

Biochemical and Molecular Actions of Nutrients

Retinoic Acid Supplementation Attenuates Ventricular Remodeling after Myocardial Infarction in Rats¹⁻³

Sergio A. R. Paiva,⁴ Luiz S. Matsubara, Beatriz B. Matsubara, Marcos F. Minicucci, Paula S. Azevedo, Álvaro O. Campana, and Leonardo A. M. Zornoff

UNESP-Universidade Estadual Paulista, Faculdade de Medicina de Botucatu-Brazil

ABSTRACT The objective of this study was to evaluate the role of retinoic acid in experimental postinfarction myocardial remodeling. Wistar rats were subjected to myocardial infarction (MI) and treated with retinoic acid (RA), 0.3 mg/(kg · d) (MI-RA, $n = 29$), or fed a control diet (MI, $n = 34$). After 6 mo, the surviving rats (MI-RA = 18 and MI = 22) underwent echocardiograms, and isolated hearts were tested for function *in vitro*. The cross-sectional area of the myocyte (CSA) and interstitial collagen fraction (IC) were measured in a cross section of the heart stained by hematoxylin-eosin and picosirius red, respectively. The CSA was smaller in the MI-RA group [229 (220,234) μm^2] [medians (lower quartile, upper quartile)] than in the MI group [238 (232,241) μm^2] ($P = 0.01$) and IC was smaller in the MI-RA group [2.4 (1.7, 3.1)%] than in the MI group [3.5 (2.6, 3.9)%] ($P = 0.05$). The infarct size did not differ between the groups [MI = 44.6 (40.8, 48.4)%, MI-RA = 45 (38.6, 47.2)%]. Maximum rate of rise of left ventricular pressure (+dp/dt) was greater in the MI-RA group (2645 \pm 886 mm Hg/s) than in the MI group (2081 \pm 617 mm Hg/s) ($P = 0.05$). The other variables tested did not differ between groups. Retinoic acid supplementation of rats for 6 mo attenuates the ventricular remodeling process after MI. *J. Nutr.* 135: 2326–2328, 2005.

KEY WORDS: • ventricular function • myocardial fibrosis • remodeling • retinoids

Early after myocardial infarction (MI),⁵ left ventricular enlargement can occur as a result of infarct expansion, which increases the surface of the infarcted area by stretching and thinning the damaged region. This regional alteration in cavity size produces left-ventricular-chamber enlargement, increasing wall stress on the remaining normal regions. This in turn stimulates the expression of altered proteins and myocyte hypertrophy. In addition, increased neurohormonal activation with endothelin, angiotensin II, and phenylephrine after MI may also stimulate collagen synthesis, thus leading to myocardial fibrosis of noninfarcted areas, remote from the infarct. This overall process of alterations in ventricular size, composition, and mass is known as ventricular remodeling (1–3).

Considering that the extent of remodeling has been related to increased risk for progressive ventricular dysfunction and cardiovascular death after MI, reversing or preventing further remodeling is the key to improve prognosis following MI.

Therefore, several strategies have been used to attenuate the remodeling process (4–7).

Retinoic acid (RA) is a derivative of vitamin A that modulates cardiac structure and function throughout life. Experimental studies suggest that RA suppresses both morphological alterations and changes in gene expression associated with hypertrophy induced by endothelin, angiotensin II, and phenylephrine (8–10). However, the effects of RA on ventricular remodeling after MI are still unknown. Thus, to address this issue, we tested the hypothesis that RA supplementation could attenuate ventricular remodeling after MI in rats.

MATERIALS AND METHODS

All experiments and procedures were performed in concordance with the NIH guidelines and were approved by the Animal Ethics Committee of “Faculdade de Medicina de Botucatu da Universidade Estadual Paulista-UNESP.”

