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PRECLINICAL RESEARCH

Defective Intercellular Adhesion Complex in Myocardium Predisposes to Infarct Rupture in Humans

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Objectives Our goal was to evaluate intercellular adhesion complex proteins in myocardium in human infarct rupture.

Background Infarct rupture, a fatal complication of myocardial infarction (MI), has been attributed to a defective cell adhesion complex in a transgenic mouse model.

Methods Heart samples were collected from autopsies from infarct rupture and control (nonrupture) MI patients. Both infarcted and remote areas were included. Cell adhesion proteins including αE-catenin, β-catenin, γ-catenin, and N-cadherin were characterized by immunohistochemistry and immunoblotting. Genetic analysis was undertaken to evaluate mutations and polymorphisms in the αE-catenin gene. In addition, infarct rupture was studied in transgenic mice heterozygous for αE-catenin C-terminal deficiency, mimicking the situation in human infarct rupture patients.

Results No αE-catenin was detected in 70% of remote samples of infarct rupture hearts compared with 20% in control MI by immunohistochemistry. The immunoblot analysis confirmed a significant reduction in remote areas, and complete absence of αE-catenin in infarct areas from infarct rupture patients. No mutation or polymorphism of the αE-catenin gene was discovered. Other cell adhesion proteins were not significantly affected in remote areas of infarct rupture hearts. Three-fourths of the heterozygous αE-catenin C-terminal truncated mice died of infarct rupture, compared with one-fourth of the wild-type littermates.

Conclusions The data show a reduced expression and defective localization of αE-catenin in the intercalated disc region in patients dying of infarct rupture. The mechanism of lower expression of αE-catenin remains to be elucidated.
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Although generally considered to be a rare complication of acute myocardial infarction (MI), myocardial infarct rupture remains a common cause of death (1–3), and accounts for approximately 10% of mortality (4,5). Rupture is most frequent when the infarct area is located in the ventricular free wall (1) and nearly always results in sudden death from massive blood loss into the pericardium, causing cardiac tamponade (1,6). Most infarct ruptures occur within the first week of MI (4,6), usually after transmural, first, or anterior MI, and in the setting of poor collateral circulation (7,8). Little is known about the molecular mechanisms of infarct rupture.

Recently, the role of defective intercellular adhesion complexes has been implicated, based on an increased incidence of infarct rupture in mice lacking the αE-catenin gene (9). αE-catenin protein is a component of the cadherin/catenin cell adhesion complex, which mediates cell adhesion in many cell types (10). Loss of cell adhesion molecules has been associated with metastasis in many human cancers (11), underscoring their role in cell adhesion. In myocardium, these cell adhesion complexes are located in the intercalated disks (12), and a weakened cardiomyocyte adhesion could facilitate inflammatory cell infiltration. Increased inflammatory cell influx and consequent up-regulation and activation of matrix metalloproteinases have previously been reported to be associated with infarct rupture in animal studies (13–15) and in humans (15).

The present study was undertaken to evaluate the role of cadherin and catenin proteins, especially αE-catenin, in patients dying of infarct rupture. In this case control study, we performed biochemical and immunohistochemical characterization of the adhesion complex proteins and compared

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with age matched post-mortem myocardial tissue specimens obtained from MI patients dying of causes other than infarct rupture (control MI). The adhesion complex proteins were characterized in both infarcted and remote areas. The analysis of the αE-catenin gene was also performed to identify mutations or polymorphisms. In addition, to mimic these observations in an animal model, we studied the impact of MI in transgenic mice heterozygously deficient for the C-terminal part of αE-catenin and found a higher incidence of infarct rupture compared with that seen in their wild-type littermates.

Abbreviations and Acronyms

ICD = intercalated disc
LV = left ventricle/ventricular
MI = myocardial infarction
PCR = polymerase chain reaction
SNP = single nucleotide polymorphism

Methods

Myocardial tissue specimens. Collection, storage, and use of human heart tissue and patient data were undertaken in compliance with the "Code for Proper Secondary Use of Human Tissue in the Netherlands." For the present study, the post-mortem myocardial tissue specimens were collected in 40 patients with MI: 20 with infarct rupture and 20 without rupture (control MI). For each patient, samples from the infarct area and from remote myocardial region were obtained (Table 1).

For the prospective part of the study, myocardial samples were obtained at autopsy (collected from 2001 to 2006). Part of the samples were snap frozen in liquid nitrogen, part were formalin fixed. Left ventricular (LV) myocardial samples were collected from 10 patients who died of infarct rupture (age 52 to 87 years; 8 men, 2 women) (infarct rupture group). For comparison, LV samples were also collected from 10 MI patients who suffered from MI 3 to 7 days before death but died from causes other than infarct rupture (age 48 to 86 years; 4 men, 6 women; control group). The 2 groups were similar according to post-MI survival times. Also, there was no significant difference in the time lapsed between death and autopsy between the 2 groups (rupture: 22.6 ± 6.1 h, control MI: 19.6 ± 5.1 h). The frozen tissue samples were stored at −80°C and subsequently used for Western blotting and deoxyribonucleic acid isolation. The formalin-fixed tissues were embedded in paraffin, and the sections stained with hematoxylin and eosin to estimate the infarct age. In addition, all formalin-fixed tissue specimens were used for immunohistochemical staining for catenin and cadherin family proteins.

