

Dilated Cardiomyopathy Is Associated With Significant Changes in Collagen Type I/III ratio

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Background—It is controversial whether myocardial fibrosis in end-stage dilated cardiomyopathy (DCM) is associated with altered collagen type I/type III (Col I/Col III) ratio.

Methods and Results—Patients with DCM (ejection fraction [EF] <50%, n=12) and with mild global left ventricular dysfunction (EF >50%, n=18) were examined. Col I, Col III, and transforming growth factors- β_1 (TGF- β_1) and - β_2 (TGF- β_2) gene expression in endomyocardial biopsies was evaluated by quantitative competitive reverse transcriptase-polymerase chain reaction (qRT-PCR). Collagen content was quantified after picrosirius red and immunohistological staining and by hydroxyproline assay. In patients with EF <50%, there was a pronounced 2- to 6-fold increase of myocardial Col I mRNA abundance ($P<0.01$), with a corresponding 1.6-fold increase at the protein level versus that found in patients with EF >50%. The Col III mRNA abundance showed a 2.0-fold increase ($P<0.04$). There was a relevant shift in the Col I/Col III mRNA ratio for DCM patients (Col I/Col III, 8.2) compared with patients with an EF >50% (Col I/Col III, 6.4). In addition, total collagen content was increased in patients with EF <50% (n=3) ($4.3\pm 0.1\%$) compared with patients with EF >50% (n=8) ($2.7\pm 0.9\%$) ($P<0.004$). The biochemically determined ratio of hydroxyproline/total protein (n=12) was correlated to the Col I mRNA abundance ($P<0.05$, $r=0.77$). TGF- β_1 and TGF- β_2 showed elevated myocardial mRNA abundances (1- to 7-fold and 4- to 5-fold, respectively) in DCM patients.

Conclusions—Differential increase of Col I and Col III leads to an increased Col I/Col III ratio in DCM myocardium. Because Col I provides substantial tensile strength and stiffness, this may contribute to systolic and in particular diastolic dysfunction in DCM. (*Circulation*. 1999;99:2750-2756.)

Key Words: cardiomyopathy ■ collagen ■ growth substances ■ remodeling ■ polymerase chain reaction

The myocardial extracellular matrix is composed of a complex network of structural protein, mainly collagen type I (Col I) and collagen type III (Col III), which provide architectural support for the muscle cells and also play an important role in myocardial function.^{1,2} Several studies of dilated cardiomyopathy (DCM) have demonstrated changes in collagen content at the protein level, characterized primarily by an accumulation of Col I.³⁻⁵ However, whether there is also an increase in Col III remains controversial. The ratio of collagen types within the heart is of significance because of their different mechanical properties.⁶ For example, tissues with predominance of Col I (such as tendon) are characterized by strength and stiffness, whereas tissues containing large amounts of Col III (such as skin) are characterized by greater elasticity. Yoshikane et al⁵ documented an increase in the number and thickness of collagen fibers in end-stage DCM by electron microscopic and immunohistological methods. A biochemical analysis by Bishop and colleagues³ showed an increase in the absolute amounts of both Col I and Col III. In contrast, Weber et al⁷ demonstrated a pronounced increase in predominantly thin collagen fibers and a decrease

in thick collagen fibers in explanted hearts from patients with end-stage DCM compared with normal myocardium. The apparent discrepancy of these studies may be explained by differences in methodology; however, other factors, such as collagen cross-linking, may have influenced the results.

Our aim was to examine changes in messenger RNA (mRNA) abundance of collagen subtypes in endomyocardial biopsy samples from patients with and without diagnosis of DCM. Furthermore, we also sought to determine whether there were changes in transforming growth factors- β_1 (TGF- β_1) and - β_2 (TGF- β_2), cytokines known to play a crucial role in mediating collagen synthesis.⁸⁻¹⁰ Furthermore, the small amount of tissue available from endomyocardial biopsy samples required that we establish quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) assays to conduct the analysis of multiple genes.

Methods

Patients

Thirty patients with the presumptive clinical diagnosis of DCM were analyzed in this study. All patients underwent cardiac catheterization

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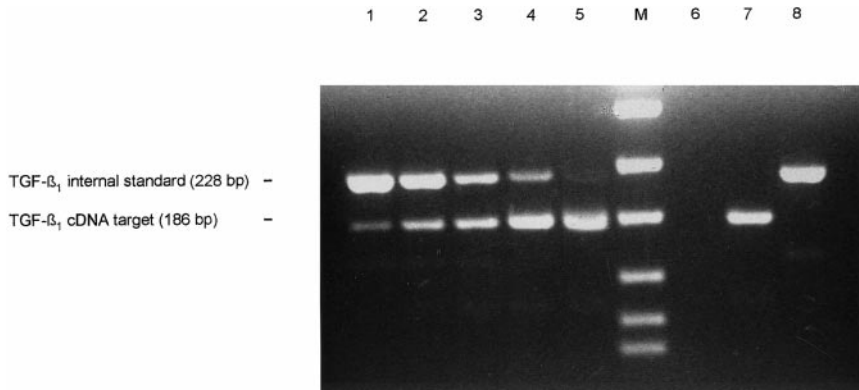


