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Cardiotonic steroids induce anti-angiogenic and anti-proliferative profiles in first trimester extravillous cytotrophoblast cells☆

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ABSTRACT

Objective: Preeclampsia (preE), is characterized by abnormal placental invasion and function. Marinobufagenin (MBG), a cardiotonic steroid (CTS), inhibits cytotrophoblast (CTB) cell functions that are critical for normal placental development. This study tests the hypothesis that CTSs induce anti-angiogenic and anti-proliferative effects in CTB cells.
Methods: Human extravillous CTB cells of the line Sw-71, derived from first trimester chorionic villus tissue, were incubated with 0, 0.1, 1, 10, and 100 nM of each of three CTSs (MBG, cinobufatalin (CINO) and ouabain (OUB)) for 48 h. Thereafter, levels of pro-angiogenic (vascular endothelial growth factor (VEGF165), placental growth factor (PlGF)) and anti-angiogenic (soluble fms-like tyrosine kinase-1 (sFlt-1), soluble endoglin (sEng)) factors were measured in culture media using ELISA kits. Expression of three receptors (VEGF receptor 1 (VEGFR1), angiogenic angiotensin type 1 receptor (AT_1) and anti-angiogenic angiotensin type 2 receptor (AT_2)) were assayed using immunoblotting (western blots) in cell lysates.
Results: sFlt-1 and sEng secretion were increased while VEGF165 and PlGF were decreased in the culture media of CTB cells treated with 1 nM or more of each CTSs ($p < 0.01$ for each). The AT_2 receptor expression was up-regulated ($p < 0.05$) in CTB cells treated with 1 nM or more of MBG and CINO and with 100 nM OUB, while AT_1 and VEGFR1 expressions decreased ($p < 0.05$) with 1 nM or more of MBG and 10 nM or more of CINO and OUB.
Conclusions: CTSs influence extravillous CTB cells to induce an anti-angiogenic and anti-proliferative profile.

1. Introduction

Preeclampsia (preE) is a disorder of pregnancy characterized by *de novo* development of hypertension and proteinuria after 20 weeks gestation. This disorder affects about 1 in 20 pregnancies and is a leading cause of maternal and fetal morbidity and mortality [1–5]. Potential multi-system impacts and sequelae of preE include eclampsia, pulmonary edema, thrombocytopenia and fetal growth restriction [6–9]. Early diagnosis and treatment are not yet available and strategies to prevent the symptom cascade have only minimal success. The exact mechanism(s) leading to preE continues to elude investigators, but several theories have been proposed. One theory invokes a role for bufadienolides, a subgroup of cardiotonic steroids (CTSs). The most studied of this group, marinobufagenin (MBG), is elevated in both animal models of volume expansion and patients with preE [2,10–12]. Evaluation of MBG serum levels prior to the onset of hypertension and proteinuria in women implicates MBG in the pathogenesis of preE [1,2,4,11,12]. MBG also has been shown to interfere with proliferation, migration and invasion of CTB cells [13–15], and specifically to disrupt endothelial cell junctional complexes [9,16]. MBG is increased in a rat model of preE and pregnant rats treated with MBG develop preE symptoms [17].

Angiotensin II (Ang II), a bioactive effector component of the renin-angiotensin system (RAS), influences cell growth, cell contraction, hypertrophy, inflammation, fibrosis, and progression to cellular damage in certain cell types [18,19]. The biological

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actions of Ang II are mediated via two G-protein coupled receptors, Angiotensin receptor type 1 (AT_1) and Angiotensin receptor type 2 (AT_2) [20]. Changes in AT_1 and AT_2 expression, in the presence of Ang II, can result in vasoconstriction, cell proliferation, and tissue-remodeling through stimulation of AT_1 [21] or vasodilatation, cell growth inhibition and activation of apoptosis through AT_2 [22] depending on the balance between expression of these two receptors. Angiotensin (1–7) (Ang (1–7)) is a heptapeptide hormone

