The basement membrane of intramyocardial capillaries is thickened in patients with acute myocardial infarction

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ABSTRACT

Background: Atherosclerotic epicardial coronary arteries are a major cause of acute myocardial infarction (AMI). Recently, we found that intramyocardial capillaries may also play a role in AMI induction. We now have evaluated intramyocardial capillaries using ultrastructural analysis in AMI patients.

Methods: 43 AMI patients (with AMI in the left ventricle) and 27 controls were studied. No patient included in either group had diabetes mellitus. Basement membrane (BM) thickness of intramyocardial capillaries was determined using electron microscopy. BM thickness was also studied in a rat AMI model.

Results: BM thickness in the left ventricle of AMI patients was significantly higher than in controls (102 ± 9 nm vs. 77 ± 4 nm; p=0.016). This increase was not found in the right ventricle. In AMI patients, BM thickness was already increased in recent infarcts and did not increase further with infarct age. No correlation was found between BM thickness and the amount of stenosis or atherosclerotic plaque stability of epicardial coronary arteries. In addition, BM thickness did not differ between control rats and AMI rats.

Conclusions: These results suggest that BM thickening constitutes significant changes in the intramyocardial capillaries in patients that develop AMI. Also these changes are likely to occur prior to the induction of AMI.
INTRODUCTION

Acute myocardial infarction (AMI), remains a leading cause of morbidity and mortality worldwide. It is common knowledge that AMI is mainly caused due to alterations or abnormalities in coronary artery structure or function, especially epicardial coronary arteries, which can cause abrupt changes in regional blood flow and provoke acute ischemia, contributing to arrhythmia and sudden death. AMI involving the left anterior descending coronary artery is found to be a strong determinant of increased myonecrosis, reduced left ventricular function and higher mortality, compared with infarction in other vascular territories. In a previous study, however, we have found evidence for pre-existing accumulations of Nε-(carboxymethyl)lysine (CML), an advanced glycation end product (AGE), in intramyocardial small arteries in patients with AMI but without diabetes mellitus (DM) that was not related to stenosis of epicardial coronary arteries. These results suppose that pre-existing aberrations in the intramyocardial (micro) vasculature may contribute to the induction of AMI as well.

DM is also a major risk factor for coronary artery disease and coronary artery events, and it is related to the formation of AGEs resulting in vascular stiffening. In a former study, we showed accumulation of the AGE CML in intramyocardial small arteries in patients with DM. It is known that high levels of AGEs can cause increased thickening of the basement membrane (BM) in the diabetic kidney probably caused by the accumulation of plasma proteins or structural proteins, which eventually causes dysfunction of the filtration process. In line with this, BM thickness of capillaries was found to be significantly increased in endomyocardial biopsy specimens in patients with DM compared to control patients. However, to our knowledge a putative relationship between BM thickness of intramyocardial capillaries and AMI independent of DM has not been analyzed. The present study demonstrates that such relation exists.

MATERIALS AND METHODS

Human heart tissue

Human hearts were obtained at autopsy (n = 70) as soon as possible (at most 24 hours after death). We included 43 patients who died of AMI, of which one patient had an infarction 3 hours before he died by drowning, and 27 control patients who died from a cause not related to cardiac disease (table 1). None of these patients had DM. Fifteen patients suffered from hypertension (10 patients with AMI and 5 controls) and were treated with antihypertensive drugs. Four patients with AMI had angina pectoris, but the majority of the patients with AMI had no cardiovascular history.
Table 1: Clinical data of patients included in the study.

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<tr>
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<th>Controls (n=27)</th>
<th>Acute myocardial infarction (n=43)</th>
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<tr>
<td>Mean age (range), years</td>
<td>49 (24 – 92)</td>
<td>55 (22 – 83)</td>
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<tr>
<td>Male/female</td>
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<td>28/15</td>
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<td>Cause of death</td>
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<td>Intra-uterine infection (n = 1)</td>
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AMI, acute myocardial infarction; CVA, cerebro vascular accident; DC, decompensatio cordis; MOF, multiple organ failure; PHT, pulmonary hypertension; RI, respiratory insufficiency.

