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Alpha-tocopherol succinate increases cyclooxygenase-2 activity: Tissue-specific action in pregnant rat uterus in vitro

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ABSTRACT

Aims: Lipid soluble vitamin E plays a role in several physiological mechanisms, however, the mechanism of this action is controversial. We investigated how tocopherol (α-tocopherol acid succinate) influences the effects of cyclooxygenase inhibitors (COXi) in the smooth muscles.

Main methods: The contractility of the samples from 22-day-pregnant myometrium and non-pregnant myometrium and trachea was determined in an isolated organ bath in vitro. The activity of cyclooxygenase enzymes (COX) was also measured in the tissues.

Key findings: Diclofenac (10^{-9}–10^{-5} M) and rofecoxib (10^{-10}–10^{-5} M) decreased the contractions in non-pregnant and 22-day-pregnant uteri. Tocopherol (10^{-7} M) increased the relaxant effect only in pregnant uteri. Both diclofenac (10^{-9}–10^{-5} M) and rofecoxib (10^{-10}–10^{-5} M) reduced the tracheal tones, while they were slightly intensified by pretreatment with tocopherol (10^{-7} M). Tocopherol enhanced the contractions of pregnant uteri. Tocopherol (10^{-7} M) itself can induce the cyclooxygenase activity and shift the COX-1 and COX-2 ratio to COX-2. The lowest COX activity was found in non-pregnant uteri, while the highest one was in the trachea.

Significance: The COX enzymes, especially COX-2, play an important role in the contraction of pregnant uteri in rat. Tocopherol has a tissue specific COX-2 activity increasing effect in pregnant rat uterus but has no such action in non-pregnant uteri or tracheal tissue. Hereby, tocopherol may intensify selectively the uterine relaxing effect of COX-2 inhibitors in preterm contractions. However, tocopherol can enhance the contractile response of pregnant uterus that may increase the risk of premature contractions.

1. Introduction

Vitamin E is a lipid-soluble compound with high peroxyl radical scavenger ability. Vitamin E includes eight natural analogs, such as α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols, however, their activity is quite various [1]. α-tocopherol is the most frequently used analog. Over the past thirty years, many studies have been published on the biological effects and functions of vitamin E, e.g. it is needed for reproduction [2] and may help to prevent Alzheimer's disease, non-alcoholic fatty liver diseases (NAFLD and NASH) of non-diabetic patients, atherosclerosis and certain types of cancer [3–7]. The mechanism of action of vitamin E has not yet been fully clarified. It can get across the cell membrane and can bind to many intracellular receptors and enzymes and may lead to modifying their functions [8]. For example, tocopherols can increase the activity of estrogen receptor β (ERβ) [9], inhibit activities of protein kinase C alpha [10], phospholipase A2 [11] and protein tyrosine kinases [12]. Moreover, vitamin E can also influence the action of other drugs by its antioxidant effect that shows tissue specificity. The tracheal tone-reducing effect of β_2-agonist terbutaline was decreased in female estrous rat by pretreatment with α-tocopherol, while in pregnant uterus the pretreatment was inefficient. This difference was explained by the various oxidative states of smooth muscles [13].

The interaction between tocopherols and cyclooxygenase enzymes (COX) was investigated in a few studies. Wu et al. [14] reported vitamin E inhibited the activity of cyclooxygenase enzymes in human aortic endothelial cells. Moreover, vitamin E can affect the different steps of the arachidonic acid cascade, but this effect may be diverse in tissues. According to literature, prostaglandin E2 (PGE_2) production was reduced in mouse [15,16] and rat [17] macrophages by vitamin E. Analogs of tocopherols have different effects on COX. Alpha-tocopherol succinate inhibited more efficiently the LPS-stimulated PGE_2 production in macrophages and the COX activity in human lung epithelial cells than the other analogs [18,19].

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit COX-1 or COX-2 enzymes, hereby decrease the liberation of prostaglandins (PG).

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The low level of PGE_2 induces the relaxation of the uterine smooth muscle, although the clinical use of NSAIDs for the prevention of preterm birth is limited because of the side effects [20]. Non-COX selective NSAIDs, especially acetyl-salicylic acid, are responsible for aspirin-induced asthma (AIA), while COX-2 selective compounds seem to be helpful not to exacerbate asthmatic symptoms [21].

We hypothesize that tocopherols modifies the COX activity and the effect of NSAIDs that may have significance in smooth muscle contractions. Accordingly, the aim of this study is to investigate how α-tocopherol acid succinate (tocopherol) influences the effects of non-selective and selective COX inhibitors (COXi) in the non-pregnant and pregnant uterine, and tracheal smooth muscle contractions in rats in vitro.

2. Materials and methods

All experiments involving animal subjects were carried out with the approval of the Hungarian Ethical Committee for Animal Research (permission number: IV/198/2013). The animals were treated in accordance with the European Communities Council Directives (86/609/ECC) and the Hungarian Act for the Protection of Animals in Research (Article 32 of Act XXVIII).

2.1. Housing and handling of the animals

Sprague-Dawley rats were procured from INNOVO Ltd. (Gödöllő, Hungary) and were kept at a controlled temperature (22 ± 3 °C), with 30–70% relative humidity, on a 12 h dark-light cycle. The animals were fed with standard rodent pellet diet (Charles-River Laboratories, Budapest, Hungary) and given tap water ad libitum.

