The effect of leukocyte reduction filters on inflammatory mediator release during coronary artery bypass grafting

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Abstract

Background: Extracorporeal circulation used during coronary artery bypass grafting triggers systemic inflammatory response with neutrophil activation which adversely affects ischaemic/reperfused myocardium. One method of myocardial protection during cardiac surgery is the use of blood cardioplegia. Its protective effect is related to cardiac cooling and metabolism reduction, oxygen supply from erythrocytes, and reactive oxygen species scavenging. However, blood cardioplegia is also associated with myocardial damage induced by undesirable morphotic blood elements.

Aim: To evaluate the effect of the use of leukocyte reduction filters on the activity of polymorphonuclear neutrophils (PMN) in patients undergoing surgical myocardial revascularisation. PMN activity was evaluated based on measurements of plasma activity of granulocyte enzymes, lysozyme and beta-glucuronidase.

Methods: We studied 40 patients who underwent myocardial revascularisation using extracorporeal circulation. Patients were randomly assigned to two equal groups: in Group I, blood cardioplegia was administered using leukocyte reduction filters, and in Group II, leukocyte reduction filters were not used for blood cardioplegia. Measurements were performed in plasma of arterial and coronary sinus blood samples collected before aortic clamping, immediately after unclamping, and after 25 min of reperfusion. In addition, blood cardioplegic solution samples were collected in Group I from the lines proximal and distal to the filter during first and last administration. Plasma levels of lysozyme and beta-glucuronidase were determined using previously described methods.

Results: We found a significant decrease in PMN count in filtered blood cardioplegic solution during its first administration (0.27 ± 0.07 G/L) compared to samples collected before filter passage (1.73 ± 0.049 G/L). Also during last administration, PMN count in filtered blood cardioplegic solution was decreased compared to samples collected before filter passage (0.66 ± 0.35 G/L vs. 3.64 ± 1.14 G/L, respectively). Significantly lower (p < 0.02) plasma beta-glucuronidase levels were found in arterial blood samples in Group I compared to Group II (5.59 ± 1.63 µg/mL immediately after aortic unclamping and 6.59 ± 1.98 µg/mL after 25 min of reperfusion in Group I vs. 10.19 ± 2.66 and 12.83 ± 1.88 µg/mL, respectively, in Group II). Beta-glucuronidase levels in coronary sinus blood samples collected after aortic unclamping and at the end of reperfusion were significantly higher in Group II compared to Group I (p < 0.04). In Group I, plasma lysozyme levels in arterial and venous blood samples did not show significant changes during the surgery. In contrast, plasma lysozyme level in coronary sinus blood samples at the end of reperfusion in Group II was significantly higher compared to that in pre-clamping samples (p < 0.014).

Conclusions: With the use of leukocyte reduction filters, we found significantly lower beta-glucuronidase levels in arterial and coronary sinus blood samples. These findings seem to confirm reduced PMN activation and/or reduced myocardial infiltration by activated PMN. Plasma levels of lysozyme, a characteristic product of PMN degranulation, did not show significant differences between the study groups.

Key words: ischaemia/reperfusion, coronary artery bypass grafting, myocardial protection

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INTRODUCTION
Despite rapid advances in minimally invasive techniques, extracorporeal circulation (ECC) has remained a safe method allowing cardiac surgery for more than 50 years. In addition, the use of cardioplegia provides still and clear operating field, allowing precise surgical manoeuvres. However, cardiac arrest during surgery is associated with temporary myocardial ischaemia and triggers inflammatory response involving polymophonuclear neutrophils (PMN) [1]. Adverse effects of PMN include leukocyte aggregation and their adhesion to endothelial cells, which may result in occluded microcirculation and release of reactive oxygen species and enzymes that exert a direct toxic effect on the myocardium [1]. Previous studies also showed myocardial PMN degranulation triggered by ischaemia and reperfusion [2].

The most commonly used approach for myocardial cytoprotection during total ischaemia is the use of cardioplegia. Intracoronary infusion of a cardioplegic solution results in cardiac arrest, and together with cardiac cooling this leads to largely reduced myocardial energy requirements. Comparative studies by Guru et al. [3] and Braathen and Tønnesen [4] showed an advantage of blood cardioplegia, in which blood is used as a carrier of a high potassium load, over a crystalline cardioplegic solution in patients undergoing cardiac surgery. In addition, blood cardioplegic solution has natural reactive oxygen species scavenging properties [5]. However, blood cardioplegic solution contains not only red blood cells which serve as oxygen carriers but also platelets and PMN which may contribute to post-reperfusion myocardial damage. Thus, it seems reasonable to remove from the cardioplegic solution these cellular elements that may increase myocardial damage during ischaemia and reperfusion. One proposed approach is to provide the cardioplegic solution infusion line with leukocyte reduction filters that remove leukocytes, including activated PMN. In a study by Suzuki et al. [6], a significant reduction in cardiac troponin T (TnT) and creatine kinase-MB (CK-MB) was found in patients in whom blood cardioplegic solution was administered through leukocyte reduction filters.

