Applications of Anticardiac Myosin Monoclonal Antibodies in the Diagnosis of Heart Disease

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We have developed anticardiac myosin antibodies, especially monoclonal antibodies, for helping in the diagnosis of heart disease. Our investigations were divided into three research projects.

1. We visualized the distribution of myosin isozymes in human atrial and ventricular myocardium by an immunofluorescence staining method using monoclonal antibodies specific for individual human cardiac myosin isozymes. We also revealed the redistribution of these cardiac myosin isozymes in an overloaded condition. The isozymic pattern of cardiac myosin was changed from the atrial type to the ventricular type in the overloaded atrium. This isozymic redistribution can be considered as physiological adaptive mechanism to meet increased cardiac work during overload.

2. We developed a new method of imaging for myocardial infarction by single photon emission tomography using labelled monoclonal antibody specific for cardiac myosin heavy chain. Specific localization of the labelled antibody was demonstrated in the infarcted area and no accumulation of radioactivity was shown in the bone matrix as observed in ^{99m}Tc pyrophosphate images.

3. We developed a sensitive radioimmunoassay of cardiac myosin light chain I (LCI) and demonstrated that peak serum levels of LCI in the patients with acute myocardial infarction correlated well with the left ventricular ejection fraction. Furthermore, LCI release from the infarcted myocardium was not affected by coronary reperfusion due to intracoronary thrombolysis. Thus, serial determinations of serum LCI better quantify the extent of myocardial damage even after coronary reperfusion in acute myocardial infarction.

MONOCLONAL antibodies which react with specific antigenic determinants on the protein molecule have been widely used in various biomedical fields to discriminate a specific protein among structurally similar molecules. Furthermore, these antibodies have the advantage that they can be produced on a large scale with

relative ease. Recently, we have developed monoclonal antibodies specific for cardiac myosin which is one of the major structural and contractile proteins in cardiac cells and paid attention to the application of these monoclonal antibodies to the diagnosis of heart disease.

Our investigation in this field was divided into three research projects. The first project was the application of anticardiac myosin monoclonal antibodies to discriminate human cardiac myosin isozymes to elucidate the pathophysiology of cardiac hypertrophy. Secondly, we developed a new method of imaging of myocardial infarction

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with labelled antibodies to produce high quality images for infarct sizing. Thirdly, we established a radioimmunoassay for cardiac myosin light chain in serum which is liberated from damaged myocardial cells into the blood stream to provide a sensitive and quantitative diagnosis of acute myocardial infarction.

In this paper, we report our findings and demonstrate how the application of anticardiac myosin monoclonal antibodies provides advantages in the diagnosis of heart disease.

MATERIALS AND METHODS

Preparation of Anticardiac Myosin Antibodies

Myosins for immunization were prepared from human heart muscles. Human tissues were obtained from patients who died without heart disease at autopsy. Myosins were extracted by a modified dilution method.¹ Myosin heavy chains and light chains were separated individually from whole myosin molecules by guanidine denaturation.² Immunization and the preparation of hybridomas producing antimyosin monoclonal antibodies were carried out as previously reported.³ Briefly, BALB/c mice were immunized with five intraperitoneal injections at two-week intervals with myosin emulsified in Freund's adjuvant. Animals exhibiting high serum titer were killed and their spleen cells were isolated and fused with cells of the myeloma line (P3 $\times$ 63 Ag8U1) according to the procedure of Köhler and Milstein.⁴ Antimyosin activity in the medium from hybridoma colonies was screened by enzyme-linked immunosorbent assay (ELISA).

Immunohistochemical Study of Human Cardiac Myosin Isozymes

The distribution of myosin isozymes in the human atrial and ventricular myocardium was investigated by immunofluorescence assay. Human myocardial specimens were obtained from surgical operations and autopsy. Specimens obtained from surgical operations were those from patients with ischemic heart disease and valvular heart disease including mitral stenosis and/or regurgitation and tricuspid regurgitation. Cardiac catheterization had been performed in all patients within 4 weeks prior to cardiac surgery, and right atrial pressure and pulmonary capillary wedge pressure were recorded in each patient. Postmortem specimens were obtained from patients without heart disease. For the immunohistochemical study, the specimens were washed

with saline and frozen in liquid nitrogen. Cryostat tissue sections were first incubated with anti-myosin antibodies specific for individual human cardiac myosin isozymes, then treated with biotinylated goat antimouse IgG, and stained with fluorescein isothiocyanate-labelled avidine.

