Type A dissection and chronic dilatation: tenascin-C as a key factor in destabilization of the aortic wall

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Abstract

OBJECTIVES: Tenascin-C plays an important role in myocardial and vascular remodelling. We hypothesized that tenascin-C is a key factor in the development of degenerative disease of the ascending aorta, leading to chronic dilatation and acute aortic dissection.

METHODS: Ascending aortic wall specimens were obtained during surgery for chronic dilatation ($n = 52$) and acute Type A dissection ($n = 30$). Patients ($n = 12$) undergoing aortic valve replacement served as controls. Tenascin-C expression was evaluated by immunostaining and semi-quantitatively assessed using the ImageJ software. TN-C levels in peripheral blood were determined by enzyme-linked immunosorbent assay.

RESULTS: Histological examination showed a clear difference between chronic dilatation and acute dissection. In chronic dilatation, tenascin-C staining was homogenously distributed throughout the media parallel to vascular smooth muscle cells. In acute dissection, a strong staining with a heterogenous and spotty distribution was detected. Control aortas showed no tenascin-C staining. Tenascin-C expression was significantly higher in Type-A dissection compared with chronic dilatation. This was accompanied by a significant elevation of tenascin-C levels in peripheral blood in acute dissection. There was no statistical correlation between the tenascin-C level in peripheral blood and the aortic diameter either in dissection or in dilatation.

CONCLUSIONS: Tenascin-C is a marker of progressive destabilization of the aortic wall independent of size in chronic dilatation and acute dissection. Therefore, it might be a valuable tool in guiding intervention strategies in patients with disease of the ascending aorta.

Keywords: Aortic aneurysm • Aortic dissection • Aorta/aortic pathology

INTRODUCTION

Acute Stanford Type A aortic dissection with an incidence of about 3–5 cases per 100 000 people per year still remains a life-threatening event with a high mortality and significant long-term morbidity [1]. Particularly in an elderly patient population without any known inherited connective tissue disorder, only little is known about the pathological mechanisms leading to aortic dissection. Further difficulties in therapeutic management and prevention arise from the fact that dissection may occur in addition to a thoracic aortic aneurysm or, as in most of the cases, without any pre-existing aneurysm [2].

Gene expression studies have shown that, in thoracic aortic disease, the expression pattern of genes regulating extracellular matrix (ECM) remodelling, apoptosis, cell survival and cell proliferation are altered [3]. This results in the major histopathological characteristics of thoracic aortic disease: degradation and disruption

of collagen and elastic fibres, and abnormalities of vascular smooth muscle cells (VSMCs) within the media [4].

The matricellular glycoprotein tenascin-C (TN-C) is a key factor in physiological and pathological conditions. These are modification of cell–matrix interaction and degradation of pre-existing matrix components as seen in embryonal development, wound healing and tumour invasion [5]. In cardiovascular pathology, TN-C is involved in post-myocardial infarction remodelling, neointimal hyperplasia and restenosis after percutaneous transluminal coronary angioplasty and bypass grafting, pulmonary vascular disease and hypertension [6–11]. In thoracic aortic aneurysms associated with Marfan syndrome and bicuspid aortic valve, an abnormal ECM protein transport with intracellular accumulation and reduction in extracellular distribution of TN-C could be detected [12].

The aim of the present study was to evaluate whether TN-C plays a role in degenerative disease of the ascending thoracic aorta, leading to dilatation and/or dissection.

In a second step, we wanted to assess whether TN-C plasma levels are elevated in patients with an ascending thoracic aortic aneurysm and Type A dissection, respectively, and therefore might serve as a surrogate marker for destabilization of the aortic wall.

