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Genetic predisposition in patients undergoing cardiopulmonary bypass surgery is associated with an increase of inflammatory cytokines ☆

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Abstract

Objective: Cardiopulmonary bypass (CPB) surgery induces a transient rise in pro-inflammatory cytokines typically released by activated monocytes. The E4 variant of apolipoprotein E is a recognized risk factor for atherosclerosis. It has recently been shown that apolipoprotein E affects monocyte functions in vitro and leads to higher levels of median lipoprotein (a) in humans. The aim of the study is to investigate if the E4 genetic variant of apolipoprotein E affects cytokine release after CPB surgery. **Methods**: 22 patients were operated on with standard coronary artery bypass grafting. Concentrations of interleukin 8 (IL-8) and tumor necrosis factor (TNF-α) were measured by automated Immulite immunoassay at regular intervals within 48 h after surgery. Total apparent cytokine outputs were calculated as area under the curve. Results are expressed as mean ± standard deviation and compared by unpaired *t*-test. **Results**: In the presented patient population 6 (27%) carried the E4 allele. Sixteen (63%) showed no E4 allele. Mean cross clamp time (CCT) was 56.2 ± 13.5 min versus 55.7 ± 12.1 min and CPB time was 91.8 ± 17.5 versus 93.5 ± 15.7 min. No statistical difference between E4-carriers and E4 non-carriers regarding CCT and CPB was observed. The total amount of IL-8 and TNF-α was higher in patients carrying the E4 genetic variant of apolipoprotein E in comparison to E4 non-carriers ($P < 0.08$, $P < 0.039$). **Conclusion**: The presence of the E4 allele is associated with increased release of IL-8 and TNF-α after CBP surgery. The preoperative determination of E4 in patients undergoing cardiac surgery may lead to additional perioperative measures for the treatment of an increased systemic inflammatory response.

Keywords: Cardiac surgery; Ischemia/reperfusion; Adhesion molecules; Cytokines; Apolipoprotein E

1. Introduction

While in 1989 13% of our patients admitted for myocardial revascularisation were 70–80 years old, 10 years later (in 1999) this age group represented 28% of our coronary artery bypass (CAB) population. From this shift towards older patients, one may expect that a standard CAB patient is much more prone to be multi-morbid, being on dialysis due to renal insufficiency, restricted to low level exercise due to decreased lung function and showing signs of a progressed general arteriosclerosis.

In order to optimise the anesthetic and surgical management in time the operative risk of a patient has to be individually determinate, to make the surgical procedure as safe as possible. Besides known risk factors for arteriosclerosis like hypertension, smoking, hypercholesterolemia, there may be some other factors responsible for the outcome of patients. The individual way a body is coping with situations such as low oxygen content (cardiac tissue under cardiac arrest), or a systemic infection, for example, might be even determined by polymorphic genes.

The apolipoprotein E (ApoE) is a 34 kDa glycosylated protein which plays a major role in the cholesterol metabolism as a transport protein and is known to be anti-atherogenic by redistribution of cholesterol from the arterial wall. The apolipoprotein E gene is polymorphic: there are three major alleles described in the literature (ε2, ε3 and ε4), which differ by single amino acid exchanges at residues 112 and 158 (ε2: Cys_{112} Cys_{158}; ε3: $Cys_{112}Arg_{158}$; ε4: $Arg_{112}Arg_{158}$) [1] resulting in six different genotypes (ε2/2, ε2/3, ε2/4, ε3/3, ε3/4 and ε4/4). The E4 variant of apolipoprotein E is recognized as a risk factor for atherosclerosis and correlates to the severity of coronary artery diseases [2–4]. This E4 protein increases the blood cholesterol concentration

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[5–8] which may lead to coronary artery wall lesions [9]. But might the Apo E4 protein even play a role in acute cardiac ischemia (e.g., during cardiac arrest intra-operatively)?

Cardiac ischemia leads to a release of pro-inflammatory cytokines (TNF-α, IL-6, IL-8) which may be a major cause for morbidity and mortality in the cardiac surgery patient population. Clinical and experimental studies revealed that pro-inflammatory cytokines may lead to myocardial dysfunction based on an increase of adhesion-molecules followed by invasion of leukocytes to the myocardium and an increase of myocardial apoptosis [10].