Imaging of Myocardial Infarction

Anterior myocardial infarction was produced by ligation of the left anterior descending coronary artery in the dog. Antimyosin antibodies were labelled with ¹³¹I by the chloramine-T method and injected intravenously 4 hours after coronary ligation. Twenty-eight hours later, scintigrams were taken by gamma cameras. Sagittal single photon emission computed tomography of the images was produced by reconstruction using filtered back-projection algorithms and a ramp Hanning 0.5 smooth filter. ^{99m}Tc pyrophosphate images were also examined 48 hours after coronary ligation.

Determinations of Serum Cardiac Myosin Light Chain I in Patients with Acute Myocardial Infarction

Serum light chain I (LCI) concentrations were determined by a sensitive radioimmunoassay as previously reported.^{5,6} Separation of bound from free LCI was performed by a two antibody technique. Amounts of 0.2 ng/ml of LCI were measurable. Cross-reactivity between LCI and total skeletal LC was 10.2%.

Fifty-six patients with acute myocardial infarction admitted to the coronary care unit within 12 hours after the onset of the attack were examined for serial determinations of LCI and CPK. In 30 of the patients, intracoronary thrombolysis was successful. Left ventriculography was performed in every patient about 4 weeks after the onset. Left ventricular ejection fraction (LVEF) was calculated using the area-length formula. Blood samples for CPK were obtained every 3–4 hours after the onset and those for LCI were drawn once a day for at least one week.

RESULTS AND DISCUSSION

Distribution of Myosin Isozymes in Human Atrial and Ventricular Myocardium

Recently, the presence of two types (α and β) of cardiac myosin has been revealed by electrophoretic studies.^{7,8} $\alpha(V_1)$ type cardiac myosin shows high ATPase activity and fast shortening

Fig.1. (A) Fresh-frozen cryostat section of normal human atrial myocardium stained with CMA 19. All but a few myocytes are brightly stained. (B) Section of normal atrial myocardium stained with HMC 14. 20–40% of the myocytes are stained with variable intensity. (C) Section of normal human ventricular myocardium stained with HMC 14. All myocytes are strongly stained. (D) Section of normal ventricular myocardium stained with CMA 19. Only few interspersed myocytes are stained.

velocity of contraction but low energy efficiency compared with those of $\beta(V_3)$ type myosin. α and β type myosin are composed of two α and β heavy chains (HC α and HC β , respectively). However, it has not been conclusively determined that these isozymes exist in human cardiac myosin, since electrophoretic analysis failed to separate the individual isozymes of human cardiac myosin. Immunofluorescence studies with specific monoclonal antibodies visualized the distribution of cardiac myosin isozymes in the human atrial and ventricular myocardium.

We selected two hybridoma clones, CMA 19 and HMC 14, which secreted monoclonal antibodies specific for human atrial and ventricular myosins, respectively.⁹ Neither CMA 19 nor HMC 14 reacted with light chains. Since human atrial and ventricular myosin showed enzymatic properties similar to those of α and β type myosin, respectively,¹⁰ CMA 19 and HCM 14 appeared to react specifically with α and β types of myosin heavy chains (HC α and HC β). The immunohistochemical study using these antibodies revealed that 100% of ventricular myocytes were

strongly and uniformly stained by HMC 14 and most of them were completely unreactive to CMA 19. Only few interspersed myocytes gave a positive reaction to CMA 19 (Fig. 1D, C).

In contrast, in the atrium, more than 95% of myocytes were brightly stained by CMA 19. However, wide variation was observed in staining intensity by HMC 14 among individual atrial myocytes, ranging from a completely negative to a strong positive reaction, and myocytes giving a positive reaction comprised from 20 to 40% of total myocytes (Fig. 1B). Furthermore, all myocytes which did not react with CMA 19, were stained by HMC 14 when examined in serial sections. These observations revealed that the normal isozymic pattern of myosin was exclusively β type dominant in the human ventricle, and that the genes operating the synthesis of HC α were expressed in only a few ventricular myocytes. On the other hand, the normal isozymic pattern of myosin appeared to be α type dominant in the human atrium. However, variation in the staining intensity with HMC 14 suggested that a relative amount of β type

Fig.2. Correlations between the total scores per 1,000 myocytes containing α type (HC α) or β type (HC β) myosin and mean atrial pressure.

myosin coexisted in the atrium and that individual atrial myocytes contained different amounts of HC β .

