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SHORT COMMUNICATION

Catechol Estrogens as Inhibitors of Leukotriene Synthesis

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ABSTRACT. Estrogens have a beneficial effect on atherosclerosis and osteoporosis after menopause, but their exact mechanism of action is still unknown. The aim of the present study was to investigate the effects of estradiol and its metabolites catechol estrogens on arachidonic acid metabolism *in vitro*. Estradiol had no effect on arachidonic acid metabolism up to 33 μM in A23187-stimulated human whole blood. All catechol estrogens (2-hydroxyestradiol, 2-hydroxyestrone, 4-hydroxyestradiol and 4-hydroxyestrone) had similar kinds of actions on arachidonic acid metabolism, being over ten times more potent inhibitors of leukotriene synthesis (IC_{50} values 0.044–0.16 μM) than thromboxane (IC_{50} values 0.99–2.1 μM) and prostaglandin E_2 synthesis (IC_{50} values 0.84–5.5 μM). It is suggested that some of the protective actions of estrogens—e.g., on atherosclerosis and osteoporosis—may be related to the inhibition of leukotriene synthesis by catechol estrogens. BIOCHEM PHARMACOL 55;1:101–104, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. estrogen; estradiol; leukotriene; prostaglandin; thromboxane

Extensive epidemiological studies have shown that after the menopause rates of cardiovascular mortality in women who receive estrogens are one-third to one-half those in untreated women [1]. In addition, acute administration of estradiol has a beneficial effect on exercise-induced myocardial ischemia in women with coronary artery disease [2]. Estradiol also has antioxidant actions, thereby inhibiting the oxidation of low-density lipoproteins (LDL)¶ in postmenopausal women [3]. Inhibition of LDL oxidation does not correlate with plasma estradiol concentration.

Nakano et al. [4] have demonstrated that catechol estrogens (2-hydroxyestradiol and 2-hydroxyestrone)—the metabolites of estrogens—are more effective antioxidants than α-tocopherol in preventing membrane phospholipid peroxidation. In addition to their antioxidant actions; there is abundant evidence that catechol estrogens modulate prostaglandin (PG) synthesis and $PGE_2/PGF_{1\alpha}$ ratio in uterus and blastocyst [5–9]. Rosenkrans et al. [8] have proposed that the effects of estradiol on prostaglandin synthesis are mediated via catechol estrogens.

The aim of the present study was to test the effects of estradiol and its metabolites catechol estrogens on the cyclooxygenase and 5-lipoxygenase pathways of arachidonic acid metabolism, keeping in mind that compounds with catecholic structure have antioxidant properties and are able to modulate prostanoid/leukotriene ratio [10].

MATERIALS AND METHODS

Calcium ionophore A23187, 17-beta-estradiol, 2-hydroxyestradiol, 2-hydroxyestrone, 4-hydroxyestradiol and 4-hydroxyestrone were from Sigma Chemical Co.

Whole Blood Incubation

The incubation was performed as previously described [11]. Freshly drawn heparinized venous blood was obtained from healthy volunteers who had not taken any drugs for at least two weeks. Experiments were carried out by aliquoting 1 mL whole blood into polystyrene tubes; and the test compounds were added in ethanol. The final concentration range was 0.033 μM to 33 μM for estradiol and catechol estrogens. The eicosanoid synthesis of whole blood was immediately triggered by calcium ionophore A23187 (final concentration 10 μM). The incubation was carried out for 60 min at 37°. Plasma was separated by centrifugation 1600 × *g* for 10 min at +4°.

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¶ *Abbreviations:* LDL, low-density lipoproteins; LT, leukotriene; PG, prostaglandin; RIA, radioimmunoassay; TX, thromboxane.

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Eicosanoid Assays

PGE_2 was measured using a direct ^{125}I-PGE_2 radioimmunoassay (RIA) [10]. The RIA kits were from the Institute of Isotopes Co. Thromboxane (TX)B_2 was measured using a direct ^{3}H-TXB_2 RIA [12]. The antiserum was a generous gift from Prof. C. Taube (Martin Luther University, Halle, Germany), ^{3}H-TXB_2 was purchased from Amersham International and unlabelled TXB_2 from Upjohn Diagnostics.

