor and leukotriene B_4, may also contaminate blood units in amounts of micrograms per liter [13].

HNE [19, 20], also known as elastase-2 (ELA2), makes up at least 5% of the granulocyte dry weight and is found in the dense azurophilic granules of neutrophil granulocytes [21, 22]. It also occurs to some extent in macrophages and endothelial and pancreatic cells. HNE is a general, powerful serine protease enzyme with rather low substrate specificity, hydrolyzing numerous proteins at neutral pH. After release from neutrophil granules, free HNE is rapidly bound to the α_1-proteinase inhibitor (formerly called α_1-antitrypsin) and a smaller fraction to α_2-macroglobulin, which inhibit all HNE activity in plasma within milliseconds [23]. The complexed HNE [20] has a half-life of 60 min in plasma, and HNE is usually assayed by testing the serum concentration of the HNE/α_1-proteinase-inhibitor complex [24]. HNE may damage the host [23] by enzymatic degradation of connective tissue macromolecules, such as collagen and elastin, and many other proteins including coagulation and complement factors.

The release of large quantities of HNE during the early posttraumatic reaction is considered to be a host defense mechanism and is frequently used as an indicator of the systemic inflammatory response [23, 25-27]. The HNE serum concentration increases rapidly within hours of trauma [23, 25, 28-31]. A severe clinical course in patients suffering from multiple trauma correlates significantly with increased HNE concentration [29-31]. In patients who survive or suffer lower grade trauma, HNE tends to decrease within 1 week [23, 28]. HNE was found to correlate with mortality in one study [32], but not in others [31].

Like HNE, other leukocyte products accumulate in the serum of severely injured patients. For example, C-reactive protein [29, 33], interleukin (IL)-6 [31], IL-8 [31], phospholipase A_2 [28, 29, 32], neopterin [28, 29], and C3a [28, 34], have been associated with complications and outcome. Upon neutrophil activation, these bioactive substances may be immediately released by degranulation without de novo synthesis [35-37]. Such mediators are used in clinical studies for patient monitoring, because the activation of polymorphonuclear neutrophils early after trauma is considered to contribute to the systemic inflammatory response [38-40]. The mediators of sensitized leukocytes may enhance host resistance and control infection. They may also depress the function of remote organs and cause tissue injury by a systemic inflammatory response [38, 41]. To classify the severity of injury, leukocyte products may be included in trauma scores, which are of critical clinical importance to guide decisions for diagnostic procedures, therapeutic interventions, or interhospital transfer of trauma patients.

Our study was prompted by the possibility that massive transfusions may be a iatrogenic source of inflammatory mediators from polymorphonuclear leukocytes. We checked HNE as a parameter representing the release of bioactive substances from the granules of neutrophilic granulocytes. We systematically measured the HNE concentrations in the blood components

used in current supportive therapy of, for example, trauma patients. Storage time, white blood cells (WBC), and granulocytes were associated with HNE contamination. Without leuko-depletion, the HNE content in red cell units is considerable and may confound HNE testing in polytransfused patients. The clinical relevance of the inadvertent infusion of large quantities of granulocyte enzymes in critically ill patients is unknown.

Materials and Methods

Preparation of Blood Components

Whole blood (500 ml) was collected from volunteers donors into 70 ml of citrate-phosphate-dextrose (CPD) stabilizer in primary containers of a 'top and bottom' system made of polyvinylchloride with 2-diethylhexylphtalate (2-DEHP) plasticizer in accordance with the large-scale routine procedures as described previously [11]. Whole blood units were not further processed. To prepare buffy-coat-reduced red cells and plasma, the whole blood was centrifuged (slow acceleration, 0-3,900 *g* for 4 min and 3,900 *g* for 12 min; Roto Silenta RP, Hettich, Tuttlingen, Germany) within 18 h. The supernatant (plasma) and the infranatant (red cells) were transferred to integrally attached, secondary satellite containers by using an automatic blood separation device (Optipress 1; Baxter, München, Germany). The buffy-coat-reduced red cells were suspended in additive solution (SAG-Mannitol). By filtration of these buffy-coat-reduced red cells with a polyester filter (BPF4-BBSd; Pall, Dreieich, Germany), residual WBC and platelets were removed and leukocyte- and platelet-reduced (filtered) red cell units with additive solution were obtained. Platelets were produced from buffy coats by a soft-spin method [11]. During production and storage, the temperature was maintained at 4±2 °C for red cells and 22±2°C for platelets. Within 18 h of collection, the plasma was separated and immediately flash-frozen then stored at -40 °C for 60 days. WBC and granulocyte counts after preparation were checked with a cell counter (Onyx; Coulter, Krefeld, Germany). For quality control when WBC counts were below the detection limit of the cell counter, routine preparations were visually checked by microscopy in Neugebauer chambers.

