European Journal of Obstetrics & Gynecology and Reproductive Biology 142 (2009) 12–17

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Influence of the magnesium aspartate hydrochloride administration to the maternal circuit on the aspartate concentration of the fetal circuit under in vitro perfusion of human placenta

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ARTICLE INFO

Article history:
Received 7 May 2008
Received in revised form 13 July 2008
Accepted 26 August 2008

Keywords:
Magnesium
Aspartic acid
Placental transport
Human
In vitro

ABSTRACT

Objectives: Magnesium aspartate hydrochloride (Magnesiocard®, Mg–Asp–HCl) is proposed as a substitute of magnesium sulfate for the treatment of preeclampsia and premature labor. After an i.v. administration of a dose equivalent to that used in the treatment of preeclampsia to nonpregnant volunteers, a 10-fold increase of aspartic acid (Asp) over the physiological level was observed. Animal experiments have demonstrated that highly increased fetal levels of acidic amino acids such as Asp could be associated with neurotoxic damage in the fetal brain. The influence of such an elevation of Asp concentration in the maternal circuit on the fetal level, using the in vitro perfusion model of human placenta, was investigated.

Study design: After a control phase (2 h), a therapeutic dose of Mg combined with Asp (Magnesiocard®, Mg–Asp–HCl) was applied to the maternal circuit approaching 10 times the physiological level of Asp. The administration was performed in two different phases simulating either a peak of maximum concentration (bolus application, 2 h) or a steady state level (initially added, 4 h).

Results: In four experiments, during experimental phases (6 h) a slow increase in concentration in the fetal circuit was seen for Mg, AIB (alpha-aminoisobutyric acid, artificial amino acid) and creatinine confirming previous observations. In contrast, no net transfer of Asp across the placenta was seen. A continuous decrease in the concentration of Asp on both maternal and fetal side suggests active uptake and metabolization by the placenta. Viability control parameters remained stable indicating the absence of an effect on placental metabolism, permeability and morphology.

Conclusion: Elevation of Asp concentration up to 10 times the physiological level by the administration of Mg–Asp–HCl to the maternal circuit under in vitro perfusion conditions of human placenta has no influence on the fetal level of Asp suggesting no transfer of Asp from the maternal to fetal compartment. Therefore, the administration of Mg–Asp–HCl to preeclamptic patients would be beneficial for the patients without any impact on placental or fetal physiology.

1. Introduction

Magnesium (Mg) is commonly used in preeclampsia to prevent convulsions and also for tocolysis in preterm labor [1]. The inhibitory effect of magnesium on spontaneous and induced myometrial contractility [2,3] is explained by competitive inhibition of calcium channels [3–5]. In addition, Mg was shown to have a neuroprotective effect in perinatal asphyxia and may prevent cerebral palsy [6–8]. Many studies have shown that Mg can limit neuronal injury resulting from hypoxia, ischemia and traumatic brain damage in vitro and in vivo [9–12]. The neuroprotective properties of magnesium are apparently due to its role in decreasing excitatory effects resulting from the activation of the calcium-dependent transmitter release or the stimulation of the *N*-methyl-D-aspartate (NMDA) receptor. For the therapeutic use of magnesium, its sulfate form has been the preferred drug. Antihypertensive drugs therapy in preeclampsia is associated with some concern regarding the lowering blood pressure may compromise fetal well-being as a result of reduced placental perfusion [13]. Magnesium sulfate is the drug of choice for anticonvulsant therapy to prevent eclampsia in women with severe preeclampsia. Although the benefit of magnesium sulfate prophylaxis in mild preeclampsia is less clear,

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doi:10.1016/j.ejogrb.2008.08.004

however, its therapeutic application is also recommended [14]. Magnesium aspartate (Magnesiocard®, Mg–Asp–HCl), which is a solid drug for oral applications, is proposed as a substitute of magnesium sulfate for the treatment of preeclampsia and premature labor [15–18]. After an intravenous administration to nonpregnant volunteers of a dose equivalent to that used in the treatment of preeclampsia, magnesium showed a rapid availability in blood together with a 10-fold increase of aspartate above the physiological level (Personal communications, Verla-Pharm Company, Tutzing, Germany). Animal experiments have demonstrated that highly increased fetal levels of acidic amino acids such as aspartic acid (Asp) could be associated with neurotoxic damage in the fetal brain [19,20]. Therefore, the influence of such an elevation of maternal Asp concentration on the fetal level was investigated using the in vitro perfusion model of human placenta.

2. Materials and methods

2.1. In vitro perfusion method

For this study placentae, obtained from uncomplicated deliveries at term following normal pregnancies with an appropriately grown newborn with normal Apgar scores and cord blood gas values, were used. After either vaginal or cesarean delivery the placenta was immediately taken to the laboratory, and a cotyledon was prepared for dual in vitro perfusion as originally described by Malek et al. [21]. After cannulation of a pair of chorionic artery and vein the perfusion of the corresponding villous capillary system was started (fetal compartment). The isolated lobule was fixed in a perfusion chamber that was surrounded by a jacket connected to a warm water circuit at 37 °C. Three blunt metal cannulae were introduced into the intervillous space by penetration of the decidual plate and connected to a second circuit for perfusion of the maternal compartment.

