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Tamoxifen treatment of myocardial infarcted female rats exacerbates scar formation

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Abstract Hormonal replacement therapy in postmenopausal women was associated with an increased incidence of nonfatal myocardial infarction. Selective estrogen receptor modulators were considered an alternative pharmacological approach. However, selective estrogen receptor modulators acting via estrogen receptor-dependent and receptor-independent mechanisms may negatively influence cardiac remodeling. The present study tested the hypothesis that tamoxifen (TAM) treatment after coronary artery ligation compromised scar formation. TAM administration (10 mg kg^{-1} day^{-1} for 3 weeks) to postmyocardial infarcted (MI) female adult rats significantly increased scar surface area (TAM+MI=0.67±0.08 vs MI=0.45±0.06 cm^2) and weight (TAM+MI=0.071±0.007 vs MI=0.050±0.006 grams). In the infarct region, a significant decrease ($p<0.05$) of small calibre vessels (lumen diameter <50 μm) was observed in TAM treated post-MI rats (4.5±0.8 vessels/mm^2), as compared to untreated MI rats (7±0.7 vessels/mm^2). Consistent with the latter finding, 4-OH TAM caused a dose-dependent suppression of vascular endothelial growth factor (VEGF)-stimulated (10^{-9} mol/l) capillarity-like tubule formation by rat aortic endothelial cells in vitro via an estrogen receptor-independent mechanism. These data have demonstrated that TAM treatment of post-MI female rats exacerbated scar formation and may have occurred at least in part via the attenuation of new vessel formation in the infarct region.

Keywords Heart · Ischemia · Vessels · Reparative fibrosis

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Coronary artery disease accounts for one third of all deaths among postmenopausal women [31]. Observational studies have shown that women receiving hormone replacement therapy (HRT) had a 30 to 35% lower risk of coronary artery disease [2]. Indeed, a recent study demonstrated that estrogen therapy retarded the development of subclinical atherosclerosis [8]. However, two randomized clinical trials failed to show any benefit of HRT in postmenopausal women with established coronary artery diseases [heart and estrogen/progestin replacement study (HERS) and estrogen replacement and atherosclerosis (ERA)] [7, 10]. Moreover, in the Women's Health Initiative study, a 23% increase in cardiovascular disease and a 38% increase in stroke were detected following HRT treatment of postmenopausal women without established coronary artery disease [32]. Consequently, alternative pharmacological approaches are required, which offer postmenopausal women the advantages of estrogen therapy while minimizing nefarious secondary effects.

Tamoxifen (TAM) is the prototype of a group of nonsteroidal compounds possessing a triphenylbutene core and basic side chain and a member of a class of drugs referred to as selective estrogen receptor modulators (SERMs) [12]. SERMs have a unique pharmacological profile exhibiting both a partial estrogen receptor agonist and antagonist activity [12]. For instance, TAM was identified as an effective antagonist in the treatment of

estrogen-dependent breast cancer, whereas TAM mimicked the beneficial action of estrogen on bone resorption [12]. Furthermore, akin to estrogens, TAM reduced identifiable cardiovascular risk factors, including serum cholesterol and low-density lipoprotein [3, 19]. Interestingly, a Scottish study examining the efficacy of TAM therapy for breast cancer observed a 60% reduction in fatal myocardial infarction (MI) and a 50 to 70% reduction in nonfatal MI [14, 15]. Likewise, the Stockholm Breast Cancer Group Study highlighted a similar reduction in cardiac morbidity in >2,000 women treated with TAM [26]. By contrast, in the breast cancer prevention trial, TAM therapy was not associated with either beneficial or harmful cardiovascular effects when administered to breast cancer patients with or without heart disease [24]. The disparate observations regarding the efficacy of TAM on cardiovascular disease may be in part related to differences in the study design [24]. Consequently, additional studies that examine the effectiveness of SERMs on cardiovascular disease as the primary endpoint are required.

In ovariectomized post-MI female rats, 17-beta-estradiol (17βE) administration increased infarct size, as compared to placebo-treated ovariectomized post-MI rats [27]. The latter data suggest that SERMs may likewise cause adverse cardiac remodeling post-MI via an estrogen receptor-dependent mechanism. Moreover, SERM-mediated cellular effects may also occur via an estrogen-receptor independent mechanism [11]. Indeed, acting independently of estrogen receptor activation, TAM attenuated calcium uptake by the sarcoplasmic reticulum and inhibited cardiac myocyte and fibroblast growth [4, 18]. Collectively, these observations suggest that TAM acting via either an estrogen receptor dependent and/or independent mechanism may negatively influence cardiac remodeling following damage. The present study tested the hypothesis that TAM treatment compromised scar formation in female adult rats following complete coronary artery ligation.

