Myocardial Mechanics and Collagen Structure in the Osteogenesis Imperfecta Murine (oim)

Sara M. Weis, Jeffrey L. Emery, K. David Becker, Daniel J. McBride, Jr, Jeffrey H. Omens, Andrew D. McCulloch

Abstract—Because the amount and structure of type I collagen are thought to affect the mechanics of ventricular myocardium, we investigated myocardial collagen structure and passive mechanical function in the osteogenesis imperfecta murine (oim) model of pro-α2(I) collagen deficiency, previously shown to have less collagen and impaired biomechanics in tendon and bone. Compared with wild-type littermates, homozygous oim hearts exhibited 35% lower collagen area fraction (P<0.05), 38% lower collagen fiber number density (P<0.05), and 42% smaller collagen fiber diameter (P<0.05). Compared with wild-type, oim left ventricular (LV) collagen concentration was 45% lower (P<0.0001) and nonreducible pyridinoline cross-link concentration was 22% higher (P<0.03). Mean LV volume during passive inflation from 0 to 30 mm Hg in isolated hearts was 1.4-fold larger for oim than wild-type (P=NS). Uniaxial stress-strain relations in resting right ventricular papillary muscles exhibited 60% greater strains (P<0.01), 90% higher compliance (P=0.05), and 64% higher nonlinearity (P<0.05) in oim. Mean opening angle, after relief of residual stresses in resting LV myocardium, was 121±9 degrees in oim compared with 45±4 degrees in wild-type (P<0.0001). Mean myofiber angle in oim was 23±8 degrees greater than wild-type (P<0.02). Decreased myocardial collagen diameter and amount in oim is associated with significantly decreased fiber and chamber stiffness despite modestly increased collagen cross-linking. Altered myofiber angles and residual stress may be beneficial adaptations to these mechanical alterations to maintain uniformity of transmural fiber strain. In addition to supporting and organizing myocytes, myocardial collagen contributes directly to ventricular stiffness at high and low loads and can influence stress-free state and myofiber architecture. (Circ Res. 2000;87:663-669.)

Key Words: heart ■ ventricle ■ collagen ■ stiffness ■ residual stress

Collagen fibers in ventricular myocardium are thought to contribute tensile stiffness, especially at higher cavity volumes, while maintaining the architecture of myocytes, myofiber bundles, and sheets. The cardiac collagen extracellular matrix consists of ~85% type I collagen, arranged into a hierarchy of fibers. Large coiled perimysial collagen fibers provide tensile stiffness, whereas smaller endomysial fibers surrounding and interconnecting individual myocytes are thought to prevent myocyte slippage and maintain unloaded ventricular geometry.

Many cardiac disorders are associated with an accumulation, depletion, or restructuring of the collagen matrix. Correlating mechanical alterations with changes in the size, amount, and structure of collagen fibers in these pathologies has provided insight into the functional role of myocardial collagen. Indeed, most of our knowledge of the structural contribution of collagen to ventricular wall mechanics has been deduced either from comparative studies using animal models of complex, multifactorial pathologies or from tissue preparations in which collagen has been proteolytically degraded or synthesis has been inhibited.

Human osteogenesis imperfecta (OI) is a heritable disease, resulting from deletions, insertions, or exon splice errors in the genes encoding type I collagen pro-α1 and pro-α2 chains. In most cases, the mutation is unknown and diagnosis is made by clinical assessment of symptoms, which include bone fragility, defective skeletal development, smaller stature, and blue sclera. Few data are available regarding implications of OI on cardiac mechanics or structure, although clinical descriptions of aortic dissection, left ventricular (LV) rupture, and aortic or mitral valve incompetence have been reported in OI patients.

The present study uses a mouse model of OI, the OI murine (oim), to investigate structure-function relationships in the heart. The oim model of moderate OI results from a guanine deletion on the Cola-2 gene encoding the pro-α2(I) chain of type I collagen, causing a loss of functional α2(I) chains in homozygous oim mice. The oim mouse exhibits many symptoms similar to human OI, namely defective skeletal development, smaller stature, and skeletal fragility.

