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Original Article

Different effects of different phosphodiesterase type-5 inhibitors in pre-eclampsia

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ABSTRACT

Objectives: We aimed to determine the effects of sildenafil and vardenafil in human umbilical artery preparation taken from pre-eclamptic or normal pregnant women, also to investigate underlying mechanisms in these effects.

Study design: Fifteen pregnant women with pre-eclampsia and 15 healthy pregnant women were involved. Relaxation responses of sildenafil and vardenafil in presence and absence of nitric oxide synthase inhibitor, N-[omega]-nitro-L-arginine methyl ester (L-NAME), and soluble guanylyl cyclase inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), were compared between the pre-eclampsia group and control group.

Results: Sildenafil induced relaxation responses were significantly attenuated in the presence of pre-eclampsia, L-NAME and QDO. Similarly, pre-eclampsia, L-NAME or ODQ incubation also shifted vardenafil-induced relaxation responses to rightwards. However, in all set of experiments a maximal relaxation response was achieved by vardenafil unlike sildenafil. In conclusion vardenafil seems to relax human umbilical artery stronger than sildenafil in both pre-eclampsia and normal pregnancies.

Conclusion: These data indicate that vardenafil might affect vascular responsiveness of human umbilical artery through the involvement of NO/cGMP-dependent and independent pathways while sildenafil-induced responses were seemed to be completely NO/cGMP-dependent. Further investigations are needed to clarify the mechanisms.

1. Introduction

Pre-eclampsia is a syndrome, defined as gestational hypertension combined with an onset of maternal renal, hepatic, haematological, neurological dysfunction or fetal growth restriction [1,2]. It remains one of the largest single causes of maternal/fetal mortality and morbidity. Moreover, it has been reported that pre-eclampsia accounts for 14% of direct maternal deaths and 18% of fetal or infant deaths [3]. The underlying aetiology and pathophysiology of pre-eclampsia are still unknown and despite recent improvements, no current treatment cures this devastating condition. Therefore, a novel therapeutic strategy for pre-eclampsia is desirable.

The umbilical artery is a crucial component of feto-placental circulation [4]. Umbilical blood vessels are the unique mammalian arteries that lack autonomic innervation. For this reason, feto-placental blood flow is predominantly influenced by the action of local autocrine vasoactive substances. Local mediators such as serotonin (5-HT) and nitric oxide (NO) or some ions such as potassium and calcium are essential in determining the tonus of the umbilical artery [5]. The understanding of the intracellular mechanisms modulating human umbilical artery contractility, may offer therapeutic value for the treatment of some pathologies as pre-eclampsia.

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NO/cyclic guanosine monophosphate (cGMP) signaling pathway controls a variety of physiological processes in the human fetal placental circulation [6,7]. NO is synthesized from L-arginin by nitric oxide synthase. It regulates vascular tone and the feto-placental circulation through activation of soluble guanylate cyclase (GCs) and formation of cGMP. An impaired activity of the NO–cGMP pathway has been associated with pre-eclampsia [8–10]. In a rat pre-eclampsia model or in a pregnant rat model, it has been shown that agents increasing cGMP levels produce artery relaxation [11,12].

Synthesis of cGMP in tissues is catalyzed by GCs enzyme family [13]. cGMP level in the whole cell or in specific intracellular pools is primarily determined by the balance between activities of the GCs and cyclic nucleotide phosphodiesterases (PDEs) that break down cGMP. It has been known that intracellular increased cGMP is rapidly inactivated to GMP by the activity PDEs [14]. There are 11 isoenzymes of PDEs, with different distributions throughout the body. Although cGMP and cAMP compete for catalytic sites of PDEs: PDE5, PDE6 and PDE9 have much higher affinity for cGMP than cAMP. In particular, PDE5 is located in vascular tissue such as blood vessels, platelets and vascular smooth muscle [15].

Costa et al. showed that there was an increased serum phosphodiesterase activity in women with pre-eclampsia [16]. Moreover, Turgut et al. proposed that, in pre-eclampsia, inhibitors of the cGMP-degrading PDE5 such as sildenafil increase cGMP levels, enhance endothelial function and may be useful for protection [11]. Similarly, Santos-Silva et al. observed that PDE5 inhibition increased sodium nitroprusside-induced relaxation in 5-HT-contracted human umbilical arteries [17]. For this reason, selective PDE5 inhibitors including sildenafil, vardenafil or tadalafil may represent a therapeutic tool for the treatment of some pathologies as pre-eclampsia and hypertension in pregnancy.

