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ENDOTHELIN IN HUMAN PLACENTA

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SUMMARY. Endothelin-1 (ET-1) is a member of endothelin family consisted of three peptides, ET-1, ET-2 and ET-3 which are encoded by a distinct gene on different chromosomes. Endothelins play the central role in the vasomotor control of blood vessels. The best-known functions additional to vasomotor effects are the regulation of cell processes as invasion, proliferation, migration and apoptosis. Compared to the non-pregnant state, normal pregnancy is associated with about 60% lower plasma concentrations of ET-1 as the result of down-regulation of ECE. In pregnancies complicated by pre-eclampsia, the increased levels of ET-1 in maternal blood correspond to non-pregnant values. The expression of ETRs in all compartments of human placenta, strongly suggests the various functions of ET-1. The presence of both endothelin receptors in extravillous and villous cytotrophoblast suggests the possible growth factor role of ET-1 in the early placenta. The crucial tasks of the cytotrophoblast in the first trimester of pregnancy are proliferation and invasion. This includes the replacement of endothelium and destruction of elastic and muscular media. ET-1 stimulates the proliferation and invasion of the first trimester trophoblasts in vitro in a receptor-specific manner. The relative decrease of ETR expression in cytotrophoblasts during pregnancy and lack of ET-1/ETRs in syncytiotrophoblasts suggest the effect of ET-1 predominately on the proliferation and invasion in the first trimester of pregnancy. The expression of ET-1 and both ETRs in placenta was shown to be changed in the opposite manner in mild and severe early onset pre-eclampsia. However, a decreased secretion of ET-1 from pre-eclamptic trophoblasts in vitro does not depend on the severity of disease or gestational age. Furthermore, it could not be affected by common drugs used in the prevention or therapy of pre-eclampsia.

Endothelin-1

Endothelin-1 (ET-1) is a member of endothelin family, discovered in 1988 by Yanagisawa et al.¹ This family of three peptides, ET-1, ET-2 and ET-3 is encoded by a distinct gene on different chromosomes, identified from human genomic DNA.² All three isoforms (ETs) consist of 21 amino acids with an identical hydrophobic C-terminal peptide and are stabilized by two disulfide bridges. The signalling is mediated by specific *G-proteins coupled receptors* (ETRs), endothelin receptor-A (ETR-A) and -B (ETR-B), two distinct molecules with 60% homology.³ Basal ET-1 secretion is controlled by intracellular Ca²⁺, which results (the rise or drop of Ca is for inhibition responsible?) in the inhibition of secretion.⁴ The circulating levels of ET-1 are increased in many conditions associated with the cell damage such as injury, ischemia, thrombosis and sheer stress and correlate positive with angiotensin II, thrombin, bradykinin and norepinephrin or negative with prostacyclin and nitric oxide.^{5,6} Endothelins are expressed in endothelial and smooth muscle vascular cells playing the central role in the vasomotor control of blood vessels. However,

the expression in many other locations of human body such as brain, kidney, macrophages, parathyroid cells, pituitary cells, stromal cells, decidua, trophoblast cells and cancer cells suggests many other functions. There are only few cells in human body which are not able to produce ET-1, the isoform predominant in humans. The best-known functions additional to vasomotor effects are the regulation of cell processes as invasion, proliferation, migration and apoptosis. All actions are cell and receptor specific, for example the binding to the ETR-A receptors on the vascular smooth cells causes vasoconstriction, whereas the activation of ETR-B receptors in endothelium induces the release of nitric oxide and prostacyclin resulting in vasodilatation.⁷

The potency and complexity of ET-1 in regulation of vascular tone and various cell processes is implicated by the limited use of receptor selective and unselective endothelin antagonists in many hypertensive diseases and oncology, due to serious side effects. Future therapeutic implications could be associated with the interaction of cell-signalling including post-transcriptional regulation.