For the retrospective part of the study, myocardial tissue samples were obtained from Maastricht Pathology Tissue Collection registry; autopsies were performed between 1996 to 2000. After review of the clinical histories, paraffin-embedded tissue samples from different areas of the heart were obtained from 10 patients, who had died of infarct rupture. The age of the patients (4 men and 6 women) ranged from 63 to 84 years; all 10 patients had sustained rupture of the LV free wall in the index episode and had no evidence of previous transmural MI. In addition, post-mortem myocardial specimens were also collected from 10 control patients (5 men and 5 women) who had suffered from transmural MI in the past, but had died of unrelated causes. These patients revealed pathological evidence of an old infarct with adequate infarct healing. The age of these patients ranged from 63 to 85 years and was similar with the patients in the infarct rupture group.

Table 1 Characteristics of Patients Included in This Study

	Infarct Rupture		Control MI	
	Prospective (n = 10)	Retrospective (n = 10)	Prospective (n = 10)	Retrospective (n = 10)
Men:women	8:2	4:6	4:6	5:5
Age (yrs)	67 ± 10	75 ± 6	70 ± 13	75 ± 7
MI localization				
Posterior	4	3	5	4
Anterior	5	7	4	4
Lateral	1	0	1	2
MI age	1 to 6 days	1 to 6 days	1 to 6 days	3 to 216 months
1-vessel disease	5	6	2	1
Thrombolysis	1	1	0	0
Rescue PCI	4	2	4	1
CABG*	0	0	1	6
Smoker	5	1	1	2
Diabetes	1	3	2	3
Hypertension	3	3	3	4
Previous MI	1†	3†	2	10‡

*None of the coronary artery bypass grafts (CABGs) were performed in the acute phase of myocardial infarction (MI); †small and nontransmural MI; ‡selected for previous, well-healed, transmural MI.
PCI = percutaneous coronary intervention.

Immunohistochemistry. From paraffin-embedded tissue, 4 μm-thick sections were cut, mounted on glass slides coated with 3-aminopropyltriethoxysilane (APTS, Sigma-Aldrich, St. Louis, Missouri) and dried overnight in an incubator at 37°C. The sections were deparaffinated in xylene, rehydrated, and endogenous peroxidase was blocked by incubation in methanol containing 0.3% hydrogen peroxide for 15 min, and rinsed with phosphate-buffered saline. For antigen retrieval of β-catenin, γ-catenin, and N-cadherin, sections were boiled twice for 5 min in 10 mmol/l citrate buffer (pH 6.0). For αE-catenin, sections were boiled in citrate buffer for 10 min at 110°C in an autoclave. The following primary antibodies were used: β-catenin monoclonal antibody (dilution 1:500, Transduction Labs, Lexington Kentucky), γ-catenin polyclonal antibody (dilution 1:50, Santa Cruz Biotechnology, Santa Cruz, California), and pan-cadherin monoclonal antibody (dilution 1:250, Sigma-Aldrich). This pan-cadherin antibody was raised against the C-terminal cadherin sequence, which is common in many cadherins including N-cadherin, the main cardiac form. Immunohistochemistry for αE-catenin was performed using 2 different polyclonal antibodies raised against the C-terminal part of the αE-catenin protein (dilutions 1:50, Santa Cruz Biotechnology and 1:100, Neomarkers, Fremont, California, respectively). All sections were incubated overnight with the antibody at 4°C. The following secondary antibodies were used: biotinylated multilink swine-anti-goat, -mouse, and -rabbit (dilution 1:400, DAKO, Glostrup, Denmark) and rabbit-anti-goat (dilution 1:600, DAKO). The Vectastain ABC kit (Vector Labs Inc., Burlingame, California) was used according to the manufacturer's instructions to visualize the binding of the primary antibodies. Sections were briefly counterstained with hematoxylin before mounting with Entellan (Merck, Darmstadt, Germany).

Four sections per patient, obtained from different parts of the heart, were analyzed in a blinded way by 2 independent observers. The sections were included into 1 of 3 categories: detectable staining of intercalated discs (ICDs) in 3 or more sections per patient (+), detectable staining of ICD in 1 or 2 sections per patient (±), or no detectable staining of ICD (−).