In 1996, we produced more than 150,000 buffy-coat-reduced red cell units. For quality control, 153 of these were evaluated at random and found to contain 1.2 x 10^9 WBC per liter (mean, range: <0.01-10). Of 8,000 leukocyte-depleted red cell units, 48 were checked and contained 5.9 x 10^5 WBC per liter (range: <0.01-39); of 26,000 platelet units, 167 contained 0.31 x 10^9 WBC per liter (range: < 0.01-4.4), and of 40,000 plasma units, 66 contained 1.6 x 10^7 WBC per liter (range: < 0.01-10). We studied 10 whole blood units, 10 buffy-coat-reduced and 10 leukocyte-depleted red cell units, 8 platelet concentrates derived from buffy coats, and 11 plasma units. The blood components tested in this study were representative of routine preparations; the only exception were whole blood units, which are not produced for clinical use at our institution, but are not infrequently transfused elsewhere. On the final sampling day, we checked all blood components of this study for bacterial contamination, and by cell counter for leukocyte content. All were sterile after a culture period of at least 15 days.

Sampling Procedure

Before sampling, the units were gently agitated for 5 min. By sterile puncture with a long needle through a latex adapter attached to each blood bag, samples of about 5 ml were drawn at each time point selected. The samples were immediately centrifuged and the supernatant stored frozen at -80 °C in aliquots. Shipment was on dry ice. Aliquots were tested in batches. Testing was performed immediately after thawing of any aliquot.

HNE Determination

HNE [19] (EC 3.4.21.37) was determined in plasma as an HNE/α1-proteinase-inhibitor complex by a 'two-site' sandwich enzyme-linked immunosorbent assay (ELISA) (PMN Elastase, 1.12589; Merck, Darmstadt, Germany) [24]. The detection limit was <0.26 µg/l. The reference range was 20-180 µg/l in plasma [42].

HNE Recovery

As control, to prove that a quantitative detection of HNE was possible with our assay system, we added 5,000 ng purified human 32-kD neutrophil elastase-2 enzyme (EC 3.4.21.37, lot No. FA107131443; Merck, Darmstadt, Germany) in 50 µl 0.9% NaCl to 5 ml samples drawn from each type of blood component at day 1 resulting in a calculated concentration of 1,000 µg/l. After centrifugation, supernatants were sampled for HNE testing.

HNE Release after Neutrophil Activation

At day 1 we added 100 µl cytochalasin B (10 mmol; Sigma, St. Louis, Mo, USA) dissolved in dimethylsulfoxide (DMSO) per 100 ml volume of cellular components prewarmed to 37 °C (about 300 ml total volume for red cells and about 70 ml for platelets). After 5 min incubation during agitation, we added 100 µl of N-formyl-*L*-methionyl-*L*-leucyl-*L*-phenylalanine chemotactic peptide (1 mmol f-MLP in DMSO; Serva GmbH, Heidelberg, Germany) per 100 ml volume. Sampling was done after 5 min incubation during agitation. Additional samples were drawn on day 5 for platelets and on days 10 and 21 for red cells.

Statistics

HNE and WBC concentrations were given as median (range). We performed a statistical analysis by using the two-sided nonparametric Kruskal-Wallis rank sum test with Bonferroni correction for multiple testing.

Results

HNE in Red Cells Preparations

The HNE concentrations in whole blood and buffy-coat-reduced red cells units increased considerably during storage (fig. 1). The baseline HNE concentration was about 50 µg/l at day 1. The accumulation of HNE was particularly pronounced after day 6. On expiration of the units, the median HNE contamination exceeded 1,000µg/l. In contrast, the median HNE contamination of leukocyte-depleted red cell units was below the detection limit of <0.26 µg/l throughout their storage period.

The HNE contamination appeared to be lower in buffy-coat-reduced red cells compared with whole blood units up to day 21, when the whole blood units expired (table 1). The differences in HNE supernatant concentrations were statistically significant at days 3 and 6 and would appear even more pronounced, if the total HNE amounts were considered, given about 250 ml supernatant (plasma) in whole blood but only about 120 ml in buffy-coat-reduced red cell units (110 ml additive solution and about 10 ml plasma). However, HNE concentrations and even total amounts of HNE in buffy-coat-reduced red cells beyound day 21 may well exceed HNE in whole blood units at expiration day 21. The highest HNE contaminations were frequently in buffy-coat-reduced red cells rather than whole blood.