Recently, several mouse models of OI have been used to investigate effects of collagen mutations on structure and

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mechanics in bone and tendon. Tail tendon in the oim model had ~60% less collagen and ~40% lower tensile strength than wild-type controls, and femur strength was reduced in heterozygous and homozygous oim. Experiments in several other strains of mice harboring OI-type mutations suggest a general decrease in collagen but no consistent changes in elastic stiffness.

We tested the hypothesis that the Cola-2 mutation in the oim model is associated with altered myocardial collagen content and consequent alterations in ventricular mechanics. We studied hearts from wild-type (+/+), heterozygous (+/−), and homozygous oim (−/−) mice. Our results suggest smaller, less abundant perimysial collagen fibers and lower passive uniaxial and ventricular stiffness in oim are accompanied by altered transmural myofiber angle distribution and increased residual stresses, which may be beneficial adaptive responses.

Materials and Methods

Genotyping

The oim model is available from the Jackson Laboratory (Bar Harbor, Maine). A polymerase chain reaction method was used to determine mouse genotype.

Echocardiography

Transathoracic two-dimensional M-mode and Doppler echocardiography was performed on intact mice under intraperitoneal avertin anesthesia (2.5%, 15 to 17 μL/g).

Acute Surgery

All experimental protocols were approved by the University of California, San Diego Animal Subjects Committee. A total of 62 male and 37 female 11- to 16-week-old mice were anesthetized intraperitoneally using 100 mg/kg ketamine and 8 mg/kg xylazine. Male and 37 female 11- to 16-week-old mice were anesthetized intraperitoneally using 100 mg/kg ketamine and 8 mg/kg xylazine. All experimental protocols were approved by the University of California, San Diego Animal Subjects Committee. A total of 62 male and 37 female 11- to 16-week-old mice were anesthetized intraperitoneally using 100 mg/kg ketamine and 8 mg/kg xylazine. Hearts were arrested in diastole with hyperkalemic cardioplegic solution containing 30 mmol/L 2,3-butanedione monoxime (BDM) to prevent muscle contraction.

Biochemistry

Left ventricles were isolated and lyophilized, and dry weights were recorded. Hydroxyproline and nonreducible collagen cross-link concentrations were determined.

Histology

Hearts were fixed, processed with graded alcohols, embedded in paraffin, sectioned 10 μm or 15 μm thick, and stained with picrosirius red. To enhance contrast between myocytes and collagen, 15-μm-thick sections for confocal microscopy were treated with phosphomolybdic acid. Sections were imaged using a laser scanning confocal system (BioRad MRC 1024) with a ×60 objective. Collagen area fraction and fiber number density were measured. Average collagen fiber diameter was calculated from 7 to 10 measurements along each fiber.

Myofiber Angle and Zero-Stress State

Myofiber angle was measured from stained sections of paraffin-embedded hearts fixed at zero pressure. Myofiber angle was defined as the angle between the myofibers and a reference line parallel to the equatorial LV axis. To ensure this reference was identical for all hearts, care was taken to align the axis between the two. There were no differences observed for heart shape between genotypes that may have led to systematic errors in obtaining a slice from a consistent location between hearts. Residual strain was estimated by measuring the resulting angle from a radial cut through the LV free wall in the presence of a low calcium buffer containing BDM.

Papillary Muscle Uniaxial Testing

Suture was tied to the distal end of a right ventricular (RV) papillary muscle and attached to a 1-g capacitive force transducer for constant muscle stretch at 3 mm/min. Surface marker motion in the muscle central portion was recorded, and two-dimensional finite strain was computed from video images. Fiber stress was computed as force per unit cross-sectional area. After 3 preconditioning runs, loading consisted of slow stretch to 10 mN/mm². Perfsusate was replaced with cardioplegic solution containing 2 mmol/L calcium and no BDM to confirm specimen contractile viability when field-stimulated by platinum electrodes.