Materials and methods

Ovariectomized and myocardial infarct rat models

Female Sprague-Dawley rats (7–9 weeks old; Charles Rivers, St. Constant, Quebec, Canada) were anesthetized with a ketamine (50 mg kg^{-1})/xylazine (10 mg kg^{-1}) mixture and underwent either a sham surgery or bilateral ovariectomy (OVX). Three weeks after OVX, MI was induced by ligating the left anterior descending coronary artery as previously described [21] and was killed 3 weeks later. In a separate group of animals, rats underwent either a sham operation or coronary artery ligation and were randomized into 4 groups: (1) sham-operated (n=7); (2) sham-operated + TAM (n=8); (3) MI (n=24); and (4) MI + TAM (n=21). In the MI and MI + TAM groups, five rats from each group were used to assess vessel formation in the infarct region by immunofluorescence (please see below). In the latter two rat groups, heart weight and scar size were not determined, and mean arterial pressure and left ventricular function were measured only in two MI + TAM rats (data included in Table 3). To examine the effect of TAM, a dose of 10 mg kg^{-1} per day was added to standard rat chow (12–24 h postsurgery), and continued for a period of 3 weeks. The dose of TAM employed in the present study was previously shown to normalize the exaggerated cerebral artery response to norepinephrine in the ovariectomized female rat [29]. During the 3-week protocol, rats were weighed every 2 days to adjust dosage according to changes in body weight (BW). Regardless the infarct size, all MI-, MI + TAM-, and OVX + MI-treated rats were analyzed. Lastly, the use and care of laboratory rats was according to the Canadian Council for Animal Care and approved by the Animal Care Committee of the Montreal Heart Institute.

Hemodynamic measurements

Left ventricular function was measured, as previously described [21]. Following hemodynamic measurements, the heart was removed and separated into the left ventricle or noninfarcted left ventricle, septum, right ventricle, and scar. The tissue was immediately weighed, and stored at −80°C. The scar surface area was calculated by planimetry, as previously described [20].

Immunofluorescence

In a separate series of experiments, the heart was excised, immersed directly in 2-methyl butane (temperature maintained at −80°C), and stored at −80°C. Immunofluorescence on tissue (cryostat sections of 14 μm thickness; n=5 for both MI- and TAM-treated MI rats) was performed, as previously described [5]. Vessels were identified in the infarct region via α–smooth muscle actin (mouse monoclonal; 1:200; Sigma) and VE–cadherin (rabbit polyclonal; 1:200; Serotec) immunoreactivity on vascular smooth muscle cells and endothelial cells, respectively. Secondary antibodies used were a goat antimouse immunoglobulin G (IgG) conjugated to rhodamine (1:250–500; Molecular Probes) and a donkey antirabbit IgG conjugated to fluorescein isothiocyanate (1:500; Molecular Probes). Vessels were visualized with either a 10X- or 63X-oil 1.4 NA DIC plan apochromat objective mounted on a Zeiss Axiovert 100M confocal microscope in a field of 0.7–0.84 mm^2. Four transverse sections of the infarct region from each MI and MI + TAM rat was examined, and the total number of

vessels normalized to the surface area (mm^2), as calculated by the program LSM 5 Image Browser (Zeiss). The vessel calibre was determined by the lumen diameter (long axis), and three distinct groups were identified: (1) <50 μm, (2) 50> 100 μm, and (3) >100 μm.

In vitro angiogenesis

Normal rat aortic endothelial cells (RAEC) were isolated from adult Sprague-Dawley rats. RAECs were cultured in phenol-red free Dulbecco's modified Eagle's medium (DMEM; Life Technologies) containing 5% fetal bovine serum (Hyclone Laboratories), and antibiotics (penicillin and streptomycin, Sigma). RAEC (first–fourth passage) were resuspended at a density of 5×10^4 cells/ml in phenol red-free DMEM. Matrigel (250 μl; BD Biosciences) was added to 24-well plates (Costar) and subsequently incubated at 37°C for 30 min for gelation. Thereafter, cells were seeded in solidified Matrigel (BD Biosciences) with or without vascular endothelial growth factor (10^{-9} mol/l) in the absence or presence of 17βE (10^{-9} to 10^{-7} mol/l) or 4-OH TAM (10^{-9} to 10^{-7} mol/l). Plates were incubated for 24 h and tubular formation was assessed. Results were expressed as the mean number of junctions at 20× original magnification per well. Tubular formation experiments were performed on 2–3 independent preparations of RAEC. The stock solution of 4-OH TAM was dissolved in dimethyl sulfoxide (DMSO). At the highest concentration of 4-OH TAM (10^{-6} mol/l) tested, the vehicle DMSO (1:10,000) had no effect on vascular endothelial growth factor (VEGF)-mediated tubule formation.

