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Influence of Pressure on the Endothelium of the Saphenous Vein Coronary Artery Bypass Graft

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Abstract. *The aim of this study was to evaluate the influence of pressure applied while assessing the graft's tightness on the expression of adhesion molecules. Another goal was to find a correlation between the type of fluid (heparynized blood or saline) used during preparation of the conduit and the expression of the adhesion molecules. Saphenous vein fragments were obtained from 48 patients who had undergone coronary artery surgery. Expression of the following particles was evaluated: CD 31, ICAM 1, VCAM 1 and P-selectin. Expression of the CD 31 molecule was described as a percentage of the inner surface of the vessel, showing positive immunocytochemical reaction. Expression of the remaining molecules (ICAM 1, VCAM 1, P-selectin) was assessed as the percentage of the surface, determined by CD 31 positive reaction. The expression of the adhesion molecules (ICAM 1, VCAM 1, P-selectin) was higher in the fragments of the vein exposed to pressure. In reference to VCAM 1 the difference, as compared with the control group, was: 250% in the fragments infused with blood and 270% in the fragments infused with saline, respectively. The differences for the ICAM 1 were approximately 300% in both experimental groups and 450% for the P-selectin with subtle differences between the two experimental groups. The loss of the endothelial surface (determined by the expression of the CD 31 antigen) was similar in the specimens flushed either with blood or saline, which indicates that the major cause of damage of the endothelium is influence of pressure on the conduit's wall. Mechanical widening of vessels results in the increased expression of the adhesion molecules on the surface of the endothelial cells, and, as a consequence, leads to rise in the leukocyte adhesion and loss of the functional properties of the transplanted veins.*

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Current progress in the treatment of coronary artery disease is mainly attributed to the surgical grafting of the affected coronaries. Among others, the most popular conduit remains the saphenous vein, especially due to its length on both lower extremities and simplicity of surgical harvesting. Although the saphenous vein is autologous material, in a relatively short postoperative time it displays lower mechanical resistance and ability to transport blood than arterial conduits. Veins are characterised with loss of vasomotoric properties and are more prone to atheromatic lesions and thrombosis. Damage of the vascular endothelium seems to be the primary cause of these changes (1). Mechanical damage of the endothelial surface activates the intrinsic and external coagualation pathway. Subsequently, aggregation of platelets makes various integrins and cellular adhesion molecules (CAMS) facilitate the migration of the leukocytes through the endothelial barrier and evokes local inflammatory responses (2). In a healthy organism the basic expression of CAMS remains quite low, while it increases under the influence of certain conditions, like high levels of cytokines, injuries, mechanical stress or chemical substances (3).

Preservation of the intact endothelium while harvesting is vital for inhibition of pathological processes (4). Injury of the endothelium occurs most often during surgical harvesting of the saphenous vein, storage in physiological fluids immediately before grafting of the coronary targets and while flushing the conduit with fluids in order to asses the graft's tightness. Commonly the conduits are infused with heparinized blood or normal saline under pressure to reveal any side branches which need to be tied before the vein can be used as a graft (5).

In our study, we assessed the influence of pressure on the expression of the adhesion molecules ICAM 1, VCAM 1, PECAM and P-selectin on endothelial cells. We also made an effort to investigate whether the type of fluid used to assess the graft's tightness (normal saline or heparinized blood) affects the expression of the above mentioned particles.