MATERIALS AND METHODS

Study population and aortic specimen

Between 2008 and 2010, aortic specimens were collected from patients undergoing surgery of the ascending aorta due to chronic dilatation ($n = 52$) and acute Type A dissection ($n = 30$). Patients undergoing aortic valve replacement with a non-aneurysmal and macroscopically normal ascending aorta served as a control group ($n = 12$). Patients with the bicuspid aortic valve or any known connective tissue disorder such as Marfan syndrome were excluded from the study. In every patient with chronic dilatation and Type A dissection, respectively, a computed tomography scan was performed preoperatively and the diameter of the ascending aorta was measured at the level of pulmonary artery bifurcation. In control patients, the diameter of the ascending aorta was measured intraoperatively. In the dissection group, the site of the intimal tear was marked by the surgeon and the tissue at the height of and distal from the tear was used for histological analysis. In the chronic dilatation group, tissue samples were taken from the anterior part of the ascending aorta in the most dilated region. In the control group, aortic specimens were taken from the anterior part of the ascending aorta at the time of closing the aortotomy. Full thickness aortic specimens were fixed in formalin and processed for paraffin embedding. Clinical data such as history of hypertension, smoking and diabetes were taken from the medical records.

Written informed consent was obtained from all patients, and the protocol was approved by the National Ethics Committee.

Histology and immunohistochemistry

Paraffin-embedded specimens were cut into 5 μ m sections and routinely stained with haematoxylin-eosin (HE) and resorcin-fuchsin/orcein (Elastica staining). Histological sections were assessed for the pattern of cystic media necrosis (CMN) with fragmentation of elastic fibres, loss of media myocytes and accumulation of ECM with the formation of cystic structures.

Paraffin was removed with xylene followed by pre-treatment with 0.1% pepsin. TN-C staining was performed using a primary monoclonal mouse antibody, anti-human TN-C (1:10, Clone 4F10TT; IBL). Afterwards, the sections were incubated with a peroxidase-conjugated anti-mouse antibody (1:100), and colour development was performed by diaminobenzidine. For better orientation, the sections were weakly counterstained with haematoxylin.

Semi-quantitative assessment of TN-C staining was performed using the ImageJ imaging software. At a magnification of $\times 100$ in five random areas per section within the media, the colour component of interest was quantified as percent area of the total field.

Assay of serum tenascin-C levels

In a subgroup of patients (chronic dilatation $n = 27$; acute dissection $n = 17$ and control $n = 12$) immediately before surgery, blood

samples were taken and centrifuged at 15 000 g for 15 min. The supernatant was stored at -80°C for further analysis. Serum TN-C was measured by specific enzyme-linked immunosorbent assay (ELISA) using a anti-human TN-C monoclonal mouse IgG (Human Tenascin-C Large fibronectin type III (FN III)-C Kit, Immuno-Biological Laboratories IBL Co., Ltd) according to the manufacturer's protocol. This kit can detect the TN-C high molecular weight variant containing the C-domain of FN III repeats specifically. Sensitivity of the kit is 0.01 ng/ml.

Statistical analysis

Continuous variables were given as mean $\pm$ standard deviation and compared using the analysis of variance with *post hoc* testing. Categorical variables were expressed as absolute numbers and percentages and compared using the χ^2 test and Fisher's exact test. To test if aortic diameter and plasma TN-C levels are related, Pearson r testing was used. A value of $P < 0.05$ was considered statistically significant. All statistical analyses were done using the GraphPad Prism (Version 4.03 for Windows) statistical software.

RESULTS

Clinical data

Table 1 summarizes clinical patient characteristics. Mean age did not differ significantly between patients with chronic dilatation (65 ± 12 years), acute dissection (64 ± 12 years) and control patients (63 ± 10 years). Aortic diameter was significantly lower in control patients (31 ± 7 mm, $P < 0.001$; $F = 25.29$) compared with both aneurysm and acute dissection patients (59 ± 9 and 63 ± 18 mm; $P = 0.48$). There were no significant differences concerning gender, history of hypertension, smoking, dyslipidaemia and body mass index between groups.