Real-time PCR

RNA from the normal left ventricle and noninfarcted left ventricle of 3-week postmyocardial infarct rats was isolated, as previously described [6]. Real-time polymerase chain reaction PCR was performed on 2.5 ng of cDNA template, containing the appropriate primers (300 nM) and the SYBR Green PCR master mix (Applied BioSystems), as previously described [6]. Primers for each gene were obtained from distinct exons that span an intron employing the program Ensemble Genome Browser (http://www.ensembl.org). The sequence specificity of each primer was verified with the program Blast derived from the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The primers used were the following rat atrial natriuretic peptide (ANP): forward 5′-AGAGCGGACTAG GCTGCAACA-3′ and reverse 5′ATTTGGCTGTTATCTTC GGTA-3′, and rat β-actin; forward 5′-CCCTAAGGCCAA CCGTGAA-3′ and reverse 5′-GAGGCATACAGGGACA ACACAG-3′. Appropriate negative controls were used for each experiment.

Statistics

Data were presented as the mean±SEM. Morphological and hemodynamic data were evaluated by a 2-way analysis of variance (ANOVA), and a significant difference was determined by a Neuman-Keuls post hoc test. Tubule formation in vitro and the effect of TAM and OVX on infarct size were evaluated by a 1-way ANOVA, and a significant difference was determined by a Bonnferroni post hoc test. The effect of TAM on vessel number in the infarct region as compared to untreated MI rats was evaluated by an unpaired *t* test. A p value <0.05 was considered as statistically significant.

Results

Body, uterus, and heart weight and ventricular function: the effect of TAM

Significant loss of cardiac tissue was evident 3 weeks following coronary artery ligation in adult female rats (Table 1). Despite scar formation, absolute left ventricular (LV) weight and (LV/BW) ratio in MI rats were similar to sham (Table 2). Hypertrophy of the viable myocardium was evident as ANP mRNA levels (2.8±0.7-fold increase vs sham rats; $n=8$) were increased in the noninfarcted left ventricle, as compared to sham rats. Despite the loss of left ventricular tissue, MAP, left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP) and LV +dP/dt in untreated MI rats were not different from sham rats (Table 3). The latter findings were related in part to the variable infarct size (range of infarct surface area=0.12−0.91 cm^2). Likewise, the variable infarct size in 3-week post-MI rats contributed to the absence of a significant decrease in either absolute LV weight or LV/BW ratio. Among the 3-week post-MI rats, 42% (8 of 19) had small scars as reflected by an infarct surface area <0.30 cm^2. Of the remaining animals, 26% had a medium size scar (infarct surface area; 0.30<0.60 cm^2) and 32% were identified with large infarcts (infarct surface area >60 cm^2). In post-MI rats with an infarct surface area >0.30 cm^2, LV +dP/dt (Sham=6,557±351 vs MI=5659±280 mmHg/s) was de-

Table 1 Scar weight and surface area

	Scar weight (grams)	Scar surface area (cm^2)
MI ($n=19$)	0.050±0.006	0.45±0.06
MI+TAM ($n=16$)	0.071±0.007[a]	0.67±0.08[a]
MI+OVX ($n=7$)	0.037±0.006	0.46±0.07

Data are presented as mean±SEM
MI, myocardial infarction; *TAM*, tamoxifen; *OVX*, ovariectomy
[a] represents $p<0.05$ versus MI and (n) number of rats examined

Table 2 Body, uterus and heart weights

	BW (g)	LV (g)	RV (g)	Uterus (g)	LV/BW ($\times 10^{-3}$)	RV/BW ($\times 10^{-3}$)	Uterus/BW ($\times 10^{-3}$)
Sham (n=7)	273±9	0.37±0.02	0.15±0.008	0.53±0.06	1.35±0.06	0.56±0.02	1.94±0.08
MI (n=19)	264±6	0.35±0.01	0.15±0.004	0.48±0.03	1.31±0.06	0.60±0.02	1.82±0.11
TAM (n=8)	236±7[a,b]	0.32±0.02	0.13±0.007	0.26±0.04[a,b]	1.34±0.06	0.54±0.03	1.11±0.08[a,b]
MI + TAM (n=16)	241±3[a,b]	0.26±0.02[a,b,c]	0.14±0.01	0.28±0.01[a,b]	1.10±0.07[b,c]	0.61±0.07	1.17±0.03[a,b]
OVX (n=7)	362±6[a,b]	0.51±0.03	0.19±0.01	0.11±0.01[a,b]	1.32±0.05	0.52±0.03	0.31±0.01[a,b]
MI + OVX (n=7)	339±10[a,b]	0.45±0.02	0.16±0.01	0.11±0.01[a,b]	1.32±0.07	0.48±0.04	0.32±0.02 [a,b]