Western blotting. For Western blotting, samples of the frozen heart tissue were placed in 500 μl ice-cold Laemmli buffer (6.6% glycerol, 1.5% SDS, 41.5 mmol/l Tris/HCl, pH = 8.0) and homogenized with a PRO200 tissue homogenizer (PRO Scientific, Oxford, Connecticut). After sonication and centrifugation, the supernatant was collected and protein content was measured using the BCA protein assay (Pierce Biotechnology Inc., Rockford, Illinois); 10 μg of total protein was denatured by boiling in Laemmli sample buffer (BioRad, Hercules, California), separated on a 10% SDS-PAGE gel (16), and transferred onto a Hybond C nitrocellulose membrane (Amersham Biosciences, Little Chalfont, United Kingdom). After blocking (5% nonfat dry milk [BioRad], 0.1% Tween in TBS) for 1 h, membranes were incubated overnight at 4°C with primary antibodies directed against α-catenin 1:3,000 (Sigma-Aldrich) and α-tubulin (H-300) 1:500 (Santa Cruz Biotechnology) for loading control. For this part of the study we were unable to use the αE-catenin antibodies that were used for immunohistochemistry, because these antibodies failed to produce a distinct band in our Western blot assay. The α-catenin antibody from Sigma is known to cross-react with both αE and αN-catenin, but since the latter subtype of α-catenin is not present in the heart the observed band represents αE-catenin. Anti-rabbit immunoglobulin G (PI 1000) 1:5,000 (Vector Labs Inc.) was used as the secondary antibody, and the membranes were developed using the Supersignal West Pico chemiluminescence kit (Pierce). Images of the blots were analyzed with image analysis software (Qwin, Leica, Cambridge, United Kingdom).

DNA isolation, polymerase chain reaction (PCR) amplification, and sequencing. In order to sequence the 16 coding exons of αE-catenin (CTNNA1, MIM:*116805), DNA was isolated from snap-frozen tissue samples from remote parts of the hearts of both infarct rupture patients and control MI patients. In total, we included tissue from 3 patients who died of infarct rupture and 3 patients who suffered from recent MI (3 to 7 days post-MI) but died from causes other than infarct rupture. DNA was isolated from the tissue samples using the Dneasy Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Polymerase chain reaction was performed in 25 μl reactions using PuReTaq Ready-To-Go PCR beads (Amersham). Both forward and reverse primers were used in a concentration of 10 μM (Appendix). Polymerase chain reactions were performed in the Biorad I-cycler (BioRad). The PCR products were identified on a 1% agarose gel and purified using the High pure PCR purification kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions.

Cycle sequencing was performed using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, California) according to manufacturer's instructions. The forward primers of the PCR reactions were used as sequencing primers (Appendix). In a 20 μl reaction volume, 3.2 pmol primer was added. The products of the sequence reactions were purified by ethanol precipitation and analyzed using an ABI Prism 3100 genetic analyzer (Applied Biosystems).

Experimental MI in αE-catenin deficient mice, tissue collection, and processing. ANIMALS. Heterozygous αE-catenin C-terminal deficient mice were created using a gene trap strategy. In these mice, the mutation eliminates the carboxyl-terminal third of the protein and induces a complete loss of function phenotype (17). Since homozygous mice die in the blastocyst stage (17), we only used adult, male heterozygous mice (n = 9) and compared them with their wild-type littermates (n = 11). All experiments were conducted according to institutional guidelines and conformed to The Guide of the Care and Use of Laboratory Animals published by the National Institutes of Health.

EXPERIMENTAL MI. Experimental MI was induced as described previously (18). Briefly, all mice received buprenor-

phine 0.1 mg/kg subcutaneously as pain medication before surgery. Mice were anaesthetized using 3% to 4% isoflurane, which was gradually decreased to 1.5% to 2.5% during surgery. The trachea was intubated to allow positive pressure respiration with room air (1.5 ml, 70/min). A ligature (6-0 prolene) was tied around the main left coronary artery after opening the skin, the left 4th intercostal space, and the pericardial sac. Chest and skin were closed with 5-0 silk sutures.

TISSUE COLLECTION. Mice were scheduled to be sacrificed 7 days after MI. Half of the heart was snap frozen into liquid nitrogen and stored at −80°C until further use. The other half was formalin fixed and paraffin embedded for immunohistochemistry. Ten mice died of acute rupture between 3 to 5 days, and the tissue samples were collected similarly. The same protocol for Western blotting was used as described for human tissue.

Statistical analysis. All data are presented as means ± standard error of the mean. Statistical analysis was performed using a 2-way analysis of variance with 1 repeated measures factor with Bonferroni post-hoc test as applicable. For analysis of the semiquantitative immunohistochemistry data, a chi-square test was performed. Survival was analyzed by a Kaplan-Meier curve with a log-rank test for differences between groups. A p value <0.05 was considered to indicate statistical significance. Graphpad Prism 4 software (Graphpad Software, Inc., San Diego, California) was used for statistical analysis.

Results

Immunohistochemical localization of cellular adhesion motifs. To analyze the localization characteristics of the cell adhesion complex in infarcted and remote myocardium,