Isolated Heart Inflation

A pressure transducer was used to measure intraballoon pressures for a LV balloon with 5 μL empty volume. After 3 preconditioning runs, a volume infusion pump slowly infused volume to a maximum pressure of 30 mm Hg.
Exclusion Criteria

None of the 15 hearts were excluded from the opening angle study for contracture, indicated by >20 degree change in angle after 20 minutes. Of 25 hearts studied for ventricular tissue mechanics, 6 were rejected for incompatible balloon size for ventricular dimensions and 5 were excluded for tissue contracture, evidenced as firmness to touch. Of 66 hearts studied for passive uniaxial stretch, 39 were excluded: 30 because of observation of muscles with geometry not suitable for our experiment (many RV papillary muscles exhibited a conical geometry rather than cylindrical and often were attached to the septal wall by an extensive network of trabeculae), 3 for insufficient muscle contraction after the experiment, and 6 for experimental difficulties.

Statistical Analyses

Results presented reflect mean±SE per group; 1- or 2-way ANOVA was performed with Bonferroni-Dunn post hoc comparisons. An expanded Materials and Methods section can be found in an online data supplement available at http://www.circresaha.org.

Results

The characteristics of all mice are summarized in the Table. The oim group exhibited an average smaller heart weight (HW) and body weight (BW), but ratio of HW to BW was not statistically different than in wild-type mice. Despite advances in mouse echocardiography, the signal-to-noise ratio is still not high. We acknowledge that limited resolution may prevent detection of small differences between genotypes. Echocardiograms showed no significant differences in heart rate or LV systolic and diastolic diameters (Table).

End-diastolic septal and posterior wall thickness was 12% larger in oim than wild-type mice (P<0.05), and the end-diastolic LV internal diameter tended to be larger in oim mice (P=NS), but ratios of wall thickness to LV radius were similar between groups. Systolic function was not different between genotypes. Echocardiograms showed no significant differences and 5 were excluded for tissue contracture, evidenced as firmness to touch. Of 66 hearts studied for passive uniaxial stretch, 39 were excluded: 30 because of observation of muscles with geometry not suitable for our experiment (many RV papillary muscles exhibited a conical geometry rather than cylindrical and often were attached to the septal wall by an extensive network of trabeculae), 3 for insufficient muscle contraction after the experiment, and 6 for experimental difficulties.

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wild-type mice (Figure 5). Myofiber angles for oim were 19 to 25 degrees larger at each depth, averaging 23° 6° 8 degrees (P, 0.02), but slope of myofiber angle–wall depth relation was not different (wild-type 1.1±0.1, oim 1.0±0.1; P, 0.80). For heterozygous mice, average myofiber angles were greater than for wild-type controls but less than for homozygous oim mice.

Zero-Stress State
We measured 2-fold larger opening angles (P, 0.0001) for oim compared with wild-type mice (Figure 6). The mean opening angle for heterozygous mice was intermediate.

Uniaxial Papillary Muscle Stretch
Average RV papillary muscle cross-sectional area (wild-type: 0.07±0.01 mm, oim 0.07±0.01 mm; P, 0.51) and muscle length (wild-type: 1.0±0.06 mm, oim: 0.8±0.04 mm; P, 0.14) were not different between groups. Strains were 36% to 40% higher (Figure 7A) for oim compared with wild-type mice (P, 0.01). The slope of the stress-strain relation increased with increasing stress. Mean slope for oim mice was smaller than for wild-type mice (P, 0.05) at each stress level except 10 mN/mm². A measure of nonlinearity of the stress-strain relation was calculated as ratio of slope at 10 mN/mm² to slope at a stress of 0.1 mN/mm². This ratio was 64% higher for oim than for wild-type mice: 52±16 compared with 19±3, respectively (P, 0.05). After the passive stretch experiment, most of the muscles demonstrated contractile behavior characteristic of viable tissue, but 3 (5%) were excluded for poor contraction strength, suggesting that the muscles may have undergone ischemic injury, such as contracture, which could have affected passive stiffness.