Data are presented as mean±SEM
MI, myocardial infarction; *TAM*, tamoxifen; *OVX*, ovariectomy; *BW*, body weight; *LV*, left ventricle; *RV*, right ventricle
[a] represents $p<0.05$ versus sham
[b] represents $p<0.05$ versus MI
[c] represents $p<0.05$ versus TAM and (n) represents number of rats examined

creased. Moreover, regardless infarct size, LV −dP/dt was significantly depressed in 3-week post-MI rats, as compared to sham (Table 3).

TAM treatment of sham or MI female rats significantly decreased BW and caused uterine atrophy, as compared to untreated rats (Table 2). In TAM-treated MI rats, left ventricular weight and LV/BW ratio were significantly lower as compared to sham, TAM-treated sham and untreated-MI rats (Table 2). TAM treatment did not influence the hypertrophic response of the noninfarcted left ventricle, as ANP mRNA expression (3.3±0.6-fold increase vs TAM-treated sham rats; n=8) was maintained and fold-increase was analogous to that observed in untreated MI rats. The important reduction of absolute LV weight and LV/BW ratio in TAM-treated MI rats was attributed to a significant overall increase in infarct weight (34% increase vs MI) and surface area (40% increase vs MI), as compared to nontreated MI rats (Table 1). Analysis of these data revealed that in contrast to untreated MI rats, only 13% (2 of 16 rats) of TAM-treated MI rats had an infarct surface area < 0.30 cm^2. In the remaining animals, the percentage of medium and large size infarcts was identical (43% each) and was greater than that observed in untreated MI rats. In TAM-treated sham rats, a modest nonsignificant increase of MAP, LVSP, and LV +dP/dt was observed. However, following TAM treatment of MI female rats, the greater infarct size and loss of viable left ventricle tissue were associated with a significant decrease of MAP, LVSP, LV +dP/dt and −dP/dt, as compared to TAM-treated sham rats (Table 3).

Body, uterus, heart weights and ventricular function of OVX female rats and the effect of coronary artery occlusion

The increase in infarct size documented in TAM-treated MI female rats may have occurred in part via a direct antagonism of estrogen receptor dependent cardioprotective effects and/or secondary to an ovariectomized phenotype. To concomitantly address the two latter issues, coronary artery ligation was superimposed on ovariectomized female rats (3-weeks) with established elevated blood pressure. In 6-week OVX female rats, BW was significantly increased and associated with marked uterine atrophy (Table 2). As compared to TAM-treated sham rats, uterine atrophy was greater in OVX rats (Table 2). Left ventricular (LV/BW) and right ventricle (RV)/BW ratios in OVX rats were similar to sham (Table 2). MAP, LVSP, +dP/dt and −dP/dt were significantly increased in 6-week OVX female rats, as

Table 3 Mean arterial pressure and left ventricular function

	MAP (mmHg)	LVSP (mmHg)	LVEDP (mmHg)	LV +dP/dt (mmHg s^{-1})	LV −dP/dt (mmHg s^{-1})
Sham (n=7)	110±6	122±10	8±2	6557±351	5693±428
MI (n=19)	105±5	117±4	12±2	5918±264	4287±245[a]
TAM (n=8)	124±5	142±9	9±2	7363±435	5350±496
MI+TAM (n=18)	102±4[b]	113±6[b]	12±2	55543±291[b]	3627±248[a,b]
OVX (n=7)	131±7[a]	149±10[a]	12±1	7643±494[a]	7094±341[a]
MI+OVX (n=7)	115±5	123±8	10±2	6070±233[c]	4418±270[c]

Data are presented as mean±S.E.M
MI, myocardial infarction; *TAM*, tamoxifen; *OVX*, ovariectomy; *LVSP*, left ventricular systolic pressure; *LVEDP*, left ventricular end-diastolic pressure; *RVSP*, right ventricle systolic pressure; *+dP/dt*, rate of left ventricular contraction; *−dP/dt*, rate of left ventricular relaxation
[a] represents $p<0.05$ versus sham
[b] represents $p<0.05$ versus TAM
[c] represents $p<0.05$ versus OVX and (n) number of rats examined

compared to sham rats (Table 3). Following the superimposition of coronary artery occlusion in 3-week OVX rats, the hemodynamic status was compromised as reflected by a significant decrease of LV +dP/dt and −dP/dt (Table 3). However, in contrast to TAM-treated MI rats, infarct weight and surface area of OVX + MI rats were not statistically different from nonovariectomized post-MI rats (Table 1).

