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see commentary on page 9

Calcitriol restores renovascular function in estrogen-deficient rats through downregulation of cyclooxygenase-2 and the thromboxane-prostanoid receptor

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Cardiovascular risks increase in postmenopausal women. While vitamin D is supplemented for osteoporosis, it is not known whether it protects renal arterial function during estrogen deficiency. Here we measured changes in renovascular reactivity induced by ovariectomy in rats and examined whether calcitriol, the most active form of vitamin D, was able to correct such changes. The impairment of endothelium-dependent relaxation in renal arteries from ovariectomized rats was effectively reversed by long-term calcitriol treatment. It was also corrected by acute exposure to cyclooxygenase-2 (COX-2) inhibitors and a thromboxane-prostanoid receptor antagonist, respectively. Calcitriol normalized the overexpression of COX-2 and thromboxane-prostanoid receptors in intralobal renal artery segments and aortic endothelial cells isolated from ovariectomized rats. *In vitro* exposure of the arterial segments to calcitriol for 12 h improved relaxation and downregulated thromboxane-prostanoid receptors. The attenuated nitric oxide production in ovariectomized rat aortic endothelial cells was restored following a 12-h treatment with calcitriol, COX-2 inhibition, or thromboxane-prostanoid receptor antagonism. Thus, impaired endothelium-dependent renal artery relaxation in ovariectomized rats is mediated largely through increased activity and expression of COX-2 and the thromboxane-prostanoid receptor. Calcitriol restores endothelial function through downregulating both signaling proteins during estrogen deficiency.

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Postmenopausal women experience increased cardiovascular morbidity[1] and osteoporosis[2,3] resulting from diminishing circulating levels of estrogen. Estrogen benefits cardiovascular function[4] and is essential for maintaining bone mineral density in women.[5] Calcitriol (1,25-dihydroxyvitamin D3), an active form of vitamin D that modulates calcium absorption, is a recommended medication for the treatment of osteoporosis in postmenopausal women. In fact, besides its classical action in bone mineralization, vitamin D also exerts its effect in the cardiovascular, immune, and endocrine systems owing to the universal expression of vitamin D receptor,[6,7] which regulates gene expression upon activation.[8]

Recent studies suggest an anti-inflammatory activity of vitamin D by suppressing cyclooxygenase (COX)-2 in prostate cancer cells.[9,10] COX converts arachidonic acid to release vasoactive prostanoids that exert effects by binding to their respective receptors.[11,12] Prostanoids such as thromboxane A_2 participate in proinflammatory and oxidative responses in renal dysfunction.[13,14] Thromboxane-prostanoid (TP) receptor agonists, including thromboxane A_2 and other vasoconstrictive prostanoids, contribute to vascular dysfunction, vascular smooth muscle proliferation, and platelet aggregation.[15,16] One of the conceptual strategies to limit prostanoid-induced vascular dysfunction is through

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inhibiting COX-2. COX-2 inhibition enhances vasodilatation in patients with hypertension or coronary artery disease.[17,18] However, the safety profile of COX-2 inhibitor is questioned, as the inhibition may reduce the production of COX-2-derived antithrombotic prostacyclin. Indeed, the withdrawal of Vioxx from the market in 2004 was because of the reported severe adverse cardiovascular events,[19] which is attributable to the nonspecific inhibition of prostacyclin synthase.[20] TP receptor is thus regarded as a more attractive and promising therapeutic target against prostanoid-mediated vascular inflammation, as it is more downstream in the pathway conveying the effect of constrictive and proinflammatory prostanoids.[21]

Kidneys and renal vasculature are more sensitive to vasoactive factors than the forearm and coronary arteries;[22] however, the mechanism of renovascular dysfunction associated with estrogen deficiency is incompletely understood. Although clinical[23–25] and animals studies[26–28] suggest a protective effect of vitamin D in renal function in hypertension and diabetes, the underlying mechanisms are still unclear. This study therefore aimed to define whether and how vitamin D benefits renovascular function using a well-established rodent model of estrogen deficiency, the ovariectomized (OVX) rats. We tested the hypothesis that vitamin D downregulates the COX-2/TP receptor vasoconstrictive pathway to restore endothelial function during estrogen deficiency.