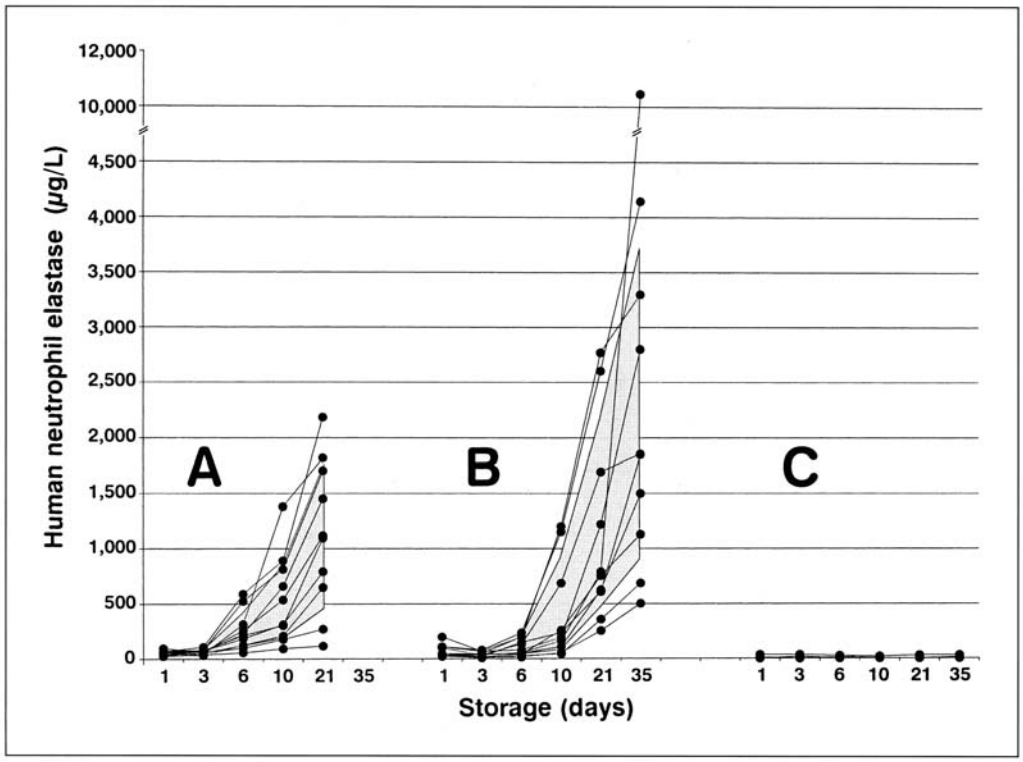

Fig. 1. HNE contamination in the supernatants (plasma or additive solution) of red cell blood components. The supernatant HNE concentrations occurring in three different types of red cell blood units during storage is shown for ten units of each type. Identical units are connected by lines. The supernatant HNE concentrations within the 25–75 percentiles are depicted as gray polygons. **A** Whole blood units. **B** Buffy-coat-reduced red cell units. **C** Leukocyte-depleted red cell units.

Table 1. HNE concentrations (median and range, in parentheses) in the supernatant (plasma or additive solution) of red cell blood components

Red cell blood components	Number	WBC, 10^9/l	HNE, µg/l					
			day 1	day 3	day 6	day 10	day 21	day 35
Whole blood units	10	5.1 (3.8–12.3)	45 (27–97)	66 (35–109)	221 (58–592)	428 (95–1,384)	1,109 (119–2,185)	ND
Buffy coat-reduced	10	2.7 (1.1–4.0)	31 (22–201)	37 (13–238)[a]	67 (13–238)[b]	216 (44–1,203)	772 (360–2,770)	1,852 (505–10,450)
Leukocyte-depleted	10	<1 (<1)	<0.3 (<0.3–34)[c]	<0.3 (<0.3–34)[c]	<1 (<0.3–24)[c]	<0.3(<0.3–18)[c]	<0.3 (<0.3–28)[c]	<0.3 (<0.3–30)[c]

Statistics were done with the Kruskal-Wallis rank sum test separately for each day with Bonferroni correction for multiple testing (n = 3). ND = Not determined.

[a] $p<0.05$ for whole blood versus buffy coat-reduced red cell units.

[b] $p<0.01$ for whole blood versus buffy coat-reduced red cell units.

[c] $p<0.001$ for leukocyte-depleted red cell versus both, whole blood and buffy coat-reduced red cell units.

HNE in Platelet and Plasma Preparations

In buffy-coat-derived platelets and in plasma the median baseline of HNE was about 50 µg/l at day 1 (table 2). A minimal HNE increase was observed until expiration of the platelets at day 5 and after thawing of plasma that had been stored at -40°C for 60 days.

Blood components	Number	WBC, 10^9/l	HNE, µg/l		
			day 1	day 5	day 60
Platelets	8	<1	54 (31–159)	66 (40–186)	ND
Plasma	11	ND	50 (37–78)	ND	56 (40–90)

ND = Not determined.