To our knowledge, the role of sildenafil and vardenafil in an umbilical artery taken from pre-eclamptic pregnancies has not been studied yet. Therefore, in our study we aimed to compare the effects of sildenafil and vardenafil-induced responses in the human umbilical artery isolated from women with pre-eclamptic or normal pregnancy. In addition we wanted to investigate the underlying mechanism in these effects.

2. Methods

The study had the approval from the Ethics Committee of Akdeniz University Medical Faculty. Over a 1-year period (September 2008–November 2009) 15 women who had a diagnosis of pre-eclampsia in accordance with Davey and McGillivray classification (maternal blood pressure above 140/90 mmHg after 20 weeks of gestation and proteinuria of at least 300 mg/24 h) and 15 women as a control group with normal, full-term pregnancy participated in our study. Women with a diabetes mellitus, renal disease, infection or other significant medical disorder were excluded. Spinal anesthesia was applied to all the parturients in both groups, and bupivacaine was used as a local anaesthetic agent.

2.1. Umbilical artery preparation

Human umbilical cords were taken from normal (N:15) and pre-eclamptic (PE:15) patients following delivery by caesarean section and placed directly into ice-cold Krebs solution of following composition (mM): NaCl 118, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1.0, $MgSO_4$ 1.2, $CaCl_2$ 2.5 and glucose 11.2. The arteries were dissected from the cord and adherent Wharton's jelly. Excess fat and connective tissue was removed from the umbilical arteries, which were cut into rings 3–4 mm in length.

2.2. Umbilical artery tension recording

Rings were mounted in 10-mL organ baths containing Krebs solution at 37 °C continuously gassed with a mixture of 95% O_2 and 5% CO_2. The rings were allowed to equilibrate for 3 h under a resting tension of 1 g with repeated washing every 15 min [18–20]. They were connected directly to an isometric force displacement transducer (FDI-05 Force Displacement Transducer, BioPac Ltd.) so that, isometric changes in force could be recorded on a MP35 Transducer Data Acquisition System, BioPac Ltd. The following drugs were used: Sildenafil was obtained from Eczacıbaşı Inc. (Istanbul, Turkey), whereas vardenafil was kindly provided by Bayer Health Care AG, Leverkusen, Germany. N-[omega]-Nitro-L-arginine methyl ester (L-NAME), 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) and serotonin (5-HT) were purchased from Sigma–Aldrich (St. Louis, MO). Stock solutions were prepared in deionized water, except sildenafil, and ODQ, which were prepared in dimethyl sulfoxide (DMSO) and stored in aliquots at −20 °C; dilutions were made in deionized water immediately before use. The final concentration of dimethyl sulfoxide did not exceed 0.1%. DMSO is used as a solvent for sildenafil and ODQ. There is not any possibility that the concentration of DMSO (under 0.1%) cause additive relaxant effect because solvent was tested alone and found to have no effect on the preparations. All experiments were performed in solutions containing the indomethacin (10^{-5} M) to suppress the synthesis of prostanoids.

After the equilibration period, the rings were challenged with 5-HT (10^{-6} M) to test their viability. Rings that induced contraction lower than 1 g when challenged with 5-HT were excluded from the study. Afterwards, the rings were contracted using 5-HT (10^{-6} M). When pre-contraction with 5-HT reached a plateau, cumulative relaxations to sildenafil or vardenafil (10^{-10}–10^{-5} M) were recorded. In some 5-HT-precontracted rings, sildenafil or vardenafil was not added and time control of arterial tension was recorded.

In another set of experiments after the equilibration period, the tissues were washed every 15 min during a 30-min additional waiting period. Before pre-contraction with 5-HT (10^{-6} M), the rings were incubated with the nitric oxide synthase inhibitor L-NAME (10^{-4} M) or soluble guanylyl cyclase (sGC) inhibitor ODQ (10^{-5} M) for 30 min; and cumulative relaxations to sildenafil or vardenafil (10^{-10}–10^{-5} M) were recorded. Only one concentration–response curve to sildenafil or vardenafil was obtained in each preparation, and experiments were performed in