The perfusate was composed of tissue culture medium NCTC-135 (Serva), diluted 1:3 with Earl's solution with addition of glucose (2 g/L final concentration), bovine serum albumin (40 g/L), dextran FP40 (10 g/L), heparin (2500 IU/L), clamoxyl (250 mg/L), mefoxitin (200 mg/L), and diflucan (5 ml/L). In the maternal and fetal circuits, a volume of 250 and 120 ml of perfusate was recirculated, respectively. Media were continuously equilibrated with 95% oxygen and 5% carbon dioxide on the maternal and 95% nitrogen and 5% carbon dioxide on the fetal side. Flow rates of 12 and 4–6 ml/min were used in the maternal and fetal circuits, respectively. The drug tested (Magnesiocard®), which is produced by Verla-Pharm (Tutzing, Germany), was kindly provided by Biomed AG (Dübendorf, Switzerland). The substance was provided as an infusion ampulla (40 mmol magnesium/50 ml H_2O).

2.2. Perfusion experiments

All experiments included a 30-min period of open perfusion (prephase) of both compartments for flushing the blood out of the intervillous space and the villous vascular compartment and to allow recovery of the placental tissue from the ischemic period after delivery before the initiation of the artificial perfusion. After a first control perfusion period of 2 h, a therapeutic dose of Mg combined with Asp approaching 10 times the physiological level of Asp was applied to the maternal circuit. The administration was performed in two different experimental modes (EP-A and EP-B) either simulating a peak of maximum concentration after infusion of a bolus with subsequent perfusion for a total of 2 h, or maintaining a constant concentration in the maternal circuit over 4 h. Each phase of the perfusion experiment was started with media exchange on the maternal and fetal sides.

In four experiments, in the first experimental phase (EP-A) a bolus of 80–120 μmol Mg–Asp–HCl + 520–680 μmol $MgCl_2$ was infused within 15–20 min into the maternal circuit. In the second experimental phase (EP-B) 30 μmol Mg–Asp–HCl + 570 μmol $MgCl_2$ was initially added to the maternal circuit. Alpha-aminoisobutyric acid (AIB) is an artificial amino acid which is not metabolized by placental tissue and is used as reference for the transplacental transfer of amino acids. ^{14}C-AIB was added in parallel to Mg–Asp combinations to the bolus fluid (125 nCi/ml, EP-A), and the EP-B (20 nCi/ml). The combined addition of $MgCl_2$ with Magnesiocard® was used to test the similar concentrations (3 mmol/L Mg, 400 μmol/L Asp) as those observed in volunteers after infusion with Mg and Asp (Mg–Asp–HCl), of a dose used for the therapy of preeclampsia and premature labor.

Antipyrine (80 mg/L) and creatinine (150 mg/L) serving as markers of permeability with different properties were added to the maternal perfusate in all experimental phases, including control phase.

2.3. Analyses

Radioactivity of ^{14}C-label was counted after adding 6 ml of scintillant to 0.1 ml sample [22]. Determination of glucose (Glucoquant-Kit, Boehringer Mannheim) and lactate (Test-Combination-Kit, Boehringer Mannheim) was performed using standard enzymatic assays. Concentrations of antipyrine [22] and creatinine (diagnostic-Kit, Sigma) were determined colorimetrically. Magnesium was determined using the flame atomic absorption spectrometry (Flammen-AAS 1100, Perkin Elmer). Amino acids were determined according to Fürst et al. [23]. For the measurement of human chorionic gonadotropin (hCG) and human placental lactogen (hPL), standard enzyme-linked immunosorbent assays (ELISA) developed in our laboratory were used [21,24]. For the ultrastructural investigation sections from perfusion fixed tissue probes performed at the end of the experiment were analyzed in a transmission electron microscope [25].

2.4. Calculations

The changes over time of lactate and glucose in total (maternal + fetal) content were used to calculate production or consumption. Results were normalized for tissue mass. For hormone release, the same calculation was applied using data from the maternal compartment only, because more than 98% of placental synthesis of both hPL and hCG is released into the maternal circulation.

3. Results

3.1. Viability of placental tissue

The integrity of placental tissue was assessed by monitoring parameters describing the metabolic activity and permeability. For the metabolic parameters, the rates of glucose consumption, lactate production and hormone release (hPL and hCG) presented in Table 1 demonstrate that these parameters showed similar rates during the control period and after the addition of Mg and Asp combinations as seen in the following two experimental phases. Similar to the metabolic parameters, the permeability markers (antipyrine and creatinine) showed no significant differences between all three perfusion phases. Antipyrine is rapidly transported from the maternal to the fetal circuit with similar profiles after the initiation of each phase (Fig. 1A). In contrast, creatinine, which is a more polar compound with a lower permeability, showed slower kinetic profiles on the fetal side