Table 2. HNE concentration (mean and range, in parentheses) in buffy coat-derived platelets and plasma

Recovery of Exogenous HNE

As a control, we tested the recovery after exogenous addition of HNE in one 5-ml sample for each type of blood component (5,000 ng HNE added to 5 ml, final concentration 1,000 μg/l). The recovery rate was generally greater than 90%. In a whole blood sample, 899 μg/l versus baseline 27 μg/l were recovered (buffy-coat-reduced red cells: 916 versus 23 μg/l; leukocyte-depleted red cells: 966 versus <0.26 μg/l; platelets 1,035 versus 47 μg/l; and plasma: 965 versus 51 μg/l.

Neutrophil Activation and HNE Release Induced by Cytochalasin B and f-MLP in Cellular Blood Components

The addition of cytochalasin B and f-MLP to units at day 1 resulted in an immediate release of HNE but did not prevent the further accumulation of HNE in the supernatant during storage. Whole blood units showed an HNE increase to 184 and 336 μg/l HNE after stimulation (n = 2; baseline: 52 and 130 μg/l at day 1) and 2,035 μg/l (n = 1; baseline 1,933 μg/l at day 10). In buffy-coat-reduced red cells, the HNE increased to 36 and 211 μg/l (n = 2; baseline: 6 and 182 μg/l at day 1) and 304 μg/l (n = 1; baseline 240 μg/l at day 10). Further increases of HNE in whole blood and buffy-coat-reduced red cell units at day 21 were minimal (data not shown). Upon stimulation of leukocyte-depleted red cells (day 1: n = 2, days 10 and 21: n = 1) and platelets (days 1 and 5: n = 3), the HNE concentrations increased minimally, if at all (data not shown). Neutrophil activation in plasma units was not tested.

Granulocyte Count in Buffy-Coat Reduced Red Cell Units

In accordance with previous observations [43], the automatic blood separation procedure preferentially removed mononuclear lymphocytes. The majority of residual WBC in our buffy-coat-reduced red cell units were neutrophil granulocytes (median 89%, range: 82-92%, n = 8; reference range in whole blood: 42-75%).

Discussion

We systematically checked the contamination by HNE in the standard blood components frequently used today for transfusion therapy. A prime source of inadvertent exposure of transfusion recipients to HNE are buffy-coat-reduced red cell units. If still used for transfusions, whole blood units would be a similar source of HNE exposure. Platelets and plasma contained low amounts of HNE, not exceeding the normal HNE plasma concentrations of healthy individuals. The median HNE contamination of leukocyte-depleted red cell units was below the detection limit of our assay throughout the storage period.

Sieunarine et al. [14] and Hertfelder et al. [18] demonstrated a >30-fold increase of HNE in whole blood units with mean concentrations of 1,100 μg/l at day 28 and in red cell concentrates. These observations were confirmed in our study showing that the median HNE concentration increased from 45 μg/l 1 at day 1 to about 1,100 μg/l at day 21 in whole blood units. For the clinical practice, the data with buffy-coat-reduced red cell units are relevant, because they contained abundant HNE, like whole blood units during the first 21 days of storage and, at expiration on day 35, perhaps exceeding that of whole blood. The maximum HNE load in buffy-coat-reduced red cell units was about 10 mg/l in 120 ml of additive solution/plasma. This

unexpected degree of contamination may be explained by the observation that the neutrophil granulocyte fraction in buffy-coat-reduced red cell units was high, because the automatic blood separation procedure may preferentially eliminate mononuclear rather than polymorphonuclear leukocytes.

The median HNE contamination in leukocyte-depleted red cell units was below the detection limit of 0.26 μg/l throughout their storage period. Leukocyte depletion by filtration not only completely prevented HNE accumulation during storage, but the filter may have absorbed naturally occurring HNE from the suspension medium. The mechanism is unknown and may not be effective with all leukocyte depletion filters [15, 17, 44]. Similar effects, however, have been reported for some, but not all, polyester filters with regard to the adsorption of C3a, C5a, IL-8 and RANTES [45]. If HNE-free red cell transfusion is an objective, prestorage leukocyte-depleted red cell units would be a first choice.

Throughout the storage period, HNE in platelets and plasma was within the reference range for plasma concentrations of healthy persons. We did not test whether leukocyte depletion of platelets might eventually reduce their residual HNE contamination. Taking our results with various blood components and reviewing the literature on their contamination by cytokines [3-7, 46] we concluded that the doses, kinetics and types of blood components affected vary considerably as to contamination by mono- and polymorphonuclear leukocyte products (table 3). There appears to be plenty of room for improvement in the production process, once the relevant parameters and efficient procedures are determined. Ample evidence points to the clinical relevance of a better quantification of blood component quality and of its consequences for transfusion therapy.

Recent in vitro and retrospective clinical studies correlated adverse effects with storage time of blood components [47, 48]. Prospective studies often have not confirmed transfusion effects that were claimed in retrospective studies, at least not in regard to their extent [49]. Nevertheless, contamination by leukocyte products is a function of storage time and could explain a less beneficial effect of stored blood components in some transfusion recipients [50]. Elimination of leukocyte products rather than reduction of viable leukocytes may contribute to the beneficial effects of leukocyte depletion [51].