Lactate dehydrogenase staining of the heart was used to determine and localize myocardial infarction. Loss of lactate dehydrogenase is indicative of an infarction of at least 3-4 hours old. All included infarcts in this study were diagnosed using the lactate dehydrogenase staining method.

We only included AMI patients that had an infarction of the left ventricular anterior wall. In AMI patients, tissue from this infarction area was studied, while in controls corresponding areas of the heart were analyzed. In addition, of each patient, tissue from the right lateral ventricular wall of the heart was investigated. The included infarctions were determined to be between 3-4 hours and two weeks old. This study was approved by and performed
according to the guidelines of the ethics committee of the VU University Medical Center, Amsterdam. Use of leftover material after the pathological examination was completed is part of the patient contract in our hospital.

**Electron microscopy**
Heart tissue from all patients was examined using the electron microscope. Heart slides were fixed in 4% formalin and refixed in 2% (v/v) gluteraldehyde for 30 min and 1.5% (w/v) osmium tetraoxide for 10 min, dehydrated with acetone and embedded in Epon 812. Ultra thin sections were collected on 300-mesh Formavar-coated Nickel grids. The sections were contracted with uranyl acetate and lead citrate and were examined in a Jeol-1200 EX electron microscope. BM thickness of intramyocardial capillaries was quantified using QPRODIT25. Per blood vessel, the highest and lowest BM thickness was measured. From each ventricle 10 intramyocardial capillaries were chosen for analysis at random by the electron microscope technician. This electron microscope technician did not know whether the tissue was from control or AMI patients or from left or right ventricle. The capillaries, therefore, were chosen at random.

**Epicardial coronary artery**
The left coronary artery (LCA) of control patients and patients with AMI was studied with respect to the percentage of stenosis after microscopical evaluation: grade 0 = 0%, 1= 0-25%, 2= 25-50%, 3= 50-75% and 4= 75-100% stenosis. In each patient, the whole LCA was taken out, and was fixated and decalcified, and then cut into cross sections of approximately 5 mm in length. These cross-sections were examined macroscopically to assess presence of occlusion. After this, 4-7 cross-sections with the highest macroscopical level of occlusion were embedded for microscopical histological analysis and occlusion scoring. For the evaluation of a putative correlation between the percentage of LCA stenosis and BM thickness of intramyocardial capillaries, the highest stenosis score of the LCA was used in each patient. In addition, histological atherosclerotic plaque (in)stability was determined within these embedded cross-sections of the LCA. Unstable plaques were identified as such in case a thin fibrous cap and/or inflammatory cells at the endothelial layer were found within the LCA. When none of these observations were found, it was identified as a stable plaque26. For further analysis, patients were divided into 2 groups, patients with unstable plaques and patients with stable plaques independent of the presence of AMI.

**The in vivo rat AMI model**
Rats were anesthetized intramuscularly with Hypnorm®/Dormicum® (fentanyl + fluanisone 0.5 ml/kg; midazolam 5 mg/kg). Hypnorm was from Janssen Pharmaceuticals B.V. (Tilburg, The Netherlands). Dormicium was from Roche Nederland B.V. (Mijdrecht, The Netherlands).
The rats were respirated (with room air) using a mechanical ventilator set to 70 breaths/min (7 ml/kg volume). To induce AMI a ligature (6.0 suture) was placed around the left coronary artery for 30 minutes, followed by 5 days of reperfusion. Hearts were then excised and prepared as described above (section electron microscopy).

**Statistical analysis**

Statistics were performed using the SPSS statistics program (windows version 11.5, SPSS Inc., Chicago, IL). Because of non-normal distribution of the data, the data were transformed to logarithmic values. Data were analyzed using a one-way ANOVA, paired t tests and independent t tests. Also post-hoc Bonferroni tests were conducted. Levene's test was used for homogeneity of variances. p values ≤0.05 were considered significant. Correlation analysis was performed using Pearson's correlation coefficient. We used the following guidelines for determining the strength of the correlation: r = 0.10 to 0.29 and -0.10 and -0.29 are a weak or small correlation, r = 0.30 to 0.49 and -0.30 to -0.49 are a medium correlation and r = 0.50 to 1.0 and -0.50 to -1.0 are a strong or large correlation. When using Pearson's correlation, many authors in this area suggest that statistical significance should be reported but ignored, and the focus should be directed at the amount of shared variance. Therefore we also calculated the coefficient of determination.