The aim of the presented study is to investigate if the E4 genetic variant of apolipoprotein E is associated with a higher pro-inflammatory cytokine (TNF-α, IL-6, IL-8) release after by cardiopulmonary bypass (CPB) surgery compared to the patient group without E4 genetic variants.

2. Material and methods

2.1. Patient groups

In a 6-month period from February to August 2000, 22 patients undergoing coronary artery bypass surgery were selected for the presented study. Informed consent was obtained from each patient according to the protocol of the Ethics committee of the University Hospital Zürich. Patients with known infection, with known neoplasm, previous coronary artery bypass surgery or inserted intra-aortic-balloon-pump were excluded from the study.

2.2. Operative techniques

Cardiopulmonary bypass was performed using a Stöckert roller pump system (Stöckert Intrumente GmbH Munich, Germany) and a Shiley–Dideco Maxima hollow fiber oxygenator (Dideco, Mirandola, Italy). Cold cristalloid Buckberg cardioplegia with an initial dose of 15 ml/kg was applied for myocardial protection. Repeated infusions of 300 ml were given every 20 min or earlier if electrical activity occurred. Before aortic declamping 500 ml warm blood cardioplegia (hot shot) was administered at 37°C for 2 min at a pressure of 50 mmHg. Using the heat-exchange oxygenator, warming blanket, and heated humidified gases to reach a rectal temperature of >34°C before terminating CPB, rewarming was achieved.

2.3. Blood sampling and analysis

For each patient 6 ml blood samples (lithium-heparinate plasma) were obtained through a central venous catheter at 12 different time points. The first sample was taken before anesthesia, the second 0.5 h after re-opening of coronary circulation (declamping the aorta), the third 1 h after re-opening of coronary circulation, the fourth 2 h after re-opening of coronary circulation, the fifth 4 h after re-opening of coronary circulation, the sixth 8 h after re-opening of coronary circulation, the seventh 16 h after re-opening of coronary circulation, the eighth 24 h after re-opening of coronary circulation, the ninth 32 h after re-opening of coronary circulation and the tenth 48 h after re-opening of coronary circulation. At the first time point an additional tube of ethylenediaminetetra acid (EDTA) blood was drawn for genotyping.

Concentration of IL-8 and TNF-α in lithium-heparin anticoagulated plasma was determined by automated enzyme-chemiluminescence immunoassay on an Immulite I system (Diagnostics Product Corporation, Los Angeles and Buehlmann Laboratories AG, Allschwil, Switzerland) according to the instructions of the manufacturer [11]. For genotyping of Apolipoprotein E, genomic DNA was extracted from 200 μl of EDTA-anticoagulated blood by the MagNA Pure LC and real time polymerase chain reaction (PCR) was performed on a LightCycler (both from Roche Diagnostics, Rotkreuz, Switzerland) with primers and hybridisation probes synthesized by TIB MolBiol (Berlin, Germany) according to Aslanidis et al. [12].

2.4. Clinical variables

Medical history and demographic data were collected prospectively, as well as the postoperative course including ICU stay, occurrence of myocardial infarction, arrhythmias, bleeding, intubation time, pulmonary infections requiring antibiotic treatment and length of hospital stay.

2.5. Statistical methods

Fischer's exact test and Student's *t*-test with mean and standard deviations were used for comparisons of percentages and means for normally distributed data. The Mann–Whitney test was applied otherwise. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Patients

Of the 22 patients enrolled in this study, six patients (27%) carried the E4 genetic variants of the apolipoprotein E (five heterozygous E3–E4, one homozygous E4–E4) 16 patients (73%) carried no E4 genetic variants. Comparison of the preoperative profile (age, sex, risk factors) of the E4-carrier group versus the E4-non-carrier group demonstrated no statistical differences (Table 1). The intra-operative management confers that in the E4- carrier versus E4-non-carrier CPB-time (93.5 ± 15.7 min versus 91.8 ± 17.5 min), aortic cross clamp time (55.7 ± 12.1 versus 56.2 ± 13.5 min), lowest body temperature on bypass (32.2 ± 2.7 versus 29.9 ± 4.5°C) and the number of performed distal anastomoses (4.8 ± 0,8 versus 4.1 ± 1.3) were not statistically significant different (Table 1).