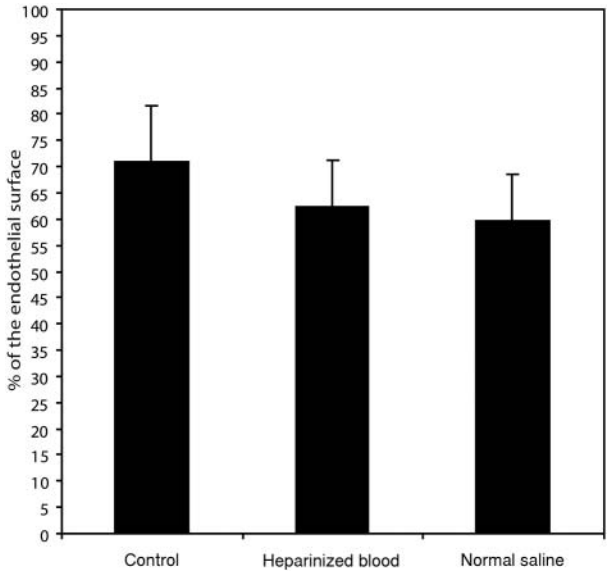

Figure 1. *Surface expression of the antigen CD 31. Control group: vessels not exposed to pressure. Heparinized blood: vessels exposed to a pressure of 300 mm Hg with the use of heparinized blood. Normal saline: the pressure applied was similar, but normal saline was used instead of blood.*

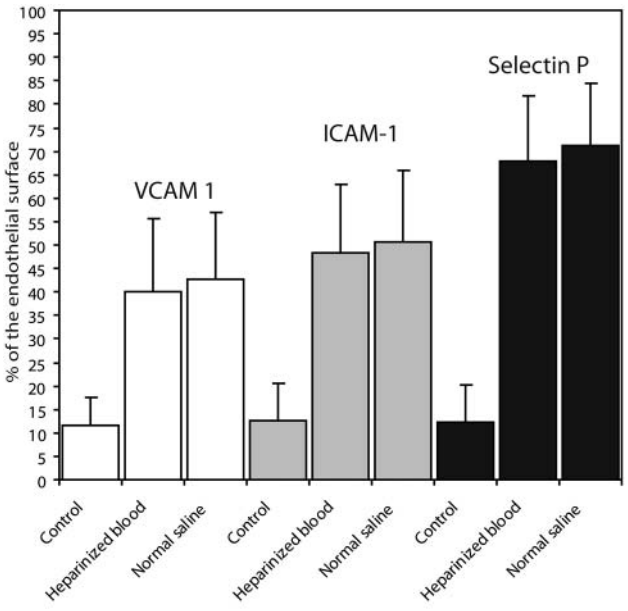

Figure 2. *Percentage values determining the expression surface of the adhesion molecules. VCAM 1, ICAM 1, P-selectin expression in the endothelium of the saphenous vein fragments either in the control group, or in the vessels exposed to pressure of 300 mm Hg for two minutes.*

Patients and Methods

Fragments of the saphenous vein were obtained from 48 patients who had undergone coronary artery grafting. The group consisted of 42 men and 6 women with a mean age of 66±7 years. Surgical procedures were performed in the Cardiac Surgery Department of the Medical University of Wroclaw, Poland. The usual length of the venous fragment was about 4 cm. These fragments remained intact either because the length of bypass required correction or because we encountered some technical difficulties which made grafting of a target vessel impossible. A written consent from each patient and approval of the Bioethics Committee were obtained.

Fragments of vessels were first flushed with buffered saline in order to remove residual blood, and then the vein was cut into three fragments of the same length. The first fragment was treated as a control, and the two remaining were exposed to infusion, of blood or normal saline, under a pressure of 300 mm Hg for two minutes. A manometer attached to the syringe to control the pressure was applied. One of the fragments was flushed with normal saline, and the second with heparinized patient's blood. All three fragments of the vein were then incubated for 60 min at room temperature, having been immersed in the normal saline or heparinized patient's blood. Incubation time corresponded to real storage time of the vein before being used as a graft under normal conditions.

After incubation, venous fragments belonging to all three groups were flushed with buffered solution of normal saline and subsequently immersed in the OTC medium and frozen at a temperature of –40˚C. The fragments were then cut into sections of 8 μm-thick each, with the use of a freezing microtome (Criostat Tissue Tek, USA). The sections were then attached to the slides, dried and fixed with a cold mixture of acetone and absolute alcohol (1:1) for 15 min. Finally, the hydrated specimens underwent routine immunocytochemical reactions, with the use of specific primary antibodies which were visualized with the EnVision™ system according to the manufacturer's protocol (Dakocytomation, Denmark).

