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Raloxifene improves vascular reactivity in pressurized septal coronary arteries of ovariectomized hamsters fed cholesterol diet

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ABSTRACT

Although vascular effects of selective estrogen receptor modulators (SERMs) have been extensively examined in conduit arteries, whether SERMs could favorably modulate myogenic response in resistance arteries is unknown. The impact of raloxifene therapy and cholesterol diet on myogenic constriction during estrogen deficiency is unresolved. This study investigated changes in vascular reactivity and myogenic responses in female ovariectomized (Ovx) hamsters fed high-cholesterol diet (HCD) with and without chronic treatment of raloxifene. Functional studies were performed on hamster septal coronary arteries cannulated in a pressure myograph. Acetylcholine (ACh)-induced dilatation was reduced in arteries from cholesterol-fed Ovx hamsters, but not in those from cholesterol-fed hamsters, while pressure-induced myogenic constriction was unaffected. Chronic treatment with raloxifene restored ACh-induced dilatation in cholesterol-fed Ovx hamsters. U46619-induced constriction was increased in arteries from cholesterol-fed Ovx hamsters but not from cholesterol-fed control hamsters, which was normalized by chronic raloxifene treatment. The pressure–diameter relationship is presented as normalized diameter versus intraluminal pressure, while the effect of ACh or U46619 is expressed as percentage of tone at 80 mmHg. Two-way analysis of variance (ANOVA) followed by Bonferroni post-tests were used for statistical evaluation among different treatment groups. $P < 0.05$ was taken as statistically significant. The present results show that chronic treatment with raloxifene could benefit myogenically active coronary arteries by (i) restoring ACh-induced dilatation and (ii) reducing U46619-induced constriction without affecting pressure-induced myogenic responses in cholesterol-fed hamsters during estrogen deficiency. If such benefit can be observed in humans, raloxifene and other SERMs may be useful to preserve endothelial function and curtail vascular hypersensitivity in resistance coronary arteries in post-menopausal women with hypercholesterolemia or hyperlipidemia, a lipid condition implicated in the pathogenesis of myocardial infarction.

1. Introduction

The development of SERMs allows the clinical use of estrogen substitutes that are devoid of side effects of hormone replacement therapy. Due to their tissue-specific and mixed estrogen-agonist/antagonist properties, SERMs such as raloxifene exhibit estrogenic activities in bone, cardiovascular and central nervous systems, while avoiding harmful effects in breast and uterus. Raloxifene exerts estrogen-like cardioprotective actions and improves coronary blood flow partly by releasing endothelial nitric oxide (NO) [1,2] in animal studies. Ovariectomized (Ovx) sheep taking raloxifene have greater diameters in their coronary arteries compared with those receiving estrogen or no treatment [3]. Chronic treatment with raloxifene may reduce cardiovascular risks in women [4] and prevents endothelial dysfunction in young and aging Ovx rats by increasing NO bioavailability [5,6]. We have recently reported that raloxifene at therapeutically relevant concentrations inhibits myogenic constriction by an NO-dependent mechanism that causally involves $[Ca^{2+}]_i$ elevation in endothelial cells and subsequent eNOS activation and raloxifene dilates resistance arteries more effectively in female rats, indicating a significant gender-related action in microcirculation [7]. Although the outcomes of the RUTH trial showed that raloxifene failed to reduce the overall risk of coronary heart disease (CHD) in

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postmenopausal women with established CHD or at increased risk for CHD [8], how SERMs could specifically benefit coronary arteries from postmenopausal women with hyperlipidemia and/or hypercholesterolaemia is still unclear and it does deserve investigation.

Diets rich in fat and cholesterol are routinely used to increase the total lipids and low-density lipoprotein cholesterol (LDL-C) in hamsters and rabbits. High plasma LDL-C is associated with increased risks of CHD, myocardial infarction and stroke [9]. In women with documented CHD in the MORE (Multiple Outcomes of Raloxifene Evaluation) study, raloxifene reduces LDL-C [10].

Although estrogen has been shown to reduce myogenic constriction in resistance arteries, it is unclear whether raloxifene could also favorably modulate myogenic responses. Therefore, this study was designed to study changes in septal coronary artery reactivity and myogenic responses of female Ovx hamsters fed high cholesterol diet with and without chronic treatment with raloxifene.

2. Materials and methods

This study was approved by the Animal Research Ethics Committee of the Chinese University of Hong Kong and conformed with *The Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH Publication No.85-23, revised 1996).