Our findings may have practical implications for transfusion practice, because of the established HNE contamination of standard red cell units. The transfusion of large amounts of HNE may cause increased plasma concentrations in the early post-traumatic (i.e. post-transfusion) period. It may also influence systemic post-injury reactions, effected by HNE alone or in conjunction with other neutrophil granule contents. First, buffy-coat-reduced red cells often carry 0.1-1.0 mg HNE per unit. In patients with severe haemorrhage receiving, for example, 15 red cell units containing 1,800 ml supernatant, nearly all the HNE in the blood samples of the early posttraumatic period is a consequence of emergency transfusion therapy. Infusions of HNE-contaminated wound drainage blood also raises the plasma HNE concentrations in patients [44]. Hence, the plasma HNE may not represent endogenous inflammatory reactions after trauma in patients transfused with HNE-tainted red cells. HNE levels range from 250 to 1,500 μg/l in the early period after severe injury [25, 29, 31, 32, 52] and may be merely a surrogate marker for transfused red cells rather than endogenous neutrophil degranulation. In emergencies, multi-transfused patients often receive older red cell units than patients with less transfusions, as in elective surgery. No meaningful interpretation can be derived from HNE laboratory data after massive transfusions. A 'clean' in vivo situation would be better and could be provided by the transfusion of leukocyte-depleted red cells.

Table 3. Characteristics of endogenous contamination during storage of blood components

Parameter	Cellular source of contamination	
	granulocytes	lymphocytes
Practical aspects		
Clinical relevance	possible	proven
Contaminated units	red cells	platelets
Standard units[a] affected	yes	no
Kinetics of contamination	delayed, after day 6	rapid, within 5 days
Prevention of contamination	possible by filtration	often not required[b]
Effect of storage temperature	+4 °C does not prevent release	+22 °C required for synthesis
Cellular and immunologic aspects		
Principal bioactive substances	enzymes	cytokines
Effector molecule	yes	no
Cellular organelle	granula	endoplasmatic reticulum
Stored in leukocytes	yes	no
de novo synthesis required	no	yes
Mode of contamination	release of stored agent	synthesis (no storage)
Availability	immediate	after synthesis
Content	milligrams	nano- to micrograms

[a] Buffy-coat-reduced red cell and buffy-coat-derived platelet units.
[b] Filtration of platelets produced by the platelet-rich-plasma method, however, may reduce cytokine contamination.

Second, the clinical effect of transfusions containing HNE is open to question, because of the potent proteinase inhibitors in human plasma. We tested the HNE/α1-proteinase-inhibitor complex, which does not represent free or active HNE. However, HNE may dissociate from the α_1-proteinase-inhibitor and bind to α_2-macroglobulin. This anti-proteinase renders HNE inactive only for large-molecular-weight substrates, whereas most of the HNE activity for low molecular weight substrates remains intact [53]. Transfused proteinase-inhibitor complexes may still affect the protein cascades of the recipient and induce cytokine synthesis. For example, cathepsin G bound to its inhibitor, α_1-antichymotrypsin, may induce fibroblasts to synthesize IL-6 [54]. Methods specific for HNE activity, e.g. testing the cleavage of *L*-pyroglutamyl-*L*-prolyl-*L*-valine-*p*-nitroanilide peptide substrate [55], have not been applied to check HNE activity in blood components. However, strong binding of HNE and cathepsin G to cell membranes does occur under physiological conditions, leaving both proteases active [56]. We cannot exclude the possibility that some membrane-bound proteases from neutrophils may be present and functionally active in blood components.

Third, we took HNE as one parameter representing a large number of bioactive substances that can be released from the granules of polymorphonuclear leukocytes. These bioactive substances comprise enzymes like cathepsin B, D and G; peroxidase and myeloperoxidase; lysozyme; and proteinase 3. They have an intracellular bactericidal role, to which end the contents of the lysosomes may be tailored, and usually occur only in small quantities outside of leukocytes. However, under pathological conditions, massive release of granule content may promote systemic effects by activating cells or plasma protein cascades [38], making many of these substances prominent effectors in pathological processes. The granule proteins can activate lymphocytes, stimulate the complement system [23], cause platelet aggregation, effect the release of histamine from mast cells [57], and facilitate the production of oxygen free radicals [58]. The observed levels of the HNE in stored blood components clearly indicate that neutrophil degranulation has occurred and implies that other WBC granule content, may be present in the additive solution, and active in vivo, after inadvertent transfusion.

