THE REACTIVITY OF CELLULAR PROTEINS EXTRACTED FROM CALCIFIED HUMAN VALVES WITH HUMAN PLASMA

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Cardiac valve aortic stenosis is a major functional abnormality frequently concomitant with degenerative calcific aortic stenosis (CSA). Age, congenital aortic valve disease (biscupid aortic valve) and post-inflammatory processes are factors influencing stenotic aortic valve degeneration. Although abnormal cellular and humoral response is involved in post-inflammatory calcification, the regulatory factors and the specific markers of CSA have yet to be characterized. Plasma was collected from four cardiac patients with diagnosed coronary artery disease (CAD). C-reactive protein (CRP), TNF-α and IL-6 were assessed to exclude acute post-infection inflammatory processes. Seven pathological valves were dissected from patients (males, mean age 60±6) undergoing elective valve replacement for calcific SA. The control group consisted of seven recipient valves (males, mean age 48±3).

To extract the proteins, each frozen valve was shattered into pieces, sonificated on ice and then ultracentrifuged. The protein concentration was determined according to Bradford. Aortic valve proteins (10 μg per spot) were immobilized to nitrocellulose membrane, and plasma-valve protein reactivity was determined using anti-human IgG and IgA polyclonal antibodies labelled with horseradish peroxidase (HRP). Polyacrylamide gel electrophoresis (PAGE), in the presence of SDS, in reducing conditions as per Laemmli, on a 12% gel, was applied to determine the protein profile. Western-blot analysis of the electrotransfered proteins was performed using the plasma as a preliminary antibody.

The spot-blot assay showed the very strong reactivity of human plasma with proteins from calcific AV. Western-blot analysis distinguished the protein MW 45 kDa reacting with human plasma and present in calcific AV. In normal valves, no specific band was observed.

The reactivity of polyvalent human plasma with proteins extracted from calcified valves was observed.

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