A Scanning Electron Microscopic (SEM) Study of the Re-Endothelialization of the Carotid End-To-Side Anastomosis in the Rat

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With 3 Figures

Summary

A carotid end-to-side anastomosis was made on 25 male Wistar rats (mean weight 197.8 g). At different time intervals, from 0 to 21 days after the operation, the animals were sacrificed. The anastomosis was exposed, the aorta was cannulated, and the animals were perfused at a constant pressure of 80 mm Hg with a 2 1/2 % glutaraldehyde solution. The anastomoses were taken out for further SEM and light microscopic (LM) study. The SEM results indicate that after the acute platelet-fibrin reaction in the first 48 hours the suture line itself becomes re-endothelialized after 4 days. On the stitches, however (EthilonR 11 x 0, 2871 G, BV 7 needle) after two days a cellular population was seen, consisting of leucocytes transforming into flattened cells. The morphology of these cells and their role in the regeneration of endothelium is discussed. This study gives evidence to the possibility of a blood-borne genesis of endothelial cells in vivo.

Introduction

Much research has been done on the reaction of the vascular wall to trauma. Most of these investigations were inspired by the question of the pathophysiology of the development of atherosclerotic lesions. A greatly varying number of mechanical and chemical experimental models have been used, and a comparison is not easy.

This work has received a new impulse in recent years from the development of microsurgical vascular techniques.

SEM is very useful for the study of cellular changes that happen at the surface of the vessel wall in cases of trauma and repair of the
endothelium. This technique was introduced by the pioneers Shima-moto, Yamashita, and Sunaga in 1969 for the study of endothelium. It is of the utmost importance to have standard methods in the preparation and fixation of the specimens in order to get uniform and reliable results and to avoid artifacts.

Gregorius and Rand described the most intense reaction to trauma in microvascular anastomosis that takes place at some distance from the suture line and at places where vascular clamps were applied. Rosenbaum and Sundt examined microvascular anastomoses in the rat, and studied the acute thrombotic reaction at the site of the anastomosis. Both papers pay attention only to the reaction of the vascular wall in the first hours after surgical trauma. The aim of this study was to pay attention to the process of re-endothelialization itself.

Materials and Methods

25 male Wistar rats (mean weight 197.8 g) were anaesthetized with Nembutal intraperitoneally. Under a Zeiss diploscope a horizontal incision was made just above the manubrium sterni. The trachea and both carotid arteries were exposed. A tracheostomy was made at the lower border of the thyroid gland, and the animal was intubated. The left carotid artery was tied off, cut tangentially just below the bifurcation, and washed out with heparin-saline. A Scoville clamp was applied to the vessel as proximal as possible. On the right common carotid artery two Scoville clamps were put at a distance of 1 cm from each other. An end-to-side anastomosis was made with interrupted stitches of 11 x 0 Ethilon (2871G, BV 7 needle). At different time intervals, from 0 to 21 days after the operation, the animals were sacrificed. The anastomosis was exposed, the aorta was cannulated, and the animals were perfused at a constant pressure of 80 mm Hg (8) with a 2\% glutaraldehyde solution. The ends of the anastomosis were tied off, and the specimen was taken out for further study. After four hours the specimen was opened and fixed on a piece of cork for 24 hours in a 2\% buffered glutaraldehyde solution, rinsed in 0.1 M phosphate buffer, and dehydrated in rising concentrations of acetone. The tissue was dried by the critical point drying method. The specimens were examined with a Cambridge Stereoscan S4.

Results

In the first two days after the operation a platelet-fibrin reaction is observed. Suture line and stitches are covered with a thin layer of coagulum consisting of flocks threads of fibrin, that caught cellular elements. This reaction markedly increases after 24 hours and stabilizes within two days. The fibrin threads and the long offshoots of the platelets are mainly arranged in the direction of the blood flow.

Some stitches are not covered by thrombus. On them the first signs of population appear after two days: platelets without offshoots and leucocytes. Some of these cells lost their spherical appearance and