The preparation processes themselves did not seem to cause neutrophil degranulation, because the HNE baseline was identical for all unfiltered blood components and within the normal range of human plasma. The induced degranulation at day 1, and also at day 10, showed that a reservoir of HNE could be released from granules immediately upon appropriate stimulation. A synthesis in red cell units at +4°C was not observed for cytokines [46] and is likewise not expected for HNE. A formal proof of this hypothesis would require further experiments with protein synthesis inhibitors. Because some granulocytes may remain viable during storage and release their granule content in vivo after transfusion, the actual HNE exposed of patients may be greater than implied by the in vitro results.

In the present study we showed, that HNE concentrations may increase more than 50-fold during storage of whole blood and buffy-coat-reduced red cell units. HNE is only one of several enzymes in the granules of neutrophils. Its presence reflects neutrophil degranulation and implies the concomitant contamination by other bioactive substances from polymorphonuclear granules. Inadvertent infusion of HNE/inhibitor complexes and other granular content may induce systemic protein cascades and enhance inflammatory processes in compromised patients who are already at high risk because of their underlying disease, such as severe trauma. Leukocyte depletion is most effective in averting HNE contamination and may limit the potentially harmful effects of transfusing HNE and other contaminants of granulocyte origin. This prophylaxis would be of prime importance for all patients in need of massive transfusions.

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References

1 Klüter H, Müller-Steinhardt M, Danzer S, Wilhelm D, Kirchner H: Cytokines in platelet concentrates prepared from pooled buffy coats. Vox Sang 1995;69:38-45.
2 Bubel S, Wilhelm D, Entelmann M, Kirchner H, Kluter H: Chemokines in stored platelet concentrates (abstract). Transfusion 1996;36:445-449.
3 Nielsen HJ, Reimert CM, Pedersen AN, Brunner E, Edvardsen L, Dybkjaer E, Kehlet H, Skov PS: Time-dependent, spontaneous release of white cell- and platelet-derived bioactive substances from stored human blood. Transfusion 1996;36:960-965.
4 Nielsen HJ, Skov F, Dybkjaer E, Reimert CM, Pedersen AN, Brunner N, Skov PS: Leucocyte and platelet-derived bioactive substances in stored blood: Effect of prestorage leucocyte filtration. Eur J Haematol 1997;58:273-278.
5 Nielsen HJ, Reimert C, Pedersen AN, Dybkjoer E, Brunner N, Alsbjorn B, Skov PS: Leucocyte-derived bioactive substances in fresh frozen plasma. Br J Anaesthesiol 1997;78: 548-552.
6 Hyllner M, Arnestad JP, Bengtson JP, Rydberg L, Bengtsson A: Complement activation during storage of whole blood, red cells, plasma, and buffy coat. Transfusion 1997;37:264-268.

7 Muylle L, Wouters E, De Bock R, Peetermans ME: Reactions to platelet transfusion: The effect of the storage time of the concentrate. Transfus Med 1992;2:289-293.
8 Heddle NM, Klama L, Singer J, Richards C, Fedak P, Walker I, Kelton JG: The role of the plasma from platelet concentrates in transfusion reactions. N Engl J Med 1994;331:625-628.
9 Muylle L, Peetermans ME: Effect of prestorage leukocyte removal on the cytokine levels in stored platelet concentrates. Vox Sang 1994;66:14-17.
10 Aye MT, Palmer DS, Giulivi A, Hashemi S: Effect of filtration of platelet concentrates on the accumulation of cytokines and platelet release factors during storage. Transfusion 1995; 35:117-124.
11 Flegel WA, Wiesneth M, Stampe D, Koerner K: Low cytokine contamination in buffy coat-derived platelet concentrates without filtration. Transfusion 1995;35:917-920.
12 Sieunarine K, Langton S, Lawrence-Brown MM, Goodman MA, Prendergast FJ, Hellings M: Elastase levels in salvaged blood and the effect of cell washing. Aust N Z J Surg 1990; 60:613-616.
13 Sieunarine K, Lawrence-Brown MM, Goodman MA, Prendergast FJ, Rocchetta S: Plasma levels of the lipid mediators, leucotriene B4 and lyso platelet-activating factor, in intraoperative salvaged blood. Vox Sang 1992;63:168-171.
14 Sieunarine K, Lawrence-Brown MMD, Brennan D, Langton S, Goodman MA, Hellings M: The quality of blood used for transfusion. J Cardiovasc Surg 1992;33:98-105.
15 Lindena J, Papastavrou S, Seidel JW: Efficacy and safety of a polyester leukocyte removal filter for whole blood and red cell concentrate filtration. J Clin Chem Clin Biochem 1989;27: 331-336.
16 Riedner C, Heim MU, Mempel W, Wilmanns W: Possibility to improve preservation of whole blood by leukocyte-depletion before storage. Vox Sang 1990;59:78-82.
17 Brecher ME, Pineda AA, Torloni AS, Harbaugh CA, Emery RL, Moore SB, Carmen R, Neslon E: Prestorage leukocyte depletion: Effect on leukocyte and platelet metabolites, erythrocyte lysis, metabolism, and in vivo survival. Semin Hematol 1991;28:3-9.
18 Hertfelder HJ, Suwer V, Popov-Cenic S, Tschesche H, Hanfland P: Leukocyte proteinase release during storage of red cell concentrates. Eur J Clin Chem Clin Biochem 1994;32:441-447.
19 Sinha S, Watorek W, Karr S, Giles J, Bode W, Travis J: Primary structure of human neutrophil elastase. Proc Natl Acad Sci USA 1987;84:2228-2232.
20 Travis J, Baugh R, Giles PJ, Johnson D, Bowen J, Reilly CF: Human leukocyte elastase and cathepsin G: isolation, characterization and interaction with plasma proteinase inhibitors; in Havemann K, Janoff A (eds): Neutral Proteases of Human Polymorphonuclear Leukocytes. München, Urban & Schwarzenberg, 1978, pp 118-127.
21 Bretz U, Baggiolini M: Biochemical and morphological characterization of azurophil and specific granules of human neutrophil polymorphonuclear leukocytes. J Cell Biol 1974; 63: 251-269.
22 Baggiolini M, Bretz U, Dewald B: Subcellular localisation of granulocyte enzymes; in Havermann K, Janoff A (eds): Neutral Proteases of Human Polymorphonuclear Leukocytes. München, Urban & Schwarzenberg, 1978, pp 3-17.
23 Weiss SJ: Tissue destruction by neutrophils. N Engl J Med 1989;320:365-376.
24 Dreher M, Gunzer G, Helger R, Lang H: An automated homogeneous enzyme immunoassay for human PMN elastase. Prog Clin Biol Res 1989;308:707-710.
25 Gando S, Kameue T, Nanzaki S, Hayakawa T, Nakanishi Y: Increased neutrophil elastase, persistent intravascular coagulation, and decreased fibrinolytic activity in patients with post-traumatic acute respiratory distress syndrome. J Trauma 1997;42:1068-1072.