The postoperative data demonstrate that the intensive

Table 1
Demographics, risk factors, coronary artery diagnosis, intraoperative data and outcome: E4 carriers vs. E4-non-carriers

	E4-non-carrier ($n = 16$) n (%)	E4-carrier ($n = 6$) n (%)	P value
Demographics			
Age	65 ± 8	63 ± 7	NS
Sex (male/female)	15/1	4/2	NS
Risk factors			
Diabetes type II	1 (6)	1 (17)	NS
Hypertension	9 (56)	3 (50)	NS
Hypercholesterolemia	15 (93)	6 (100)	NS
Smoker	10 (63)	4 (67)	NS
BMI	27.9 ± 3.4	25.4 ± 1.5	0.02
Family history	8 (50)	3 (50)	NS
COPD	3 (19)	0	NS
Coronary diagnosis			
3-KHK	10 (63)	5 (83)	NS
2-KHK	6 (38)	1(17)	NS
Myocardial infarct <7d	0	0	
Myocardial infarct >7d	7 (44)	2 (33)	NS
EF (%)	63.4 ± 11.7	67.8 ± 4.7	NS
Operation			
CPB time	91.8 ± 17.5	93.5 ± 15.7	NS
Ortic cross-clamp time	56.2 ± 13.5	55.7 ± 12.1	NS
Lowest temperature on bypass	29.9 ± 4.5	32.2 ± 2.7	NS
Number distal anastomosis	4.1 ± 1.3	4.8 ± 0.8	NS
Outcome			
ICU stay	1.6 ± 1.4	2.0 ± 1.7	NS
Hospital stay	8.6 ± 2.4	7.5 ± 1.2	NS
Infarct	0	0	
Arrhythmia			
Atrial	5 (31)	0	NS
Ventricular	2 (12)	0	NS
Block (RBBB; LBBB; AV-block)	5 (31)	3 (50)	NS
Pulmonary dysfunction	1 (6)	1(17)	NS
Rethoracotomy	1 (6)	1 (17)	NS

care unit and hospital stay did not differ between the groups. E4-carriers developed slightly more cardiac arrhythmias (right bundle branch block (RBBB); left bundle branch block (LBBB) and atrio-ventricular block (AV-block) than the E4-non-carrier (Table 1).

3.2. Cytokine release induced by cardiopulmonary bypass surgery

3.2.1. TNF-α release in E4-non-carriers versus E4-carriers

In E4-carriers a significant higher amount of TNF-α was already found at the first blood sampling (time −2: $P = 0.03$), before declamping the aorta (re-opening of the coronary circulation) (time 0: $P = 0.01$); 0.5 h ($P = 0.01$); 1 h ($P = 0.01$); 2 h ($P = 0.02$); 4 h ($P = 0.04$) and 24 h ($P = 0.001$) after declamping the aorta (Table 2). The highest levels of circulating TNF-α were found 4 h after re-opening of the coronary circulation in both groups; mean level was slightly higher in the E4-carrier group (17.1 ± 5.7 versus 11.5 ± 2.7) in the non-carrier group with no statistical significance. The total apparent cytokine output of TNF-α (area under curve) was significantly higher in patients carrying the E4 genetic variant of apolipoprotein E in comparison to E4-non-carriers (663 ± 306 pg/ml versus 361 ± 107 pg/ml; $P = 0.039$) (Table 2).