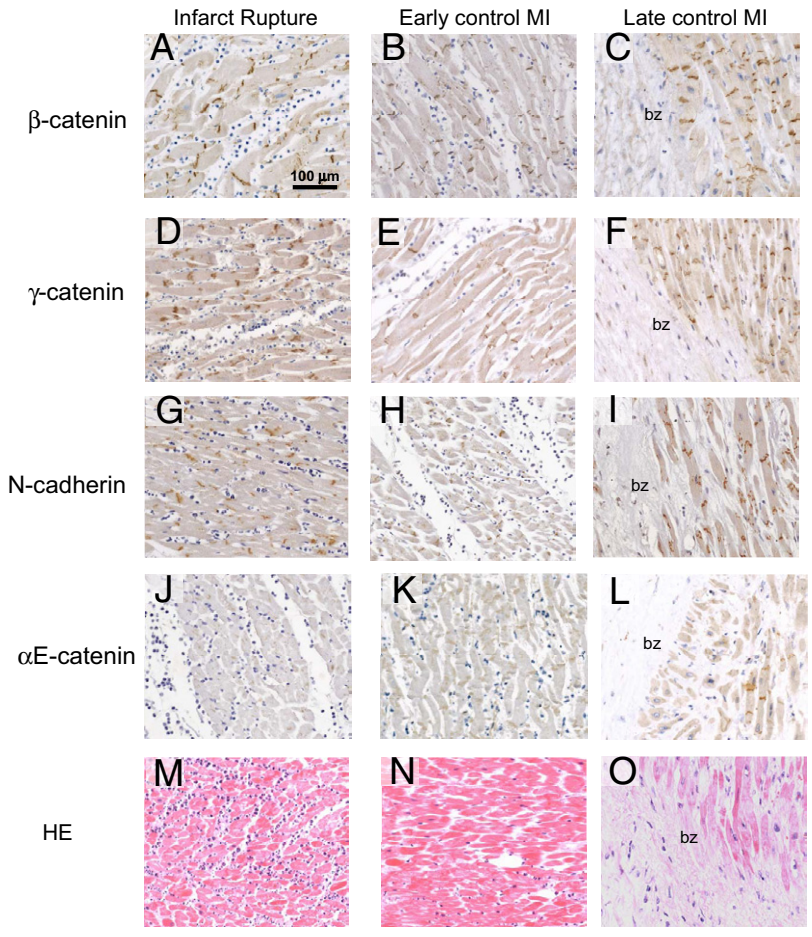

Figure 1 **Immunohistochemical Characterization of Cell Adhesion Complex Proteins in the Infarct Area**

Representative microphotographs of immunostaining for β-catenin **(A to C)**, γ-catenin **(D to F)**, N-cadherin **(G to I)**, and αE-catenin **(J to L)** in the infarct area of ruptured hearts **(A, D, G, J)**, and early **(B, E, H, K)** and late **(C, F, I, L)** control infarcts. A similar staining intensity for β-catenin, γ-catenin, and N-cadherin was observed in the intercalated disk regions of the cardiomyocytes in infarct rupture and the 2 control myocardial infarction (MI) groups. αE-catenin staining was undetectable in the infarct area of infarct rupture patients **(J)**. However, staining was observed in the early control infarcts **(K)** as also in the border zone (bz) of late control MIs **(L)**. **(M to O)** Shows hematoxylin and eosin staining of the infarct area of ruptured patients **(M)**, characterized by the necrotic cardiomyocytes and the massive influx of inflammatory cells. Hematoxylin and eosin staining of an early control MI **(N)** and the border zone of a late control MI **(O)** are also shown.

immunohistochemistry was performed using antibodies specific for various cadherin and catenin family proteins in both prospectively and retrospectively collected tissue samples. In the infarct area we observed staining for β-catenin (Figs. 1A and 1B), γ-catenin (Figs. 1D to 1E), and N-cadherin (Figs. 1G to 1H) in the cardiomyocytes of both infarct rupture and early control MI patients. In contrast, no immunohistochemical staining for αE-catenin was observed in the infarct area of the infarct rupture patients (Fig. 1J), whereas this staining was present in the early control MI group (Fig. 1K). In the late control MI group, we detected staining with all 4 antibodies in surviving cardiomyocytes in the border zone of the infarct (Figs. 1C, 1F, 1I, and 1L). The hematoxylin and eosin stained sections showed necrotic cardiomyocytes with increased numbers of inflammatory cells in the infarct area of infarct rupture patients (Fig. 1M). To analyze whether the lack of αE-catenin in the infarct rupture patients was acquired or already present before infarction, we analyzed the remote areas with all 4 antibodies (Fig. 2). β-catenin (Figs. 2A and 2B), γ-catenin (Figs. 2C and 2D), and N-cadherin (Figs. 2E and 2F) showed abundant presence within the intercalated disks in the remote myocardial regions of both the infarct rupture patients and control MI patients. No differences were observed between these 2 groups (Table 2).

The results of immunohistochemical analyses of the remote myocardium with αE-catenin antibodies (raised against the C-terminal part of αE-catenin, obtained from Santa Cruz) are shown in Figures 2G and 2H. In 70% of infarct rupture patients, no αE-catenin staining was observed in the intercalated disks in remote region, whereas in 20% the staining was found to be low. In contrast, only in 20% of the control MI patients could no αE-catenin be demonstrated in the remote region (Table 2), a statistically significant difference ($p < 0.0001$). To confirm these findings, we used another antibody directed towards the C-terminal part of αE-catenin (obtained from Neomarkers). The latter αE-catenin antibody yielded results similar to those obtained with the Santa Cruz antibody (data not shown).

Western blotting. For semiquantitative assessment of αE-catenin protein expression in infarct rupture and control MI patients, in both infarct and remote areas, we performed Western blotting (Fig. 3) on the prospectively collected tissue samples. In the remote areas, densitometric analysis showed large amounts of αE-catenin in control MI patients. The expression was almost 3-fold lower in the infarct rupture patients ($p < 0.01$). In the infarct areas of the control MI group, a slight, nonsignificant reduction in αE-catenin was detected compared with that seen in the remote areas of the same patients. In contrast, αE-catenin expression was almost completely absent in the infarct zone of infarct rupture patients (Fig. 3A).