Histological data

Light microscopic analysis showed that CMN was significantly more frequent in specimens from patients with acute dissection compared with chronic dilatation (86.6 vs 55.7%; $P = 0.006$). In

Table 1: Patient characteristics

	Chronic dilatation ($N = 52$)	Acute dissection ($N = 30$)	Control ($N = 12$)	P -value
Male gender	27 (51.9)	14 (46.6)	7 (58.3)	0.78
Age (years)	65 ± 12	64 ± 12	63 ± 10	0.77
Aortic diameter (mm)	59 ± 9	63 ± 18	31 ± 7	0.48^a $< 0.001^{b,c}$
Hypertension	40 (76.9)	18 (60)	7 (58.3)	0.19
History of smoking	10 (19.3)	7 (23.3)	2 (16.6)	0.86
Dyslipidaemia	19 (36.5)	17 (56.6)	5 (41.6)	0.13
Body mass index	26 ± 4	30 ± 4	25 ± 5	0.05

Data are given as mean $\pm$ standard deviation and absolute numbers (percentage).

^aChronic dilatation vs acute dissection.

^bChronic dilatation vs control.

^cAcute dissection vs control.

Table 2: Histological data

	Chronic dilatation (N = 52)	Acute dissection (N = 30)	Control (N = 12)	P-value
Cystic media necrosis (CMN)	29 (55.7)	26 (86.6)	0 (0)	0.006 ^a , <0.001 ^{b,c}
Atherosclerosis (AT)	23 (44.2)	9 (30)	4 (33.3)	0.41
Inflammation	8 (15.4)	7 (23.3)	0 (0)	0.17
CMN + AT	12 (23)	5 (16.6)	0 (0)	0.17
Only CMN	16 (30.8)	21 (70)	0 (0)	0.001 ^a , 0.02 ^b , <0.001 ^c
Only AT	10 (19.2)	5 (16.6)	4 (33.3)	0.46
Normal	10 (19.2)	0 (0) ^a	8 (66.6) ^b	0.002 ^{a,b} , <0.001 ^c

Data are given as mean $\pm$ standard deviation and absolute numbers (percentage).

^aChronic dilatation vs acute dissection.

^bChronic dilatation vs control.

^cAcute dissection vs control.

control aortas, CMN was completely absent. Signs of atherosclerosis were equally distributed between groups (chronic dilatation 44.2%; acute dissection 30% and control 33.3%; $P = 0.24$ chronic dilatation vs acute dissection, $P = 0.54$ chronic dilatation vs control, $P = 1.00$ acute dissection vs control). Inflammatory changes tended to be more frequent in both chronic dilatation and acute dissection compared with control, but data did not reach statistical significance.

A histological normal aortic wall was found in 19.2% of chronic dilatation and 65.5% of control patients ($P = 0.002$), whereas there was no patient with a normal histology in the dissection group ($P = 0.01$ vs chronic dilatation, $P < 0.001$ vs control). Data are summarized in Table 2.

Tenascin-C immunohistochemistry

All 30 patients with acute dissection showed strong TN-C staining with a very heterogenous and spotty distribution.

In chronic dilatation, three different staining patterns could be observed. In 38 specimens, TN-C positive areas were distributed homogenously throughout the whole media and oriented parallel to media myocytes. In 9 patients, TN-C staining was completely absent; in these patients, HE staining showed a normal aortic wall. Five patients showed a strong and spotty TN-C staining similar to the picture of acute dissection; all of these patients had signs of severe CMN.

In all 12 control patients, TN-C staining was completely absent in the media. Representative slides are shown in Fig. 1.

Semi-quantitative analysis of TN-C staining with the ImageJ imaging software revealed a significantly higher TN-C expression in acute dissection compared with chronic dilatation, whereas staining was almost absent in control aortas (Fig. 2A).

Serum tenascin-C levels

Serum TN-C levels in blood samples collected immediately before surgery were significantly higher in patients with acute dissection

compared with chronic dilatation (290 ± 121.9 vs 86.8 ± 45.9 ng/ml; $P < 0.001$; $F = 35.22$). There was no significant difference between chronic dilatation and control (86.8 ± 45.9 vs 67.8 ± 47.7 ng/ml; $P = 0.44$; Fig. 2B). There was no correlation between aortic diameter and serum TN-C levels either in patients with acute dissection or in patients with chronic dilatation (Fig. 3).