3.2.2. IL-8 release in E4-non-carriers versus E4-carriers

Highest levels of IL-8 were measured 4 h after reopening of the coronary circulation (Table 3) in both groups

Table 2
Expression of TNF-α in E4- and non- E4 carrier at different time points[a]

	TNF-α (pg/ml)		
Time (h)	E4-non-carrier ($n = 16$)	E4-carrier ($n = 6$)	P value
0	5.3 ± 2.0	8.6 ± 3.8	NS
0.5	6.4 ± 2.3	10.7 ± 2.6	0.01
1	8.4 ± 3.2	13.7 ± 3.2	0.01
2	10.4 ± 3.9	16.2 ± 4.9	0.02
4	11.5 ± 2.7	17.2 ± 5.8	0.04
8	9.5 ± 3.8	14.4 ± 6.4	NS
16	7.9 ± 4.1	11.5 ± 6.6	NS
24	6.4 ± 2.3	16.7 ± 4.4	0.001
32	6.7 ± 2.6	13.2 ± 8.7	NS
48	6.5 ± 2.8	12.3 ± 10.7	NS
Total	361 ± 107	663 ± 306	0.039

[a] Total apparent release of TNF-α was calculated as area under curve (AUC), results are expressed as mean ± standard deviation.

Table 3
Expression of IL-8 in E4- and non-E4-carrier at different time points[a]

Time (h)	IL-8 (pg/ml)		P value
	E4-non-carrier ($n = 16$)	E4-carrier ($n = 6$)	
0	6.6 ± 4.2	8.3 ± 5.6	NS
0.5	14.0 ± 11.9	16.7 ± 14.5	NS
1	14.4 ± 9.0	20.5 ± 15.4	NS
2	19.1 ± 11.4	30.8 ± 11.6	0.04
4	27.8 ± 14.1	35.3 ± 13.0	NS
8	18.7 ± 13.4	29.5 ± 11.5	NS
16	12.0 ± 9.8	17.5 ± 12.9	NS
24	10.7 ± 3.5	20.5 ± 21.5	NS
32	7.6 ± 3.0	13.2 ± 4.4	0.02
48	8.1 ± 2.6	7.3 ± 3.0	NS
Total	876 ± 400	589 ± 201	0.088

[a] Total apparent release of IL-8 within 48 h was calculated as area under curve (AUC); results are expressed as mean ± standard deviation.

(35.3 ± 13.0) in the E4-carrier group and 27.8 ± 14.1 in the E4-non-carrier group. IL-8 levels during the operation and up to 32 h after declamping the aorta were always higher in the E4-carriers, with statistical significance at time-point 2 h ($P = 0.04$) and 32 h ($P = 0.02$). The total apparent cytokine output of IL-8 (area under curve) was higher in patients carrying the E4 genetic variant of apolipoprotein E in comparison to E4-non-carrier (876 ± 400 pg/ml versus 589 ± 201 pg/ml; $P = 0.088$) (Table 3).

4. Discussion

Patients admitted for a standard CAB surgery are nowadays much more multi-morbid than 10 years ago. Improved anesthetic and surgical management have let the surgeons accept patients who would have been rejected due to a high operative risk in regard to their age and end organ function 10 years ago. To further lower the individual operative risk of patients, new factors have to be determined which might influence the postoperative course. We believe that polymorphic genes have the potential to play a role in the individual response to stress situations like an oxygen shortage.

We examined quantitatively whether the E4 variant of apolipoprotein E is connected with the release of pro-inflammatory cytokines during and after CPB surgery.

In the presented study, 27% patients possessed the apo E4 genotype. Inbal et al. [13] found an Apo E ε4 allele frequency of 9.4% in young males with an acute myocardial infarction versus 5.3% in the control group. In this study patients with Apo E4 polymorphism had a nine-fold increased estimated risk to develop an acute myocardial infarction. This risk increased up to 18-fold, if the patient was in addition a current smoker. Katzel et al. [14] demonstrate, that healthy men with the Apo E4 allele were at increased risk for exercise-induced silent ischemia.

Our results suggest that Apo E4 carriers show significant

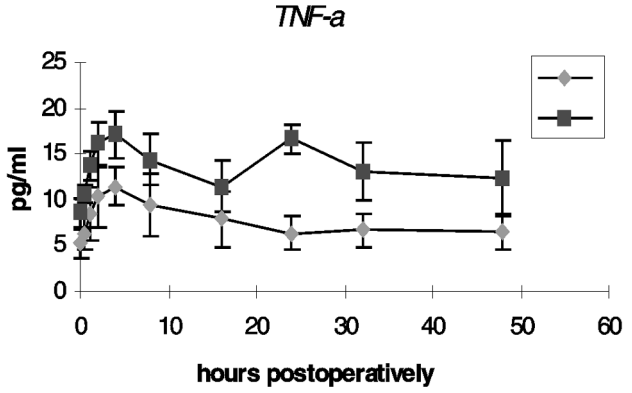

Fig. 1. Means of TNF-α at different time points (green rectangle, E4-carrier; red rhombus, E4-non-carrier).

higher TNF-α levels during and after CAB surgery than non-carriers (Fig. 1). Thus apolipoprotein E4 may regulate the production and/or release of TNF-α at the DNA or a receptor/second-messenger level.