**RESULTS**

In control patients, the mean BM thickness of intramyocardial capillaries in the left ventricle of the heart was 77 ± 4 nm, varying from 44 nm to 150 nm (figure 1A, 2). In patients with AMI (varying between 3-4 hours and 2 weeks), the mean BM thickness of the capillaries in the infarction area (left ventricle) was 102 ± 9 nm varying from 51 nm to 422 nm (figure 1B, 2). The mean BM thickness of the capillaries in the infarction area was significantly higher than the mean BM thickness in the left ventricle of control patients (p = 0.006; figure 2).

To compare BM thickness in AMI patients between infarcted tissue and non-infarcted tissue, BM thickness of capillaries was determined in the infarction area from the left ventricle and non-infarcted tissue from the right ventricle. In these patients with AMI, the mean BM thickness of the capillaries in the infarction area was significantly higher than the mean BM thickness of the right ventricle (86 ± 6 nm, p = 0.002; figure 2), while in control patients, the BM thickness did not significantly differ between left (77 ± 4 nm) and right ventricle (84 ± 7 nm). Also, the BM thickness of the capillaries of the right ventricles in AMI and control patients did not significantly differ.
Figure 1
A. Electron microscopic picture of the basement membrane (arrows) in the left ventricle of a control patient. Magnification: x15000. B. Electron microscopic picture of the basement membrane (arrows) in the infarction area within the left ventricle of a patient who died of acute myocardial infarction. Mean thickness is 422 nm. Magnification: x6000.

Figure 2
Box plots of comparison between mean basement membrane thickness of intramyocardial coronary arteries in the left and right ventricle (LV and RV) of control and acute myocardial infarction patients (AMI). The error bars represent 1.5 times the interquartile distance, the boxes represent the lower and upper quartiles and the black lines within the boxes represent the medians.

We also analyzed a putative relation between the age of the infarct and the BM thickness of the infarction area. Patients with AMI were divided into three groups: patients with an infarction of less than 6 hours old (this is the early infarction phase, no histological changes can be seen yet), those with an infarction between 6 hours and 5 days old (this is the inflammatory phase of AMI, extravascular accumulation of neutrophilic granulocytes can be seen) and those with an infarction older than 5 days (this is the remodeling phase of AMI, formation of granulation tissue is found in this phase). Already at AMI <6 hours, a significant
A possible relation between the age of the patients and the BM thickness in the left ventricle was also analyzed. Patients with AMI and controls were divided into 3 groups: 0-40 years, 41-70 years and 71-100 years old. In both AMI and control patients, no significant difference in BM thickness of the capillaries was found between the different age groups (not shown).

A putative correlation between the BM thickness of left ventricular intramyocardial capillaries and the degree of stenosis of the epicardial LCA was investigated in part of the patients included in this study, namely nine control patients and 22 patients with AMI. The mean grade score of stenosis in the LCA in control patients was 3.3 (50-75% stenosis). In patients with AMI, the mean grade score of stenosis in the LCA was also 3.3 (50-75% stenosis) and thus did not differ from that in control patients. In both control patients and patients with AMI, no correlation between the amount of stenosis in the epicardial LCA and BM thickness of left ventricular intramyocardial capillaries was found ($r = 0.046$, $p = 0.906$ and $r = 0.001$, $p = 0.996$ respectively). This means that there is no positive linear relation between the amount of stenosis and BM thickness. Also, the coefficient of determination (see ‘Materials and Methods’) was 0.21% for control patients and 0.0001% for patients with AMI. This indicates that the variation in BM thickness of intramyocardial capillaries cannot be explained by variations in the amount of stenosis of the epicardial LCA.

A possible relation between BM thickness of left ventricular intramyocardial capillaries and atherosclerotic plaque stability in the LCA was also analyzed in part of the patients included in this study. Five patients showed no signs of unstable plaques, 26 patients did have unstable plaques in the LCA. However, no significant difference in BM thickness was found between both groups ($p = 0.934$).

