Oxidative stress in twins

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Submitted: 2013-06-21 Accepted: 2013-08-30 Published online: 2013-11-10

Key words: **total antioxidant status; glutathione peroxidase; superoxide dismutase; malondialdehyde; newborn; twins**

Neuroendocrinol Lett 2013; **34**(Suppl.2):71–73 **PMID:** 24362095 NEL341013A10

Abstract

OBJECTIVES: The aim of the study was to determine the value of the total antioxidant system (TAS) and level of malondialdehyde (MDA), and the activity of glutathione peroxidase, superoxide dismutase in the case of premature twins - identical twins, monozygotic, on the 1st and 5th day of life and to compare the values between the first-born twin A and the second-born twin B.
RESULTS: We confirmed the difference between A and B twins in values of TAS and MDA, as well as the difference between the 1st and 5th day of life.
CONCLUSION: The values of TAS, which show the total activity of antioxidant enzymes in a newborn's organism, reflect the ability of protection against oxidative stress before and during delivery. In the case of twin pregnancy, the value of TAS is crucial and determines the degree and severity of consequences of asphyxia. MDA values indicate the presence of lipoperoxidation.

Abbreviations:
GPX - glutathione peroxidase
Hb - haemoglobin
MDA - malondialdehyde
SOD - superoxide dismutase
TAS - total antioxidant status

INTRODUCTION

One of the main characteristics of twins is whether they are monozygotic or dizygotic and the number of placentas. When the placentas are separate, it does not mean that the twins are dizygotic. One third of monozygotic twins are dichorionic and diamniotic. A single placenta can be joined with monoovular or polyovular twins (Benirschke 2004; Elsayes *et al.* 2009).

The intrauterine development of twins can be affected mainly by intrauterine growth retardation, congenital anomalies and premature delivery. During delivery, a higher incidence of complications occurs in the second twin (twin B): respiratory distress syndrome due to prematurity and asphyxia (premature separation of the placenta, umbilical cord anomalies, abnormal presentation). Asphyxia is even nowadays an actual problem, particularly in newborns of multiple pregnancies.

Protection of the newborn against stress during the prenatal and perinatal period depends on the value of the total antioxidant status (TAS) (Brucknerová *et al.* 2004; Upadhyaya *et al.* 2005). In the case of twins, it depends on the characteristics of the twins, their birth weight, and on the course of delivery itself (Minghetti *et al.* 2011). The optimal capacity of the TAS is beneficial for the protection against asphyxia, a condition in which the newborn's organism is exposed for a certain time to insufficient oxygen supply. The activity and func-

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tion of individual organs of the human body depends on sufficient amount of energy (Brucknerová *et al.* 2005). Severe asphyxia may result in multi-organ dysfunction and even death (Brucknerová & Benedeková 2000).

Complications of the effect of asphyxia on the organism of the newborn cover a broad gamut. Affection of the central nervous system is considered to be the most serious. The degree and nature of the damage depend on the gestational age of the newborn and on its nutritional condition. They depend also on the time of onset of asphyxia and on its intensity (Brucknerová & Benedeková 2000).

The aim of the study was to determine the value of the total antioxidant system, and level of malondialdehyde (MDA), and the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPX) in the case of premature twins - identical twins, on the 1st and 5th day of life, to assess the dynamics of changes by comparing the values of twin A and twin B.

METHODS

Patients

The group of patients consisted of 12 premature newborns admitted in the course of 24 hours of their life. The patients were hospitalized at the 1st Department of Paediatrics, School of Medicine, Comenius University in Bratislava (Slovak Republic).

Biochemical analysis

In the whole group of newborns we took samples of venous blood on the 1st and 5th day of life. Blood samples were drawn into syringes containing heparin solution. The level of TAS was analyzed in the blood samples. The antioxidant status was measured by a Randox TAS kit (Randox Ltd., England) consisting partly of an artificial production of radicals in the biological material tested. The ability of the system to stop or slow down the radical reaction is assessed. The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS® (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]) to $ABTS^{\bullet+}$ by metmyoglobin and H_2O_2. This is of a relatively stable blue-green colour, which is measured spectrophotometrically at 600 nm. Antioxidants in the added sample cause suppression of this colour production to a degree which is proportional to their concentration. For the quantitative evaluation of lipoperoxidation we used the reaction of MDA with thiobarbituric acid in an acid solution with the formation of violet product, which was measured spectrophotometrically (532–535 nm). The activity of SOD was measured spectrophotometrically (505 nm) using RANSOD kit (RANDOX Ltd., England) and was calculated to amount of haemoglobin. The activity of GPX was measured using RANSEL kit provided by RANDOX Ltd. (England) the result was calculated to the amount of haemoglobin (Hb).

Statistical analysis

One-way analysis of variance followed by LSD *post-hoc* test was used, $p \leq 0.05$ was considered to be significant. The work is complied with the guidelines for human studies. The subjects have given their informed consent and the study protocol has been approved by the committee on human research.

RESULTS

The series consisted of 12 premature twins (twin A/B: gestational age 34.3±0.42 weeks; twins A: birth weight 2033.0±47.28 g, birth length 45.0±0.52 cm; twins B: birth weight 1787.3±102.20 g, birth length 43.3±1.26 cm).