Incubation with the primary antibodies (Dakocytomation, Denmark) ICAM-1 (dilution 1:50), VCAM-1 (1:250), PECAM-1 (1:250) and P-selectin (1:100) was performed at room temperature for 30 min. In order to provide the negative control for the immunocytochemical reactions incubation was performed with buffered saline instead of the prime antibodies.

Expression of CD 31 (PECAM-1) was assessed microscopically, as a percentage of the endothelial surface showing a positive immunocytochemical reaction. Expression of the remaining molecules (ICAM-1, VCAM-1, P-selectin) was estimated as a percentage of the endothelial surface, determined by the reaction with CD 31 (6).

Statistical analysis of the obtained data was conducted with the use of multifactorial analysis of variance (ANOVA). Differences were recognized as statistically significant at the level of probability (p) lower than 0.05. Calculations were performed with the use of Statistica software (Statsoft, Poland).

Results

Expression of CD 31 (PECAM-1). The inner walls of all venous fragments (n=48) were covered with endothelium showing a positive CD 31 reaction. The percentage of stained endothelium differed between the control and experimental groups. In both the control and the

experimental groups there were regions of endothelium which showed no reaction.

In the vessels of the control group the endothelial surface showing positive reaction for the CD 31 antigen (Figure 1) was estimated as 70.94% (SD=10.55), whereas in the vessels exposed to pressure with the use of heparinized blood it was 62.29%. In the vein fragments flushed with normal saline this surface was calculated as 59.69% (SD=8.78) (Anova F=4.613, p=0.0002).

Expression of VCAM-1. Immunoreactive of the surface of endothelium in most cases was not continuous and showed different intensities.

The surface expression for the VCAM-1 molecule (calculated as the percentage of the surface of expression for CD 31 in the endothelial cells) was as follows: for the control group 11.51% (SD=6.02), for the vessels exposed to pressure with the use of the heparinized blood 40.23% (SD=15.26), and for the vessels flushed with normal saline 42.71% (SD=14.25) (Anova F=8.058, p=0.0000) (Figure 2).

Expression of ICAM-1. The majority of vessels used in our research showed an expression of ICAM-1 in the endothelial cells. The degree of staining of the endothelial surface differed between the groups and also within each group. The most significant differences were observed between the control group and remaining two experimental groups (Figure 2).

The mean value of the stained endothelial surface for the control group was 12.60% (SD=7.92), and for the experimental groups 48.42% (the blood group), (SD=14.47) and 50.63% (the normal saline group), (SD=15.22), respectively. (Anova F=2.298, p=0.0258).

Expression of the P-selectin. The extent of the endothelial surface showing positive P-selectin expression differed between the study groups. The most significant differences were observed between the control and the experimental groups (Figure 3).

The percentage values of the stained endothelial surface showing positive expression of the P-selectin were as follows: control group 12.53% (SD=7.98), for the vessels filled with blood under pressure of 300 mm Hg 67.81% (SD=13.91) and for the veins flushed with normal saline 71.25% (SD=13.27) of the endothelial surface (Anova F=2.428, p=0.024) (Figure 2).

Discussion

The results obtained in the course of the research showed that the expression of the adhesion molecules on the endothelial surface in the saphenous vein fragments differed significantly, except for the expression of the CD 31 molecule (PECAM). This molecule is highly specific for endothelial cells and has a constant expression, modified only to a minimal extent by intracellular mechanisms and external factors (6).

In the vein fragments which were not exposed to high pressure the area of endothelium showing positive reaction for CD 31 constituted 70% of the entire endothelial surface; in the experimental groups this area was approximately 10% lower (see Results). Comparing the data, we assume that the lower expression of the CD 31 in the experimental groups was caused by loss or damage of the endothelial cells due to the influence of high pressure. Taking into account that the inner surface of the native vessels is entirely covered by an endothelial layer, our results indicate that in the course of the graft preparation nearly 40% of the endothelial surface is lost. The loss of endothelium seems to be a primary cause of various pathological processes, such as local inflammatory states, clot formation and formation of the atheromatic plaques (7).