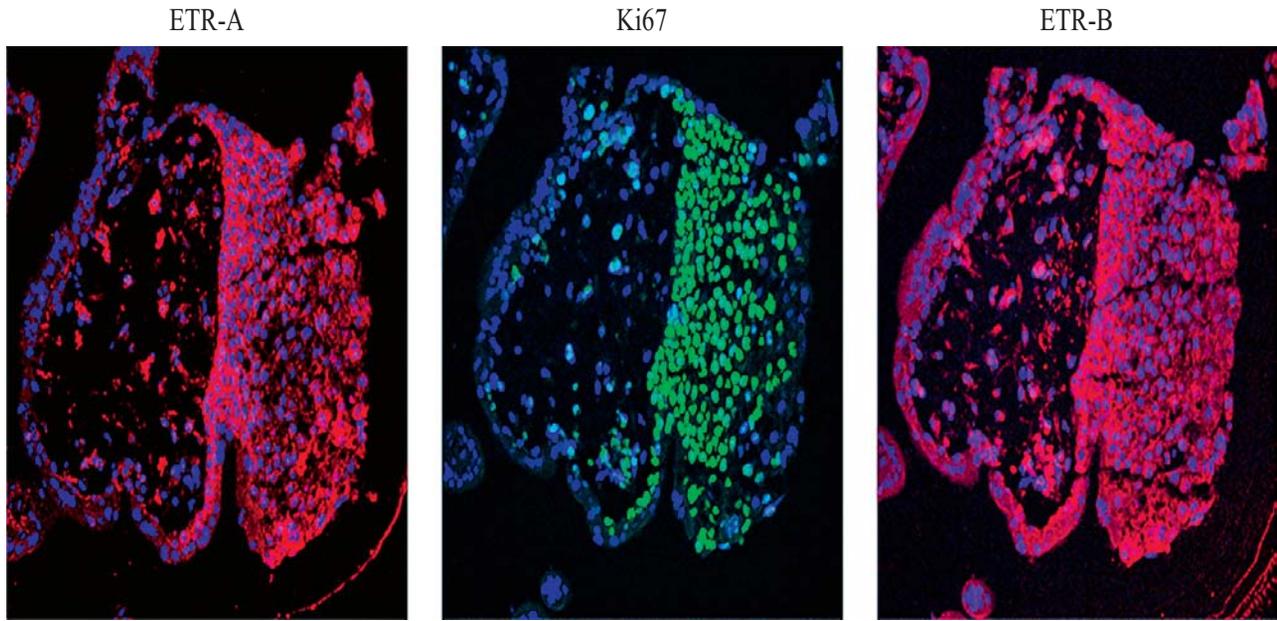


Figure 1. Distribution of ETRs in a first trimester trophoblast cell column (cc in b) attached to an anchoring villus (ac in b). Immunostaining of serial sections was performed using antibodies against ETR-A (a, red), the proliferation marker Ki67 (b, green), and ETR-B (c, red). Fluorescence immunostaining revealed that the proliferative trophoblast subpopulation showed a high expression of ETR-A in the proximal part of the cell column, while ETR-B also showed clear staining in the invasive trophoblast subpopulation of the cell column, original magnification x100. (Cervar et al. *J Clin Endocrinol Metab.* 2011;96:0000-00000.)