In all 12 premature twins we investigated the value of TAS on the 1st and 5th day of life. The mean value of TAS was on the 1st day of life 1.25±0.14 mmol/l (n=12), on the 5th day 0.97±0.08 mmol/l (n=12). The value of TAS on the 1st day of life in the group of twins A (n=6) was 1.48±0.24 mmol/l, on the 5th day of life (n=6) it was 1.17±0.08 mmol/l. The value of TAS on the 1st day of life in the group of twins B (n=6) was 1.01±0.10 mmol/l, on the 5th day of life (n=6) it was 0.78±0.07 mmol/l. The values were significantly lowered in the group of twins B and statistical analysis revealed a significant decrease of the TAS level on the 5th day after delivery [$F(1,10)=13.92$; $p=0.0039$].

The value of MDA on the 1st day of life in the group of twins A (n=6) was 1.68±0.22 μmol/l, on the 5th day of life (n=6) it was 2.52±0.53 μmol/l. The value of MDA on the 1st day of life in the group of twins B (n=6) was 1.8±0.13 μmol/l, on the 5th day of life (n=6) it was 2.61±0.44 μmol/l. Statistical analysis revealed a significant difference in MDA levels of both groups between the 1st and 5th day of life [$F(1,10)=10.22$; $p=0.0095$].

The activity of SOD on the 1st day of life in the group of twins A (n=6) was 504.04±68.01 IU/gHb, on the 5th day of life (n=6) it was 526.64±74.58 IU/gHb. The activity of SOD on the 1st day of life in the group of twins B (n=6) was 538.87±56.51 IU/gHb, on the 5th day of life (n=6) it was 524.38±59.63 IU/gHb. Statistical analysis revealed a significant difference between the 1st and 5th day of life in the group of twins A [$F(1,9)=7.72$; $p=0.021$].

The activity of GPX on the 1st day of life in the group of twins A (n=6) was 33.78±4.96 IU/gHb, on the 5th day of life (n=6) it was 28.52±3.33 IU/gHb. The activity of GPX on the 1st day of life in the group of twins B (n=6) was 28.67±2.68 IU/gHb, on the 5th day of life (n=6) it was 27.45±5.57 IU/gHb. No significant differences were observed either between groups or days in activities of GPX.

DISCUSSION

Protection of the newborn´s organism against stress during the prenatal and perinatal period depends on the TAS. In the case of twins it depends on the char-

acteristics of the twins and on the course of delivery itself. The TAS presents antioxidative protection of the organism against the influence of free radicals and their reactive metabolites. TAS is one of the most important parameters which can help in characterizing the readiness for protection of the newborn organism against consequences of asphyxia (Brucknerová *et al.* 2006). In twins, we consider the establishment of TAS to be crucial since complications are associated with the number of fetuses and the "fluent" course of delivery can protect the newborn against perinatal asphyxia.

According to the results obtained, we confirmed the differences between the group of twins A (n=6) and twins B (n=6) on the 1st and 5th day of life. A statistically significant decrease of TAS was found only in the group of twins B on the 5th day of life. We assume that the reason for the decreased TAS levels in twins B was the „utilization" of antioxidants due to maternal problems and complications during delivery (premature separation of placenta, knot on the umbilical cord) as it was confirmed by values of MDA. The values of MDA on the 5th day of life were significantly higher in both groups. The obtained results of MDA on the 1st day of life in the group of twins A were more than 2 times higher in comparison with healthy term newborns (Brucknerová *et al.* 2006). Scientific literature lacks information about TAS levels and oxidative stress in monozygotic twins. We found only the work of the authors Minghetti *et al.* (2011) who evaluated the extent of oxidative stress in neonates born from multiple pregnancies. These authors observed only changes in TAS levels on the 1st day of life, positively correlated with both birth weight and gestational age and significantly higher in term twins compared to preterm and late preterm groups. However they did not observe differences in TAS level between co-twins as we did. The reason for such a finding could be the incorporation of both monochorial (26%) and dichorial (74%) pregnancies. Our results rely on monochorial pregnancies only and are therefore specific and valuable not only for research of monozygotic twins. In our group of patients, the mean values of TAS were surprisingly higher (2–3 times higher) in comparison with healthy term and asphyxial term newborns from single pregnancies (Brucknerová *et al.* 2006). We can only assume that it concerns a normal physiological protection in a specific situation, which twin pregnancy certainly is. The obtained biochemical values can be used as a knowledge base for therapeutic influence of TAS, as e.g. in animal experiment and in cardiovascular surgery (Holomáň *et al.* 1999; Ikeda *et al.* 1999; Holomáň & Pecháň 2002; Navarová *et al.* 2004).

The main function of SOD is in detoxification of superoxide anion. We did not confirm the statistical difference between the 1st and on the 5th day of life. The obtained activity of SOD in the group of twins A were lowered in comparison with healthy term newborns (Brucknerová *et al.* 2006). There were no statistical differences between the activities of GPX on the 1st and 5th day of life. However, our previous study (Brucknerová *et al.* 2006) revealed that activities of GPX in healthy term newborns are lower when compared to monozygotic twins. In our opinion these results represent demands of the twin newborn organism to change hydrogen dioxide to water and GPX is therefore upregulated by oxidative stress and acts as a defend mechanism.

CONCLUSION

Organism of the newborn presents a uniform system of interconnected enzymatic subsystems, but before delivery the foetus is in close connection with maternal organism through placenta and umbilical cord. The establishment of physiological values of the main biochemical parameters of oxidative stress, TAS, MDA, GPX and SOD, in premature twins is a starting point for the evaluation of a newborn´s status after asphyxia.

ACKNOWLEDGEMENT

This study was supported by the grant VEGA 2/0081/11.

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