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Immunohistochemical detection of early myocardial infarction. An evaluation of antibodies against the terminal complement complex (C5b-9)

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Abstract Antibodies (abs) against the terminal complement complex (C5b-9) were used on routinely processed post mortem myocardial tissue in parallel with conventional staining methods. Both monoclonal and polyclonal abs were tested using the avidin biotin peroxidase complex (ABC), alkaline phosphatase anti-alkaline phosphatase (APAAP) methods and an ab-bridge with alkaline phosphatase. Enhancement of the diaminobenzene (DAB) end product with cobalt-nickel (ABC method) was also done. The polyclonal ab gave the most satisfactory results and the alkaline phosphate conjugated ab-bridge had a slight advantage over the ABC method. Cobalt-nickel enhancement of DAB improved the visualization, but with higher background staining. APAAP was the least satisfactory method. Comparing the immunohistochemical method with the conventional staining methods, the former showed positive reaction in 97% of areas of coagulation necrosis and in 65% of contraction band necrosis. On the other hand coagulation necrosis was seen in 44% and contraction band necrosis in 68% of C5b-9 positive areas indicating that C5b-9 abs react with ischemically damaged myocytes before visible alterations are seen in hematoxylin-eosin staining. Moreover, using C5b-9 abs, it seems possible to exclude agonal/artefactual contraction bands which show a negative reaction. Immunohistochemical detection of C5b-9, using an adequate technique could increase the possibility to demonstrate early ischemic myocardial damage.

Key words Myocardial infarction · Complement membrane attack complex · Immunohistochemistry Sudden Death

Introduction

Immunohistochemical methodology in the diagnosis of myocardial infarction and early myocardial ischemia may have important applications in forensic pathology. Leadbetter et al. (1989) tested a variety of antibodies (abs) against intra- and extracellular molecules, which were known to be present or depleted in ischemically damaged myocardium. They found that abs against myoglobin, myosin, ceruloplasmin, prealbumin and C-reactive protein, gave the most reliable results. Brinkmann et al. (1993) have shown that with hematoxylin-eosin staining in combination with immunohistochemical methods, i.e. myoglobin, fibrinogen and C5b-9 ab, the accuracy of diagnosis in cases of coronary artery disease and suspect myocardial infarction will be increased. However, in early ischemic injury many abs were found not to discriminate with certainty between true ischemic alterations and purely agonal changes (Leadbetter et al. 1990). The C5b-9 ab is thought to be specific in necrosis and to be rather insensitive to autolysis (Bhakdi and Tranum-Jensen 1983; Brinkmann et al. 1993). Schäfer et al. (1986), Hugo et al. (1990), and Thomsen et al. (1990) have shown that C5b-9 accumulates in ischemic areas of the myocardium. But there have been some technical problems with monoclonal abs especially on formalin-fixed and paraffin-embedded material (Schäfer et al. 1986; Thomsen et al. 1990). Several new poly- and monoclonal abs are now commercially available. In this study we have used one polyclonal (Calbiochem) and one monoclonal (Dakopatts) ab and compared different immunohistochemical methods. The immunohistochemical method was then compared with the hematoxylin-eosin and Mallory’s phosphotungstic acid hematoxylin (PTAH) staining methods in cases of sudden cardiac death with and without coronary artery disease.
Materials and methods

The study is based on samples from 81 autopsies performed between 1991 and 1993 at the Department of Forensic Medicine in Stockholm comprising 65 males and 16 females. The mean age was 45.8 ± 18.9 (SD) years (range 17–86 y). The cases studied could be divided into 3 groups: sudden death with coronary artery disease (n = 44), sudden unexplained death without coronary artery disease (n = 25) and controls (suicidal hangings without coronary artery disease) (n = 12).

From each autopsy 5 tissue blocks were taken from the circumference of a transversal section of the heart halfway between the valvular plane and the apex including the septum, anterior, lateral and posterior parts of the left ventricle and one block from the posterior wall of the right ventricle. Tissues were fixed in a 4% phosphate buffered formalin solution and processed routinely. The period of fixation varied between 1 and 3 d. Paraffin sections of 4 μ thickness were placed on poly-l-lysine covered slides for immunohistochemistry and parallel sections on untreated slides for routine staining with hematoxylin-eosin and PTAH.

The following immunohistochemical methods for visualization of the antibody-antigen reaction were used: the avidin biotin peroxidase complex (ABC) and the alkaline phosphatase anti-alkaline phosphatase (APAAP) methods. We also used an indirect method employing an ab-bridge conjugated with alkaline phosphatase (AP). With the ABC method we tried to improve visualization by enhancement of the diaminobenzene (DAB) reaction end product with cobalt and nickel (Co-Ni) (Adams 1981). For immunohistochemistry sections were rinsed in a Tris saline buffer at pH 7.6 and treated with pronase (SIGMA). Both the mono- and polyclonal abs were diluted 1:25 in normal rabbit serum (ABC and APAAP methods) or goat serum (AP method) and incubated on the slides overnight at +4 ° C and an additional 1 h at room temperature. The slides with polyclonal ab were treated with swine anti-rabbit IgG ab at a 1:300 dilution in 1% normal swine serum (ABC and APAAP methods) or with a bridge goat anti-rabbit IgG ab, conjugated with alkaline phosphatase (Dakopatts, D487), diluted 1:25 in 1% normal goat serum (AP method). The incubation time was 30 min in all cases. After rinsing in Tris saline buffer, ABC-kit solution (Dakopatts) and DAB (SIGMA), or APAAP-kit solution (Vector 1) and alkaline phosphatase substrate chromogen (Vector red) were applied. Enhancement of the DAB reaction product was performed in selected cases in a 1% cobalt chloride/4% nickel ammonium sulphate solution (Adams 1981). A total of 405 slides with 81 negative controls (without C5b-9 ab but otherwise identically treated) and 81 positive controls was made for every individual case. Each slide was studied blindly, once by both authors and then re-evaluated after several months. Comparisons were made between 2 sets of data and expressed in percentages of the total.

Results

Monoclonal ab against C5b-9 generally showed a weaker reaction than the polyclonal ab. With enhancement of the DAB reaction product with cobalt-nickel, a satisfactory result could be reached even with monoclonal ab. The polyclonal ab in combination with the ABC method resulted in good visualization of C5b-9, and the ABC method with cobalt-nickel enhancement increased contrast. With the AP method, giving a red coloration of infarcted areas, the result was at least equally good (Fig. 1).

The APAAP method was not satisfactory with monoclonal or polyclonal abs.

In every section an inbuilt positive control was found in the form of C5b-9 positive material in the walls of blood vessels and in mast cell granules. Artefacts were often seen and with experience easy to eliminate, mainly tiny spots that did not follow the outline of the myocytes and filamentous deposits near the edges of the sections.

In the study of separate lesions in the 405 slides from 81 hearts, polyclonal C5b-9 ab combined with the AP