Isolated Heart Mechanics
Compared with wild-type mice, average relative volume changes (Figure 7B) for oim were 1.4-fold higher during inflation pressures ranging from 0 to 30 mm Hg (R², 0.99, linear regression). Pressure-volume relation slopes were calculated from linear interpolation of data in a narrow range surrounding each pressure step. For pressures 10 to 30 mm Hg, mean slopes for wild-type mice were 1.35 times higher than for oim (R², 0.78). HW (0.17±0.01 versus 0.15±0.01 g), absolute initial volume (5.9±0.4 versus 6.1±0.4 mL), and LV estimated volume (130±6 versus 113±6 mL) of hearts in this study did not differ statistically between wild-type and oim mice, respectively.

Discussion
Our results suggest that the Cola-2 mutation in the oim model produces significant alterations in the structural and mechanical properties of ventricular myocardium. Although oim animals are smaller in general than wild-type animals, HW/BW ratio is similar and basal LV function in vivo is not significantly altered.

Figure 3. Collagen fiber diameter distribution from LV tissue measured from confocal micrographs. Average oim fiber diameter was 42% smaller than wild-type (P<0.05). n=4 animals per genotype, 20 to 30 fibers measured per animal.

Figure 4. Collagen content (hydroxyproline) was 45% less in oim heart compared with wild-type heart (P<0.0001). Collagen nonreducible cross-links (pyridinoline) were 29% higher in oim heart compared with wild-type heart (P<0.03). n=8 animals per genotype.

Figure 5. Transmural distribution of myofiber angle measured using light microscopy. Average oim fiber angle is 19 to 25 degrees higher than wild-type at all depths (P<0.02). n=6 for heterozygous and homozygous oim, n=5 for wild-type.

Figure 6. Opening angle represents LV circumferential residual strain. The oim hearts displayed a 2-fold higher opening angle compared with wild-type hearts. n=5 for each group.
The mild increase in LV wall thickness in oim mice may represent a degree of compensation for reduced collagen, although the ratio of wall thickness to chamber diameter was comparable with wild-type mice. Important differences between oim and wild-type mice were manifested as smaller myocardial collagen fiber area fraction, number density, and diameter. Hydroxyproline results support a decrease in total LV collagen content. These alterations were associated with softer passive uniaxial stress strain and ventricular pressure–volume relations in oim mice. Unexpected alterations in transmural myocardial fiber angles, residual strains, and collagen cross-link density in oim may be adaptations to these alterations in collagen amount and mechanical properties and could explain the normal cardiac function and lifespan for oim mice.

Myocardial Collagen Structure
Loss of functional α2(I) chains and deposition of α1(I) homotrimers are the main structural extracellular matrix alterations in homozygous oim. The biological effect of the mutation is probably compounded by decreased deposition of homotrimeric collagen. Altered myocardial collagen structure and size in oim are consistent with data published by Misof et al.16 reporting an ≈60% decrease in collagen fiber diameter in the oim tendon. Both collagen content and structure are important determinants of passive stiffness. A microstructural model of a single collagen fiber suggested that myocardial fiber stiffness is affected by collagen fiber density, tortuosity, and, most of all, diameter.4 The 42% smaller diameter and 38% lower number density measured for oim would suggest significant differences in LV stress-strain behavior. Other factors that may potentially influence myocardial stiffness in oim could be altered type III collagen or titin2 concentrations, homotrimeric collagen fibers comprised of three α1(I) chains,29 or altered integrin expression.30 Our biochemistry results suggest a mild increase in nonreducible cross-links, suggesting that the lower amount of collagen present is more highly cross-linked than typical myocardial collagen. The increase in cross-links could be a form of compensation for the compromised tissue stiffness in the oim heart. Without this increase, the greater material compliance measured in oim may have been even more pronounced. The mechanism by which cross-link density affects the stiffness or morphology of the relatively large perimysial collagen fibers has not been investigated in the myocardium or other tissues. Increased cross-link density directly affects adjacent collagen fibrils, which pack together to form large collagen fibers.31 It is unclear how increased cross-link density would alter the tortuosity, periodicity, or bending modulus of a large coiled perimysial collagen fiber, for example.

