Introduction

The oxygen is the term of the development and existence of life. Besides, its harmful effect is also well-known. In the course of physiological procedures of a living organism free radicals are generated and their formation is increased under pathological conditions. Reactive oxygen species disturb the balance of pro and antioxidants causing oxidative stress, wherewith increasing the sensitivity and vulnerability of the cells. Free radicals are proved to have decisive role in the pathomechanism of several diseases (e.g.: tumors, diabetes, ischemic heart disease, inflammation). The interest in free radicals is not recent- it has been already known at the beginning of the past century, that molecular oxygen has an unpaired electron- and it is not limited to the investigation of the living organism. All living and inorganic things are exposed to oxygen and so they are liable to free radical attack.

Oxidative stress is a physiological event in the fetal-to-neonatal transition, which is actually a great stress to the fetus. Adequately mature infants are able to

tolerate this drastic change in the oxygen concentration. A problem occurs when the intrauterine development is incomplete or abnormal. Preterm or intrauterine growth retarded (IUGR) neonates are typically of this kind. Beyond the level of maturity of the newborn the mode of delivery is also important in medical respect.

The aim of our study was the circumstantial investigation and hence better understanding of the pathomechanism of IUGR. In the interest of this we aimed to evaluate and compare the antioxidant status and oxidative damage in the umbilical cord of IUGR normal weight neonates.

Materials and methods

Umbilical cord blood samples were obtained from the Department of Obstetrics and Gynecology of the Medical University of Szeged, Hungary. 277 samples were analyzed. 223 full-term mature neonates of either sex, born between weeks 37 and 40 were selected, 182 of them with normal weight (3350 ± 550g) and 49 neonates with symmetrical IUGR (weight

1950 ± 450g). Apgar score of the controll samples was in the range of 8-10. For the comparison of the modes of the delivery (cesarean section, vaginal delivery, epidural analgesia) the samples of the controll groups were employed. The remaining 54 samples camefrom premature infantsand were utilized for the investigation of the role of acidosis.

Measurements from umbilical cord blood: Blood was taken from the umbilical cord before the birth of the placenta. Coagulation was inhibited by EDTA. The samples were assayed spetrophotometrically to determine the level of total and carbonyl proteins, the level of glutathione, the activities of the major antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase and the glucose-6-phosphate dehydrogenase), the level of nitrite and peroxynitrite and the level of lipid peroxidation. The latter was assayed on erytrocytes, plasma and on erytrocyte ghosts and to confirm these results deformability test were done.

Determination of DNA damage and repair was carried out using a fluorimeter.

A Thermo Spectronic Biomate 5 was used for the spectrophotometric measurements. Fluorescence analysis was carried out with dual-channel modulated fluorimeter (Hansatech, King's Lynn, UK) in the emission region of 590 nm.

Measurements from the arteria umbilicalis: RNA extraction, reverse transcription and PCR amplification were performed to assess the expression of endothelial nitric-oxide synthase (ecNOS) Approximately 100 mg of the umbilical artery was homogenized in TRI reagent (Sigma) and total RNA was prepared. To follow the expression of ecNOS gene, semi quantitative RT-PCR was performed using β-actin as an internal control. First strand cDNA was synthetized by using 5 µg total RNA as template Amplification was performed in a PTC 150-16 Minicycler-ben (MJ Research) and in PTC 200 Peltier Thermal Cycler (MJ Research). The amplified products were electrophoresed on 1.8% agarose (Sigma) gel.

Images of ethidium bromide stained agarose gels were digitalized with a GDS 7500 Gel Documentation System and analyzed with the GelBase/GelBlot™ Pro Gel Analysis Software (UVP).

Results and discussion

The aim of our work was the investigation and better understanding of the pathomechanism of IUGR, and we approached the question from a consideration of oxidative stress. The numerous samples obtained from the Department of Obstetrics and Gynaecology allowed us to take other aspects in consideration.

Many ambivalent results are available that deal with the relation of oxidative stress and the mode of delivery. Based on our results we conclude that there are some differences between the modes of delivery in the respect of oxidative stress, but these are not conclusive and non-significant. It is worthy of note that we found favourable values at the cases of epidural analgesia, so the absence of pain not only stimulates the blood-flow, but also decreases oxidative stress.

IUGR is a neonatal disorder most probably caused by abnormality of the foeto-maternal blood flow. The results of the analysis suggest that the antioxidant defense of neonates with IUGR is similar to that of premature infants. The antioxidant capacity and the level of GSH is significantly lower in this group. Moreover the level of GSH is not only low, it is rather minimal. Analysis of the whole redox cycle of the tripeptide could account for the phenomenon.

The level of enzymes responsible for GSH regeneration was very low, especially of glucose-6-phosphate dehydrogenase and the rate of GSH synthesis was very low compared to controls. Activities of enzymes using GSH as a cofactor did not change in the IUGR samples. Further analysis revealed severe nitrosative stress in the umbilical cord of IUGR neonates, which is also conducive to GSH depletion. All the parameters of oxidative damage were significantly higher in the IUGR neonates, while the antioxidant enzyme activities were significantly lower.

We also detected that ecNOS expression in IUGR neonates varied at least 2-fold greater in the umbilical

artery compared to control group. In the human foeto-maternal circulation, NO appears to contribute to the maintenance of low vascular resistance. In addition we also determined the level of nitrite, peroxynitrite, LP, GSH and SOD activity to have a more complex view on the question. We found that in IUGR neonates elevated expression of ecNOS is coupled with decreased SOD activity. SOD is responsible for superoxide decomposition. The deficiency of this activity creates increased superoxide level. NO reacts very fast with superoxide forming the highly toxic and stable peroxynitrite. It interacts with biomolecules especially lipids and GSH via direct oxidative reactions. Our results of GSH depletion and elevated LP confirm these suggestions.

Lipid peroxides may decrease the membrane fluidity, inactivate membrane-bound receptors and enzymes, and increase the membrane permeability. The integrity of the erythrocyte membranes is especially important, because it is crucial for their transfer function. If the membrane is damaged, the erythrocytes lose their shape and elasticity and may undergo haemolysis.

We conclude that the increase in NO indexes could represent a compensatory effort to improve placental blood flow and decrease the peripheral pressure, but in IUGR neonates it is coupled with inadequate antioxidant defense, resulting in significant oxidative stress. Adequate foeto-placental circulation is indispensable for the proper development of the fetus and the increased production of NO in IUGR neonates does help improving it. However NO is still a problem for these fetuses, as it enhances oxidative stress in the umbilical cord. Nevertheless it may allow a good potential for therapeutic approaches. Adequate antioxidant therapy-after confirmation of the findings through intervention studies- may decrease the indices of oxidative stress arising from the increased NO production.

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