2.2. Mating of the animals

The mature female Sprague-Dawley rats (180–200 g) were selected by the estrus cycle. The estrus cycle was measured by the vaginal impedance of rats with an Estrus Cycle Monitor EC40 (Fine Science Tools. Foster City, CA, USA). The female in estrus and sexually mature male (240–260 g) rats were placed into a special mating cage with two rooms. There was a time-controlled movable metal door between the rooms. The separating door was pulled up before dawn.

In the morning, vaginal smears were taken from the female rats. Successful copulation was indicated by the presence of sperm in the native vaginal smear or a copulation plug. This day meant the first day of pregnancy.

2.3. Isolated organ bath studies

2.3.1. Preparation of uteri

The animals were terminated by CO_2 inhalation. Two horns of uteri were excised, 5-mm-long muscle rings were sliced and mounted vertically in an organ bath containing 10 ml of de Joung solution (composition in Mm: 137 NaCl, 3 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 12 $NaHCO_3$, 4 NaH_2PO_4, 6 glucose, pH 7.4). The organ bath was heated at 37 °C and carbogen (95% O_2 + 5% CO_2) was bubbled into the chambers. After the setting of initial tension (1.5 g), myometrial rings were incubated with a buffer change every 15 min for about 1 h. Tocopherol was dissolved in mixture of ethanol 96%: Macrogol 400 (1:14) and diluted further in Macrogol 400. The control tissues have been treated with the solvent without tocopherol and results with tocopherol have been corrected with these values. The samples were equilibrated for another 60 min with tocopherol (10^{-7} M). Tocopherol was added to tissues after every wash of buffer solution. The control preparations were incubated for 1 h without tocopherol. Cumulative dose-response curves of non-selective COXi diclofenac (10^{-9}–10^{-5} M) and COX-2 selective inhibitor rofecoxib (10^{-10}–10^{-5} M) were obtained.

2.3.2. Preparation of trachea

Trachea tissues were dissected from non-pregnant estrous rats (160–260 g, n = 8), then the esophagus and blood vessels were removed. The tracheal tube was cut transversally into 4–5- mm-wide rings, which were placed in Krebs buffer (composition in mM: 118 NaCl; 4.75 KCl; 2.5 $CaCl_2$; 1.19 K_2HPO_4; 25 $NaHCO_3$; 1.2 $MgSO_4$ and 11 glucose). The tracheal rings were mounted with their longitudinal axis vertically by hooks. The initial strain was set to about 2.00 g. The samples were equilibrated for 1 h, while the buffer solution was changed in every 15 min. After that, the tissues were incubated for another 60 min with tocopherol (10^{-7} M) with further buffer change in every 15 min. Except for the control preparations, tocopherol was applied after every wash of buffer solution. Cumulative dose-response curves of non-selective COXi diclofenac (10^{-9}–10^{-5} M) and COX-2 selective inhibitor rofecoxib (10^{-10}–10^{-5} M) were obtained.

2.3.3. Measurement of COX enzymes activity

The smooth muscle samples (22-day-pregnant and non-pregnant myometrium, trachea, n = 6/group) were incubated in an organ bath as described above. After the incubated period, tissues were perfused with cold Tris Buffer pH 7.4 to remove any red blood cells and clots, frozen in liquid nitrogen and stored at − 80 °C until assay. On the day of measurement, samples were homogenized in 5 ml of cold buffer (0.1 M Tris-HCl, pH 7.8, containing 1 mM EDTA) per gram tissue, centrifuged at 10,000 × *g* for 15 min at 4 °C. The supernatant was stored on ice. The activity of COX enzymes was determined by COX Activity Assay Kit (Cayman chemicals, Ann Arbor, MI). This Kit measures the peroxidase activity of COX. The peroxidase activity is assayed with the colorimetric method by monitoring the appearance of oxidized *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm.

2.4. Statistical analysis

All data were analyzed by the Prism 5.01 (GraphPad Software, USA) computer program. The values were statistically evaluated with the unpaired *t*-test or ANOVA with Tukey Multiple Comparison Test.

3. Results

3.1. Isolated organ bath studies

Pretreatment with tocopherol significantly enhanced the contractions of the 22-day-pregnant myometrium (Fig. 1 striped columns) but did not alter it in non-pregnant uteri (Fig. 1 empty columns).

The non-selective COX inhibitor diclofenac (10^{-9}–10^{-5} M) and the selective COX-2 inhibitor rofecoxib (10^{-10}–10^{-5} M) inhibited the contractions of non-pregnant uteri in a concentration-dependent manner. In the presence of tocopherol, the relaxant effects of diclofenac and rofecoxib did not change (Fig. 2).

In 22-day-pregnant smooth muscle uteri the relaxant effect of selective COX-2 inhibitor rofecoxib was higher than that of diclofenac. After pretreatment with tocopherol, the relaxant effect was increased significantly in each concentration in the case of both compounds (Fig. 3).

Both diclofenac (10^{-9}–10^{-5} M) and rofecoxib (10^{-10}–10^{-5} M) also reduced the tone of tracheal tissues. The average tone decrease by diclofenac and rofecoxib was 46.8 ± 5.0 mg and 32.6 ± 10.4 mg, respectively. Tocopherol influenced the effect of diclofenac and rofecoxib only in lower concentrations (Fig. 4).

When one dose of selective COX-2 inhibitor rofecoxib (10^{-7} M) was applied before KCl-evoked control contractions on 22-day pregnant uteri, the activity of diclofenac stayed low, while in the presence of tocopherol it augmented slightly (Fig. 5**A**).

Besides, if we added one dose of selective COX-1 inhibitor SC-560 before eliciting control contraction, the relaxing effect of rofecoxib was enhanced and the presence of tocopherol kept on increasing this effect