The aim of the present study was to evaluate the effect of PALL BC1B leukocyte reduction filters on the composition of blood cardioplegic solution administered with the use of these filters, and the activity of enzymes released by activated PMN.

METHODS
We studied 40 patients (12 women, 28 men) aged 48 to 68 (mean 56.2) years who underwent surgical myocardial revascularisation using ECC. Surgical revascularisation was performed in patients with 3-vessel disease. Exclusion criteria included myocardial infarction within 3 months before the surgery, diabetes, cancer, and renal failure. All patients were operated under ECC using the same type of oxygenator, Dideco 703 (Dideco, Mirandola, Italy), in both groups. Duration of ECC ranged from 43 to 78 (mean 62) min, and duration of aortic clamping ranged from 18 to 42 (mean 33) min, with no significant differences between groups. Buckberg cardioplegic solution was used to induce cardiac arrest.

Patients were randomly assigned to two groups of 20 patients each: in Group I, blood cardioplegia was administered using PALL BC1B leukocyte reduction filters, and in Group II, leukocyte reduction filters were not used for blood cardioplegia. Anaesthetic and surgical technique did not differ between the two groups. After ECC was initiated, a cannula (ATC 0 11V, Research Medical, USA) for administration of cardioplegic solution was inserted into the aorta, and another cannula (RC 014, Research Medical) to allow blood sampling was inserted into the coronary sinus. When individually calculated target cardiac output was reached, aorta was cross-clamped and cardiac arrest was induced by administering blood cardioplegic solution. Initially, cold cardioplegic solution (6–12°C) was administered at 200–300 mL/min for about 4 min, followed by 2-min infusions at 200 mL/min every 20 min. The last dose of 450 mL of low-potassium cardioplegic solution was administered for about 3 min.

We evaluated leukocyte content in the cardioplegic solution and the activity of enzymes released from activated PMN, lysozyme and beta-glucuronidase. We also compared plasma activity of these enzymes during coronary artery bypass grafting (CABG) in patients in whom leukocyte reduction filters were used for administration of blood cardioplegic solution compared to those operated without the use of leukocyte reduction filters.

Plasma was obtained from arterial (A) blood and coronary sinus (S) samples collected directly before aortic clamping (A1 and S1, respectively), immediately after aortic unclamping (A2, S2) and after about 25 min of reperfusion, before discontinuation of ECC (A3, S3). In addition, samples of blood cardioplegic solution were collected directly from the infusion line proximally (F1) and distally (F2) to the filter during first and last (F3 and F4, respectively) administration of cardioplegic solution in those patients in whom leukocyte reduction filters were used. Blood and cardioplegic solution were centrifuged, and obtained plasma samples were stored at −70°C until assays.

Plasma beta-glucuronidase levels were determined using the method previously described by Gallin et al. [7], by reading phenol red extinction dilution based on an analytical curve. Results were expressed in µg/mL ± standard error of the mean (SEM). Plasma lysozyme levels were determined using the ELISA method previously described by Gallin et al. [7], by reading extinction dilution for the mixture of the analysed plasma sample and Micrococcus luteus suspension at 450 nm wavelength. Results were expressed in mg/mL as the mean value from 3 measurements ± SEM.

Due to non-normal distribution of the examined variables, as determined using the Kolmogorov-Smirnov test, nonparametric tests were used for comparisons. Differences
in the activity and level of the evaluated enzymes within groups were evaluated using the Friedman test, and the Wilcoxon test was used to compare the evaluated parameters between patients in whom blood cardioplegic solution was administered using leukocyte reduction filters and those in whom leukocyte reduction filters were not used.

RESULTS

Leukocyte count in cardioplegic solution
We found a significant decrease (p < 0.04) in PMN count in filtered blood cardioplegic solution during its first administration (0.27 ± 0.07 G/L) compared to samples collected at the same time before filter passage (1.73 ± 0.049 G/L). Also during last administration, PMN count in filtered blood cardioplegic solution was decreased compared to samples collected before filter passage (0.66 ± 0.35 G/L vs. 3.64 ± 1.14 G/L, respectively), despite an increase in PMN count in the cardioplegic solution compared to its first dose (Fig. 1).