Figure 1. Ethidium bromide-stained 1.5% agarose gel of qRT-PCR for TGF- β_1 (lanes 1 through 5: qRT-PCR products; lane M: molecular weight marker; lane 6: negative control; lane 7: positive control cDNA TGF- β_1 ; lane 8: positive control for DNA standard TGF- β_1).

for further evaluation of impaired global or regional left ventricular function initially revealed by echocardiography and by reduced exercise tolerance with a minimum duration of 6 months. Right and left heart catheterization, including standard hemodynamic measurements, was carried out in all patients in whom coronary, hypertensive, and valvular heart disease as well as restrictive or constrictive heart disease had been excluded before an endomyocardial biopsy was obtained. In addition, none of the analyzed patients had decompensated heart failure. Because of the limited number of available biopsies, a subgroup analysis for total collagen content by picrosirius red staining was performed in a subgroup of 11 patients ($n=3$, EF $<50\%$, and $n=8$, EF $>50\%$) from the total study group of 30. In addition, in another subgroup of 12 patients (mean EF, $49 \pm 11\%$) from the total study group, the ratio of hydroxyproline concentration/total protein concentration (both $\mu\text{g/mL}$) was correlated with the collagen mRNA abundance.

All procedures were performed in accordance with ethical standards and with the Helsinki Declaration of 1975. In addition, all patients gave informed consent for all of the invasive studies performed.

Biopsy

Endomyocardial biopsies were taken from the right ventricular septum in all patients with a Cordis biptome. From these small biopsy samples, we measured (1) Col I and Col III mRNA abundance, (2) mRNA abundance for TGF- β_1 and TGF- β_2 , (3) mRNA abundance for GAPDH, (4) total collagen content histologically by picrosirius red staining, (5) Col I and Col III expression immunohistologically on the protein level, (6) hydroxyproline concentration biochemically, and (7) CD3 T lymphocytes as a marker of inflammation.

RNA Extraction

Total RNA was isolated from all 30 samples by a modification of the lithium chloride method according to Auffray et al.¹¹

Reverse Transcription

For the PCR, 300 ng total RNA of each biopsy was transcribed into cDNA by use of avian myeloblastosis virus reverse transcriptase (AMV RT) and random primer in accordance with the supplier's protocol (Promega).

qRT-PCR

The qRT-PCR was based on the coamplification of a specific internal standard with the cDNA to be examined. Because of the identical primer sequences of the internal standard and cDNA, both molecules compete for the primers of the PCR mixture in the phase of primer annealing. A decreasing concentration of internal standard accounts for an increased amplification of examined cDNA (Figure 1).

The internal standard was constructed by use of the PCR mimic-constructing kit (Clontech Laboratories, Inc) with linker primers for Col I, Col III, TGF- β_1 , TGF- β_2 , and GAPDH. The standard differed in size from the amplified cDNA to distinguish between both PCR products on agarose gels. The difference in size between standard and endogenous cDNA was in the range of 10% to 20% of base length. The exogenous DNA standard was applied in qRT-PCR in 5 dilutions ranging from 0.1 to 0.16 attomol, calculated by an estimated length of 500 bp while a constant concentration of cDNA was added. The amounts of cDNA were 1.5 ng for Col I, 3 ng for Col III, 2 ng for TGF- β_1 , 6 ng for TGF- β_2 , and 0.6 ng for GAPDH.

The qRT-PCR program consisted of a denaturing phase at 94°C for 1 minute, primer annealing at 60°C for Col III, TGF- β_1 , TGF- β_2 , and GAPDH and at 72°C for Col I, each for 90 seconds, and an extension temperature of 72°C for 2 minutes. The PCR ran for 35 cycles and was still in the linear phase.

The PCR reacting volume of 25 μL consisted of 0.4 $\mu\text{mol/L}$ of each primer, 0.2 mmol/L dNTPs, 2.0 mmol/L MgCl_2 , 50 mmol/L KCl, 10 mmol/L Tris-HCl, and 4 U of *Taq* polymerase (Perkin-Elmer).

Oligonucleotides Used for qRT-PCR

Primers were selected on 2 different exons, separated by an intron to differentiate between amplified DNA and amplified reverse-transcribed RNA. The primer sequences are listed in Table 1.