TAM effect on angiogenesis in the infarct region and in vitro tubule formation

In the infarct region of untreated 3-week post-MI female rats, vessels of varying calibre were detected and identified via α-smooth muscle actin immunoreactivity of vascular smooth muscle cells and concomitant VE-cadherin staining of endothelial cells (Fig. 1). Based on the lumen diameter, vessels were classified as either <50 μm, 50>100 μm, or >100 μm and the preponderance of vessels detected were <50 μm (Fig. 1). In untreated 3-week post-MI female rats, the total number of vessels in the scar region strongly correlated with the infarct surface area (r=0.94; p=0.019; n=5 MI rats) (Fig. 2). By contrast, the total number of vessels in the scar region of TAM-treated 3-week post-MI rats did not correlate with the infarct surface area (r=0.63; p=0.25; n=5 TAM-treated MI rats). A nonsignificant reduction (p=0.1) of the total number of vessels was observed in the infarct region of TAM-treated MI rats (7.3± 0.7 vessels/mm^2), as compared to untreated MI rats (9.2± 0.7 vessels/mm^2). However, a selective significant decrease (p<0.05) of small calibre vessels (<50 μm) was detected in TAM-treated MI rats (4.5±0.8 vessels/mm^2), as compared to untreated MI rats (7±0.7 vessels/mm^2; Fig. 1).

The reduction of small calibre vessels in the infarct region of TAM-treated MI rats may have occurred via a direct