According to previous reports of incomplete endothelial cell reaction for CD 31 (6), the loss of endothelial surface could be even greater than indicated by our results, as in our research only the extent of damage of the endothelial cells with positive immunocytochemical reaction was assessed.

In studies on pig arteriovenous bypass grafts which evaluated changes in the endothelial layer exposed to a pressure of 300 mm Hg, similar to our conditions, the loss of the endothelial surface was estimated to be 20 to 33%, which is consistent with our results (8).

Despite the mechanical stress, there is a variety of factors which may lead to endothelial damage. Little *et al.* (9) revealed that vessels infused with acidic fluids (ph<7) were more prone to endothelial damage than the vessels flushed with basic or neutral solutions. Studies performed by O'Connell *et al.* (10) showed that severe loss of the endothelial layer was caused by flushing the veins with saline, whereas the use of autologous plasma resulted in very subtle changes. Similar results were obtained by other scientists who used heparinized blood (11).

The extent of the endothelial damage was similar in the vessels exposed to heparinized blood or normal saline, which indicates that the primary cause of the ongoing rearrangements is the mechanical activity of pressure. Our estimation indicates that saline and heparinized blood infused at a pressure of 300 mm Hg show similar destructive properties.

Interactions between the endothelium and leukocytes lead to a variety of pathological processes in the vessels. The presence of the CAMs on the cell surface enables leukocyte binding and their migration through the vessel walls. In the control group of vessels the expression of the VCAM-1 on the cellular surface was scarce or even absent. In contrast, vein fragments from both experimental groups showed

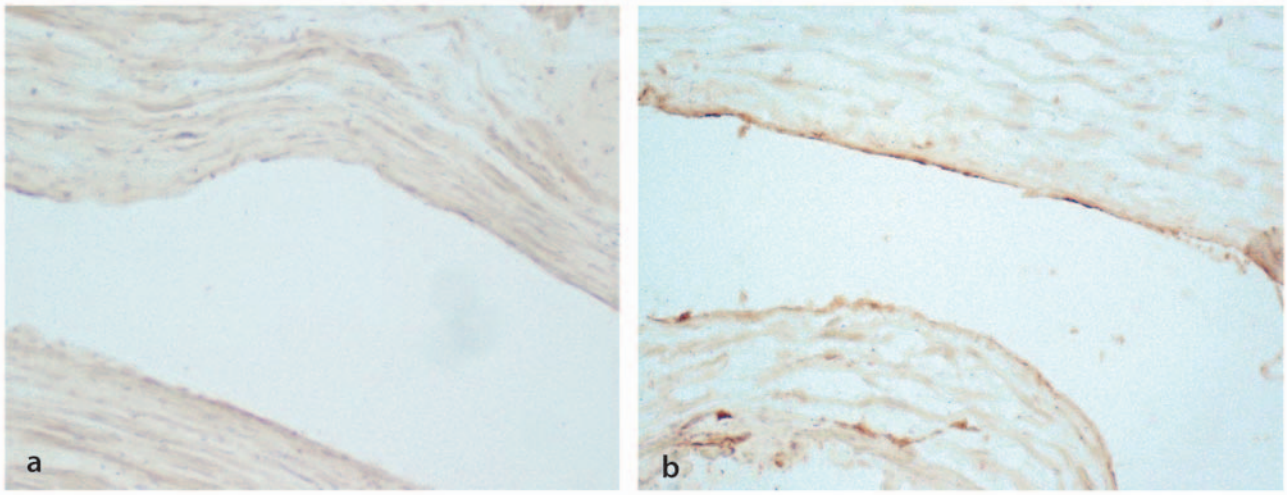

Figure 3. *Immunocytochemical location of the expression of the P-selectin. a) No reaction product in the endothelial cells of the control group. b) Significant reaction in the endothelium of the vessels exposed to a pressure of 300 mm Hg, with the use of heparinized blood. 200 x magnification.*

significantly higher VCAM-1 expression, regardless of the type of fluid used for infusion. The increase in expression of VCAM 1 in the vessels infused either with saline or with blood was nearly two-fold greater compared to the control group. Similar results were obtained for ICAM 1. More signifficant changes were observed in reference to expression of P-selectin, which was three-fold higher in the experimental groups compared to the control group.