Total cysteinyl leukotriene (LT) synthesis was determined as LTE_4-like immunoreactivity from unextracted plasma by RIA [12]. An in-house rabbit antiserum, raised against the bovine serum albumin conjugate of LTC_4/LTD_4/LTE_4, was used for the assay. The cross-reactivity values of the antibody used in LTE_4 RIA were: LTC_4 100%, LTD_4 90%, LTE_4 35%, LTF_4 120%, 5(S),6(R)-dihydroxyeicosatetraenoic acid 7.33% and to the other eicosanoids less than 0.05%. LTE_4 was determined in a cross-reactive way, using ^{3}H-LTC_4 (DuPont NEN) as the radiolabelled ligand and LTE_4 (Cayman Chemical) as the nonlabelled ligand. The unknown samples were diluted 1:50–100 in assay buffer. Possible nonspecific interference by plasma matrix was verified by adding unstimulated normal human plasma containing A23187 of identical dilution to that used in stimulated blood. As the standard curves were superimposable to those obtained in the absence of plasma matrix, nonspecific interference could be ruled out.

Statistics

One-way ANOVA was used to demonstrate significant differences between the IC_{50} values of catechol estrogens for inhibition of eicosanoid synthesis.

RESULTS

The basal A23187-stimulated synthesis of LTE_4 was 72 ± 19 ng/mL (n = 15), of TXB_2 87 ± 10 ng/mL (n = 15), and of PGE_2 2.5 ± 0.3 ng/mL (n = 22). Estradiol had no effects on arachidonic acid metabolism up to 33 μM (Fig. 1). All catechol estrogens had similar kinds of effects on arachidonic acid metabolism (Fig. 1), being over ten times more potent inhibitors of leukotriene synthesis than thromboxane and prostaglandin E_2 synthesis (Table 1). The IC_{50} values of 2-hydroxy metabolites (2-hydroxyestradiol and 2-hydroxyestrone) seem to be lower than those of 4-hydroxy metabolites (4-hydroxyestradiol and 4-hydroxyestrone) (see Table 1).

Estradiol itself did not affect arachidonic acid metabolism, while its metabolites, the catechol estrogens inhibited both arachidonic acid 5-lipoxygenase and cyclooxygenase metabolism in A23187-stimulated whole blood.

DISCUSSION

The present paper demonstrates that catechol metabolites of estrogens are able to modulate arachidonic acid 5-lipoxygenase and cyclooxygenase metabolism. Estradiol itself was ineffective. Catechol estrogens inhibited both leukotriene and prostaglandin synthesis in A23187-stimulated whole blood. Our results are in good agreement with previous studies in which catechol estrogens were shown to modulate prostanoid synthesis [5–9]. The inhibitory action of catechol estrogens on leukotriene synthesis has not been substantiated. Nishizawa *et al.* [13] have demonstrated that estradiol inhibits leukotriene formation in transformed mouse Leydig cell culture. They concluded that the effect of estradiol is mediated via a 5-lipoxygenase inhibitor. The possible role of catechol estrogens in their investigation has yet to be determined. Our findings support previous reports which conclude that the actions of estradiol on arachidonic acid metabolism are mediated via catechol estrogens [8]. According to recent reports [14, 15], the antioxidant actions of estrogens on lipid peroxidation are not attributed to the phenolic estrogens but to their catecholic metabolites. Conjugated catechol estrogens also have antioxida-

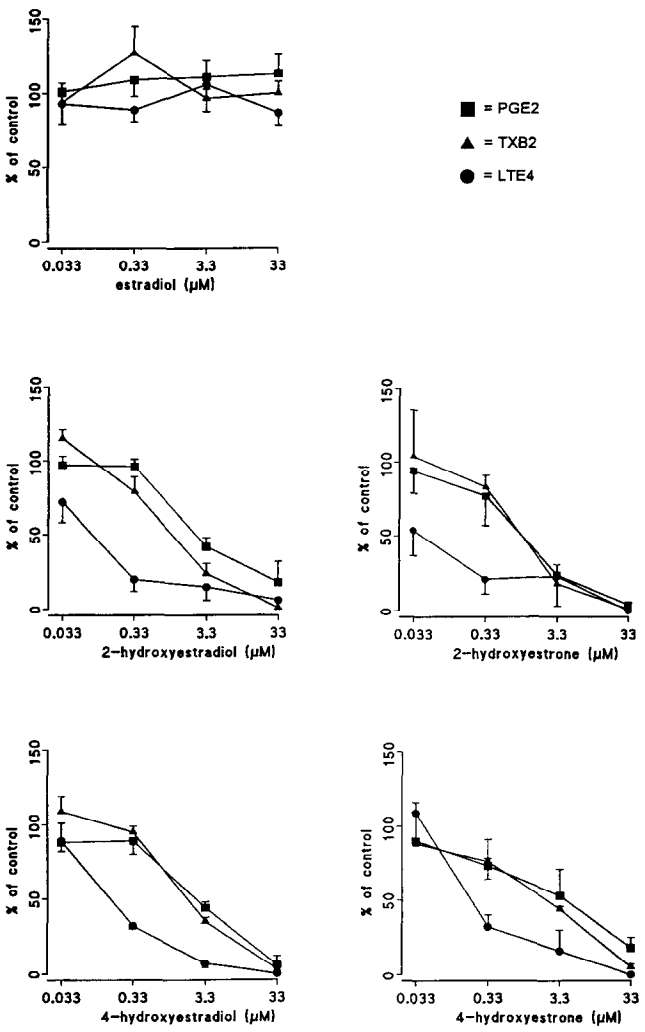

FIG. 1. Effects of estradiol and catechol estrogens on leukotriene (LTE_4), thromboxane (TXB_2) and prostaglandin (PGE_2) synthesis in A23187-stimulated human whole blood (mean ± SEM, n = 3–9).