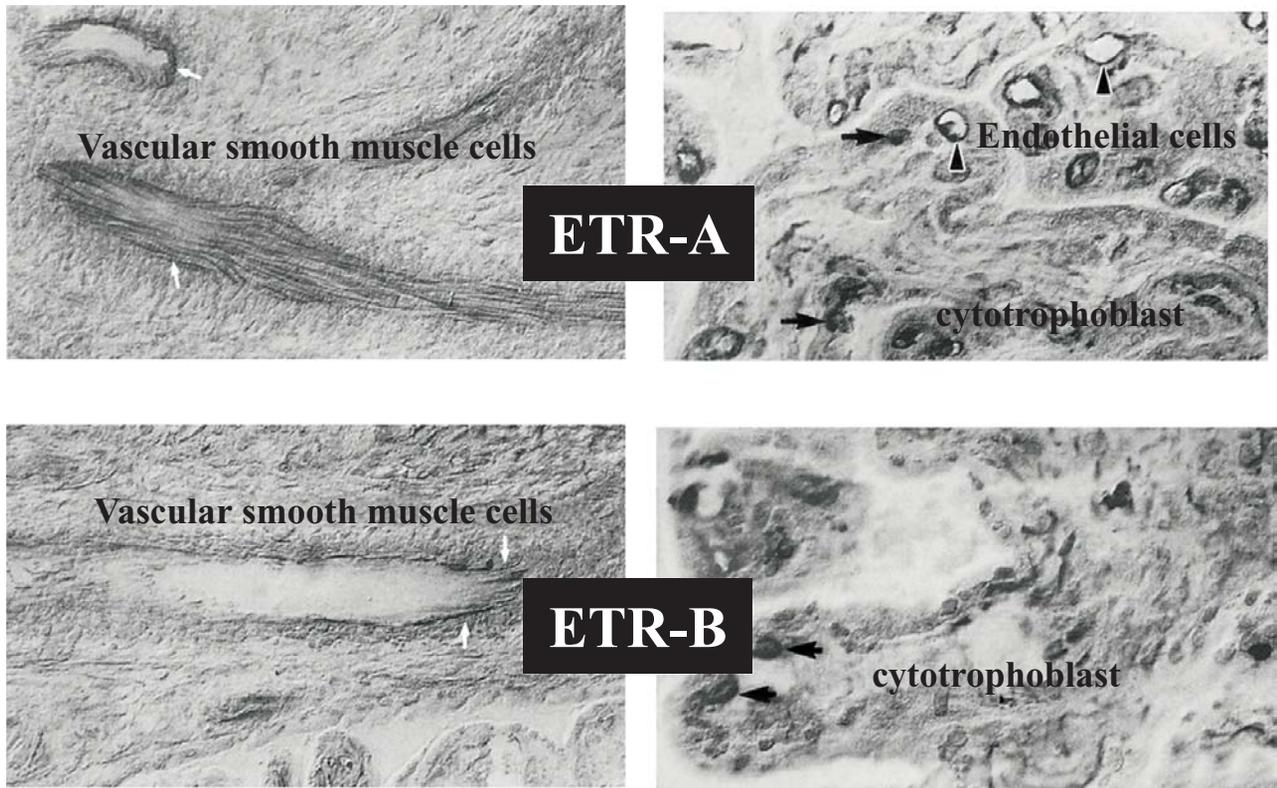


Figure 2. Immunohistochemistry of ETR-A and ETR-B in term human placenta. The vascular smooth cells (white arrows) inside stem villi and the endothelium (arrowheads) of peripheral vessels are immunolabelled with the ETR-A antibody. The syncytiotrophoblast is negative, while the cytotrophoblast shows immunoreactivity (black arrows). The endothelial cells are negative and vascular smooth cells are only weakly positive for the antibody against ETR-B. (Cervar et al. *Placenta* 2000;21:536-46.)

Endothelin-1 in human pregnancy

Endothelin-1 in maternal plasma

Compared to the non-pregnant state, normal pregnancy is associated with about 60% lower plasma concentrations of ET-1 as the result of down-regulation of ECE.⁸ ET-1 plasma levels increase during delivery over the non-pregnant concentrations and persist after delivery. In pregnancies complicated by pre-eclampsia, a pregnancy-specific syndrome characterized by general vasoconstriction, the increased levels of ET-1 in maternal blood correspond to non-pregnant values.⁸ It will be postulated, that the reduced utero-placental perfusion, the initiating event in pre-eclampsia, could be associated with an enhanced production of ET-1 causing vasoconstriction of uterine arteries followed by the systemic increase of maternal blood pressure and proteinuria, two of the leading symptoms of pre-eclampsia. Furthermore, lower uterine artery blood flow and higher ET-1 relative to nitric oxide metabolite levels in maternal blood are also associated with intrauterine growth restriction (IUGR).⁹ Moreover, elevated amniotic fluid concentrations of ET-1 could predict the development of IUGR at later stages of pregnancy. Furthermore, it will be assumed, that increased ET-1 production combined with the inhibition of nitric oxide synthesis induces fetal growth restriction in pre-eclampsia. Also, a decreased vascular response instead of normal concentration of ET-1 in maternal plasma is specific for diabetic pregnancies.¹⁰

Endothelin-1 in human placenta

Endothelin-1 and its receptors are expressed in almost all placental cells including endothelial cells, vas-

cular smooth cells, cytotrophoblasts, Hofbauer cells and fibroblasts. The expression of ETRs in all compartments of human placenta, strongly suggests the various functions of ET-1, additionally to the established vasomotor activity. The presence of both endothelin receptors in extravillous and villous cytotrophoblast suggests the possible growth factor role of ET-1 in the early placenta. The temporary disappearing of endothelin system in syncytiotrophoblast could suggest its role in apoptosis, and its presence in fibroblasts and macrophages a possible influence on immune activity, inflammation and tissue repair.

The varying distribution of endothelin system in different developmental stages of human placenta suggests different functions of ET-1 in the human placenta during pregnancy. The aging of placenta from beginning to the end of pregnancy is associated with a continuous decrease of ETR-A and increase of ETR-B expression in villous tissue and cultured trophoblasts.¹¹

The expression of ET-1 and ETRs in the placenta is altered in some pregnancy complications such as pre-eclampsia, IUGR and gestational diabetes in a very specific manner. The isolated placental up-regulation of the whole endothelin system could be observed in early onset preeclampsia with and without IUGR, in contrast to down-regulation in placentas of pre-eclampsia, gestational diabetes and isolated IUGR late in pregnancy.¹²

Endothelin-1 in the first trimester trophoblast

The crucial tasks of the cytotrophoblast in the first trimester of pregnancy are proliferation and invasion,