Densitometric Analyses of qRT-PCR Products

Each qRT-PCR product was loaded on an ethidium bromide-stained agarose gel (1.5%). For analysis of bands, the gel was photographed under 254-nm UV light with a photo documentary system (Ultra Violet Products Gel Documentation System Image Store 5000) and quantified with the NIH image program (Ultra-Violet Products Limited). All values were measured twice to avoid inaccuracy. The

TABLE 1. Primer Sequences Used for qRT-PCR

mRNA	5' Region	3' Region	Product (bp)
Col I	GGCGGCCAGGGCTCCGAC	AATTCCTGGTCTGGGGCACC	347
Col III	TGGTGTGGAGCCGCTGCCA	AGTCGAAGGAATGCCAGCTG	483
TGF- β_1	CAGAAATACAGCAACAATTCCTGG	TTGCAGTGTGTTATCCCTGCTGTC	186
TGF- β_2	TCCAAAGATTTAACATCTCCAACC	TCCCACTGTTTTTTTCTAGTGG	310
GAPDH	TGAAGGTCGGAGTCAACGGATTGGT	CATTGTGGCCATGAGGTCCACCAC	983

abundances of all different mRNA species were related to GAPDH mRNA to increase accuracy.

Histology

Picrosirius red staining was used for the histological assessment of the total collagen content by use of computer-assisted morphometry (Lucia software program). Formalin-fixed tissue was processed for paraffin embedding, sectioned at a thickness of 5 μ m, and stained with picrosirius red (Polyscience, Inc). The collagen content of the sections was measured when viewed with circularly polarized light according to previously published methods.¹² For each sample, a total of 10 fields were analyzed with a $\times 20$ objective lens. Histological analyses of endomyocardial biopsies for myocardial inflammation were performed in a standard manner and evaluated according to the Dallas classification.¹³

Immunohistology

Endomyocardial biopsies were directly embedded in OCT (Miles Laboratories, Inc) and frozen at -70°C . With a cryostat, the biopsies were cut into 5- μ m thin sections, mounted on a slide, and fixed in acetone for 10 minutes.

Antibodies

Biopsies were incubated with monoclonal mouse anti-human antibodies against Col I (Oncogene Research Products) and Col III (Sigma Chemical Co), with a working solution for Col I of 1:300 and Col III of 1:150. Monoclonal antibodies directed against CD3 T lymphocytes were used according to Kühl et al.¹⁴

Immunohistological Staining

Each primary antibody was added to a section of 6 slices and incubated for 45 minutes at room temperature in a humidified chamber. After incubation, unbound antibodies were removed by 2 washing steps with PBS for 5 minutes. Then, peroxidase-conjugated rabbit anti-mouse antibody (Dianova GmbH), diluted at 1:200 in PBS containing 10% FCS, was added and likewise incubated for 45 minutes at room temperature. After another 2 washing steps with PBS, the specifically bound antibodies were detected by a stain reaction with carbazole for 12 minutes in the dark. For quantification of stained areas, the sections were viewed with a light microscope at a magnification of $\times 40$ and digitized. With the Lucia software program (Nikon), the relation between stained and unstained areas was calculated and the Col I and Col III protein was measured as percentage proportion. For each biopsy and antibody, 6 sections were measured and averaged. The quantification of CD3 T lymphocyte content was performed at a magnification of $\times 400$ according to Kühl et al.¹⁴

Hydroxyproline Determination

The endomyocardial biopsies were homogenized in 330 μ L of 6 mol/L hydrochloric acid for 15 seconds. Of this homogenate, 30 μ L was used for the determination of the total protein content in the analyzed tissue sample. Afterward, 300 μ L of the 6 mol/L hydrochloric acid homogenate was heated at 120°C overnight to hydrolyze collagen into its component amino acids. Hydroxyproline concentration was measured in an amino acid analyzer in standard fashion. In addition, the total protein concentration of the analyzed biopsy was measured with the bicinchoninic acid protein quantification kit (Pierce). The concentration of tissue hydroxyproline was expressed as the ratio of hydroxyproline concentration/total protein concentration (both $\mu\text{g/mL}$) for each biopsy. The hydroxyproline concentration was applied to the total protein content, because with the very small size of endomyocardial biopsies, a weight-adjusted standardization of the hydroxyproline content would be not accurate enough.

