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ORIGINAL ARTICLE

Melatonin 1A and 1B Receptors' Expression Decreases in the Placenta of Women with Fetal Growth Restriction

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Abstract

Melatonin and its metabolites prevent oxidative stress and apoptosis, and it is actively produced by the placenta during pregnancy. Melatonin 1A and 1B receptors are present in human villous trophoblastic cells. We aimed to investigate the expression of melatonin 1A and 1B receptors in human placental tissue in the case of placental insufficiency manifested as the intrauterine growth restriction syndrome of the fetus (IUGR). Thirty-two pregnant women aged 18–36 with placental insufficiency manifested at the term 36 weeks of gestation as the IUGR syndrome (the estimated fetal weight less than the 3rd percentile) were included in the experimental group; all their babies had the diagnosis confirmed at birth, which occurred after 37 weeks of gestation. The control group consisted of 30 women with uncomplicated pregnancy of the same term. Pieces of the placental tissue were obtained after deliveries, and melatonin 1A and 1B receptors were immunoassayed; the richness of melatonin receptors in the placental tissue was estimated on the basis of immunohistochemical (IHC) staining of receptors, calculated in the IHC image score. The optical density of melatonin 1A receptors in the placentas obtained from women whose pregnancies were complicated with IUGR was significantly lower than that in the placentas from uncomplicated pregnancies: generally in the trophoblast, it was 0.095 ± 0.0009 IHC image score (in the control group, $0.194 = 0.0015$, $p < 0.0001$); in the apical parts of the syncytiotrophoblast, 0.108 ± 0.0016 IHC image score (in the control group, $0.221 + 0.0013$, $p < 0.0001$); and in the stromal cells of placental villi, $0.112 + 0.0013$ IHC image score (in the control group, $0.156 + 0.0011$, $p < 0.0001$). The optical density of melatonin 1B receptors in placentas obtained from women whose pregnancies were complicated with IUGR was also lower than that in the placentas from uncomplicated pregnancies: generally in the trophoblast, it was $0.165 = 0.0019$ IHC image score (in the control group, $0.231 + 0.0013$, $p < 0.0001$), and in the apical parts of the syncytiotrophoblast, $0.188 + 0.0028$ IHC image score (in the control group, $0.252 + 0.0009$, $p < 0.0001$). There was no difference found in the optical density of melatonin 1B receptors in the stromal cells of placental villi between the two groups: in the experimental group, 0.109 ± 0.006 IHC image score, and in the control group, $0.114 = 0.0011$ ($p = 0.65$). Melatonin receptors 1A and 1B are significantly less expressed in the placental tissue in the case that pregnancy is complicated with placental insufficiency, manifested as the intrauterine growth restriction syndrome of the fetus.

Keywords Placenta · Melatonin · IUGR · Melatonin 1A receptors · Melatonin 1B receptors

Introduction

The human placenta is considered to be the key organ in the development of pregnancy. It plays a crucial role in gas and nutrient exchange between the mother and fetus, and it maintains fetal excretion as well [1]. The endocrine function of the human placenta is limited to the production not only of reproductive hormones [2, 3] but also of melatonin [4–6]. The roles of melatonin in human pregnancy and labor are being widely studied. For example, melatonin promotes embryo implantation [7, 8], regulates labor activity [9], improves placental efficiency and birth weight [10], and increases pregnancy rates in both animals and humans [7, 11, 12]. Melatonin is a lipophilic hormone; due to this property it is broadly distributed in the body [13]. This indolamine controls biological rhythms and possesses a strong anti-oxidant effect and other anti-inflammatory properties [5, 14–16]. Melatonin and its metabolites prevent oxidative stress and apoptosis [17–20]; it plays a significant role in fetal neuroprotection [21]. Our previous investigations demonstrated that decrease of blood melatonin concentrations caused by light exposure in pregnant rats was accompanied with significant elevation of pro-inflammatory interleukin-6 [22]. Moreover, in pregnant women with the intrauterine growth restriction syndrome of the fetus (IUGR), lowered melatonin levels in the blood are combined with the increase of pro-inflammatory interleukin-1-β, interleukin-6, and tumor necrosis factor-α [23]. Melatonin reduces secretion of a preeclampsia-associated molecule—soluble fms-like tyrosine kinase-1 (sFLT)—from the primary trophoblast as well [24].