We also performed Western blotting for β-catenin (Fig. 3B), γ-catenin (Fig. 3C), and N-cadherin (Fig. 3D). The

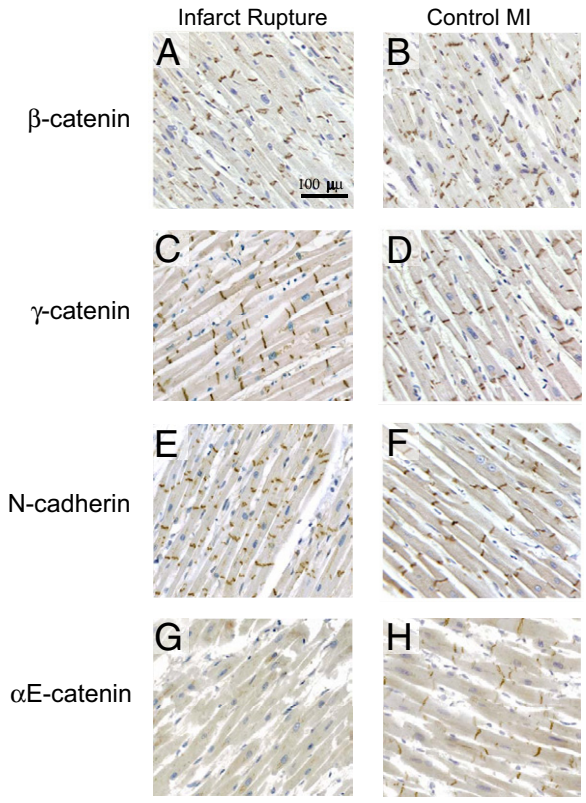

Figure 2 **Immunohistochemical Characterization of Cell Adhesion Complex Proteins in the Remote Area**

Representative images of immunostaining for β-catenin **(A, B)**, γ-catenin **(C, D)**, N-cadherin **(E, F)**, and αE-catenin **(G, H)** in remote parts of ruptured hearts **(A, C, E, G)** and control myocardial infarction (MI) hearts without rupture **(B, D, F, H)**. Abundant and similar staining for β-catenin, γ-catenin, and N-cadherin was observed in the intercalated disk regions of the cardiomyocytes in infarct rupture and control MI groups. For αE-catenin, abundant staining of intercalated disks between cardiomyocytes was observed in the control MI hearts **(H)**. On the contrary, 90% of the patients from the infarct rupture group showed either weak or no detectable αE-catenin staining **(G)**.

remote tissue from both the infarct rupture and control MI patients demonstrated similar amounts of these cell adhesion molecules. Also, no significant difference was observed in β-catenin, γ-catenin, and N-cadherin content in the infarct area of infarct rupture patients compared with that seen in control MI patients. Furthermore, in the infarct rupture group, β-catenin, γ-catenin, and N-cadherin all decreased significantly in the infarct area compared with remote myocardium.

Sequencing. To evaluate potential mutations or polymorphisms of the αE-catenin gene in the infarct rupture patients that could lead to expression of a dysfunctional protein (i.e., unable to localize to the ICDs), we sequenced each of the 16 coding exons of αE-catenin (CTNNA1) in 3 infarct rupture and 3 control MI patients with transmural infarcts. Although more than 20 single nucleotide polymorphisms (SNPs) have been described for the αE-catenin gene, we only observed a single SNP in exon 16 (rs1059110,

Table 2 Immunohistochemical Distribution of Adhesion Complex Proteins in Myocardial Specimens Obtained From Patients With and Without Infarct Rupture

	Control MI (n = 20)			Infarct Rupture (n = 20)		
Detectable Staining (%)	+	±	−	+	±	−
β-catenin	100			100		
γ-catenin	100			100		
Pan-cadherin	80	20		80	20	
αE-catenin	60	20	20	10	20	70

Detectable staining of the intercalated disc region was designated as + or ±, when staining was observed in ≥3 or 1 to 2 sections, respectively, per 4 sections examined. Immunohistochemical staining was designated as (−) when no detectable staining was observed in all 4 sections.
MI = myocardial infarction.

G to A) in this study. In the rupture group we observed the following variants: 1 patient was GG, 1 patient was GA, and 1 patient was AA. In the control group 2 patients were GG and 1 patient was AA. Based on these limited results, we conclude that there is no evidence for a role of this SNP in infarct rupture.

αE-catenin in experimental MI. Heterozygous αE-catenin C-terminal deficient mice were used to study the effect of reduced αE-catenin content on infarct rupture, to mimic clinical scenario in ruptured MI patients. After induction of MI, only 25% of the heterozygous mice were alive 7 days post-MI, as compared with 75% of their wild-type littermates (Fig. 4A). All mice died of infarct rupture, diagnosed by massive blood loss in the thoracic cavity. Kaplan-Meier analysis demonstrated significantly higher mortality in the heterozygous mice ($p = 0.038$). Interestingly, no evidence of spontaneous heart muscle disease was obtained in noninfarcted heterozygous mice up to 70 weeks after birth (data not shown).