Statistics

For statistical analysis, the SPSS statistical software package was used. Statistical analyses were done with the Mann-Whitney *U* test, and the mRNA values of each group are shown with their respective

TABLE 2. Clinical and Hemodynamic Parameters of the Enrolled Patients

	EF<50% (n=12)	EF>50% (n=18)	P
Age, y	47 \pm 11	40 \pm 14	NS
Sex, F/M	3/9	7/11	NS
Medication			
ACE inhibitors	7/12	6/18	NS
Diuretics	7/12	2/18	<0.05
Digitalis	6/12	1/18	<0.05
β -Blocker	3/12	3/18	NS
EF, %	39 \pm 9	64 \pm 7	<0.001
CI, L \cdot min $^{-1}$ \cdot m $^{-2}$ BSA	3.1 \pm 1.0	4.3 \pm 1.0	<0.004
LVEDP, mm Hg	11 \pm 5	8 \pm 2	NS
PCWP, mm Hg	9 \pm 5	6 \pm 2	NS
LVEDD, mm	63 \pm 8	51 \pm 7	<0.003

CI indicates cardiac index; BSA, body surface area; LVEDP, left ventricular end-diastolic pressure; PCWP, pulmonary capillary wedge pressure; and LVEDD, left ventricular end-diastolic dimension.

medians and the corresponding interquartile distances. Statistical significance was accepted at the level of $P \leq 0.05$. The other values are expressed as mean \pm SD.

Results

Patients and Hemodynamic Parameters

The patients were assigned to 2 groups on the basis of cardiac function. Twelve patients (3 women, 9 men) 34 to 60 years old (mean, 47 \pm 11 years) had global left ventricular dysfunction (EF <50%) and were classified as having DCM. Eighteen patients (7 women, 11 men) 17 to 71 years old (mean, 40 \pm 14 years) had only mild or regional left ventricular dysfunction (EF >50%) and were classified as having "latent" DCM. The other hemodynamic and clinical data, including the medications, are listed in Table 2.

Collagen Quantification

Gene Expression Studies

The mRNA abundance of Col I, measured as Col I/GAPDH mRNA ratio (0.45 \pm 0.49) in the group of patients with an EF <50%, was increased significantly, by 2.6-fold ($P < 0.01$), versus patients with an EF >50% (0.172 \pm 0.23) (Figure 2). The mRNA ratio of Col III/GAPDH increased significantly, by 2.0-fold ($P < 0.04$), from 0.027 \pm 0.026 in patients with mild regional dysfunction and EF >50% versus 0.055 \pm 0.066 in patients with global dysfunction and EF <50% (Figure 2). These changes resulted in a shift in the ratio between Col I and Col III at the mRNA level for patients with DCM (ratio, 8.2) compared with the gene expression ratio of Col I/Col III in the group with an EF >50% (ratio, 6.4).

The growth factors TGF- β_1 and TGF- β_2 showed a trend toward an increased mRNA abundance in the group with EF <50% (TGF- β_1 /GAPDH, 0.048 \pm 0.027; TGF- β_2 /GAPDH, 0.018 \pm 0.014) versus the group of patients with EF >50% (TGF- β_1 /GAPDH, 0.029 \pm 0.041; TGF- β_2 /GAPDH, 0.004 \pm 0.014).

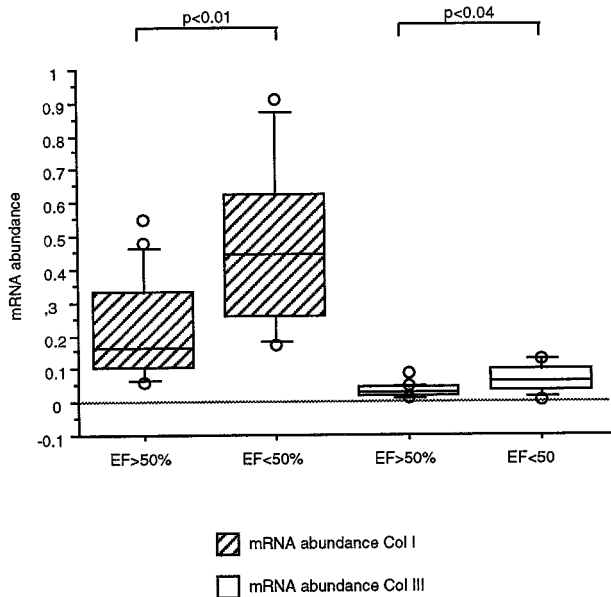


Figure 2. Significantly increased myocardial mRNA abundance of Col I (hatched bars) and Col III (open bars) in patients with EF<50% compared with patients with EF>50%.

Histology and Immunohistology of Collagen

The results of gene expression for Col I were confirmed on the protein level. A trend toward a greater accumulation of the percentage proportion of stained and unstained areas of Col I was detected in patients with DCM (Col I, $20 \pm 11\%$) compared with patients with a normal EF (Col I, $12 \pm 8\%$). However, a trend toward a decrease of Col III was found (patients with EF <50%, $30 \pm 13\%$; patients with EF >50%, $36 \pm 11\%$). The histological analysis of the total collagen content by picrosirius red staining visualized in circularly polarized light also showed a significant increase in fibrosis in patients ($n=3$) with EF <50% ($4.3 \pm 0.1\%$) compared with patients ($n=8$) with EF >50% ($2.7 \pm 0.9\%$) ($P < 0.0035$; see Figure 3). This increase in collagen content appeared to be uniform throughout the biopsy samples.