DISCUSSION

To the best of our knowledge, this is the first study to show that, in degenerative disease of the ascending aorta, the matricellular glycoprotein TN-C shows a distinct expression pattern dependent on the status of the disease. That is, a significantly higher TN-C expression with different distribution could be seen in emergency patients with acute Type A dissection compared with those with aneurysms of the ascending aorta scheduled for elective surgery. These histological findings were accompanied by higher TN-C levels in peripheral blood. In this study, we wanted to focus on patients with aortic aneurysm and dissection, respectively, due to degenerative disease of the thoracic aorta as a consequence of long-lasting hypertension, smoking and atherosclerosis, and therefore those with bicuspid aortic valve and with a known connective tissue disorder were excluded.

TN-C plays a role in both physiological as well as pathological remodelling of blood vessels. Its expression is triggered by factors like transforming growth factor β , interleukin-1, interleukin-4, tissue necrosis factor α , matrixmetalloproteinases (MMPs), angiotensin II and mechanical stress [13]. In the normal vasculature of normotensive rats, TN-C is expressed weakly, but homogenously distributed in the media with an increase in intensity with decreasing vessel size. The strongest staining was observed at branching sites to smaller arteries both in the media and the basement membrane implicating higher TN-C expression at sites of mechanical stress. In hypertensive rats, TN-C expression was significantly increased in foci scattered through the media and is supposed to contribute to the pathological process of hypertensive vascular remodelling [14].

Aneurysms of the ascending aorta are characterized by changes of the ECM and VSMCs, including overexpression of MMPs, degradation of collagens and elastic fibres, an increase in TN-C, fibronectin and osteopontin and a decrease in laminin [15, 16]. In this setting, there is an interplay between matricellular proteins, MMPs and collagen as TN-C itself can activate MMPs leading to collagenolysis and vice versa. Collagen I fragments and MMPs enhance TN-C synthesis promoting tissue degradation [17]. Furthermore, there are reciprocal influences of an altered ECM environment on VSMCs, which change from the contractile to the synthetic phenotype being the main source of TN-C synthesis in aortic aneurysms [15, 18]. Aforementioned changes of the ECM environment may even play a causative role in the apoptosis of VSMCs in aortic aneurysms [15]. Under normal circumstances, the physiological function of TN-C in vessel development, response to injury and increased mechanical stress is the promotion of survival and proliferation [17]. In an environment of MMP overexpression, however, TN-C degradation produces molecular fragments, which are proapoptotic for VSMCs [19]. These data indicate that TN-C as a proliferative and anti-apoptotic factor may be part of a compensatory mechanism and 'healing response' in the development of aortic aneurysms. If the disease progresses with increasing destruction of the tissue by inflammatory factors and MMPs, the anti-apoptotic and

Figure 1: Representative images in hematoxylin-eosin (HE), elastica staining and tenascin-C (TN-C) immunohistochemistry (magnification $\times 100$). **(A)** In acute dissection, HE and elastica staining show the typical picture of cystic media necrosis (CMN) with loss of media myocytes, accumulation of extracellular matrix (ECM) with the formation of cystic structures and disruption of elastic fibres. TN-C staining is strong with a very heterogenous and spotty distribution. **(B1–B3)** show the three different staining patterns found in chronic dilatation: In **(B1)**, signs of CMN in HE and elastica staining are present with homogenous distribution of TN-C staining throughout the media oriented parallel to the media myocytes. In **(B2)**, severe signs of CMN in HE and elastica staining are accompanied by a strong and spotty TN-C staining similar to the picture in acute dissection. **(B3)** shows a normal aortic wall in HE and elastica staining without TN-C positive staining of the media. All control patients **(C)** have a normal aortic wall in HE and elastica staining with a TN-C negative media.

proliferative effect of TN-C turns into the opposite, further promoting tissue destruction.

In a mouse model of abdominal aortic aneurysm, Kimura *et al.* [20] found a correlation between TN-C expression, growth rate of the diameter and histological destruction of the aortic wall. In specimens of human abdominal aortic aneurysms, a strong and patchy TN-C staining was observed in the most dilated region of the aneurysm accompanied by severe tissue destruction similar to the histological pattern we found in acute Type A dissection at the site of the intimal tear and in some of the patients with chronic dilatation at the site of maximum diameter [20]. These data imply that TN-C expression correlates with the severity of aortic wall destabilization and might therefore serve as a biomarker to assess the activity of the disease.