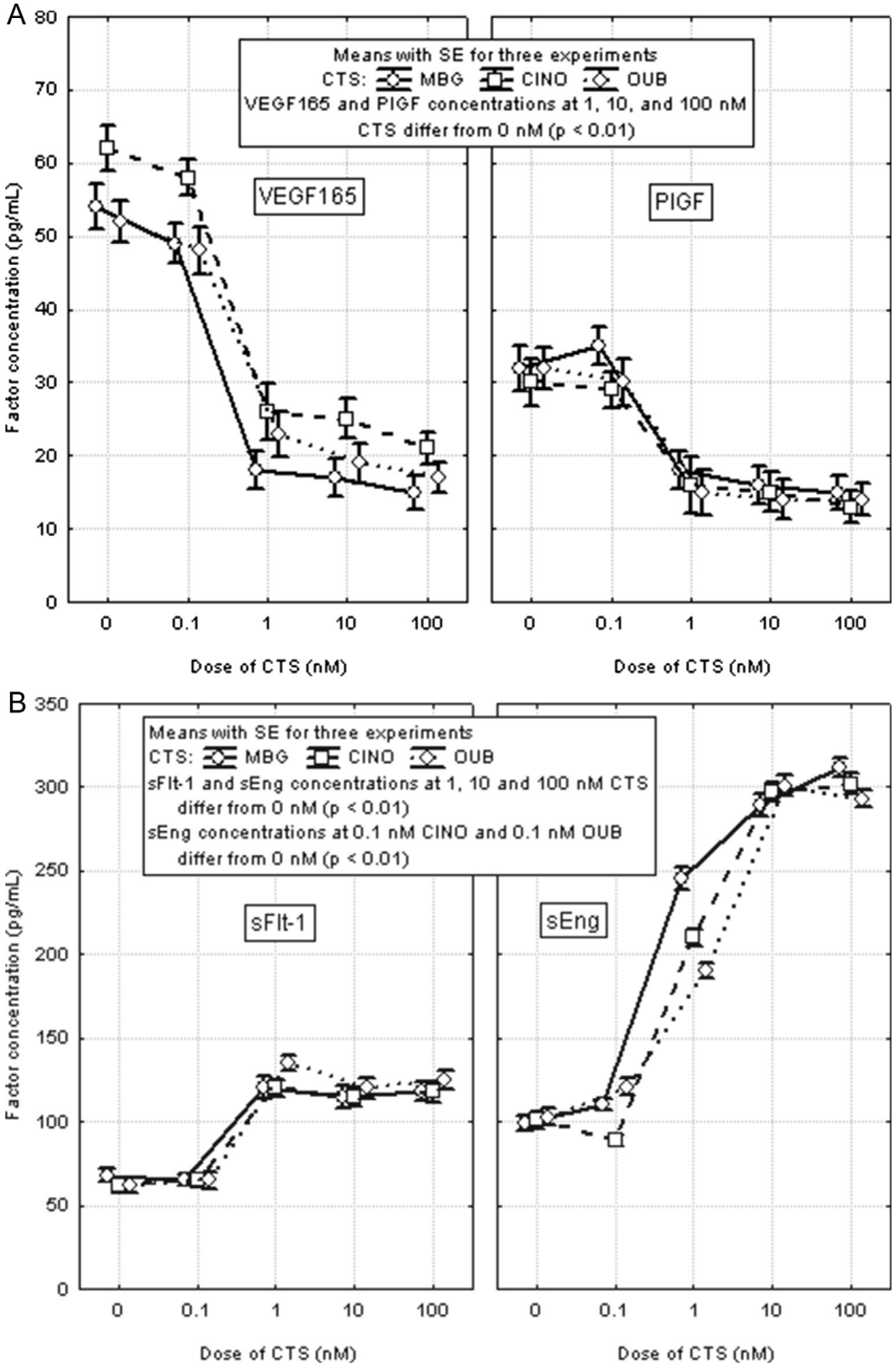

Fig. 1. A and B. Graph presents means with SE of three experiments for the secretion of 2 pro-angiogenic factors (VEGF165 and PlGF) (A) and 2 anti-angiogenic factors (sFlt-1 and sEng) (B) by CTB cells treated with 5 doses (0, 0.1 nM, 1 nM, 10 nM, and 100 nM) of 3 CTSs (MBG, CINO, and OUB) for 48 h. Comparisons with basal (0 nM) doses were performed using repeated measures analysis of variance with Dunnett's post-hoc test. All of 3 CTSs at levels of 1 nM or greater decreased the secretion of VEGF165 and PlGF and increased the secretion of sFlt-1 by CTB cells. CINO and OUB at levels of 0.1 nM altered the secretion of sEng. The sEng dose response curves varied between the 3 CTSs such that MBG differed ($p < 0.01$) from those of CINO and OUB, while CINO and OUB are similar ($p = 0.88$). For the other 3 secreted factors (VEGF165, PlGF, and sFlt-1), all 3 CTSs (MBG, CINO and OUB) produce similar response curves ($p > 0.4$).