2.1. Animals

Twenty-nine female Syrian golden hamsters (~12-month old), supplied by the Laboratory Animal Service Center at Chinese University of Hong Kong, were randomly divided into 5 groups: normal diet (ND, $n = 7$), high cholesterol diet (HCD, $n = 5$), high cholesterol diet with raloxifene treatment (HCD + Rf, $n = 5$), high cholesterol diet plus ovariectomy (HCD + Ovx, $n = 6$), and high cholesterol diet plus ovariectomy with raloxifene treatment (HCD + Ovx + Rf, $n = 6$). Hamsters had free access to either ND or HCD and maintained under controlled humidity (~48%) and temperature (20 °C), and a 12-h light cycle for 15 weeks. For OVX rats, ovariectomy was performed before HCD feeding. Table 1 shows the composition of HCD and ND. Body weights and food consumption were measured weekly and twice a week, respectively, while wet weight of uteri was taken at the time of sacrifice.

2.2. Raloxifene administration

HCD + Rf and HCD + Ovx + Rf were subjected to raloxifene treatment. Hamsters were anesthetized with a 3-ml mixture of ketamine and xylaxine (1.5–1, v/v) per kg body weight and a raloxifene-filled Silastic® tubing (Dow Corning) was inserted subcutaneously in the back of each hamster [11]. The average daily raloxifene release in each hamster was calculated by weight difference of raloxifene resided in the tubing before and after treatment, which was 0.0986 μg/kg body weight/day.

Table 1
Composition of normal and high cholesterol diets.

	Normal diet	High cholesterol diet
Ingredient	(%)	(%)
Casein	20	20
Lard	5	20
Starch	53.7	38.6
Sucrose	10	10
Mineral mix	4	4
Vitamin mix	2	2
DL-Methionine	0.1	0.1
Pure cholesterol	0	0.1
Gelatin	2	2
Fibre	3.2	3.2
Total	100	100

2.3. Ovariectomy

For HCD + Ovx and HCD + Ovx + Rf groups, ovariectomy was performed before HCD feeding. During ovariectomy, the hamsters underwent anesthesia followed by aseptic bilateral ovariectomy via a mid-abdominal route [6]. Raloxifene treatment started 2 weeks after ovariectomy in HCD + Ovx + Rf group.

2.4. Coronary artery preparation

After 15-week treatment with of raloxifene, hamsters were euthanized by CO_2 inhalation. The hearts were removed and placed in ice-cold Krebs solution. Septal coronary arteries (~250 μm in outer diameter) were dissected and cleaned of surrounding connective tissues. Each artery was cut into 2–3 mm long ring segments which were mounted on a pressure myograph (Danish Myo Technology, Denmark). Changes in vessel diameter were measured at various intraluminal pressures. Briefly, each artery was cannulated between two cannulae (outer diameter of ~100 μm) in a 10-ml chamber filled with Krebs solution which was continuously gassed with 95% O_2–5% CO_2 and maintained at 37 °C (pH 7.4) monitored by a temperature probe. One end of the artery was first secured on a cannula with two fine nylon sutures and then perfused with Krebs solution at a pressure difference <20 mmHg to remove residual intraluminal blood. The other end of the artery was then cannulated. Glass cannulae were prepared using a micropipette puller (model P-87, Shutter Instrument Company, Novato, CA). Both cannulae were connected to separate Krebs solution-containing bottles seated on a pressure regulator that controlled flow rate and intraluminal pressure. Myogenic constriction developed under no-flow conditions. A video camera attached to a light-inverted microscope (Zeiss Axiovert 40 Microscope, Model 110P) was used to visualize the artery and data were recorded and stored on a computer. Changes in outer diameter and luminal pressure were determined using Myo-View software (version 1.1 P, Photonics Engineering, Denmark).

2.5. Experimental protocols

2.5.1. Development of myogenic constriction

After mounting in pressure myograph, intraluminal pressure was increased from 20 to 100 mmHg at room temperature in 20 mmHg increment each 5 min. After this procedure, intraluminal pressure was maintained at 80 mmHg and superfusate was warmed up gradually to 37 °C using a built-in heating device. The chamber solution was kept at 37 °C for the remaining part of the experiment. When the superfusate temperature reached 37 °C, myogenic response developed sharply, as recorded by a decrease in the vessel diameter that stabilized after 10–20 min.

2.5.2. Pressure–diameter relationships

To compare difference in myogenic responses in various treatment groups, the pressure–diameter relationships of arteries were obtained by stepwise increment of intraluminal pressure from 20 to 120 mmHg in Krebs solution at 37 °C. At the end of the experiment, arteries were superfused with a Ca^{2+}-free and 2 mM EGTA-containing Krebs solution and pressure–diameter curve was repeated again to obtain the maximum passive vasodilatation at each pressure step (Fig. 1).