TABLE 1. IC_{50} **values of catechol estrogens for inhibition of leukotriene (LTE_4), thromboxane (TXB_2) and prostaglandin (PGE_2) synthesis in A23187-stimulated human whole blood (mean ± SD, $n = 3$).**

	IC_{50} (μM)			
	LTE_4 mean (SD)	TXB_2 mean (SD)	PGE_2 mean (SD)	
2-hydroxyestradiol	0.073 (0.041)	1.4 (0.48)	3.0 (0.73)	$P < 0.01$
2-hydroxyestrone	0.044 (0.023)	0.99 (0.11)	0.84 (0.20)	$P < 0.01$
4-hydroxyestradiol	0.15 (0.015)	1.8 (0.35)	2.4 (0.75)	$P < 0.01$
4-hydroxyestrone	0.16 (0.010)	2.1 (0.60)	5.5 (5.2)	$P = 0.17$
	$P < 0.01$	$P < 0.05$	$P = 0.27$	

One-way ANOVA was used to evaluate the significance of differences between the IC_{50} values of catechol estrogens, and the corresponding P-values are given at the end of each column.

tive properties [14], which increases the physiological relevance of the described findings.

The actions of catechol estrogens on arachidonic acid metabolism seem to be related to their catecholic structure, i.e., to the orthoposition of hydroxyl groups. Estradiol in itself, i.e., without catecholic hydroxyl groups, had no effect on arachidonic acid metabolism. A similar structure-action relationship is seen with other phenolic compounds [10]. Catechol estrogens are 10–1000 times more potent 5-lipoxygenase inhibitors than the water-soluble catecholic antioxidants (catechol, catecholamines and Trolox C) [11, 16, 17], which makes them possible physiological candidates as modulators of lipoxygenases *in vivo*. The key to the inhibition of leukotriene synthesis is the way catecholic compounds (catechol estrogens) inhibit 5-lipoxygenase by reducing the catalytically active ferric enzyme to the catalytically inactive ferrous form [18]. The inhibition of cyclooxygenase may be due to the capability of catechol estrogens to remove the essential intermediate radical for the cyclooxygenase mechanism [19].

Considering the possible physiological role of the present findings, both leukotrienes and prostanoid/leukotriene ratio may be logically linked with cardiovascular actions of estrogen. Vasodilatory prostanoids have been considered a potentially antiatherogenic factor, while leukotrienes may be regarded as unfavourable. Leukotriene synthesis increases in unstable angina pectoris and acute myocardial infarction [20], and in acute and chronic lower limb ischemia [21]. In addition, atherosclerotic human myocardial arteries show an increased sensitivity to the contractile action of leukotrienes [22]. The beneficial effect of estradiol on exercise-induced myocardial ischemia in women with coronary artery disease [2] might be explained by the inhibition of leukotriene synthesis by catechol estrogens. Moreover, the inhibition of LDL oxidation by estradiol in postmenopausal women *in vivo*, not correlated with the plasma estradiol concentration [3], could be related to catechol estrogens. In addition to atherosclerosis, the inhibition of leukotriene synthesis by catechol estrogens may have relevance for the development of osteoporosis. In fact, leukotrienes are among the most potent stimulators of bone resorption [23].

Free 2-hydroxyestrone concentration in plasma is *ca.* 0.5–2 nM, but conjugated catechol estrogen concentrations are 10–20 times higher than those of free catechol estrogens [24]. The IC_{50} of 2-hydroxyestrone for inhibition of leukotriene synthesis in the present study was 44 nM. The action of catechol estrogens on arachidonic acid metabolism is probably additive. Thus, the total concentration of all catechol estrogens is greater than that of any single one. The concentrations of catechol estrogens needed to modify arachidonic acid metabolism can be regarded physiologically relevant, although the concentrations necessary to inhibit leukotriene synthesis *in vitro* are somewhat higher than those measured in plasma *in vivo*.

Although the physiological and pathophysiological relevance of the findings is unclear, it can be concluded that some of the effects of estrogens might be related to catechol estrogens via the inhibition of leukotriene synthesis.

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