BRIEF COMMUNICATIONS

CELLULAR DEOXYRIBONUCLEIC ACID FRAGMENTATION DURING OPERATIVE COURSE IN HUMAN SAPHENOUS VEIN GRAFTS

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Medial and endothelial integrity can be damaged by the surgical preparation techniques for coronary bypass grafts.¹ To obtain further insight into the cell injury that can occur during operation, we studied the deoxyribonucleic acid (DNA) fragmentation^{2,3} in human aorta-coronary saphenous vein grafts during the time required to conduct the first anastomosis.

Saphenous veins were harvested by the no-touch technique from 15 patients (12 male and three female, mean age 63 ± 5 years) referred for coronary revascularization. Group I segments (10 mm length) were excised immediately and group II segments were excised after 85 ± 10 minutes, when the first anastomosis was terminated. Group I and II segments were soaked in 1 ml Roswell Park Memorial Institute cell culture medium after resection and transported to the laboratory in less than 5 minutes.

For quantitative determination of the internucleosomal DNA cleavage in endothelial cells, group I and II segments were cut longitudinally ($n = 12$ each) and inserted between two polystyrene plates, with the upper plate bearing two perforated wells⁴ and each exposing 20 mm^2 endothelial surface to reagents. Mouse monoclonal antibodies to histones and peroxidase-labeled antibody to DNA (Cell Death Detection Enzyme-Linked Immunoassay [ELISA] Kit POD; Boehringer, Mannheim, Germany) were used for the photometric measurement of histone-associated DNA fragments in sandwich-enzyme immunoassay. A standard calibration curve was created by inducing apoptosis with camptothecin ($5 \mu\text{g/ml}$) in 2.5×10^3 to 1.5×10^4 cultured human saphenous vein endothelial cells at 37°C for 4 hours.

For immunohistochemical detection of DNA damage, group I and II segments ($n = 5$) were fixed in 3% formaldehyde in phosphate-buffered saline solution, embedded in low-melting point paraffin wax, and cut to $5 \mu\text{m}$

transverse sections. The 3'-OH ends liberated in the DNA strand breaks were revealed with terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) reaction with in situ Cell Death Detection ELISA Kit POD.

A low level of DNA fragmentation determined by ELISA on the luminal surface (Fig. 1) and counterstaining of the von Willebrand factor-positive lining with methyl green characterized endothelium at the time of venous resection in group I segments. During the first anastomosis, the increase in endothelial cells with DNA damage was significant, as shown for group II segments in Fig. 1. The representative labeling patterns of the DNA breaks on histologic sections are shown in Fig. 2. Only rare endothelial cells and a few cells in the subendothelial layers exhibited positive staining in group I segments (Fig. 2, A). In group II segments, the frequency of labeled cells increased and an extensive DNA breakdown appeared in the adventitia (Fig. 2, B). Hemalum staining indicated the preservation of tissue integrity, and in agreement the counterstaining with methyl green revealed high cell density that was not detected by TUNEL in group II segments. Accordingly, the TUNEL-positive cells accounted for a sparse scattered population in the media and endothelium in group II segments.

Fig. 1. Lethal endothelial DNA fragmentation in human saphenous vein grafts. Segments excised at the termination of venous resection (I) and of the distal anastomosis (II). Results are means $\pm$ standard error of the mean ($p < 0.01$, Student's t test).

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Fig. 2. Immunohistochemical detection of DNA fragmentation in human saphenous vein grafts. At the termination of venous resection (**A**) and the distal anastomosis (**B**). Arrows indicate endothelium.

Discussion. An increase in injured cells in terms of DNA damage was detected in coronary saphenous vein grafts during the period of about 90 minutes required for the first anastomosis. It was difficult to apply ELISA to the cells surrounded with extracellular matrix in the media and adventitia. An immunohistochemical technique was therefore also used to visualize the DNA breaks.³ The DNA fragmentation increased throughout the graft with time required for completion of distal anastomosis (Fig. 2, B). The diffuse and patchy labeling in the adventitia reflects the release of DNA breaks from the lysed cells. The internucleosomal cleavage of DNA detected by ELISA in the endothelial cells and by the TUNEL reaction on histologic sections resembles DNA damage observed in apoptosis.⁵ Programmed cell death eliminates cells that have developed in excess, cells that have developed improperly, and cells that have completed their function.² Although apoptotic DNA cleavage as result of tissue turnover cannot be ruled out, the normal venous cells in the graft were not destined for this physiologic cell suicide.

In the interpretation of our results, it should be taken into account that certain morphologic criteria of cell injury can be induced *in vitro* by acting on cellular processes as cytoskeletal protein organization or transmembrane ion transport.^{2,5} Our results can also be related to the strong increase in DNA damage after only 30 minutes in cells exposed to hypoxic injury *in vitro*.⁵ Our

results suggest that signals to trigger or transduce DNA damage can be generated during the grafting and can lead to lethal cell injury before reperfusion. The identification of factors involved in endonuclease-mediated DNA fragmentation in the vein grafts deprived of circulating blood during the operative course remains a challenging problem.

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