Saphenous vein fragments, which are used as grafts in coronary surgery, are exposed to a variety of stimuli which enhance the expression of the adhesion molecules. These are, among others, activity of various types of cytokines released after the procedure, increase of the vessel tone due to exposure to high blood pressure and the loss of the inner surface integrity as a result of previous mechanical injuries (12).

The influence of mechanical stimulation on expression of the adhesion molecules has been reported in numerous articles. Chappel *et al.* (13) revealed that oscillatory flow of fluid in an *in vitro* model evoked an increase in the expression of the adhesion molecules in the endothelial cells of the rat umbilical cord. The authors suggested that the endothelium *in vivo* showed an increase in the expression of VCAM, ICAM and selectins due to exposure to oscillatory flow. Another study by Golledge *et al.* (14) displayed that in a saphenous vein model exposed to arterial type of flow *in vitro* the rise in the expression of the ICAM-1 was two-fold higher as compared with the vessels stabilized with an external stent.

Taking into consideration that there were no significant differences between the extent of adhesion molecule expressions between our experimental groups, we suggest that the major factor responsible for the increase of the adhesion molecules is the mechanical influence of pressure applied to the endothelial surface during the assessment of the conduit's tightness. Moreover, heparinized blood used to inflate the vein fragments, consists of numerous cytokines and inflammatory mediators, which should affect the expression of the adhesion molecules. Nevertheless, endothelial cells themselves control the transfer of various substances and cell types in the intracellular spaces.

Endothelial cells take active part in the process of leukocytes extravasation (15). Recent studies indicate that endothelial cells not only form a kind of barrier but also receive a number of mechanical signals and transmit them into the cells, which results in modifying the expression profile of various molecules, including adhesion molecules (16). Intracellular mechanisms leading to increased expression and release of various molecules in response to mechanical signals have not been clearly explained and deserve further studies.

It is worth mentioning the differences obtained between the expression of VCAM-1 and ICAM-1 on one hand, and P-selectin on the other. In our studies, the endothelial surface with P-selectin positive reaction was almost two-fold larger than the area of endothelium showing an expression of VCAM-1 and ICAM-1. The results reflect different strategies of mobilization of VCAM-1, ICAM-1 and P-selectin. The rise in the VCAM-1 and ICAM-1 expression is caused by initiation of transcription and synthesis of the molecules *de novo*, as a response to cytokine release and mechanical or chemical stimuli. Increased expression of P-selectin is a result of mobilization of the already existing molecules stored in the Wiebel-Palade's bodies in the

cytoplasm. Activation of cells leads to immediate translocation of the molecule, and subsequently, adhesion of the leukocytes (17). Incubation time (60 min) was supposed to correspond to the storage of the graft before it is used for transplantation, but it is likely to have been too short for the adhesion molecules (VCAM-1 and ICAM-1) to reach their maximum expression. The process of protein synthesis from the initiation of transcription to complete product lasts about 4 to 6 hours (18), which may explain the differences between the expression of the VCAM-1, ICAM-1 and the P-selectin in our research.

Our results indicate that pressure applied to the endothelial surfaces of the venous conduits, prepared to be used in the coronary artery bypass grafting, contributes to loss of the endothelial layer of the grafts. Mechanical widening of vessels results in the increased expression of the adhesion molecules on the surface of the endothelial cells, and, as a consequence, would lead to a rise in the leukocyte adhesion and loss of the functional properties of the transplanted veins.

