Chapter 57

EXPRESSION OF PROLIFERATING CELL NUCLEAR ANTIGEN IN RAT HEARTS SUBJECTED TO TRANSIENT ISCHEMIA FOLLOWED BY REPERFUSION

Tomiyasu Koyama*, Zhonglin Xie, Jun'ichi Suzuki & Kazuhiro Abe
Third Dept. of Anatomy, School of Medicine, Hokkaido University, 060 Sapporo, Japan; *064-0821 Sapporo, N-I W-25 C.I. Ms516.

Abstract: Early mechanisms involved in improving capillarity and oxygen transport to cardiac tissue exposed to transient coronary ischemia followed by reperfusion were studied in rats. Under ether anaesthesia, the left coronary artery was mechanically occluded for 3 min after which it was released, and the rats allowed to recover. After 2, 24 or 48 h the rats were sacrificed and the hearts frozen in liquid nitrogen. Frozen cross-sections were stained immunohistochemically for proliferating cell nuclear antigen (PCNA) and for the growth factors, VEGF and bFGF. No reaction for PCNA was seen in sections of sham-operated hearts but an inhomogeneous reaction occurred in annular structures in the occluded hearts at 48 h reperfusion. The stain appeared to be located in proliferating nuclei, and in the cytosol of endothelial cells. It is suggested that PCNA is stimulated by the increase in growth factors that is known to occur within 2 h after the end of the coronary occlusion. It is concluded that the increase in capillarity, indicated by the nuclear proliferation of endothelial cells, will improve the transport of oxygen to the cardiac tissues.

Key words: heart, ischemia/reperfusion, proliferating cell nuclear antigen, capillarity, vascular growth factors

1. INTRODUCTION

In previous studies on rat hearts the effects on ventricular capillarity produced by transient ischemia have been investigated. Ischemia was induced either by intravenous injection of vasopressin [1] or by mechanical
occlusion of the left coronary artery [2] and was followed by reperfusion. Both procedures produced an increase in capillary density in the left ventricle tissue within one month; this change would increase the oxygen supply to the ventricular tissues. These long-term effects on capillarity were preceded by expression of basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) in the early phase of reperfusion, immunohistological staining for both proteins being increased within 2 h of the vasopressin injection or of the onset of reperfusion following the mechanical occlusion. While it is possible that these growth factors are involved in endothelial cell proliferation, direct evidence of proliferation and of capillary neogenesis has not been available.

In the present immunohistochemical investigation we examined the expression of proliferating cell nuclear antigen (PCNA), which is involved in DNA synthesis and functions as a cofactor for DNA polymerase-δ [3]. PCNA has been used as a marker for regenerating cells in brain tissue [4], proliferation of tumor cells [5], and uterine endothelial cells [6] and of changes in capillarity in thyroid-treated rat hearts [7]. PCNA staining can thus be expected to provide evidence for endothelial cell proliferation in rat hearts subjected to mechanical ischemia/reperfusion.

2. METHODS AND MATERIALS

Twelve male 9-week-old Wistar rats given standard rat chow and water ad libitum were used as ischemia/reperfused group (n=9) or the Sham-operated (n=3) group. All procedures followed the institutional guidelines for care and use of laboratory animals. Rats were anesthetized with ethyl ether, intubated with a polyethylene tube and mechanically ventilated with a rodent ventilator using room air, being added intermittently as a low concentration of ethyl ether as necessary. The surgical procedures and method of coronary occlusion have been described previously [2]. In brief, a left thoracotomy was performed via the fourth intercostal space, and the pericardium opened. The left atrial appendage was raised and a small curved needle, threaded with fine silk, was passed through the ventricular myocardium to encompass the left coronary artery. The thread was then tied in an overhand knot (an occluder). Two other threads were tied to the main knot (releasers) [8]. The ligature could be tightened or loosened by pulling on the relevant threads. The left coronary artery was occluded for 3 min, and the ligature then released. In the Sham-operated group a thread was looped around the coronary artery but not tightened. After the treatment the left lung was inflated, the pneumothorax evacuated and the chest closed. Three rats of the occluded group, concomitantly one Sham-operated rat,