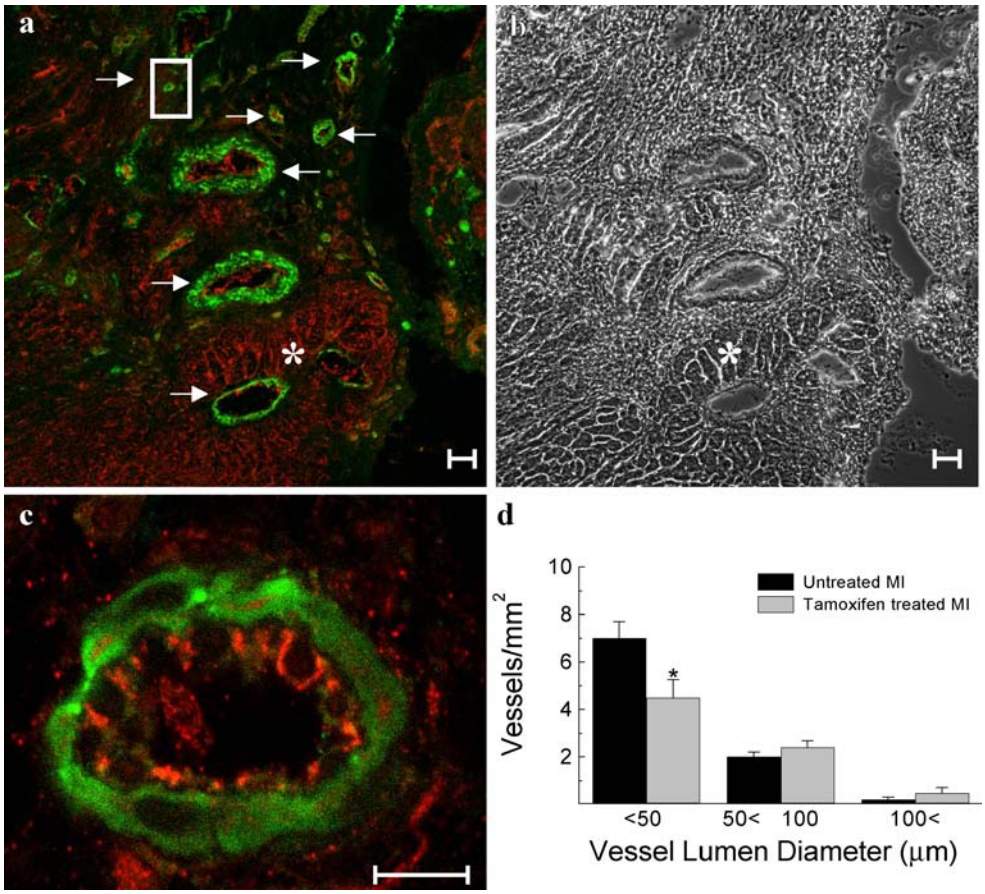

Fig. 1 Vessel identification in the infarct region and the effect of tamoxifen on blood vessel growth **a** In the infarct region of untreated MI rats, numerous vessels (indicated by *arrow*) of varying calibre (lumen diameter; 11–116 μm) were detected via α-smooth muscle actin immunoreactivity (green fluorescence) of vascular smooth muscle cells and VE-cadherin staining (red fluorescence) of endothelial cells. Endothelial cell staining was not evident in small calibre vessels at low magnification. The *asterisk* reflects a weak non-specific signal in cardiac myocytes residing in the infarct region. **b** Phase contrast picture of **a**. **c** The small calibre vessel highlighted in the box of **a** was magnified and VE-cadherin immunoreactivity (red fluorescence) of endothelial cells, and concomitant α-smooth muscle actin staining (green fluorescence) of vascular smooth muscle cells were evident. **d** Bar graph depicting vessel calibre and number in transverse sections (please see Materials and methods) of the infarct region of untreated (n=5) and tamoxifen-treated MI rats (n=5). *Asterisk* depicts p<0.05 versus untreated MI rats. Scale bar; **a** and **b**, 50 μm; **c**, 10 μm

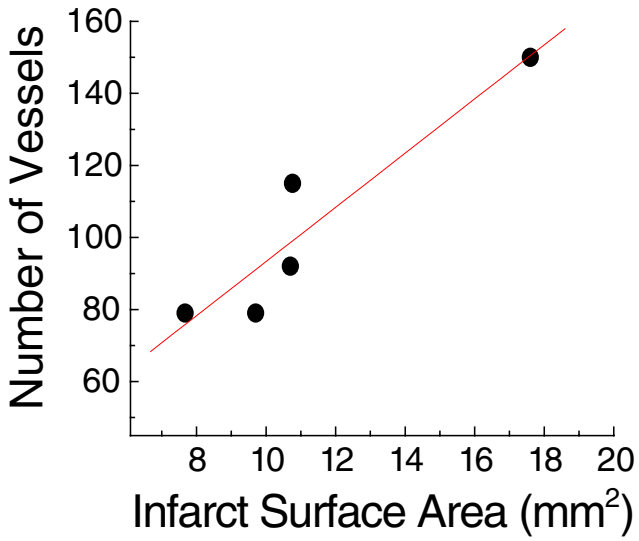

Fig. 2 Relationship between vessel number and infarct region in 3-week post-MI rats Vessel number were quantified in four transverse sections of the infarct region of each MI rat. Total number of vessels (regardless of lumen diameter) detected in the scar region of 3-week post-MI rats strongly correlated with the infarct surface area (r=0.94; p=0.019; n=5 MI rats)

angiostatic action. In the Matrigel assay, tubule formation by RAEC was observed following a 24-h treatment with VEGF (10^{-9} mol/l) (Fig. 3). The coadministration of 4-OH TAM (n= 3 separate experiments) resulted in a dose-dependent inhibition of tubule formation with a maximal effect observed at 10^{-7} mol/l (Fig. 2). By contrast, VEGF-mediated capillary tubule formation by RAEC was unaffected by the coadministration of 17βE (n=2 separate experiments; Fig. 3).

Discussion

The increased incidence of cardiovascular disease in postmenopausal women following HRT has provided the impetus to examine alternative therapeutic approaches. Indeed, consideration was given to selective estrogen-receptor modulators (SERMs). However, acting either via an estrogen receptor-dependent and/or receptor-independent pathway, several studies support a maladaptive effect of TAM on the damaged myocardium [4, 18, 27]. Thus, the present study examined whether TAM treatment compromised scar formation following coronary artery occlusion in adult female rats. TAM administration 24 h after coronary artery occlusion and continued for 3 weeks increased infarct weight, surface area and reduced the number of small calibre vessels in the scar, as compared to untreated MI rats. In vitro, 4-OH TAM inhibited VEGF-mediated capillary tubule formation by RAEC via an estrogen receptor-independent mechanism.

Despite the loss of ventricular tissue following coronary artery ligation, neither MAP, LVSP, nor +dP/dt were decreased in 3-week post-MI female rats. These latter findings were attributed to variable scar size (range of infarct surface area=0.12−0.91 cm^2). However, LV +dP/dt was reduced in MI rats with an infarct surface area >30 cm^2. Moreover, regardless infarct size, a significant decrease of LV −dP/dt was detected in 3-week post-MI female rats. As previously demonstrated, TAM treatment of female sham rats partially mimicked the phenotype of ovariectomized rats as reflected by significant uterine atrophy and modest increase of MAP, LVSP, and dP/dt [23]. By contrast, TAM promoted a significant loss of BW, whereas a weight gain was observed in ovariectomized rats. Collectively, these data highlight the partial estrogen agonist and antagonistic properties of TAM in the adult female rat [17, 27]. Following the treatment of female MI rats with TAM, infarct weight and surface area were significantly increased, as compared to untreated MI rats. Consistent with these data, a statistically significant reduction of absolute LV weight was observed in TAM-treated MI rats, as compared to sham, TAM-treated sham, and untreated MI rats. The important loss of viable left ventricular tissue in TAM-treated MI rats was associated with a significant decrease of MAP, LVSP, +dP/dt, and −dP/dt, as compared to TAM-treated sham rats.