Immunohistochemical characterization revealed less intense staining of the intercalated disks in the heterozygous mice compared with that seen in wild-type littermates (Fig. 4C). On the contrary, other components of the cell adhesion complex proteins revealed no differences between wild-type and transgenic mice (data not shown). Similarly, Western blotting also demonstrated a significant decrease in αE-catenin protein expression in the heterozygous mice compared with that seen in their wild-type littermates (Fig. 4D) ($p < 0.05$). The reduction of αE-catenin in the heterozygous mice was similar to that observed in the infarct rupture patient group. Other

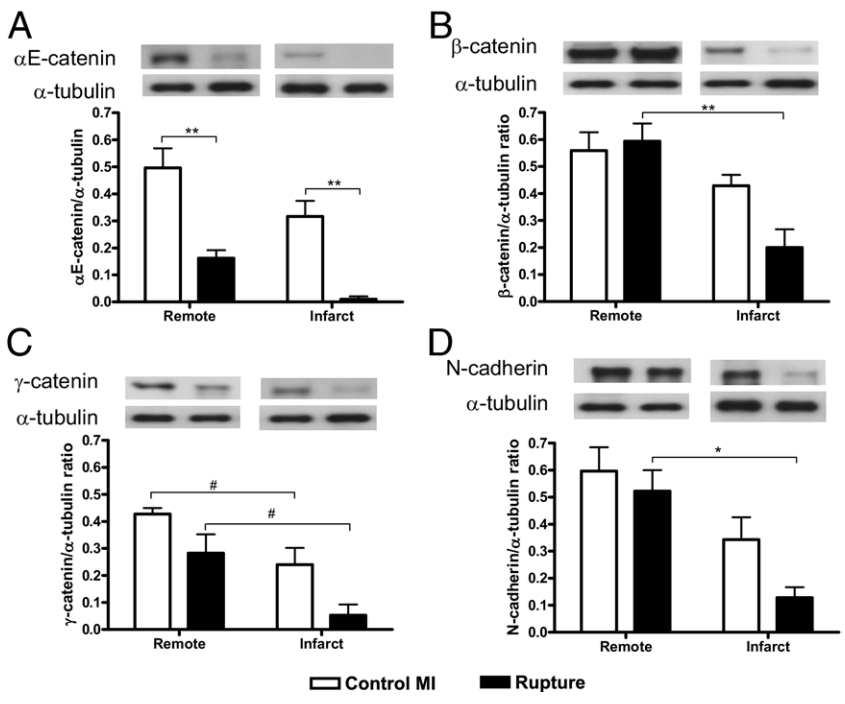

Figure 3 Immunoblotting Studies for Characterization of Cell Adhesion Complex Proteins

(Top) Representative Western blots of tissue obtained from infarct rupture patients and control myocardial infarction (MI) patients, both from remote (nonischemic) and infarct areas. **(A)** αE-catenin, **(B)** β-catenin, **(C)** γ-catenin, and **(D)** N-cadherin; α-tubulin was used for normalization of the samples. **(Bottom)** Quantitative analysis of adhesion complex proteins, expressed as protein/α-tubulin ratio. Significantly less αE-catenin was detected in the remote area of infarct rupture patients (n = 10) compared with that seen in control MI patients (n = 10). In the infarct area, αE-catenin was undetectable in the infarct rupture group but readily detectable in the control MI group. Similar levels of β-catenin, γ-catenin, and N-cadherin were observed in the remote areas of infarct rupture and control MI groups, although more extensive degradation of these proteins was detected under ischemic conditions. *$p < 0.01$; **$p < 0.001$; #$p < 0.05$.

adhesion complex proteins in the transgenic mice remained unaffected (Figs. 4E to 4G). After MI, however, a reduced γ-catenin and N-cadherin expression was observed in the infarct zone of the heterozygous mice, compared with that seen in uninfarcted tissue obtained from interventricular septum ($p < 0.01$).