In a second step in order to evaluate whether TN-C might be a suitable biomarker in clinical practice to monitor disease activity

and possible risk of rupture in patients with degenerative disease of the ascending aorta, we assessed TN-C levels in plasma samples collected immediately before surgery. These data correlated well with histological findings and showed significantly higher TN-C levels in patients with acute dissection compared with those with chronic dilatation and control patients. TN-C levels, however, did not correlate with aortic diameter either in patients with acute dissection or in those with chronic dilatation. It is known that size is not a good predictor of the risk of rupture or dissection as more than 50% of acute dissections occur in an ascending aorta of <5 cm in diameter [2]. So one may speculate that a high TN-C expression is a marker for destabilization of the aortic wall independent of size.

However, before TN-C can be used as a tool in guiding clinical intervention strategies, several issues have to be clarified: (i) according to the existing data, we cannot say whether TN-C levels in patients with acute dissection were already elevated

Figure 2: (A) Tenascin-C (TN-C) expression by semi-quantitative assessment of TN-C immunohistochemistry in five random areas per section using the ImageJ software. Data are given as percent area of the total field. $**P = 0.002$ dissection vs dilatation; $P = 0.25$ dilatation vs control; $P = 0.03$ dissection vs control; $F = 0.04$ (B) TN-C levels in peripheral blood immediately before surgery assessed by ELISA. $**P < 0.001$ dissection vs dilatation, $P = 0.44$ dilatation vs control, $P = 0.002$ dissection vs control. Data are depicted as boxplots, boxes represent the inter-quartile range and whiskers define the minimum to maximum, line at median.

immediately before the event of dissection or TN-C was spilled over in the circulation as a consequence of dissection. Therefore, to highlight this question, the histological staining pattern in a larger group of patients with chronic dilatation has to be compared with plasma levels to see whether there is a correlation between a strong and patchy staining and high plasma levels. Additionally, samples of patients with chronic Type B dissection have to be evaluated, and the correlation between the extent of dissection and height and time course of TN-C levels in peripheral blood have to be determined. If TN-C was liberated from the media only during the constitution of the two lumina, its determination would be useless as a prognostic marker. Then, however, it might serve as a diagnostic tool under emergency conditions to approach the diagnosis of acute dissection. Thereby in combination with markers for myocardial ischaemia, it might accelerate and guide further diagnostic steps in patients with acute chest pain.

Figure 3: There is no statistical correlation between aortic diameter and plasma tenascin-C (TN-C) levels immediately before surgery neither in chronic dilatation (A) nor in acute dissection (B) according to Pearson r testing ($\alpha = 0.05$).

(ii) There was no difference in TN-C levels between chronic dilatation and control plasma, which was collected from patients with cardiovascular disease, i.e. aortic stenosis. It has been demonstrated that TN-C plasma levels are elevated as a consequence of cardiovascular disease and remodelling [6, 21]. Therefore, it might be possible that these 'control patients' had an elevation of TN-C levels as a consequence of cardiac remodelling and for both possible clinical applications of TN-C—either as a prognostic or as a diagnostic tool in the emergency setting—it will be necessary to determine a kind of 'TN-C baseline'. In a subsequent study in a larger patient cohort with cardiovascular disease with and without dilatation of the aorta, blood samples have to be evaluated and compared with healthy volunteers.

In conclusion, TN-C is a key factor in degenerative disease of the ascending aorta, with the highest expression levels in patients with acute Type A dissection, indicating severe destruction of the aortic wall. As plasma levels in dissection patients might be elevated as a consequence of dissection, further research is needed on whether TN-C might be a useful biomarker in clinical practice to guide intervention strategies in patients with dilatation of the ascending aorta, or might serve as a marker under emergency conditions to approach the diagnosis of Type A dissection more quickly.

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