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Intrauterine growth restriction is associated with structural alterations in human umbilical cord and decreased nitric oxide-induced relaxation of umbilical vein

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ABSTRACT

Introduction: Intrauterine growth restriction (IUGR) affects ~8% of all pregnancies and is associated with major perinatal mortality and morbidity, and with an increased risk to develop cardiovascular diseases in adulthood. Despite identification of several risk factors, the mechanisms implicated in the development of IUGR remain poorly understood. In case of placental insufficiency, reduced delivery of oxygen and/or nutrients to the fetus could be associated with alterations in the umbilical circulation, contributing further to the impairment of maternal–fetal exchanges. We compared the structural and functional properties of umbilical cords from growth-restricted and appropriate for gestational age (AGA) term newborns, with particular attention to the umbilical vein (UV).

Methods: Human umbilical cords were collected at delivery. Morphological changes were investigated by histomorphometry, and UV's reactivity by pharmacological studies.

Results: Growth-restricted newborns displayed significantly lower growth parameters, placental weight and umbilical cord diameter than AGA controls. Total cross-section and smooth muscle areas were significantly smaller in UV of growth-restricted neonates than in controls. Maximal vasoconstriction achieved in isolated UV was lower in growth-restricted boys than in controls, whereas nitric oxide-induced relaxation was significantly reduced in UV of growth-restricted girls compared to controls.

Conclusion: IUGR is associated with structural alterations of the UV in both genders, and with a decreased nitric oxide-induced relaxation in UV of newborn girls, whereas boys display impaired vasoconstriction. Further investigations will allow to better understand the regulation of umbilical circulation in growth-restricted neonates, which could contribute to devise potential novel therapeutic strategies to prevent or limit the development of IUGR.

1. Introduction

Numerous epidemiological studies have associated adverse events occurring during the perinatal period with the development of chronic diseases in adulthood. Intrauterine growth restriction (IUGR), defined as a failure of the fetus to reach its full growth potential, is a common complication, affecting approximately 8% of all pregnancies. It is the second leading cause of perinatal mortality, after prematurity. Besides perinatal complications, growth-restricted newborns are at increased risk of long-term neurological and developmental disorders. Moreover, they displayed a higher incidence of chronic diseases later in life, like coronary heart disease, systemic hypertension, stroke and non-insulin-dependent diabetes mellitus [1–6].

The mechanisms implicated in the development of IUGR are poorly understood, even though some maternal risk factors have been identified, such as maternal diseases (e.g. systemic arterial hypertension, lupus, renal insufficiency), malnutrition, mother's stress, strenuous work, as well as tobacco, alcohol and drug abuse [7]. Etiologies of IUGR are multiple and can be of fetal, placental, or maternal origins [8]. Placental insufficiency is associated with a decrease in maternal–fetal exchanges and is present in almost all

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cases of IUGR. Adequate fetal growth is primarily determined by nutrient availability and IUGR is associated with a reduction in oxygen and nutrients supply across the placenta [9]. The placenta can adapt to maternal environment by changing its size, structure and function, hence its contribution to fetal programming [10]. Placental insufficiency induces also adaptive mechanisms in the fetus, including modifications in fetal circulation, metabolism and endocrinology [11].

There is currently no efficient way to prevent or treat IUGR, but only potential preventive approaches to reduce risk factors, mainly by modifying mother's health behavior [7,12]. Identifying high-risk pregnancies is key in order to prevent or alleviate the consequences of poor fetal growth. Prenatal IUGR screening is based on ultrasonographic biometrical measurements of the fetus, as well as Doppler velocimetry of some relevant vessels [13–16]. Management of IUGR is mainly based on careful monitoring of fetal growth and biophysical profile. Premature delivery is often the only issue when fetal adaptation is overwhelmed, contributing to further increased risk of perinatal mortality and morbidity. Additional understanding of the mechanisms implicated in the development of IUGR and in the fetal programming of adult diseases is necessary to develop novel strategies to prevent or limit IUGR and its consequences.

The fetus survival and development depend mainly on the functional integrity of the maternal-placental-fetal circulation. In humans, umbilical arteries (UA) and vein (UV) are extremely long and muscular, contributing largely to the total umbilical-placental vascular resistance, whereas the placental microcirculation is characterized by low resistance [17]. IUGR has been linked to decreased umbilical blood flow and abnormal umbilical circulation, as shown by Doppler velocimetry [16,18–20]. Umbilical vascular tone is regulated by numerous vasoactive factors, such as nitric oxide (NO), calcitonin gene-related peptide, endothelin-1 and thromboxane [17]. Umbilical vasoreactivity was shown in vitro to vary along the umbilical cord (UC) [21], and to depend on gestational age [22] and experimental conditions, particularly the level of oxygenation [23–25].

We therefore hypothesized that IUGR could be related to structural and functional alterations in UC. Placental insufficiency and the resultant reduction in oxygen and/or nutrients delivery to the fetus could be associated with alterations in the regulation of umbilical vascular tone. Altered umbilical vasoreactivity could be either a direct consequence of placental insufficiency, or play a role among the global components of underlying pathologies resulting in decreased maternal–fetal exchanges and finally IUGR.

This study therefore focused on identification of structural and functional alterations occurring in UC of growth-restricted newborns, with particular attention to UV, which is the only vessel to conduct the blood from the placenta to the fetus. In the present report, structural modifications were assessed by morphometrical measurements, whereas NO-induced relaxation was used to evaluate functional properties.

2. Methods

2.1. Samples collection – inclusion and exclusion criteria

The present study was approved by the ethical committee of the Faculty of Biology and Medicine of the University of Lausanne (protocol number 134/08).