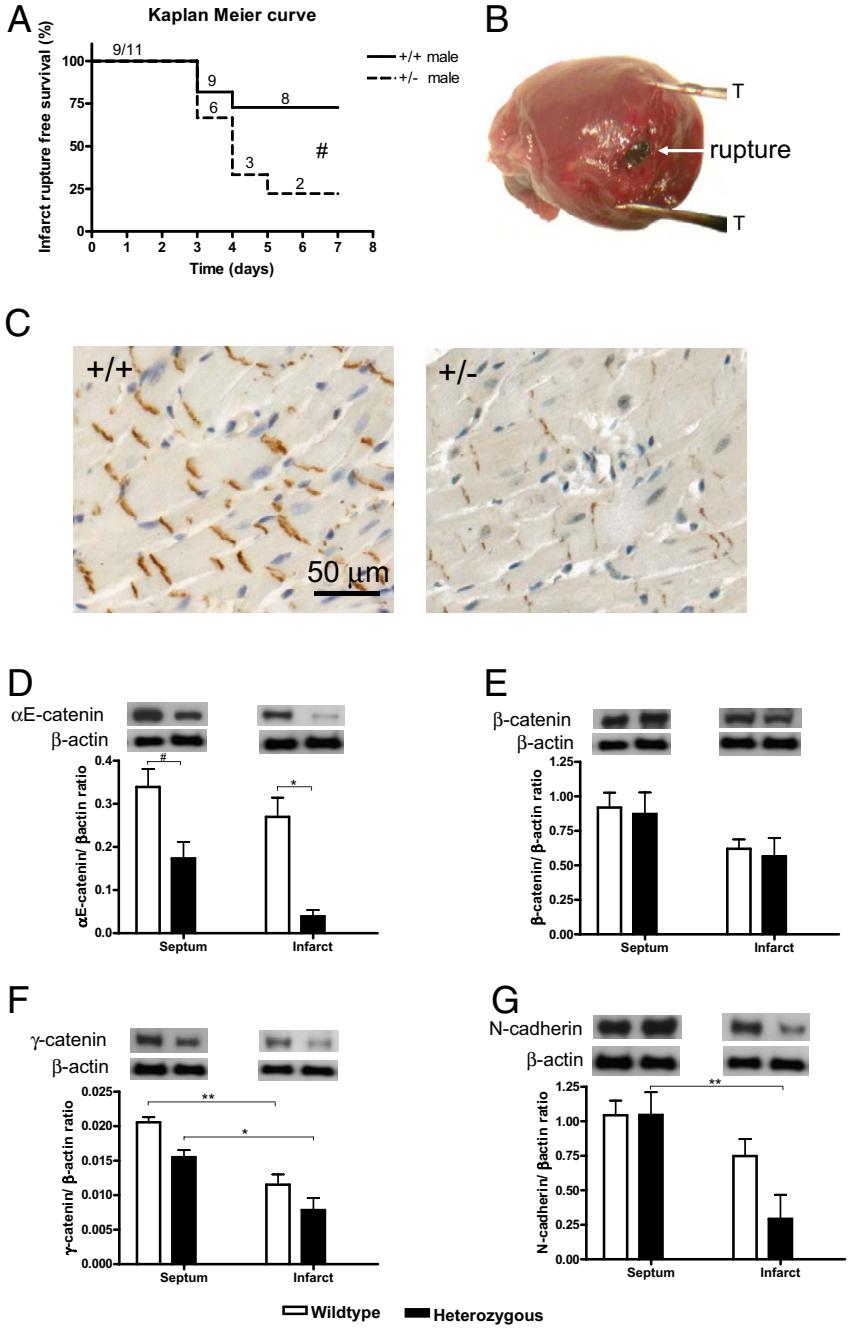

Figure 4 **Heterozygous αE-catenin C-Terminal Deficient Mice Show Susceptibility to Infarct Rupture Post-MI**

(A) Kaplan-Meier curve analysis of heterozygous αE-catenin C-terminal deficient mice (n = 9) showed significantly lower rupture-free survival post-myocardial infarction (MI) (#p = 0.038) compared with their wild-type littermates (n = 11). **(B)** Macroscopic image of infarct rupture (T = tweezers). The **arrow** points at the tear in the infarct area, showing the blood loss through the ventricular wall. **(C)** Representative images of immunohistochemical analysis of αE-catenin, showing less intense staining of the intercalated disks in the heterozygous mice compared with staining in the wild-type littermates. **(D to G) (Top)** Representative Western blot analysis for αE-catenin **(D)**, β-catenin **(E)**, γ-catenin **(F)**, and N-cadherin **(G)** in heterozygous C-terminal αE-catenin deficient mice and their wild-type littermates, both from septum (nonischemic) and infarct tissue; β-actin was used for normalization of the samples. **(Bottom)** Quantitative analysis of adhesion complex proteins, expressed as protein/β-actin ratio, demonstrating significantly lower αE-catenin in mice heterozygously deficient for C-terminally truncated αE-catenin (n = 9) compared with that in their wild-type littermates (n = 11). The other cell adhesion proteins were unaffected, although N-cadherin showed more degradation under ischemic conditions in the heterozygous mice. *$p < 0.01$; **$p < 0.001$; #$p < 0.05$.

Discussion

Characterization of the cell adhesion complex proteins in the myocardial specimens obtained from infarct rupture patients demonstrates a significantly reduced expression of αE-catenin and a lack of localization of this protein in the intercalated disks of both the infarct area and the remote, nonischemic parts of infarct rupture hearts. These observations suggest that infarct rupture patients have an intrinsic defect in their cell adhesion complex, which may have little effect on normal cardiac function, but may predispose the myocardium to rupture after transmural MI. The defective expression and localization of adhesion complex protein is not determined genetically. We sequenced the 16 exons that form the entire coding region of αE-catenin in infarct rupture patients and compared these sequences with those obtained from control MI group. A single SNP was observed; however, this SNP was not consistently linked to the infarct rupture group. Therefore, mutations that affect αE-catenin at the protein level are unlikely to explain the reduced amounts and lack of localization in infarct rupture patients. However, this does not rule out that αE-catenin mutations that affect messenger ribonucleic acid splicing and stability are present in infarct rupture patients. These mutations have been shown to reduce the stability of the cadherin/catenin complex in human cancer cells (11). Unfortunately, messenger ribonucleic acid obtained from postmortem tissue samples is severely damaged and cannot be used to study this hypothesis.