The underlying mechanism(s) attributed to the exacerbated infarct size in TAM-treated post-MI female rats remains presently undefined. The antagonism of estrogen receptor-dependent cardiac protective effects and/or secondary to a pharmacologically induced ovariectomized phenotype may have independently or cooperatively contributed to the increase of infarct size in TAM-treated MI rats [22, 25]. To concomitantly address these issues, coronary artery occlusion was superimposed on 3-week ovariectomized female rats characterized by increased mean arterial pressure and marked uterine atrophy [17, 23]. Previous studies have documented an antiapoptotic action of 17βE on cardiac myocytes [22, 25]. Thus, it was anticipated that the superimposition of MI on ovariectomized rats would exacerbate scar formation. However, in ovariectomized female rats that underwent coronary artery ligation, infarct weight and surface area were not statistically different from nonovariectomized female MI rats. Likewise, scar formation was not exacerbated in ovariectomized female mice subjected to coronary artery occlusion [9]. However, a significant reduction of dP/dt indices was observed in ovariectomized MI female rats, as compared to ovariectomized female rats. Collectively, these data suggest that the increased infarct size documented in TAM-treated MI rats was not directly related to either an antagonism of estrogen receptor-dependent cardioprotective effects or secondary to a pharmacologically induced ovariectomized phenotype. Thus, additional studies are required to assess