Hydroxyproline Content

Because of the limited availability of endomyocardial biopsies, these biochemical analyses were performed in a subgroup of 12 patients (EF <50%, $n=6$; EF >50%, $n=6$; mean EF, $49 \pm 11\%$). The ratio of hydroxyproline concentration/total protein concentration (both $\mu\text{g/mL}$) was significantly correlated with the mRNA abundance of Col I ($r=0.77$; $P < 0.05$) (see Figure 4) but not with Col III.

Histology and Immunohistology of Myocardial Inflammation

Active myocarditis according to the Dallas classification was excluded in all patients. The distribution of CD3 T lymphocytes differed between groups; patients with global dysfunction and EF <50% showed a significant ($P \leq 0.05$) increase of CD3 T lymphocytes of 1.8 ± 0.7 lymphocytes per visual field, compared with the group of patients with EF >50%, who showed a value of 1.3 ± 0.6 CD3 T lymphocytes per visual field.

Discussion

The study revealed that myocardium obtained from patients with a diagnosis of DCM and an EF <50% had an increase in the ratio of Col I/Col III mRNA abundance versus that found in patients with an EF >50%. The total collagen content quantified by computer-assisted morphometry of picrosirius red-stained histological slices in circularly polarized light showed a significant increase in patients with EF <50% compared with patients with EF >50%. Furthermore, these changes in mRNA levels were paralleled by immunohistologically observed increases in Col I in the patients with DCM as well as a significant correlation between the biochemically determined ratio of hydroxyproline concentration/total protein concentration and the mRNA abundance of Col I. In addition, these changes in collagen expression and deposition were matched by a trend toward increased expression of TGF- β_1 and TGF- β_2 in combination with a significantly increased CD3 T-lymphocyte count. Thus, we have demonstrated that it is possible to determine from small endomyocardial biopsies various structural and humoral factors that may play key roles in the development of functional abnormalities.

Collagen mRNA Abundance

The shift in the Col I/Col III ratio from 6.4:1 to 8.4:1 in the patients with DCM may derive from different molecular mechanisms. Either there could be a stimulation of collagen mRNA expression through the activation of various cytokines or there could be a reduced turnover of Col I and Col III mRNAs. Although we found no significant increase in the mRNA abundance of either TGF- β_1 or TGF- β_2 , 2 cytokines intimately involved with collagen production, this does not rule out the former pathway. It is possible that we missed the peak in TGF- β_1 and TGF- β_2 expression and detected only its consequences. In addition, there are other possible cytokines that may be equally or even more important.^{9,10}

Our study showed a trend toward increased myocardial TGF- β_1 and TGF- β_2 mRNA abundance in DCM. This, however, could possibly translate into a significant increase in collagen gene expression, because both the promoters of Col I and Col III contain TGF- β -activatable elements.¹⁵⁻¹⁷ Because the Col I promoter also contains several SP-1 binding sites, which are not found in the Col III promoter, the differential gene expression levels of Col I and Col III might rely on their respective promoters.^{18,19} Stimulation of myocardial TGF- β expression itself may result from a number of factors that are not known at present. Because Hinglais et al²⁰ demonstrated the colocalization of myocardial fibrosis and predominantly T helper lymphocytes and macrophages in spontaneously hypertensive rats, the local myocardial expression of TGF- β_1 and TGF- β_2 could have been stimulated by the significantly increased CD3 T lymphocytes cell count as seen in our DCM patients with EF <50%.^{21,22}

Collagen Protein Detection

The increase in collagen mRNA that we found was mirrored by an increase in collagen detected at the protein level by histochemistry (picrosirius red staining) and immunohistochemistry. We were able to document a significant increase