Interestingly, phosphorylation of the Tyr^{148} residue of α-catenin has been shown to enhance its interaction with β-catenin at the adherens junction (19). Moreover, several phosphorylation sites on β-catenin ($Tyr^{142, 489}$ and ^{654}) and cadherin ($Ser^{684, 686}$ and ^{692}) have been proposed to play a role in the formation of the adherens junction complex (10,20,21). Analysis of the effect of these post-translational modifications on cell adhesion complex formation in cardiomyocytes and their relation to infarct rupture is desirable.

To test the role of defective adhesion protein complex in infarct rupture in an animal model, we used mice heterozygous for C-terminal truncated αE-catenin. Deletion studies have shown that the C-terminus is required for strong cell adhesion (22). The immunohistological and Western blotting characteristics of the cell adhesion molecules in these mice were remarkably similar to those observed in human infarct rupture victims: significantly reduced αE-catenin expression was observed localized to intercalated disk region in remote myocardium in comparison with normal expression and distribution of other components of the cell

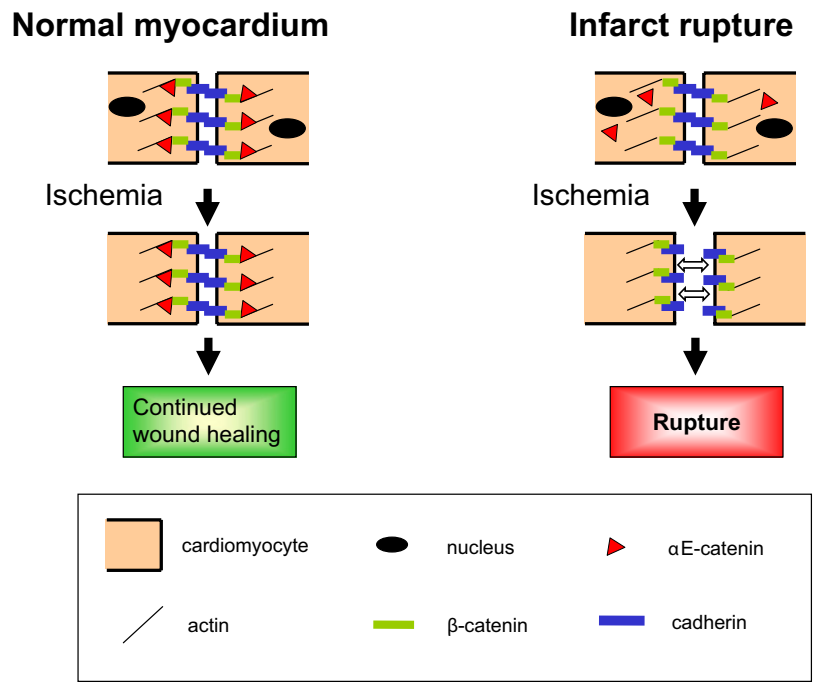

Figure 5 Cartoon Depicting the Role of a Defective Intercellular Adhesion Complex in Infarct Rupture in Humans

In the **left** part of the cartoon, the normal components of the cell adhesion complex of cardiomyocytes from patients with normal infarct healing are shown. In the uninjured cardiomyocytes of normal hearts, αE-catenin is localized in the intercalated disks. Ischemia induces death of the cardiomyocytes in the infarct area, as shown by the loss of their nucleus. However, the integrity of the infarct area is sufficiently preserved by the adhering cardiomyocytes, and the wound healing and granulation tissue formation continue. As shown in the **right side** of the cartoon, αE-catenin is not localized in the intercalated disks of the cardiomyocytes in rupture-prone hearts. After ischemia, this abnormal cell adhesion complex causes the dead cardiomyocytes in the infarct area to loosen up their connections, causing further deterioration of the integrity of the infarct area and subsequently leading to infarct rupture.

adhesion complex (β-catenin, γ-catenin, and N-cadherin). Induction of MI in these mice resulted in a significantly higher frequency of infarct rupture than their wild-type littermates. Interestingly, when not exposed to cardiac ischemia, these heterozygous mice did not show any signs of cardiac malfunction for up to 70 weeks of age.

The alterations in the cell adhesion complex, observed in the infarct rupture patients and animals, seems to affect the integrity of myocardial skeleton in the infarct area, even though most of the cardiomyocytes are necrotic (Fig. 5). A reduced cardiomyocyte adhesion could facilitate the migration of inflammatory cells into the infarct area, explaining the high numbers of these cells reported in ruptured infarcts; attendant up-regulation and activation of proteolytic enzymes may further facilitate infarct rupture (14,15,23).

Conclusions

The present study demonstrates that a decreased αE-catenin cell adhesion protein expression and localization predispose to infarct rupture. The results of this study lead to the hypothesis that infarct rupture is associated with an abnormality of the cell adhesion complex of the cardiomyocytes. Further research on the mechanisms that control the localization of cell adhesion molecules in the intercalated disks will be needed to better understand this proposed molecular mechanism of infarct rupture.

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APPENDIX

For a list of the primers used for PCR and sequencing of αE-catenin, please see the online version of this article.