RESULTS

Basic physical and biochemical parameters

Successful ovariectomy was validated by a significant reduction in the weight of the uterus (Supplementary Figure S1a online). Oral gavage of calcitriol to OVX rats resulted in an elevated serum calcitriol level (Supplementary Figure S1b online). Blood pressure remained similar among groups (Supplementary Figure S1c online), whereas body weight was increased in OVX rats compared with sham rats and was unaffected after calcitriol treatment (Supplementary Figure S1d online).

Calcitriol alleviates systemic inflammation under estrogen deficiency. As detected by Suspension Antibody Array-Based Multiplex Immunoassay, OVX rats had higher levels of serum monocyte chemoattractant protein-1 and tumor necrosis factor-α, which were normalized by calcitriol treatment (Supplementary Figure S2a and b online). For renal function, the level of albumin excretion in the urine from sham-operated rats and OVX rats with or without calcitriol treatment was examined. Results showed that there was no significant difference between these three groups of rats (Supplementary Figure S2c online). This may indicate that impairment in renovascular reactivity may precede deterioration of renal function, which was not yet obvious in our 10.5-month-old OVX rodent model.

Hematoxylin and eosin staining showed that there was no change in the media-to-lumen ratio in the renal arteries from sham-operated rats and OVX rats with or without calcitriol treatment (Supplementary Figure S3a and b online). Isometric force measurement showed that the contractions to 60 mmol/l KCl or phenylephrine were not modified (Supplementary Figure S3c and d online) among all three groups of rats, indicating that the renal arteries, especially the media (vascular smooth muscle layer), were not structurally altered. These data ruled out the possibility that the effects of calcitriol were attributed by an alteration in vascular geometry.

In vivo calcitriol treatment enhances endothelium-dependent relaxations in renal arteries from OVX rats

Acetylcholine (ACh)-induced relaxations were impaired in renal arteries from vehicle-treated OVX rats (pD_2: 6.53 ± 0.10, E_{max}: $39.6 \pm 4.9\%$ in OVX + vehicle, $n = 5$ vs. pD_2: 6.80 ± 0.17, E_{max}: $64.6 \pm 10.5\%$ in sham control, $n = 4$). Chronic treatment with calcitriol (150 ng/kg per day for 4.5 months) improved the relaxations (pD_2: 6.30 ± 0.09, E_{max}: $68.6 \pm 3.9\%$ in calcitriol-treated OVX, $n = 8$; Figure 1a and b). In contrast, sodium nitroprusside–induced endothelium-independent relaxations were not different between groups (Figure 1c).

The improvement of endothelium-dependent vasodilatation by calcitriol was translated to enhanced renal blood flow examined using magnetic resonance imaging (Figure 2a). Renal blood flow in OVX rats was reduced in both kidneys, and the flow was restored by calcitriol treatment (Figure 2b and c).

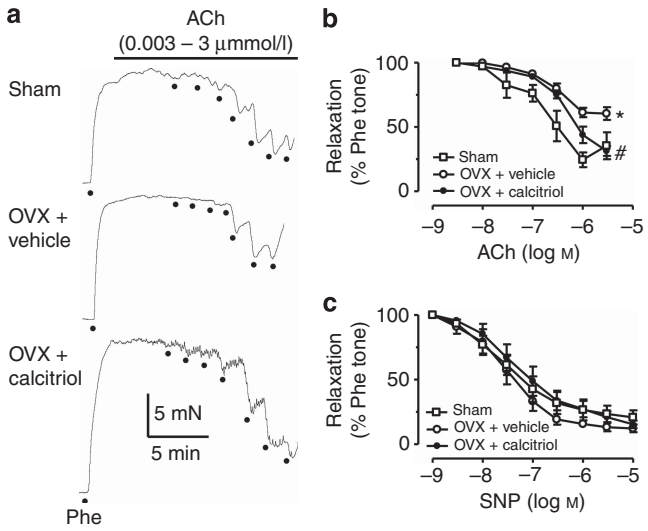

Figure 1 | Chronic calcitriol treatment improves endothelium-dependent relaxations during estrogen deficiency. (**a**, **b**) Renal arteries from ovariectomized (OVX) rats exhibited impaired acetylcholine (ACh)-induced relaxations, which were partially restored by oral treatment with calcitriol ($n = 8$). (**c**) Endothelium-independent sodium nitroprusside (SNP)-induced relaxations were similar in all groups ($n = 5$). Data are means ± s.e.m. of 4–8 experiments. $^{*}P < 0.05$ vs. sham; $^{\#}P < 0.05$ vs. OVX + vehicle. % Phe tone, percentage of tension with phenylephrine contraction.