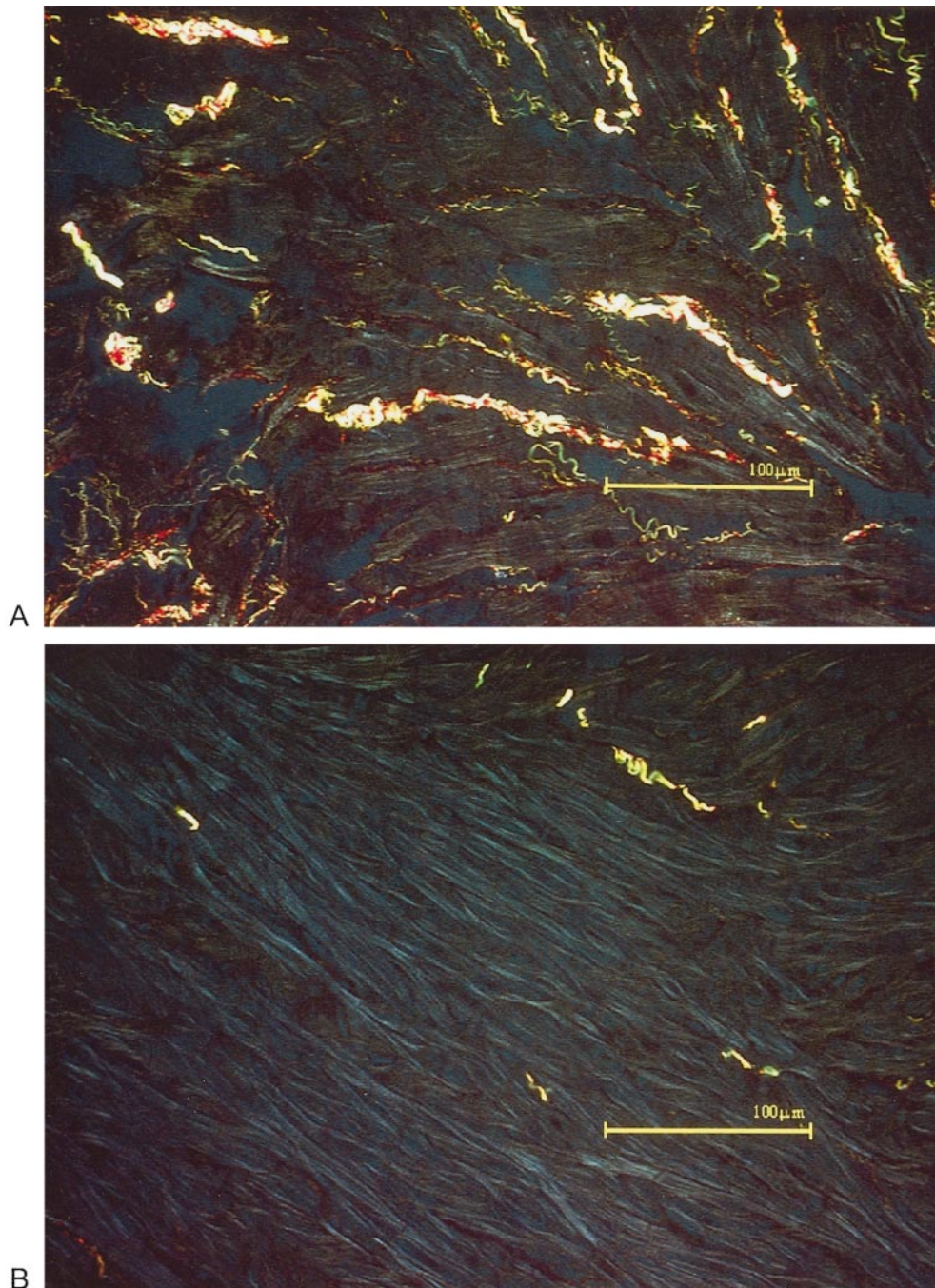


Figure 3. Histological analysis of the total collagen content by picrosirius red staining visualized in circularly polarized light in patients with EF<50% (A) and EF>50% (B).

of fibrosis in patients with DCM because of a trend toward an increase in Col I but not Col III in patients with DCM, which is in line with earlier reports by Marjjanowski et al.⁴ This increase in collagen content appeared to be uniform throughout the biopsy samples. The inability to detect an increase of Col III by immunohistochemical techniques as well might be influenced by the availability of Col III epitopes for antibody binding. If the Col III fibers are covered by type I collagen, it may be difficult to accurately measure the amount of Col III in these biopsies. Despite this principal methodological problem of immunohistological staining, the immunohistochemical results are consistent with the mRNA findings and

indicate a shift in the Col I/Col III ratio on both the mRNA and protein levels. In addition, the biochemical results of the significant correlation between the ratio of hydroxyproline concentration to total protein concentration and the mRNA abundance of Col I emphasize these findings. Although the increase in collagen detected at the protein level may reflect an increase in synthesis, it is also possible that differences in degradation may play a role. Differences concerning protein levels of Col I and Col III may depend not only on differential expression but also on differential degradation. Two different matrix metalloproteinases (MMPs) are described as being active in degrading collagens. MMP 1 is synthesized by