In an isolated rat heart model ischemia induced local release of TNF-α leads to a depression in myocardial contractility and coronary flow as well as to an increase in end-diastolic pressure and creatine kinase production [10], therefore E4 carrier might be at a higher risk to develop a more severe myocardial dysfunction during coronary artery bypass surgery.

It cannot, however, be excluded that the effect is caused by another gene polymorphism in linkage disequilibria with the Apo E4 genetic variant. In any case, our results warrant confirmation in a larger study.

In addition our patients with the Apo E4 allele had a higher level of IL-8 during and up to 32 h after cardiac surgery (Fig. 2). However, the aortic cross clamp and the CPB time did not differ between groups, we suggest that Apo E4 carriers might be more prone to myocardial cell damage than the E4-non-carriers, or Apo E4 directly or

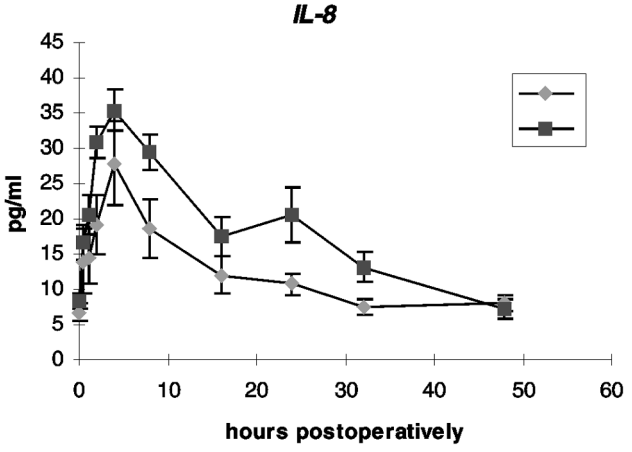

Fig. 2. Means of IL-8 at different time points (green rectangle, E4-carrier; red rhombus, E4-non-carrier).

indirectly stimulates IL-8 release unrelated to the extent of injury.

While IL-8 also works as a potent activator of neutrophils as well as of T-lymphocytes [15] when released from the myocardium during ischemia, it is known that Apolipoprotein E also has the ability to suppress lymphocyte proliferation, generate cytolytic T-cells and stimulate cultured neutrophils [1], effects could be exponentiated. De Bont and co-workers also showed that Apo E −/− mice are more susceptible than control C57BL/6 mice to a *Klebsiella pneumoniae* infection. The absence of Apo E may render these mice more susceptible, since this protein is of importance in the detoxification of lipopolysaccharide of Gram-negative bacteria. On the other hand, the phagocytic capacity of granulocytes also seems to be decreased in Apo E −/− mice, resulting in increased mortality [16].

In conclusion patients carrying the apolipoprotein E4 allele variant can be identified with a simple blood genotyping test, and the anesthetic and surgical management may be individually changed in time. While we demonstrated that these patients are prone to a higher TNF-α release during and after CPB surgery, we suggest to consider additional perioperative measures for the treatment of the increased systemic inflammatory response, i.e. modified ultrafiltration (MUF), since Grünenfelder et al. [17] could show that circulating cytokines like TNF-α and IL-8 can be filtered out of the circulation by using modified ultrafiltration. Perioperative steroid application has also shown to lower cytokine release after cardiopulmonary bypass [18]. The surgical management has to be discussed once these results have been confirmed in a larger patient population.

References

[1] Weisgraber KH. Apolipoprotein E: structure-function relationships. Adv Protein Chem 1994;45:249–302.