Umbilical cords of 285 newborns delivered at the Maternity of the University Hospital CHUV in Lausanne (Switzerland) were collected between June 2009 and July 2013. Demographic and medical data were prospectively collected for each patient in our digital medical record system.

Inclusion criteria were term (≥37 accomplished weeks of gestation) singleton pregnancies of either IUGR or AGA fetuses.

Exclusion criteria were fetal abnormalities, genetic syndromes, mothers presenting with HIV, hepatitis A, B or C, preeclampsia, single umbilical artery, and neonates with a birth weight >P90.

Based on measurements of body weight at birth, newborns were dichotomized into two categories: IUGR and AGA controls. Samples were assigned to the control group when birth weight was between P10 and P90, and to the IUGR group when birth weight was <P10. The percentile classification was determined according to growth charts endorsed by the Swiss Society of Pediatrics [26]. When available, prenatal data (e.g. estimated fetal weight) were used to determine whether a growth curve break occurred, namely a significant change in percentiles, in order to distinguish "IUGR" from constitutive "Small for Gestational Age", as proposed by Barker et al. [27]. Male and female neonates were studied separately.

Umbilical cords were collected at delivery, and used within 24 h. Because vasoreactivity was shown to vary along the UC [21], we decided to focus on the segment closer to the fetus. Umbilical cords were cut as close as possible to the fetus, and a 10–15-cm segment was kept at 4 °C in deoxygenated modified Krebs–Ringer solution (118.3 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25.0 mM $NaHCO_3$, and 11.1 mM glucose) until dissection.

Before dissection, UC diameter (UCD) was measured at three different places of the considered portion to calculate the average UCD for each patient.

For technical reasons, it was not possible to perform all types of experiments in each UC. However, we checked that each subset of samples used for the various experiments was representative of the entire population included in this study.

2.2. Histomorphological studies

A 1-cm length segment of UC was harvested, fixed in paraformaldehyde (PFA 4%, 2 h, 4 °C) and paraffin embedded. Five-μm microtome sections were stained according to standard procedures using hematoxylin/eosin, as well as Masson's trichrome or resorcin/fuchsin to unequivocally distinguish UV from UA in tissue sections. Histomorphometrical measurements were performed using a Leica stereomicroscope (Leica Microsystems, Heerbrugg, Switzerland) and ImageJ software (National Institutes of Health) to determine total cross-sectional area (CSA) of the cord, umbilical vessels' CSA, the luminal and smooth muscle (SM) areas of each vessel, and the area of Wharton's jelly (WJ) [28]. Three sections were analyzed for each patient, by two independent experimenters. Histomorphological data represent the mean of these measurements.

2.3. Pharmacological studies

The vascular reactivity of UV was investigated by isolated vessel tension studies, adapted from our previous experiments [29]. Briefly, UV was carefully dissected and cut into small rings (4–5 mm length). Each ring was suspended into an organ chamber filled with modified Krebs–Ringer solution, with two stirrups (0.35 mm diameter) passed through the lumen. Preliminary experiments, performed as previously described [30], displayed that a 2-g stretch tension allowed umbilical vessels to achieve maximal contractile response to 100 mM KCl. A 2 g stretch tension was therefore applied to each vessel ring, followed by 20-min equilibration before washing. Stretch/equilibration/wash steps were repeated still three times, in order to get the vessels to their optimal resting tension (RT). After equilibration, N^G-nitro-L-arginine (10^{-4} M) and indomethacin (10^{-5} M) were added in order to exclude possible interference of endogenous NO and prostanoids, respectively. Preliminary experiments determined that 10^{-5} M serotonin (5-HT) or 10^{-6} M U46619, an analog of thromboxane A_2, induced a sustained contraction in umbilical vessels. Vascular rings were pre-contracted by 5-HT or U46619, and relaxant response to cumulative doses of the NO-donor 2-(N,N-Diethylamino)-diazenolate-2-oxide (DEA/NO) (10^{-8}–10^{-4} M) was then tested. Change in tension induced by DEA/NO was expressed as percent of the initial contraction induced by the vasoconstrictor (residual tension, RDT). Non-linear regression analysis was performed to calculate E_{max} and EC_{50} for each dose–response curve.

Because umbilical vessels' reactivity was shown to depend on the partial pressure in oxygen (PO_2) and pH [24,25], *in utero* conditions (PO_2 23–29 mm Hg and pH 7.35 in UV) [31,32] were reproduced as closely as possible. Cords were therefore harvested in a deoxygenated physiological solution and UV vasoreactivity was tested in the presence of low PO_2 and pH near the fetal physiological pH. We obtained such conditions by bubbling 21% O_2 and 5% CO_2, balanced with nitrogen, in organ chambers filled with modified Krebs–Ringer solution maintained at 37.5 °C. PO_2 was assessed using an in-line electrode as previously described [33]. In our system, such experimental conditions led to PO_2 values of 25–27 mm Hg and pH of 7.32–7.38. As comparison, bubbling with 95% N_2 and 5% CO_2 in the same conditions led to PO_2 of 6–7 mm Hg with in-line electrode.

2.4. Data analyses

Statistical analyses were performed using InStat 3.0 or Prism 4.0 (GraphPad Software, San Diego, CA). Unless otherwise specified, data observed in growth-restricted newborns were compared to controls using the Mann–Whitney test. Two-way ANOVA was performed to compare the dose–response curves in isolated vessel tension studies. The difference was considered statistically significant when $P < 0.05$.

The efficiency (E_{max}) and sensitivity (EC_{50}) related to DEA/NO-induced relaxation were calculated by non-linear regression analysis of the dose–response curves, using Prism 4.0.