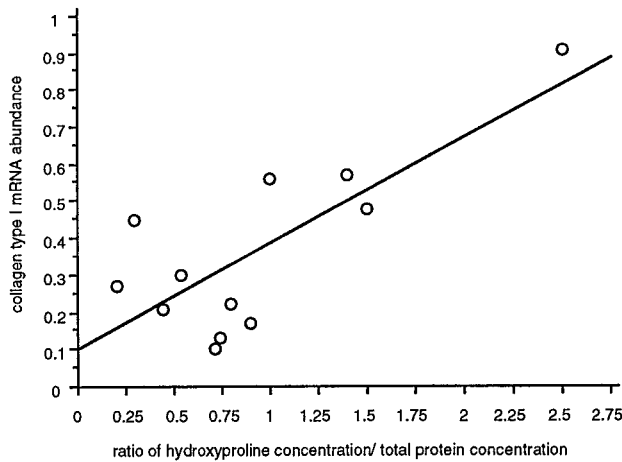


Figure 4. Significant correlation between the mRNA abundance of Col I and the ratio of hydroxyproline concentration/total protein concentration (both $\mu\text{g/mL}$) ($r=0.77$; $P<0.05$).

fibroblasts and shows equal affinity for Col I and Col III degradation. MMP 8, however, which is synthesized by neutrophils, has a higher affinity for Col III. Therefore, either differential activities or differential expression levels of MMP may also contribute to changes in myocardial Col I and Col III content, in addition to the changes in gene expression shown in our own study.^{23–25}

Potential Significance of Collagen Changes

Col I and Col III are essential components of the myocardium, maintaining its structural and functional integrity. Because of their different physical properties, the altered Col I/Col III ratio may therefore have a major impact on the diastolic and systolic function of the heart. Whereas Col III forms an elastic network storing kinetic energy as an elastic recoil, Col I represents a stiff fibrillar protein providing tensile strength. Therefore, increasing Col I protein levels might impose increasing myocardial stiffness, compromising diastolic and systolic function of the myocardium.⁶

Limitations

A limitation of this study is that only endomyocardial biopsies from patients with different degrees of impaired left ventricular function were analyzed, and not explanted hearts with end-stage heart failure. Therefore, the analysis of regional distribution of collagen content (septal versus posterior region, endocardial versus epicardial region, right ventricle versus left ventricle), which would have strengthened our study, was not possible. However, this type of analysis of regional matrix remodeling is possible only in explanted hearts with end-stage heart failure, which was not the target group of patients to be evaluated in our study. The aim of the study was to analyze, in particular, the collagen remodeling in endomyocardial biopsies of patients with mild to severe left ventricular dysfunction and not in terminally failing explanted hearts. In addition, postmortem studies by Baandrup et al²⁶ demonstrated identical collagen contents in the right ventricular septum and the left ventricle as assessed in histological studies of autopsy samples of normal hearts. Therefore, the use of right ventricular endomyocardial biop-

sies from the right ventricular septum should also be representative for left ventricular matrix remodeling. Further studies will be necessary to determine whether the collagen changes reported here are specific for DCM or whether similar changes occur in heart failure as a result of other cardiac pathology. Another limitation of this study is that we did not obtain endomyocardial biopsies from normal hearts for ethical reasons. However, the mean total collagen content in the group of patients with mild left ventricular dysfunction in this study measured by picrosirius red staining in circularly polarized light is similar to the total collagen content measured with the same technique in normal hearts from the right ventricular septum²⁷ as well as from the free left ventricular wall.²⁸ Therefore, we also did not analyze the collagen expression in normal hearts. The reported differences in collagen expression between DCM and the patients with mild left ventricular dysfunction are relevant and may have major implications for systolic and in particular diastolic dysfunction as seen in DCM.

Summary

Our results showed increased mRNA abundances for Col I and Col III in endomyocardial tissues of patients with DCM. In addition, a significant shift of the Col I/Col III ratio was found both at the level of gene expression and at the protein level. Finally, the increased mRNA abundances of TGF- β_1 and TGF- β_2 in DCM suggest that these growth factors could play a role in the myocardial matrix remodeling seen in DCM. An interesting secondary finding was the possibility to establish a method for the analysis of mRNA abundances of 5 different genes in one single human endomyocardial biopsy with a wet weight of 3 to 5 mg.

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References

1. Borg TK, Caulfield JB. The collagen matrix of the heart. *Fed Proc.* 1981; 40:2037–2041.
2. Weber KT. Cardiac interstitium in health and disease: the fibrillar collagen network. *J Am Coll Cardiol.* 1989;13:1637–1652.
3. Bishop JE, Greenbaum R, Gibson DG, Yacoub M, Laurent GJ. Enhanced deposition of predominantly type I collagen in myocardial disease. *J Mol Cell Cardiol.* 1990;22:1157–1165.
4. Marijjanowski MM, Teeling P, Mann J, Becker A. Dilated cardiomyopathy is associated with an increase in the type I/type III collagen ratio: a quantitative assessment. *J Am Coll Cardiol.* 1995;25:1263–1272.
5. Yoshikane H, Honda M, Goto Y, Morioka S, Ooshima A, Moriyama K. Collagen in dilated cardiomyopathy: scanning electron microscopic and immunohistochemical observations. *Jpn Circ J.* 1992;56:899–910.
6. Lapiere CM, Nussgens B, Pierard GE. Interaction between collagen type I and type III in conditioning bundles organization. *Connect Tissue Res.* 1977;5:21–29.
7. Weber KT, Pick R, Janicki JS, Gadodia G, Lakier JB. Inadequate collagen tethers in dilated cardiomyopathy. *Am Heart J.* 1988;116:1641–1646.
8. Villarreal FJ, Dillmann WH. Cardiac hypertrophy-induced changes in mRNA levels for TGF-beta 1, fibronectin and collagen. *Am J Physiol.* 1992;262:H1861–H1866.
9. Bornstein P, Sage H. Regulation of collagen gene expression. *Prog Nucleic Acid Res Mol Biol.* 1989;37:67–106.
10. Kovacs EJ. Fibrogenic cytokines: the role of immune mediators in the development of scar tissue. *Immunol Today.* 1991;12:17–23.