Coronary artery ligation. Male Wistar rats weighing 200–250 g, from the University of Campinas-UNICAMP animal facilities, were used in the study. Myocardial infarction was produced as previously described (11,12). In brief, the rats were anesthetized with ether, and after a left thoracotomy, the heart was exteriorized. The left atrium was retracted to facilitate ligation of the left coronary artery with 5–0 mononylon between the pulmonary outflow tract and the left atrium. The heart was then returned to the thorax and the lungs inflated by positive pressure as the thoracotomy was closed. After surgery, the rats were housed in a temperature-controlled room (24°C) with a 12-h light:dark cycle. Rats consumed food and water *ad libitum*.

Groups and treatment. Two days after the surgical procedure, survivors were divided into 2 groups: rats that consumed the commercial diet [MI group, $n = 34$; fully described by Paiva et al. (12)]

¹ Presented in abstract form: Paiva SAR, Matsubara BB, Matsubara LS, Correa C, Bruno MB, Campana AO, Zornoff LAM. [Efeito do ácido retinóico sobre a remodelação ventricular após o infarto experimental em ratos]. Anais do VII Congresso da Sociedade Brasileira de Alimentação e Nutrição-SBAN 2003;1: 194–5.

² Supported in part by a grant from Fundação de Amparo à Pesquisa do Estado de São Paulo and CNPq.

³ Supplemental Tables 1–3 are available as Online Supporting Material with the online posting of the paper at www.nutrition.org.

⁴ To whom correspondence should be addressed.
E-mail: paiva@fmb.unesp.br.

⁵ Abbreviations used: +dp/dt, maximum rate of rise of left ventricular pressure; CSA, cross-sectional area of the myocyte; IC, interstitial collagen fraction; LV, left ventricle; MI, myocardial infarction; RA, retinoic acid supplementation; RAR, retinoic acid receptor; RXR, retinoid X receptor.

and those that consumed that diet supplemented with 0.3 mg all *trans*-RA/kg (Sigma); each of these rats ingested $\sim 24 \mu\text{g}/(\text{kg body wt} \cdot \text{d})$ of RA (MI-RA group, $n = 29$). Treatments were started 48 h after surgery because before this time, bleeding, pneumothorax, and anesthesia may cause death not related to the infarct itself or treatment administration. The RA dose used in this protocol was the same as that used in a previous study and shown to be biologically effective (13). The planned observation period was 180 d.

Echocardiographic study. After 6 mo, all rats were weighed and subjected to a transthoracic echocardiographic exam. The exams were performed as described by Paiva et al. (12) using an echocardiographic machine SONOS 2000 (Hewlett-Packard Medical Systems) equipped with a 7.5-MHz phased array transducer. M-mode images of the left ventricle (LV), left atrium, and aorta were recorded on a black-and-white thermal printer (Sony Up-890MD) at a sweep speed of 100 mm/s. All measurements were manually obtained by the same observer.

In vitro left ventricular function. Two days after the echocardiographic study, the rats were anesthetized with thiopental sodium (50 mg/kg, i.p.) and administered heparin (2000 UI, i.p.). The chest was entered through a median sternotomy under artificial ventilation. The entire heart was quickly removed from the chest and transferred to a perfusion apparatus (model 830 Hugo Sachs Electronick-Greenstasse). The ascending aorta was isolated and cannulated for retrograde perfusion with filtered and oxygenated Krebs-Henseleit solution, which was maintained at a constant temperature and perfusion pressure (37°C and 75 mm Hg, respectively). All hearts were paced at 200 beats/min. The procedures and measurements were executed following a previously described method (12,14).

Morphometric analysis. At the completion of the functional study, the right and left ventricles (including the interventricular septum) were dissected, separated, and weighed. The morphometric analysis [myocyte cross-sectional area (CSA) and interstitial collagen volume fraction (IC)] of the myocardium was performed as described previously (15). Intramyocardial coronary arteries ranging from 30 to 160 μm were selected for analysis. A mean of 3 arteries was used for each rat. Perivascular collagen was measured and normalized to the vessel luminal areas as proposed by Lu et al. (16). Infarct size was calculated as described by Spadaro et al. (17).