26 Viljoen M, Roux LJ, Pretorius JP, Coetzee IH, Vilioen E: Hemostatic competency and elastase-alpha-1-proteinase inhibitor levels in surgery, trauma, and sepsis. J Trauma 1995; 39: 381-385.
27 Roumen RM, Redl H, Schlag G, Zilow G, Sandtner W, Koller W, Hendriks T, Goris RJ: Inflammatory mediators in relation to the development of multiple organ failure in patients after severe blunt trauma. Crit Care Med 1995; 23:474-480.
28 Kellermann W, Frentzel-Beyme R, Weite M, Jochum M: Phospholipase A in acute lung injury after trauma and sepsis: Its relation to the inflammatory mediators PMN-elastase, C3a, and neopterin. Klin Wochenschr 1989;67:190-195.
29 Waydhas C, Nast-Kolb D, Jochum M, Trupka A, Lenk S, Fritz H, Duswald KH, Schweiberer L: Inflammatory mediators, infection, sepsis, and multiple organ failure after severe trauma. Arch Surg 1992; 127:460-67.
30 Donnelly SC, MacGregor I, Zamani A, Gordon MW, Robertson CE, Steedman DJ, Little K, Haslett C: Plasma elastase levels and the development of the adult respiratory distress syndrome. Am J Respir Crit Care Med 1995; 151:1428-1433.
31 Nast-Kolb D, Waydhas C, Gippner-Steppert C, Schneider I, Trupka A, Ruchholtz S, Zettl R, Schweiberer L, Jochum M: Indicators of the posttraumatic inflammatory response correlate with organ failure in patients with multiple injuries. J Trauma 1997;42:446-454.
32 Uhl W, Beger HG, Hoffmann G, Hanish E, Schild A, Waydhas C, Entholzner E, Muller K, Kellermann W, Vogeser M, et al: A multicenter study of phospholipase A in patients in intensive care units. J Am Coll Surg 1995;180: 323-331.
33 Moore EE, Moore FA, Franciose RJ, Kim FJ, Biffl WL, Banerjee A: The postischemic gut serves as a priming bed for circulating neutrophils that provoke multiple organ failure. J Trauma 1994;37:881-887.
34 Hecke F, Schmidt U, Kola A, Bautsch W, Klos A, Kohl J: Circulating complement proteins in multiple trauma patients. Correlation with injury severity, development of sepsis, and outcome. Crit Care Med 1997;25:2015-2024.
35 Becker EL: The short and happy life of neutrophil activation. J Leukoc Biol 1990;47:378-389.
36 Kuijpers TW, Tool AT, van der Schoot CE, Ginsei LA, Onderwater JJ, Roos D, Verhoeven AJ: Membrane surface antigen expression on neutrophils: A reappraisal of the use of surface markers for neutrophil activation. Blood 1991;78:1105-1111.
37 Vissers MC, Day WA, Winterbourn CC: Neutrophils adherent to a nonphagocytosable surface (glomerular basement membrane) produce oxidants only at the site of attachment. Blood 1985;66:161-166.
38 Cioffi WG, Burleson DG, Pruitt BA: Leukocyte responses to injury. Arch Surg 1993; 128: 1260-1267.
39 Hogasen AK, Wurzner R, Abrahamsen TG, Dierich MP: Human polymorphonuclear leukocytes store large amounts of terminal complement components C7 and C6, which may be released on stimulation. J Immunol 1995; 154:4734-4740.
40 Tanaka H, Sugimoto H, Yoshioka T, Sugimoto T: Role of granulocyte elastase in tissue injury in patients with septic shock complicated by multiple-organ failure. Ann Surg 1991;213: 81-85.
41 Nuytinck JK, Goris JA, Redl H, Schlag G, van Munster PJ: Posttraumatic complications and inflammatory mediators. Arch Surg 1986; 121:886-890.
42 Neumann S, Gunzer G, Hennrich N, Lang H: 'PMN-elastase assay': Enzyme immunoassay for human polymorphonuclear elastase complexed with alpha-1-proteinase inhibitor. J Clin Chem Clin Biochem 1984;22:693-697.
43 Wagner HJ, Kluter H, Kruse A, Bucsky P, Hornef M, Kirchner H: Determination of the number of Epstein-Barr virus genomes in whole blood and red cell concentrates. Transfus Med 1995;5:297-302.