It is advisable to minimize the exposure of the veins to high pressure during the graft's preparation, in order to diminish the risk of pathological processes, thus providing better durability of the venous grafts and decreasing the need for reoperation.

References

1 Bryan AJ and Angelini GD: The biology of saphenous vein graft occlusion: etiology and strategies for prevention. Curr Opin Cardiol *9*: 641-69, 1994.

2 Cook JM, Cook CD, Marlar R *et al*: Trhombomodulin activity in human sapheonus vein grafts preparet for coronary artery bypass. J Vasc Surg *14*: 147-151, 1991.

3 Jang Y, Lincoff AM, Plow EF and Topol EJ: Cell adhesion molecules in coronary artery disease. J Am Coll Cardiol *24*: 1591-1601, 1994.

4 Motwani JG and Topol EJ: Aortocoronary saphenous vein graft disease: pathogenesis, predisposition, and prevention. Circulation *10*: 916-931, 1998.

5 Bush HL Jr, Jakubowski JA, Curl GR, Deykin D and Nabseth DC: The natural history of endothelial structure and function in arterialized vein grafts. J Vasc Surg *3*: 204-215, 1986.

6 Chester AH, Morrison KJM and Yacoub MH: Expression of vascular adhesion molecules in saphenous vein coronary bypass grafts. Ann Thorac Surg *65*: 1685-1689, 1998.

7 Stoica S, Goodard M and Large SR: The endothelium in clinical cardiac transplantation. Ann Thorac Surg *73*: 1002-1008, 2002.

8 Angelini GD, Bryan AJ, Williams HM, Morgan R and Newby AC: Distention promotes platelet and leukocyte adhesion and reduces short-term patency in pig arteriovenous bypass grafts. J Thorac Cardiovasc Surg *99*: 433-439, 1990.

9 Little JH, Cooper P Sarwat A *et al*: Factors influencing endothelial injury and vascular thrombosis after perfusion. J Surg Res *14*: 221-227, 1973.

10 O'Connell TX, Sanchez M, Mowbray JF and Fonkalsrud EW: Effect on arterial intima of saline infusions. J Surg Res *16*: 197-203, 1974.

11 Abbott WM, Wieland S and Austen WG: Structural changes during preparation of autogenous venous grafts. Surgery *76*: 1031-1040, 1974.

12 Asimakopoulos G and Taylor M: Effects of cardiopulmonary bypass on leukocyte and endothelial adhesion molecules. Ann Thorac Surg 66: 2135-2144, 1998.

13 Chappell DC, Varner SE, Nerem RM, Medford RM and Alexander RW: Oscillatory shear stress stimulates adhesion molecules expression in cultured human endothelium. Circ Res *82*: 532-539, 1998.

14 Golledge J, Tumer RJ, Harley SL, Springall DR and Powell JT: Development of an *in vitro* model to study the response of saphenous vein endothelium to pulsatile arterial flow and circumferential deformation. Eur J Vasc Endovasc Surg *13*: 605-612, 1997.

15 Luscinskas F, Ma S, Nusrat A, Parkos C and Shaw S: The role of endothelial cell lateral junctions during leukocyte traticking. Immunol Rev *186*: 57-67, 2002.

16 Chiu YJ, Kusano K, Thomas TN and Fujiwara K: Endothelial cell-cell adhesion and mechanosignal transduction. Endothelium *14*: 59-73, 2004.

17 Weller A, Isenmann S and Vestweber D: Cloning of the mouse endothelial selectins. Expression of both E- and P-selectin is inducible by tumor necrosis factor. J Biol Chem *267*: 15176-15183, 1992.

18 Asimakopoulos G, Thompson RD, Nourshargh S, Lidington E, Mason JC, Haskard DO, Ratnatunga RC, Taylor KM and Landis RG: An anti-inflammatory property of aprotinin detected at the level of leukocyte extravasation. J Thorac Cardiovasc Surg *120*: 361-369, 2000.

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