The ratio of collagen types I/III can affect myocardial stiffness and is altered in some models of heart disease.32,33 It is possible that there was a compensatory increase in type III collagen in the oim. Hydroxyproline measurements showed a markedly lower total collagen concentration in oim myocardium, suggesting that any changes in type III collagen did not appreciably affect total collagen. The oim model has also been reported to assemble homotrimeric collagen I molecules that may also affect collagen fiber and tissue stiffness.35 Biochemical measurements in the present study only addressed differences in total collagen between genotypes, whereas the histological measurements only addressed structural changes in perimysial collagen fibers. A more complete biochemical investigation, including collagen type distribution as well as finer levels of histology using electron microscopy, would help to better characterize the determinants of altered stiffness in this model. But in light of the abnormality of type I collagen in the oim and the uncertain specificity of available polyclonal antibodies against type III collagen (a highly conserved protein), we elected not to measure the collagen I/III ratio in the present study.

Uniaxial and Ventricular Chamber Stiffness
Because myofiber and collagen fiber axes coincide with the papillary muscle long axis,34 uniaxial stretch reveals properties along the perimysial collagen fiber axis. Although papillary muscle stiffness was reduced ≈40% in oim mice, LV chamber stiffness was only ≈20% lower than in wild-type mice. This may indicate that perimysial collagen contributes more to stiffness in the fiber direction than transverse directions, which are also strained during filling. In that case, anisotropy in oim myocardium would also be reduced. The relationship between collagen fiber tortuosity and strain may also be affected by the oim mutation, because the smaller average fiber diameter measured in oim may affect the bending and uncoiling of the perimysial collagen fibers.35 Investigators have used various experimental models exhibiting alterations in collagen content to study LV structure-function relationships. Decreased collagen has been achieved through treating with bacterial collagenase5 and 5,5′-dithio-2-nitrobenzoic acid,36 whereas increased collagen has been measured in hypertension,37 pressure-overload hypertrophy,38 and exercise.39 Changes in myocardial stiffness for these models were varied, and some differences were attributed to other factors, such as edema, altered collagen cross-links, or hypertrophy. Alterations in resting sarcomere length, integrin expression, collagen cross-linking, adaptive

Figure 7. A, Mean stress-strain relations during passive uniaxial stretch of RV papillary muscle. Stains for oim mice are higher at all pressures compared with wild-type mice, indicating lower stiffness under uniaxial tension. B, Pressure-relative volume change during passive inflation of isolated hearts. Volumes for oim are higher at all pressures compared with wild-type, suggesting a softer ventricle under passive loading.
mechanisms, or ventricular geometric remodeling may counterbalance the 35% reduction in collagen and 42% smaller fiber diameter in oim hearts to approximate normal function. Even if sarcomere lengths in the zero-stress state were not different, the differences in residual strain would make their transmural distributions different in the unloaded (zero-pressure) state, and this could alter the mechanics of ventricular filling.

Zero-Stress State

A 2-fold higher opening angle in oim indicates increased circumferential residual strain gradients in the LV wall, suggesting that decreased collagen causes increased residual stresses. In the tightskin mouse heart with twice as much type I collagen, the opening angle was only 7±6 degrees.22 These results suggest that collagen may influence the distribution of residual stresses. In normal myocardium, residual stress is compressive at the endocardium and tensile at the epicardium.23 Higher strains may induce higher residual stresses until circumferential fiber strain gradients are normalized.41 Minimal transmural gradients of sarcomere length have been observed in the stress-free state, whereas large gradients occurred in the unloaded state.40 This sarcomere length gradient may offset an opposite sarcomere extension gradient during filling and promote more uniform end-diastolic sarcomere length distribution and, hence, more uniform systolic force development. Increasing residual stress would additionally increase sarcomere length gradient and thus balance higher stress and strain gradients transmurally. Regardless of mechanism, increased residual strain in oim may be an adaptation to higher ventricular strains measured during isolated heart inflation and higher fiber strains measured during uniaxial stretch, which are consequences of less stiff material properties.