44 Arnestad JP, Bengtsson A, Bengtson JP, Johansson S, Redl H, Schlag G: Release of cytokines, polymorphonuclear elastase and terminal C5b-9 complement complex by infusion of wound drainage blood. Acta Orthop Scand 1995;66:334-338.
45 Geiger TL, Perrotta PL, Davenport R, Baril L, Snyder EL: Removal of anaphylatoxins C3a and C5a and chemokines interleukin 8 and RANTES by polyester white cell reduction and plasma filters. Transfusion 1997;37:1156-1162.
46 Grunenberg R, Kruger J: Analysis of cytokine profiles of stored CPDA-I whole blood. Infusionsther Transfusionsmed 1995;22:292-294.
47 Purdy FR, Tweeddale MG, Merrick PM: Association of mortality with age of blood transfused in septic ICU patients. Can J Anaesthiol 1997;44:1256-1261.
48 Mynster T, Dybkjoer E, Kronborg G, Nielsen HJ: Immunomodulating effect of blood transfusion: Is storage time important? Vox Sang 1998;74:176-181.
49 Wagner FF, Flegel WA, Kubanek B: Blood transfusion: influcence of transfusion therapy on outcome. Curr Opin Anaesthesiol 1998;11:167-175.
50 Avall A, Hyllner M, Bengtson JP, Carlsson L, Bengtsson A: Postoperative inflammatory response after autologous and allogeneic blood transfusion. Anesthesiology 1997;87:511-516.
51 van de Watering LM, Hermans J, Houbiers JG, van den Broek PJ, Bouter H, Boer F, Harvey MS, Huysmans HA, Brand A: Beneficial effects of leukocyte depletion of transfused blood on postoperative complications in patients undergoing cardiac surgery: A randomized clinical trial. Circulation 1998;97:562-568.
52 Ensenauer R, Puttmann M, Quintel M, Kattermann R, Aufenanger J: Comparison of serum phospholipase A2, polymorphonuclear granulocyte elastase, C-reactive protein and serum amyloid A with the APACHE II score in the prognosis of multiple injured patients. Clin Invest 1994;72:843-849.
53 Twumasi DY, Liener IE, Galdston M, Levytska V: Activation of human leukocyte elastase by human alpha-2-macroglobulin. Nature, 1977;267:61-63.
54 Kurdowska A, Travis J: Acute phase protein Stimulation by alpha-I-antichymotrypsin-cathepsin G complexes. Evidence for the involvement of interleukin-6. J Biol Chem 1990; 265:21023-21026.
55 Tanaka H, Shimazu T, Sugimoto H, Yoshioka T, Sugimoto T: A sensitive and specific assay for granulocyte elastase in inflammatory tissue fluid using *L*-pyroglutamyl-*L*-prolyl-*L*-valine-*p*-nitroanilide. Clin Chim Acta 1990;187:173-180.
56 Bangalore N, Travis J: Comparison of properties of membrane bound versus soluble forms of human leukocytic elastase and cathepsin G. Biol Chem Hoppe Seyler 1994;375:659-666.
57 Janoff A: Elastase in tissue injury. Annu Rev Med 1985 ;36:207-216.
58 Ambruso DR, Johnston RB: Lactoferrin enhances hydroxyl radical production by human neutrophils, neutrophil particulate fractions, and an enzymatic generating System. J Clin Invest 1981;67:352-360.