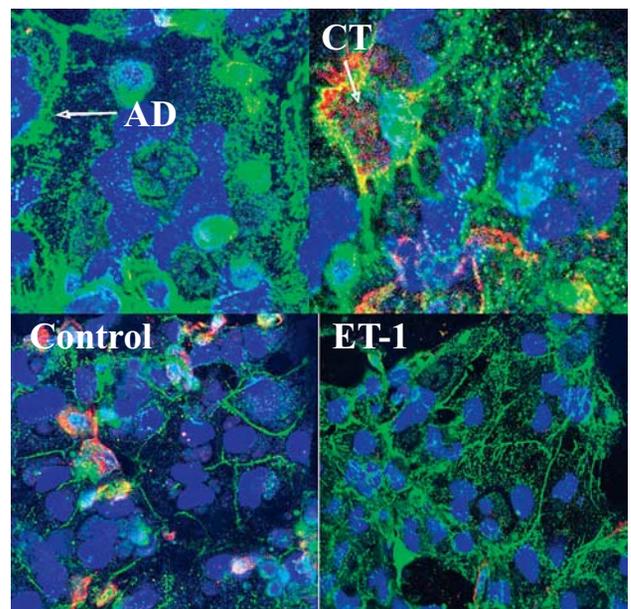
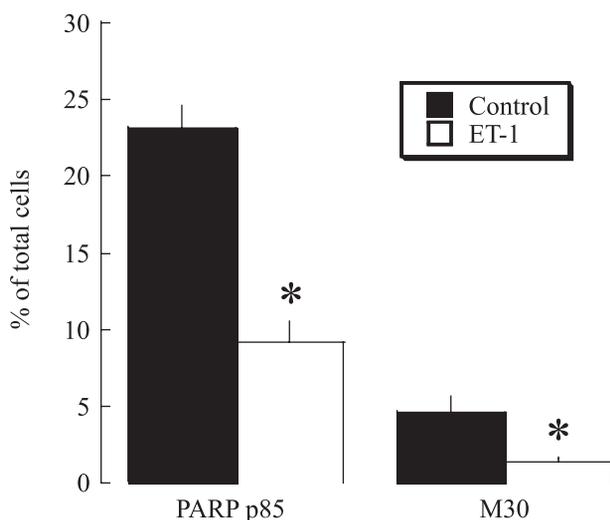


Figure 3. The effect of endothelin-1 (ET-1) on apoptosis detected by immunocytochemical staining of 5 primary cultures of trophoblasts exposed to 20% oxygen and serum-free conditions for 72 hours. Cells immunostained for cleaved poly-ADP-ribose polymerase (PARP) or cytokeratin 18 (cyt18), were quantified in vehicle control cultures, designated by the minus sign, or in cultures exposed to 100 pmol/mL ET-1, designated by the plus sign. The mean \pm standard deviation percentage of cells positive for each marker in cytotrophoblasts (CT) and PARP positive in syncytiotrophoblasts (white arrow) at 72 hours was normalized to total nuclei, as detected by To-Pro staining. Note that the scale for the y-axis is less than 100%. * $P < .01$ compared to vehicle control. (Červar *et al. Reprod Sci* 2007;14(5):430-9).

both of them controlled in a special extravillous unit of early placenta, called cell column. In the distal part of cell column are the invasive extravillous cytotrophoblasts, which invade into maternal decidua and uteroplacental arteries. The subsequent events including replacement of endothelium and destruction of elastic and muscular media elements lead to dilatation of vessels, necessary to enhance uterine and intravillous blood flow in pregnancy. The process of trophoblast invasion is completed until the 14th week of pregnancy and after this period only the differentiation of villous cytotrophoblasts in syncytiotrophoblasts can occur.

Endothelin-1 and its two receptors are present in the first trimester placenta (Fig. 1).^{11,13} Compared with term placenta, both receptor subtypes are more expressed in the first trimester, suggesting a specific role of ET-1 in early pregnancy. As a matter of fact, it has been shown that ET-1 stimulates the proliferation and invasion of the first trimester trophoblasts *in vitro* in a receptor-specific manner. The proliferation is mediated by both receptor subtypes whereas the invasion is mediated by ETR-B only.^{13,14} The crucial processes for adequate development and function of feto-placental unit during pregnancy depend on the regulation of trophoblast proliferation and its invasion in maternal decidua and spiral arteries. The lack of adequate control could lead to miscarriages, pre-eclampsia and fetal growth restriction. The over-expression of ETR-A in the first trimester placental tissue of delayed miscarriages has already been shown.¹⁵