Transmural Myofiber Angle

Myofiber angles in normal hearts,42 disease models,43 between systole and diastole,44 and from our experience with rodents, porcine, and canine models show small variation over a wide range of conditions and between individuals or species. Thus, the ±20-degree shift in transmural myofiber angles in oim hearts from wild-type mice throughout the LV wall thickness is quite remarkable. Myofiber angle is an important determinant of torsion, which results from myocardial anisotropy in systole and diastole. Both torsion and myofiber angle distribution may act to reduce transmural strain gradients.45 Mathematical models in which fiber angles were allowed to redistribute show fiber angles assume a physiological configuration to minimize transmural strain or fiber strain gradients transmurally. Regardless of mechanism, increased residual strain in oim may be an adaptation to higher ventricular strains measured during isolated heart inflation and higher fiber strains measured during uniaxial stretch, which are consequences of less stiff material properties.

Changes in fiber orientation hardly affect pressure-volume behavior but significantly affect distribution of active muscle fiber stress and sarcomere length.46 The myofiber angle shift in oim hearts may be an adaptation to altered mechanical properties. Mathematical models have shown torsional deformation contributes to equalizing transmural fiber length changes by increasing epicardial fiber lengthening and decreasing endocardial fiber lengthening during filling.26,45 Without torsion, fiber strains would be larger at the endocardium than the epicardium. During systole, twist is generated by anisotropic muscle fiber contraction. During passive filling, torsion is also the reflection of anisotropic myocardial stiffness,45 which is greater in the fiber direction and can be partially attributed to alignment of large coiled collagen fibers with myofibers. The loss of myocardial collagen in oim may be associated with greater decreased stiffness in the myofiber direction and material properties that are more isotropic compared with normal. This reduced anisotropy would produce a consequent loss of twist.45 Yet our data suggest normal epicardial shear strains in oim isolated hearts during passive inflation (wild-type: −0.015, oim: −0.019). We propose that realignment of myofibers in oim is an adaptation to restore normal myocardial twisting in the setting of reduced passive anisotropy. This adaptation serves to neutralize transmural stress and sarcomere length gradients from endocardium to epicardium.45 Similarly, lower fiber stiffness would increase end-diastolic volume and strain and thus increase stress and sarcomere length gradient between endocardium and epicardium. We propose that oim hearts develop larger residual strains that compensate for these higher gradients and, in addition to the realignment of myofibers, promote a normal gradient of stresses through the wall thickness.45

Alternatively, collagen itself may be required for normal myofiber patterning. Neonatal cardiac myocytes express in vivo–like phenotypes and spread parallel to a substrate of aligned type I collagen, and the cardiac αβ integrin complex can detect and transduce phenotypic and positional information stored within the structure of the surrounding matrix.8 The oim α2(I) mutation may impede normal myofiber development by interfering with this pathway of signal transduction.

Although this study investigated collagen structure, collagen biochemistry, fiber stiffness, LV chamber stiffness, myofiber angle distribution, and zero-stress state, other factors were not investigated. Integrin expression and titin concentration affect tissue stiffness but were not measured. If they were increased in oim, they may partially compensate for loss of type I collagen. The endomysial collagen structure is undetectable with light or confocal microscopy; thus, our conclusions and discussion reflect only differences between genotypes measured for perimysial collagen fibers. The major advantage of our approach is the ability to relate how α2(I) collagen deficiency in the heart affects more than structure and myocardial stiffness but results in altered fiber angle morphology and increased residual stress.

In conclusion, we have measured significant structural alterations in the heart resulting from the oim model of α2(I) collagen deficiency. Reductions in passive uniaxial and ventricular chamber stiffness were accompanied by substantially altered myofiber angles and residual strain, which may be beneficial adaptations. Our work supports the hypothesis that in addition to providing a structural role by supporting and organizing myocytes, myocardial collagen directly contributes to mechanical stiffness at high and low loads and may indirectly influence stress-free states. The present study serves as the first characterization of the cardiac phenotype for the oim model of OI. These results can provide a basis to examine structural and mechanical roles of collagen in other tissues and can be combined with a diseased state, such as myocardial ischemia, to investigate how collagen contributes to cardiac mechanics in health and disease.

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