Redistribution of Cardiac Myosin Isozymes in Overloaded Human Atrial Myocardium

Evidence has accumulated to suggest that relative proportions of these two basic isoforms of myosin are changed in rat and rabbit ventricles during development and during cardiac overload.^{11,12} By immunofluorescence assay, we also observed striking changes in the response of overloaded human atrial myocardium to the antibodies. The number of atrial myocytes reactive with HMC 14 was markedly increased when in an

overloaded condition, whereas the myocytes reactive with CMA 19 were proportionally decreased. Atrial myocytes unreactive with CMA 19 were observed more frequently. To demonstrate the changes in the amounts of individual myosin isozymes more quantitatively, we counted the myocytes which reacted with each antibody and observed a significant correlation between atrial pressure and the number of these myocytes (Fig. 2). In the patients whose atrial pressure markedly rose, HC α was almost completely converted to HC β . When β type myosin is increased, it has been reported that tension dependent heat per unit tension and oxygen consumption are reduced.^{13,14} Thus, isozymic redis-

Fig.3. Planar images of myocardial infarction using labelled HMC48 in the dog. The hot spots of the antibody uptake were localized in the infarcted area.

tribution can be considered to be a physiological adaptive mechanism to meet increased cardiac work during overload. Isozymic changes in cardiac myosin from α to β isoform in the overloaded atrium, may constitute a compensatory mechanism by which the mechanical function of the atrium changes from volume work to pressure work.

Imaging of Myocardial Infarction with Labelled Antimyosin Monoclonal Antibody

Myocardial damage resulting from ischemia initiates a sequence of cellular changes. The rapid appearance of alterations is observed in sarcolemma, allowing leakage of macromolecules such as CPK and GOT. Conversely, intravenously administered antimyosin antibodies could enter into the myocytes and combine with myosin molecules in the myofilaments. Thus, if the antibodies are labelled with isotope, radioactivity could be seen to accumulate in the damaged myocardial tissue and hot spots of antibody uptake could be sharply delineated by scintigrams.

We examined the imaging of myocardial infarction experimentally in the dog using anti-cardiac myosin heavy chain antibody, HMC 48,

Fig.4. Sagittal tomography of the images reconstructed by single photon emission computed tomography revealed very specific localization of radioactivity in mainly apical areas extending to the anterior wall.

Fig.5. Correlations between peak light chain I (LCI) and left ventricular ejection fraction (LVEF) in patients with acute myocardial infarction without or with coronary reperfusion (control or reperfusion, respectively).

Fig.6. Correlations between peak CPK and left ventricular ejection fraction (LVEF) in control or reperfusion group.

of which the affinity constant was 1.8×10^{-9} M. Images of myocardial infarction were produced with ^{131}I labelled HMC 48 (1 mg, 1 mCi) given intravenously. Specific localization of HMC 48 uptake was demonstrated in the infarcted area (Fig. 3). In contrast to $^{99\text{m}}\text{Tc}$ pyrophosphate images, no accumulation of radioactivity was observed in the bone matrix. Images of thyroid glands were observed but did not interfere with the discrimination of images of the infarcted area. Single photon emission computed tomography revealed very specific and distinctive localization of radioactivity mainly in the apical area extending to the anterior wall (Fig. 4). We also observed that the concentration of HMC 48 was much greater in the endocardial region than in the epicardial region. These results suggest

that the concentration of the antibody rose with increasing severity of myocardial damage due to ischemia and showed an inverse relationship to regional blood flow in the infarcted myocardium. Thus, imaging of myocardial infarction using labelled HMC 48 may provide an accurate and sensitive noninvasive method for visualization of the infarcted myocardium.

Serum LCI Levels and Left Ventricular Function in Patients with Acute Myocardial Infarction

In most patients, LCI from the infarcted heart first appeared in the serum within 6 hours of the onset of infarction or on admission, reached peak levels between 2 and 5 days and remained elevated for more than 10 days; whereas serum CPK concentrations had returned to normal by this

time. This distinctive pattern of appearance of LCI in the serum has not been observed in time-course studies of many cardiac enzymes. Since exogenous LC injected intravenously disappeared rapidly from the circulation with a half-time of disappearance of about 4.5 hours, the prolonged elevation of LCI cannot be attributed to accumulation but reflects the continuous liberation of LCI from damaged myofibrils within the infarcted myocardium.

Peak LCI levels correlated to LVEF in the patients with or without coronary reperfusion by thrombolysis ($r = -0.57$ and -0.82 , respectively) (Fig. 5). On the other hand, peak CPK levels were related to LVEF only in the patients without coronary reperfusion ($r = -0.52$, $p < 0.05$) (Fig. 6). No significant correlation was observed in the patients with coronary reperfusion. In addition, most LCI levels of 1st-5th day after the onset related well to LVEF. Peak appearance time of CPK in patients with coronary reperfusion was remarkably earlier than in patients without coronary reperfusion. However, that of LCI was almost equal in both groups. The abrupt CPK release after coronary reperfusion is probably because of washout by reperfused blood flow¹⁵ but LCI release does not appear to be affected by reperfusion. Thus, the serial determinations of serum LCI better quantify the extent of myocardial damage even after coronary reperfusion in acute myocardial infarction, and it is unique biochemical index for evaluation of the clinical effects of intracoronary thrombolysis.

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