Statistical analysis. Groups were compared using Student's *t* test (normally distributed variables) or the Mann-Whitney U test. Data are expressed as means \pm SD or medians (lower quartile, upper quartile). Data analysis was carried out with SigmaStat for Windows v2.03 (SPSS). Survival was analyzed using Kaplan-Meier curves with the Cox regression model. Differences were considered significant at $P < 0.05$.

RESULTS

After 6 mo, 12 MI rats and 11 MI-RA rats had died ($P > 0.05$).

The CSA was smaller in the MI-RA group [229 (220,234) μm^2] than in the MI group [238 (232,241) μm^2] ($P = 0.01$) and IC was smaller in the MI-RA group [2.4 (1.7, 3.1)%] than in the MI group [3.5 (2.6, 3.9)%] ($P = 0.05$). The other morphological variables tested did not differ between the groups (Supplemental Table 1).

The infarct size did not differ between the groups [MI = 44.6 (40.8, 48.4)%, MI-RA = 45 (38.6, 47.2)%]. The other echocardiographic variables tested did not differ between groups (Supplemental Table 2).

Maximum rate of rise of LV pressure (+dp/dt) was greater in the MI-RA group (2645 \pm 886 mm Hg/s) than in the MI group (2081 \pm 617 mm Hg/s) ($P = 0.05$). Retinoic acid supplementation did not affect the other functional variables measured in the isolated heart study (Supplemental Table 3).

DISCUSSION

The purpose of this investigation was to analyze the effects of chronic RA supplementation on ventricular remodeling

after MI in rats. Our results indicate that RA supplementation may attenuate the ventricular remodeling process after MI.

Several experimental studies analyzed the effects of RA on cardiac structure and function. Indeed, during the early stages of cardiogenesis, excess RA produces congenital defects related to septation of the outflow tract and cardiac chambers (18). Vitamin A-deficient animals and receptor knockout models present a thinner myocardial wall, which is associated with heart failure (19). Also, overexpression of retinoic acid receptors (RAR or RXR) induced dilated cardiomyopathy, depressed cardiac function, and congestive heart failure (20–22). In cultured neonatal rat cardiomyocytes, RA antagonized endothelin-stimulated hypertrophy (8). In vitro data suggest that RA suppresses both morphological alterations and changes in gene expression associated with hypertrophy induced by phenylephrine and angiotensin II (9,10). In spontaneously hypertensive rats, chronic treatment with RA prevented myocardial and renal artery thickening (16). In the same model, RA treatment for 1 mo increased gene expression of angiotensin-converting enzyme 2, resulting in a reduction in blood pressure and attenuation of myocardial damage (23). In addition, we demonstrated previously that RA supplementation induced myocardial hypertrophy, ventricular enlargement, and enhanced myocardial function in normal rats (13). The cardiac effects of RA are still poorly understood. The reasons for these disparities are not clear, but the different animal models utilized, differences in the dosage of RA, and differing pathologic and/or physiologic conditions that existed during exposure in different developmental periods, could contribute to these discrepancies in the cardiovascular response to RA.

The main finding of the present study was that the RA supplementation attenuated the LV morphological changes induced by coronary occlusion. This phenomenon was characterized mainly by a decrease in LV CSA compared with controls. Alterations in ventricular mass, volume, and geometry after cardiac injury can be interpreted as an expression of the remodeling process. In fact, regardless of the complexity of the remodeling process, measurements to assess cardiac remodeling include heart size, shape and mass, and ventricular volumes (1–3). Therefore, our data would indicate that retinoic acid supplementation decreases the hypertrophic process after MI.