Beta-glucuronidase levels
Significantly lower (p < 0.02) plasma beta-glucuronidase levels were found in arterial blood samples in Group I compared to Group II (5.59 ± 1.63 µg/mL immediately after aortic unclamping and 6.59 ± 1.98 µg/mL after 25 min of reperfusion in Group I vs. 10.19 ± 2.66 and 12.83 ± 1.88 µg/mL, respectively, in Group II) (Fig. 2). Plasma beta-glucuronidase levels in coronary sinus blood samples in Group I were as follows: S1: 6.27 ± 1.89 µg/mL; S2: 4.26 ± 1.14 µg/mL; S3: 4.96±1.58 µg/mL, and in Group II were as follows: S1: 8.55 ± 2.12 µg/mL; S2: 5.93 ± 1.46 µg/mL; S3: 7.89 ± 1.54 µg/mL (Fig. 3). No significant difference in plasma beta-glucuronidase level in S1 samples was found between the two groups. In contrast, beta-glucuronidase levels in coronary sinus blood samples collected after aortic unclamping and at the end of reperfusion were significantly higher in Group II compared to Group I (p < 0.04). In addition, plasma beta-glucuronidase levels showed a downward trend during
the surgery in Group I but these changes were not significant. Beta-glucuronidase levels in S2 and A2 samples and in S3 and A3 samples did not show any significant differences.

**Lysozyme levels**

In Group I, plasma lysozyme levels in arterial blood samples did not show significant changes during the surgery and were as follows: A1: 3.6 ± 0.36 mg/mL; A2: 4.26 ± 0.28 mg/mL; A3: 4.28 ± 0.4 mg/mL (Fig. 4). Lysozyme levels in coronary sinus blood samples also did not change significantly during the surgery and were as follows: S1: 4.46 ± 0.2 mg/mL; S2: 3.95 ± 0.32 mg/mL; S3: 4.46 ± 0.34 mg/mL (Fig. 5).

In Group II, plasma lysozyme levels in arterial blood samples did not change significantly during the surgery and were as follows: A1: 3.6 ± 0.23 mg/mL; A2: 3.84 ± 0.26 mg/mL; A3: 3.90 ± 0.3 mg/mL (Fig. 4). In contrast, plasma lysozyme level in coronary sinus blood samples at the end of reperfusion in Group II was significantly higher compared to that in pre-clamping samples (p < 0.014) (Fig. 5).

**DISCUSSION**

During cardiac surgery using ECC, a systemic inflammatory response is triggered which may contribute to many postoperative complications including bleeding, thrombosis, embolism, and temporary multiorgan failure [8–10]. Release of inflammatory mediators leads to PMN activation [11]. In the study by Farah et al. [12], two factors were identified that led to PMN activation during CABG under ECC after aortic clamping. The first of these factors is ECC itself, resulting in PMN activation due to their contact with artificial surfaces of the cannulae and tubing connecting the patient and the ECC system. The other factor is myocardial ischaemia followed by reperfusion after aortic unclamping. Although blood cardioplegic solution is the most physiologic oxygen carrier used for myocardial protection during cardiac surgery, containing many antioxidant substances, it is also a source of activated PMN. Blood present in the cardioplegic solution passes through the ECC system and thus it may be assumed that leukocytes present in blood are activated by direct contact with artificial surfaces of the blood reservoir and connecting tubes [11]. During cardiac surgery, the cardioplegic solution reaches the myocardium through a cannula that is usually placed in the aortic root or directly in the coronary artery ostia, and thus activated PMN may exert their potential adverse effects directly on the reperfused myocardium. Leukocyte reduction filters used for administering blood cardioplegic solution reduce its PMN content by more than 90%, as shown in our studies and by other authors [6]. Benefits of the use of leukocyte reduction filters were confirmed in clinical studies. Significantly reduced TnT [6] and CK-MB [13] levels were shown in patients administered a cardioplegic solution with a reduced PMN content, indicating less myocardial damage related to perioperative ischaemia and reperfusion. In the study by Palatinos and Balentine [14], a significant reduction in the rate of postoperative ischaemia, low cardiac output syndrome, and perioperative ischaemia was found in the leukocyte reduction filter group compared to patients in whom filters were not used. However, these data contrast with other studies showing higher CK-MB and CK levels in the non-filtered cardioplegic solution group only immediately after aortic unclamping but without significant differences seen 6 h after the surgery [15].
Based on histopathological examination of myocardial biopsy specimens, Sawa et al. [13] found a significant reduction in the number of PMN adhering to coronary capillary endothelium in patients in whom blood cardioplegic solution was administered through leukocyte reduction filters. With lower myocardial PMN infiltration with the use of filters, lower myocardial or coronary sinus blood levels of activation products of these cells may be expected.