To determine whether AMI can induce BM thickening, the BM thickness of left ventricular intramyocardial capillaries was measured in an in vivo rat AMI model. For this, we used six control rats and five AMI rats with an infarction of 5 days old. However, we did not find a significant difference in BM thickness between the control and AMI rats in the left ventricle of the heart ($p = 0.343$; figure 3).
DISCUSSION

To the best of our knowledge, this is the first paper analyzing BM thickening in intramyocardial capillaries in patients with AMI. We have found that in the infarction area of patients with AMI the BM thickness of capillaries was significantly higher compared to corresponding heart tissue in control patients (102 ± 9 nm vs. 77 ± 4 nm, p = 0.006) and compared to non-infarcted areas of those patients with AMI (86 ± 6 nm, p = 0.002).

The BM thickness of intramyocardial capillaries in autopsy hearts of control patients measured here is in accordance with reported BM thickness measurements in endomyocardial biopsies of living patients in non-DM, non-AMI patients, which were, respectively, 80 nm \(^2\) and 75 ± 15 nm \(^2\). This thus indicates that BM thickness is not influenced by changes after death. In another study, no effect of hypertension on BM thickness of capillaries in the heart was found (67 ± 8 nm) \(^2\). We also did not find that this increase in BM thickness was related to the age of the patients, in agreement with previous studies\(^{28,29}\). In patients with DM, but without AMI, increased BM thickness in the heart has been described between 98 and 153 ± 48 nm \(^2\). Therefore, we excluded DM patients in the present study.

Notably, the duration of the infarction was not related to the aberrant BM thickness of intramyocardial capillaries within the infarction area in patients with AMI. The BM thickness in the infarction area was already significantly increased in patients with AMI less than 6 hours old and did not increase further with infarct age. In addition, we show in the rat AMI model that AMI followed by 5 days of reperfusion and the concomitant inflammatory reaction did not result in BM thickening. Not a lot is known about the timeframe in which BM thickening can manifest itself. However, in diabetic rat models BM thickening only became significant
after months\textsuperscript{30}, whereas in another rat model, the earliest extra cellular matrix alterations involved in BM thickening were detected 48 hours after VEGF injection\textsuperscript{31}. These data suggest that it is unlikely that a significant increase in BM thickness can manifest itself within 6 hours, thereby making it likely that the BM thickening in AMI hearts occurred prior to the AMI and not as a consequence.

The exact mechanism of the BM thickening in patients with AMI is unknown. However, in patients with DM, BM thickening in the kidney is caused by matrix expansion which is due to expression of extracellular matrix (ECM) components collagen, fibronectin and laminin\textsuperscript{32,33}. Fibrotic factors such as TGF-β1 and CTFG were found to play a key role in the BM thickening and increased ECM in patients with DM\textsuperscript{34}. These fibrotic factors are known regulators of ECM accumulation and were found to stimulate the production of collagen type IV, fibronectin and laminin in diabetic patients\textsuperscript{35-37}.

It is unclear how BM thickening of the intramyocardial arteries can contribute to AMI. One of the possibilities is that the thickened basement membrane limits the exchange of oxygen, inducing or aggravating hypoxic stress.

We also found in patients with AMI that variation in BM thickness of intramyocardial capillaries was not dependent on the amount of stenosis, nor on atherosclerotic plaque stability of the epicardial coronary artery. In addition, in another study (Baidoshvili et al.\textsuperscript{15}) we recently found that the AGE CML was present in intramyocardial small arteries in patients with AMI. These data suggested that the CML depositions in these intramyocardial small arteries preceded the onset of AMI and were independent of epicardial coronary artery stenosis.

In conclusion, these results suggest that BM thickening and also our previous finding of the formation of AGEs\textsuperscript{15} constitute significant changes in the intramyocardial capillaries in patients who develop AMI. Also, these changes are likely to occur prior to the induction of AMI and are independent of the condition of the epicardial coronary arteries. These data imply that prior to the development of AMI, there are substantial aberrations in the intramyocardial blood vessels. Whether these aberrations are in a causal conjunction with the induction of AMI remains to be established.
REFERENCES