The myocardial extracellular matrix surrounds and interconnects muscle fibers, cardiac myocytes, and myofibrils. Accordingly, interstitial collagen influences the size and shape of the cardiac chambers as well as ventricular function (24–26). After MI, ventricular remodeling is also associated with alterations in the structure of the interstitial matrix. The presence of multiple foci of replacement fibrosis was observed in combination with interstitial fibrosis in both the right and noninfarcted LV. Importantly, this collagen accumulation is associated with myocardial dysfunction and poor prognosis (24–26). In our study, retinoic acid supplementation resulted in a smaller amount of collagen (IC) compared with the control group.

Initially, ventricular remodeling is a compensatory process influenced by hemodynamic overload or neurohormonal activation (1–3). The morphological adaptations are a key process to preserving cardiac function after several injuries. However, chronic ventricular remodeling is now recognized as an important pathologic process, which results in progressive ventricular dysfunction and clinical presentation of heart failure or sudden death (1–3). In agreement with this concept, in the RA supplementation group, the attenuation of the remodeling process improved cardiac function as assessed by +dp/dt.

An important concept to keep in mind is that cardiac remodeling is related to major clinical outcomes, such as cardiovascular death, which represent the natural history of heart failure syndrome. Therapeutic interventions in humans that slow down or attenuate the remodeling process, such as angiotensin-converting enzyme inhibitors or β -blockers, were proven to decrease mortality after MI (27). Therefore, we contend that there is a role for RA supplementation in the overall management following MI.

In conclusion, retinoic acid supplementation for 6 mo attenuates cardiac remodeling after experimental MI in rats.