11. Auffray C, Rougeon F. Purification of mouse immunoglobulin heavy-chain messenger RNAs from total myeloma tumor RNA. *Eur J Biochem.* 1980;107:303–314.
12. Whittaker P, Kloner RA, Boughner DR, Pickering JG. Quantitative assessment of myocardial collagen with picosirius red staining and circularly polarized light. *Basic Res Cardiol.* 1994;89:397–410.
13. Aretz HT, Billingham ME, Edwards WD, Factor SM, Fallon JT, Fenoglio JJ, Olsen EGJ, Schoen FJ. Myocarditis: a histopathologic definition and classification. *Am J Cardiovasc Pathol.* 1987;1:3–14.
14. Kühl U, Noutsias M, Seeberg B, Schultheiss H-P. Immunohistological evidence for a chronic intramyocardial inflammatory process in dilated cardiomyopathy. *Heart.* 1996;75:295–300.
15. Ritzenthaler JD, Goldstein RH, Fine A, Lichtler A, Rowe DW, Smith BD. Transforming growth factor beta activation element in the distal promoter regions of the rat alpha 1 type I collagen gene. *Biochem J.* 1991;280:157–162.
16. Brenner DA, Rippe RA, Rhodes K, Trotter JF, Breindl M. Fibrogenesis and type I collagen regulation. *J Lab Clin Med.* 1994;124:755–760.
17. Rossi P, Karsenty G, Roberts AB, Roche NS, Sporn MB, de Crombrughe B. A nuclear factor 1 binding site mediates the transcriptional activation of a type I collagen promoter by transforming growth factor-beta. *Cell.* 1988;52:405–414.
18. Briggs MR, Kadonaga JT, Bell SP, Tijan R. Purification and biochemical characterization of the promoter-specific transcription factor SP 1. *Science.* 1986;234:47–52.
19. Gunzburg WH, Salmons B, Schlaefli A, Moritz-Legrand S, Jones W, Sarkar NH, Ullrich R. Expression of the oncogenes *mil* and *ras* abolishes the in vivo differentiation of mammary epithelial cells. *Carcinogenesis.* 1988;9:1849–1856.
20. Hinglais N, Heudes D, Nicoletti A, Mandet C, Laurent M, Bariety J, Michel J-B. Colocalization of myocardial fibrosis and inflammatory cells in rats. *Lab Invest.* 1994;70:286–294.
21. Roberts AB, Sporn MB. Transforming growth factor-beta: potential common mechanisms mediating its effects on embryogenesis, inflammation-repair, and carcinogenesis. *Int J Rad Appl Instrum B.* 1987;14:435–439.
22. Sporn MB, Roberts AB. Transforming growth factor- β : recent progress and new challenges. *J Cell Biol.* 1992;119:1017–1021.
23. Arthur MJ. Matrix degradation in the liver. *Semin Liver Dis.* 1990;10:47–55.
24. Overall CM, Wrana JL, Sodek J. Transcriptional and post-transcriptional regulation of 72-kDa gelatinase/type IV collagenase by transforming growth factor- β 1 in human fibroblasts. Comparisons with collagenase and tissue inhibitor of matrix metalloproteinase gene expression. *J Biol Chem.* 1991;266:14064–14071.
25. Woessner JF. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J.* 1991;5:2145–2154.
26. Baandrup U, Florio RA, Olsen EGJ. Do endomyocardial biopsies represent the morphology of the rest of the myocardium? A quantitative light microscopic study of single v. multiple biopsies with the King's biptome. *Eur Heart J.* 1982;3:171–178.
27. Pickering JG, Boughner DR. Fibrosis in the transplanted heart and its relation to donor ischemic time. *Circulation.* 1990;81:949–958.
28. Chello M, Mastroberto P, Romano R, Perticone F, Marchese AR. Collagen network remodelling and left ventricular function in constrictive pericarditis. *Heart.* 1996;75:184–189.