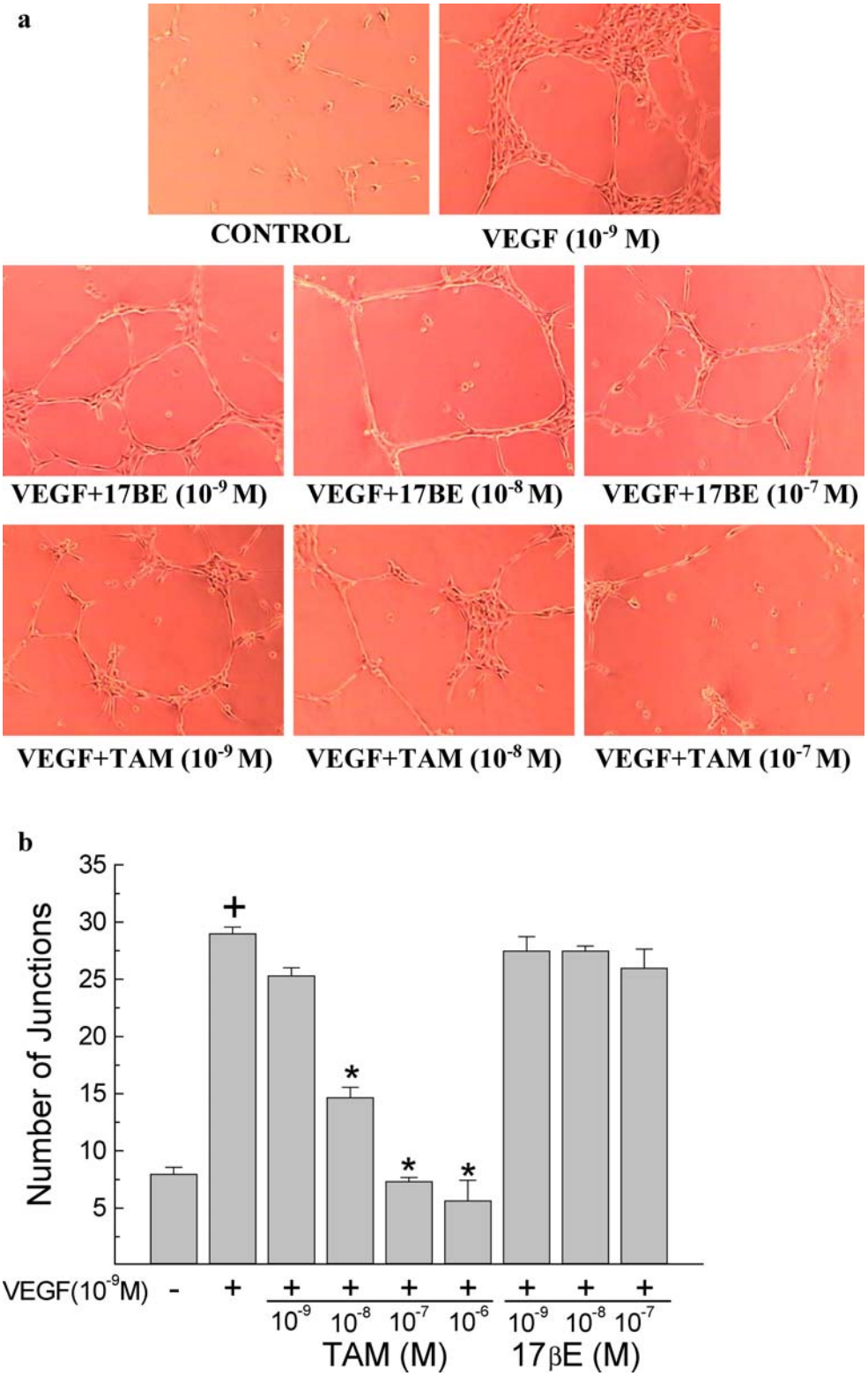

Fig. 3 The effect of tamoxifen on vascular endothelial growth factor (VEGF)-mediated capillary tubule formation. **a** Phase contrast pictures depicting the dose dependent inhibition of VEGF-mediated capillarity-like tubule formation of rat aortic endothelial cells by 4-OH tamoxifen (TAM), whereas no effect was observed with 17-beta-estradiol (17βE). **b** Quantitative analysis of tubule formation was assessed by the number of junctions formed by VEGF (10^{-9} M; 24-h stimulation) and the subsequent effect of 17-beta-estradiol (17βE; n=2) and 4-OH tamoxifen (TAM; n=3). *Plus sign* depicts $p<0.05$ versus untreated and *asterisk* depicts $p<0.05$ versus VEGF alone

the contribution of estrogen receptor-independent effects of TAM on scar remodeling post-MI. Alternatively, pharmacologically elevating estrogenic activity with TAM administration may be deleterious in post-MI female rats. The study by Smith et al. [27] reported increased infarct size in ovariectomized post-MI female rats that received continuous estrogen therapy, as compared to nontreated ovariectomized post-MI rats.

Collateral vessel formation in the noninfarcted left ventricle and angiogenesis in the infarct region were

reported in the rat model of MI [16]. The exogenous administration of either hepatocyte growth factor or VEGF in the post-MI rat enhanced the angiogenic response and concomitantly reduced infarct size [1, 16, 28]. Based on these observations, attenuating either collateral vessel growth and/or angiogenesis during the early phase of cardiac remodeling post-MI would contribute to further scar expansion. Thus, to assess whether TAM can impede vessel growth in the MI rat, the potential angiostatic action of the SERM was examined in the infarct region. Consistent with previous studies, numerous vessels (α-smooth muscle actin; vascular smooth muscle cells, VE-cadherin; endothelial cells) of varying size were detected in the infarct region of 3-week post-MI rats and the total number of vessels strongly correlated with the scar surface area. Vessels were subsequently characterized by lumen diameter, and the majority detected were of small calibre (lumen diameter <50 μm). In TAM-treated MI rats, total vessel number did not correlate with infarct surface area, and a significant reduction of small calibre vessels was detected, as compared to untreated MI rats. The latter data were consistent with previous studies reporting the angiostatic action of TAM in vivo [13, 30]. Furthermore, the in vivo findings were confirmed in the in vitro Matrigel assay, as TAM administration caused a dose-dependent inhibition of VEGF-mediated tubule formation by RAEC. Moreover, the angiostatic property of TAM was not related to its partial estrogenic activity, as VEGF-mediated capillary tubule formation was unaffected by the coadministration of 17βE. Collectively, an impaired angiogenic response in the infarct region of TAM-treated MI rats may have contributed to increased scar expansion and the in vitro data further suggest that the angiostatic action of the SERM occurred via an estrogen receptor-independent mechanism.

The detrimental effect of HRT on the incidence of cardiovascular disease in post-menopausal women has spawned a search for alternative pharmacological approaches. The class of compounds referred to as SERMs possess partial estrogen activity and mimic the beneficial effect of the endogenous hormone on bone resorption and circulating HDL levels [3, 12, 19]. In addition, TAM therapy normalized the exaggerated cerebral vascular reactivity to norepinephrine in ovariectomized female rats [29]. However, during the early phase of remodeling in post-MI female rats, TAM administration increased infarct size and the underlying mechanism may have been related in part to the attenuation of new vessel growth. Additional studies are required to elucidate whether other members of the SERM family mimic the maladaptive effect of TAM on scar formation following experimental MI.

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