LITERATURE CITED

- Pfeffer JM, Pfeffer MA, Braunwald E. Influence of chronic captopril therapy on the infarcted left-ventricle of the rat. *Circ Res*. 1985;57:84–95.
- Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction: experimental observations and clinical implications. *Circulation*. 1990;81:1161–72.
- Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling—concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. *J Am Coll Cardiol*. 2000;35:569–82.
- Pfeffer MA, Braunwald E, Moye LA, Basta L, Brown EJ Jr, Cuddy TE, Davis BR, Geltman EM, Goldman S, Flaker GC, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. Results of the survival and ventricular enlargement trial. The SAVE Investigators. *N Engl J Med*. 1992;327:669–77.
- Oie E, Bjonerheim R, Groggaard HK, Kongshaug H, Smiseth OA, Attramadal H. ET-receptor antagonism, myocardial gene expression, and ventricular remodeling during CHF in rats. *Am J Physiol Heart Circ Physiol*. 1998;275(3 Pt 2):H868–77.
- Bristow MR. Beta-adrenergic blockade in chronic heart failure. *Circulation*. 2000;101:558–69.
- Pfeffer MA, Pfeffer JM, Steinberg C, Finn P. Survival after an experimental myocardial infarction: beneficial effects of long-term therapy with captopril. *Circulation*. 1985;72:406–12.
- Wang HJ, Zhu YC, Yao T. Effects of all-*trans* retinoic acid on angiotensin II-induced myocyte hypertrophy. *J Appl Physiol*. 2002;92:2162–8.
- Wu JM, Garami M, Cheng T, Gardner DG. 1,25 (OH)₂ vitamin D-3 and retinoic acid antagonize endothelin-stimulated hypertrophy of neonatal rat cardiac myocytes. *J Clin Invest*. 1996;97:1577–88.
- Zhou MD, Sucov HM, Evans RM, Chien KR. Retinoid-dependent pathways suppress myocardial-cell hypertrophy. *Proc Natl Acad Sci U S A*. 1995;92(16):7391–5.
- Zornoff LA, Matsubara BB, Matsubara LS, Paiva SA, Spadaro J. Early rather than delayed administration of lisinopril protects the heart after myocardial infarction in rats. *Basic Res Cardiol*. 2000;95:208–14.
- Paiva SAR, Novo R, Matsubara BB, Matsubara LS, Azevedo PS, Minicucci MF, Campana AO, Zornoff LAM. β -Carotene attenuates the paradoxical effect of tobacco smoke on the mortality of rats after experimental myocardial infarction. *J Nutr*. 2005;135:2109–13.
- Paiva SAR, Zornoff LA, Okoshi MP, Okoshi K, Matsubara LS, Matsubara BB, Cicogna AC, Campana AO. Ventricular remodeling induced by retinoic acid supplementation in adult rats. *Am J Physiol Heart Circ Physiol*. 2003;284:H2242–6.
- Zornoff LA, Paiva SA, Matsubara BB, Matsubara LS, Spadaro J. Combination therapy with angiotensin converting enzyme inhibition and AT1 receptor inhibitor on ventricular remodeling after myocardial infarction in rats. *J Cardiovasc Pharmacol Ther*. 2000;5:203–9.
- Matsubara LS, Matsubara BB, Okoshi MP, Cicogna AC, Janicki JS. Alterations in myocardial collagen content affect rat papillary muscle function. *Am J Physiol Heart Circ Physiol*. 2000;279:H1534–9.
- Lu L, Yao T, Zhu YZ, Huang GY, Cao YX, Zhu YC. Chronic all-*trans* retinoic acid treatment prevents medial thickening of intramyocardial and intrarenal arteries in spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol*. 2003;285(4):H1370–7.
- Spadaro J, Fishbein MC, Hare C, Pfeffer MA, Maroko PR. Characterization of myocardial infarcts in the rat. *Arch Pathol Lab Med*. 1980;104:179–83.
- Shenefelt RE. Morphogenesis of malformations in hamsters caused by retinoic acid: relation to dose and stage at treatment. *Teratology*. 1972;5:103–18.
- Dyson E, Sucov HM, Kubalak SW, Schmidtschonbein GW, Delano FA, Evans RM, Ross J Jr, Chien KR. Atrial-like phenotype is associated with embryonic ventricular failure in retinoid-X receptor- α $-/-$ mice. *Proc Natl Acad Sci U S A*. 1995;92(16):7386–90.
- Colbert MC, Hall DG, Kimball TR, Witt SA, Lorenz JN, Kirby ML, Hewett TE, Klevitsky R, Robbins J. Cardiac compartment-specific overexpression of a modified retinoic acid receptor produces dilated cardiomyopathy and congestive heart failure in transgenic mice. *J Clin Invest*. 1997;100:1958–68.
- Colbert MC, Kirby ML, Robbins J. Endogenous retinoic acid signaling colocalizes with advanced expression of the adult smooth muscle myosin heavy chain isoform during development of the ductus arteriosus. *Circ Res*. 1996;78:790–8.
- Subbarayan V, Mark M, Messadeq N, Rustin P, Chambon P, Kastner P. RXR α overexpression in cardiomyocytes causes dilated cardiomyopathy but fails to rescue myocardial hypoplasia in RXR α -null fetuses. *J Clin Invest*. 2000;105:387–94.
- Zhong JC, Huang DY, Yang YM, Li YF, Liu GF, Song XH, Du K. Upregulation of angiotensin-converting enzyme 2 by all-*trans* retinoic acid in spontaneously hypertensive rats. *Hypertension*. 2004;44:907–12.
- Janicki JS, Brower GL. The role of myocardial fibrillar collagen in ventricular remodeling and function. *J Card Fail*. 2002;8(6 Suppl):S319–25.
- Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. *Circulation*. 1991;83:1849–65.
- Janicki JS, Matsubara BB. Myocardial collagen and left ventricular diastolic dysfunction. In: Gaash W, LeWinter M, editors. *Left ventricular diastolic dysfunction and heart failure*. Philadelphia: Lea & Febiger, 1994. p. 125–40.
- Udelson JE, Patten RD, & Konstam MA. New concepts in post-infarction ventricular remodeling. *Rev Cardiovasc Med*. 2003;4(Suppl 3):S3–12.