The presence of melatonin receptors 1A (also known as MT1 or MTNR1A) and 1B (also known as MT2 or MTNR1B) in human villous trophoblastic cells was demonstrated by Lanoix et al. and Soliman et al. [4, 6]. Melatonin was suggested to play an important role in the human placental function. Expression of melatonin receptors 1A and 1B, as well as melatonin production, is evidenced to be significantly reduced in the case of preeclampsia compared with that in normotensive patients [17]. The cellular anti-oxidant defenses in the placental tissue are realized through melatonin receptor 1A– and receptor 1B–dependent pathways [17, 25, 26] and are reportedly impaired in the case of preeclampsia [17]. Moreover, in the human placenta, melatonin was found to significantly reduce trophoblast apoptosis through its receptor-dependent pathway [27].

IUGR is commonly associated with perinatal morbidity and mortality [28]. However, the expression of melatonin receptors in placentas of IUGR patients compared with placentas maintaining normal fetal growth has not been widely studied. The aim of this study was to investigate the expression of melatonin 1A and 1B receptors in human placental tissue in the case of placental insufficiency manifested as IUGR.

Methods

Patients

Between April 2017 and January 2019, 32 Caucasian pregnant women aged 18–36 with placental insufficiency (PI) were recruited in the experimental group. PI manifested at the term 36 weeks of gestation as the IUGR syndrome: the estimated fetal weight was less than the 3rd percentile at ultrasound fetometry [29, 30]. The "Formula C" of Hadlock was used for calculation of the estimated fetal weight [31, 32], and the reference ranges recommended by the Fetal Medicine Foundation [33] were applied. The control group consisted of 30 women with uncomplicated pregnancy of the same term. Only patients with singleton pregnancies were involved in the groups. Pregnancy terms were calculated based on the date of the last menstrual period and based on the crown-rump embryo length in the 1st trimester (10–12 weeks of pregnancy) ultrasound scan. There were no significant discrepancies between the two calculated dates for each patient. Women who had severe extragenital pathology (cardiovascular disorders, including chronic arterial hypertension, preexisting diabetes mellitus, hepatic diseases, obesity), as well as certain complications of pregnancy (preeclampsia, gestational diabetes, immune conflicts, major fetal anomalies, and prematurely ruptured fetal membranes), and also patients with the ultrasound signs of possible intrauterine infectious contamination of the fetus (polyhydramnios, oligohydramnios, hyperechogenic bowels, ascites) [34] were excluded from the study. Smoking and drug abuse were also criteria of exclusion. The diagnosis "IUGR syndrome" was re-confirmed after birth by the presence of the birth weight below the 3rd percentile for the respective pregnancy term in all newborns from mothers included in the experimental group. Percentiles were calculated using the online calculator http://medicinafetalbarcelona.org/calc. All the pregnancies in both groups were completed at 37 weeks or more. The study took place in Chernivtsi Municipal Clinical Maternity Hospital #1 (Chernivtsi, Ukraine).

Ethical Approval

The study was approved by the Biological and Medical Ethics Committee of the Higher State Educational Establishment of Ukraine "Bukovinian State Medical University" (the minutes no. 3 dated March 30, 2017) and was carried out strictly in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Sample Processing

After deliveries, 4 randomly selected pieces of the placental tissue from each patient were obtained and preserved in neutral 10% formalin solution, buffered according to Lillie [35] for 24 h; then, they were dehydrated through an ethanol series and placed in paraffin at a temperature of 58 °C. Histological sections 5 mcm thick were used for performing the immunohistochemistry method based on primary antibodies against melatonin receptors 1A and 1B. The pathologists who examined the placental samples were blinded for the clinical diagnoses and outcomes.

Determination of the daytime of the delivery was conducted using the timer on the website weather.com with established the geolocation as Chernivtsi, Ukraine.

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