Our other findings do not allow clear conclusions regarding the effect of leukocyte reduction filters on the level of enzymes released from PMN in patients undergoing CABG. We found significantly lower beta-glucuronidase levels in arterial and coronary sinus blood samples in the leukocyte reduction filter group, which seems to confirm reduced PMN activation and/or reduced myocardial infiltration by activated PMN. On the other hand, plasma levels of lysozyme, a characteristic product of PMN degranulation, did not show significant differences between the study groups, which may suggest that PMN contact with the filter membrane does not result in PMN activation and release of granulocytic enzymes. In addition, a reduction in granulocyte elastase level was reported in the literature [16] in patients administered cardioplegic solution through a leukocyte reduction filter compared to the unfiltered cardioplegic solution group, which also suggests decreased PMN activation with the use of filter.

CONCLUSIONS

Our findings clearly confirm that leukocyte reduction filters effectively decrease leukocyte count in blood cardioplegic solution, thus limiting the number of activated PMN that reach the myocardium. PMN adhesion to the filter does not result in significant activation of these cells, as evidenced by unchanged or even lower levels of enzymes released during degranulation of PMN. The ability to eliminate PMN from blood cardioplegic solution without concurrent activation of these cells may suggest an advantage of this form of cardio-protection.

Conflict of interest: none declared

References

Wpływ filtrów leukocytarnych na uwalnianie mediatorów zapalenia podczas operacji pomostowania aortalno-wieńcowego

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Streszczenie


Cel: Celem niniejszej pracy była ocena wpływu zastosowania filtrów leukocytarnych na aktywność garnulocytów obojętnych (PMN) u chorych poddanych chirurgicznej rewaskularyzacji mięśnia sercowego. Aktywność neutrofilów oceniono na podstawie pomiaru osoczowych aktywności enzymów granulocytarnych — lizozymu i beta-glukuronidazy.


 Wyniki: Stwierdzono istotny statystycznie spadek liczby leukocytów w kardiopleginie filtrowanej pobranej podczas pierwszego podania 0,27 ± 0,07 G/L w stosunku do kardiopleginy pobranej przed filtrem 1,73 ± 0,049 G/L. Również podczas ostatniego podania stwierdzono spadek liczby leukocytów w kardioplegnie po przejściu przez filtr, odpowiednio 0,66 ± 0,35 G/L i 3,64 ± 1,14 G/L. W grupie I zanotowano znacząco niższe osoczowe stężenia beta-glukuronidazy w porównaniu z grupą II. W osoczu krwi tętniczej pobranej bezpośrednio po odkleszczeniu aorty, stężenie beta-glukuronidazy wynosiło 5,59 ± 1,63 µg/ml, a w osoczu pobranym po 25 min reperfuzji 6,59 ± 1,98 µg/ml, co stanowiło wartości istotnie niższe (p < 0,02) niż w osoczu uzyskanym z krwi tętniczej pobranej w tym samym czasie u chorych, u których nie zastosowano filtrów leukocytarnych; analogicznie: 10,19 ± 2,66 i 12,83 ± 1,88 µg/ml. W grupie II stężenie beta-glukuronidazy w próbkach osocza z zatoki wieńcowej pobranych po odkleszczeniu aorty i na końcu reperfuzji było istotnie wyższe (p < 0,04) niż w grupie I. W próbkach z zatoki wieńcowej w grupie II stwierdzono znamienny statystycznie wzrost stężenia lizozymu (p < 0,014) pod koniec reperfuzji w porównaniu z próbami pobranymi przed zakleszczeniem aorty.

Wnioski: Stwierdzono znacząco niższe stężenia beta-glukuronidazy w osoczu żylnym, tętniczym i uzyskanym z krwi z zatoki wieńcowej. Wydaje się to potwierdzać fakt mniejszej aktywacji PMN i/lub zmniejszonej infiltracji mięśnia sercowego przez aktywowane granulocity. Osoczowe stężenia lizozymu, będącego charakterystycznym produktem degranulacji PMN, nie wykazywały istotnych różnic między gruppami.

Słowa kluczowe: niedokrwienie/reperfuzja, pomostowanie aortalno-wieńcowe, protekcja mięśnia sercowego

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