[2] Laskowitz DT, Lee DM, Schmechel D, Staats HF. Altered immune responses in apolipoprotein E-deficient mice. J Lipid Res 2000;41:613–620.

[3] Lindahl G, Mailly F, Humphries S, Seed M. Apolipoprotein E phenotype and lipoprotein(a) in familial hypercholesterolaemia: implication for lipoprotein(a) metabolism. Clin Investig 1994;72:631–638.

[4] Arras M, Hoche A, Bohle R, Eckert P, Riedel W, Schaper J. Tumor necrosis factor-alpha in macrophages of heart, liver, kidney, and in the pituitary gland. Cell Tissue Res 1996;285:39–49.

[5] Sing CF, Davignon J. Role of the apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. Am J Hum Genet 1985;37:268–285.

[6] Ehnholm C, Lukka M, Kuusi T, Nikkila E, Utermann G. Apolipoprotein E polymorphism in the Finnish population: gene frequencies and relation to lipoprotein concentrations. J Lipid Res 1986;27:227–235.

[7] Lehtimaki T, Moilanen T, Viikari J, Akerblom HK, Ehnholm C, Ronnemaa T, Marniemi J, Dahlen G, Nikkari T. Apolipoprotein E phenotypes in Finnish youths: a cross-sectional and 6-year follow-up study. J Lipid Res 1990;31:487–495.

[8] Dallongeville J, Lussier-Cacan S, Davignon J. Modulation of plasma triglyceride levels by apoE phenotype: a meta-analysis. J Lipid Res 1992;33:447–454.

[9] Stary HC. The histological classification of atherosclerotic lesions in human coronary arteries. In: Fuster V, Ross R, Topol EJ, editors. Atherosclerosis and coronary artery disease, Philadelphia, PA: Lippincott-Raven Publishers, 1996. pp. 463–474.

[10] Meldrum DR, Cleveland Jr JC, Cain BS, Meng X, Harken AH. Increased myocardial tumor necrosis factor-alpha in a crystalloid-perfused model of cardiac ischemia-reperfusion injury. Ann Thorac Surg 1998;65:439–443.

[11] Berthier F, Lambert C, Genin C, Bienvenu J. Evaluation of an automated immunoassay method for cytokine measurement using the Immulite immunoassay system. Clin Chem Lab Med 1999;37:593–599.

[12] Aslanidis C, Schmitz G. High-speed apolipoprotein E genotyping and apolipoprotein B3500 mutation detection using real-time fluorescence PCR and melting curves. Clin Chem 1999;45:1094–1097.

[13] Inbal A, Freimark D, Modan B, Chetrit A, Matetzky S, Rosenberg N, Dardik R, Baron Z, Seligsohn U. Synergistic effects of prothrombotic polymorphisms and atherogenic factors on the risk of myocardial infarction in young males. Blood 1999;93:2186–2190.

[14] Katzel LI, Fleg JL, Paidi M, Ragoobarsingh N, Goldberg AP. ApoE4 polymorphism increases the risk for exercise-induced silent myocardial ischemia in older men. Arterioscler Thromb 1993;13:1495–1500.

[15] Wan S, Leclerc JL, Vincent JL. Cytokines responses to cardiopulmonary bypass: lessons learned from cardiac transplantation. Ann Thorac Surg 1997;63:269–276.

[16] de Bont N, Netea MG, Demacker PN, Kullberg BJ, van der Meer JW, Stalenhoef AF. Apolipoprotein E-deficient mice have an impaired immune response to *Klebsiella pneumoniae*. Eur J Clin Invest 2000;30:818–822.

[17] Grünenfelder J, Zund G, Schoeberlein A, Maly FE, Schurr U, Guntli S, Fischer K, Turina M. Modified ultrafiltration lowers adhesion molecule and cytokine levels after cardiopulmonary bypass without clinical relevance in adults. Eur J Cardio-thorac Surg 2000;17:77–83.

[18] Yilmaz M, Ener S, Akalin H, Sagdic K, Serdar OA, Cengiz M. Effect of low-dose methyl prednisolone on serum cytokine levels following extracorporeal circulation. Perfusion 1999;14:201–206.