Choriocarcinoma cells are usually used to study the proliferation and invasion, as a system with extreme properties. Choriocarcinoma is a malignant tumor originating from trophoblasts, consisting of the embryonic cytotrophoblasts in the central part and surrounded by syncytiotrophoblast on the periphery of cancer. However, there are some differences between normal trophoblasts and cancer cells. The investigations in JAR, JEG-3 and BeWo choriocarcinoma cell lines showed an autocrine regulation of ET-1 and ETRs with the predominance of ETR-B receptor subtype and decreased secretion of ET-1 compared with normal term trophoblasts.^{16,17}

Endothelin-1 in term trophoblast

The relative decrease of ETR expression in cytotrophoblasts during pregnancy and lack of ET-1/ETRs in syncytiotrophoblasts suggest the effect of ET-1 predominantly on the proliferation and invasion in the first trimester of pregnancy. The apical distribution of both endothelin receptor subtypes in syncytiotrophoblasts of the first trimester villi disappears in the third trimester villous tissue completely, remaining only in cytotrophoblasts (Fig. 3).¹¹ However, the relative constant expression of ETR-B in villous tissue until the end of pregnancy could be associated with the protective role of endothelin-1 against trophoblast apoptosis, which is mediated by the decrease of p53, a tumor suppressor gene and regulator of the death response to cell stress.¹⁸ The

lack of this protective effect could be due to conditions associated with hypoxic injury accompanied by enhanced trophoblast apoptosis, higher levels of p53. Also with general down-regulation of endothelin system in placenta of pre-eclampsia, gestational diabetes and IUGR at term.¹² Opposite results could be observed in pre-eclampsia in the early third trimester of pregnancy. Instead of decreased binding capacity of placental tissue for ET-1 in this severe form of disease, trophoblast affinity to bind ET-1 is increased compared to normal pregnancies and could be inhibited *in vitro* by vasodilator hydralazine.¹⁹ Furthermore, also the expression of ET-1 and both ETRs in placenta was shown to be changed in the opposite manner in mild and severe early onset pre-eclampsia.¹² However, a decreased secretion of ET-1 from pre-eclamptic trophoblasts *in vitro* does not depend on the severity of disease or gestational age.²⁰ Furthermore, it could not be affected by common drugs used in the prevention or therapy of pre-eclampsia.²¹

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ENDOTELIN U LJUDSKOJ POSTELJICI

Pregled

Ključne riječi: posteljica, endotelin, preeklampsija, trofoblast

SAŽETAK. Endotelin – 1 (ET-1) je član obitelji endotelina koja se sastoji od tri peptida, ET-1, ET-2 i ET-3, a kodirana je genima na različitim kromosomima. Endotelini imaju središnju ulogu u regulaciji tonusa krvnih žila. Osim spomenute, najpoznatije druge funkcije su regulacija staničnih procesa poput invazije, proliferacije, migracije i apoptoze. U odnosu na netrudno stanje, normalna trudnoće povezana je s oko 60% nižom koncentracijom ET-1 u plazmi. U trudnoća kompliciranih preeklampsijom povećana je razina ET-1 u krvi majke što odgovara vrijednostima u netrudnom stanju. Zastupljenost ET receptora u svim dijelovima ljudske posteljice nedvojbeno ukazuje na različite funkcije ET-1. Prisutnost oba receptora endotelina u ekstraviloznom području te citotrofoblastu sugerira moguću ulogu ET-1 kao čimbenika rasta u posteljici tijekom ranog razdoblja trudnoće. Ključni zadaci citotrofoblasta u prvom tromjesečju trudnoće su širenje i invazija. Spomenuto uključuje zamjenu endotela i uništavanje elastičnog i mišićnog sloja krvnih žila. ET-1 stimulira proliferaciju i invaziju trofoblasta tijekom prvog tromjesečja in vitro, na receptor – specifičan način. Relativno smanjenje zastupljenosti ET receptora u citotrofoblastu napredovanjem trudnoće i nedostatak ET-1/ET receptora u sinciotrofoblastu upućuju na to kako ET-1 ima učinak na proliferaciju i invaziju pretežno u prvom tromjesečju trudnoće. Zastupljenost ET-1 te ET receptora u posteljici je promijenjena u slučaju ranog nastanka bilo blage bilo teške preeklampsije. Međutim, u slučaju preeklampsije, smanjeno izlučivanje ET-1 iz trofoblasta u in vitro uvjetima nije povezano s intenzitetom bolesti i gestacijskom dobi. Isto tako, uobičajeni lijekovi nemaju utjecaj u prevenciji ili liječenju preeklampsije.