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Stem cell source

Arylsulfatase-A in umbilical cord blood: gestational age and mode of delivery do not influence enzyme activity

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Summary:

The possibility of using umbilical cord blood for transplantation in several enzyme deficiencies has received increasing attention because of the availability of cord blood, the reduced incidence of post-transplantation complications, such as graft-versus-host disease and the possible accomplishment of good corrective results following transplantation, even in cases of greater HLA disparity. The use of hematopoietic stem cells from unrelated donors is even more highly recommended for the treatment of inherited enzyme deficiencies, because it might reduce the risk of the transplanted cells originating from a carrier of the defect, which might have an inadequate corrective ability. Our study was designed to elucidate whether the gestational age and mode of delivery influences the arylsulfatase-A activity in the umbilical cord blood. Enzyme activities proved to be similar in the four populations studied (full-term normal spontaneous vaginal delivery, full-term caesarean section, preterm normal spontaneous vaginal delivery and preterm caesarean section). Therefore, umbilical cord blood samples seem to be suitable for transplantation in metachromatic leukodystrophy, regardless of gestational age and mode of delivery. Moreover, our results are the first published data on normal values for arylsulfatase-A activity in human umbilical cord blood.

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Human lysosomal arylsulfatase-A (ASA) is a member of the highly conserved sulfatase gene family. It is synthetized as a 507 amino acid precursor and is processed in the endoplasmic reticulum to yield a mature 489 amino acid protein. Each sulfatase is characterized by high substrate specificity. Of 10 of the different human sulfatases which are known today, six are located in the lysosomes where they are responsible for the degradation of glycosaminoglycans and sulfolipids. Besides their physiological substrates arylsulfatases also degrade synthetic chromogens and fluorogens.[1] ASA's major natural substrate is cerebroside 3-sulfate which will accumulate if there is a deficiency in ASA, resulting in a lysosomal storage disorder, known as metachromatic leukodystrophy.[2]

Recently, umbilical cord blood has received increasing attention as a source of unrelated hematopoietic stem cells for transplantation. Engraftment using umbilical cord blood has proven effectiveness in treating lysosomal diseases, as indicated by normalization of the defective enzymatic activity in such important clinical entities like globoid cell leukodystrophy, metachromatic leukodystrophy, mannosidosis, fucosidosis, aspartylglucosaminuria, Hurler, Maroteaux-Lamy and Sly syndromes and Gaucher disease type III.[3] The use of umbilical cord blood has several advantages over the well-known bone marrow transplantation from unrelated donors, such as the ready availability of donor cells and the lower incidence of graft-versus-host disease. The latter provides the opportunity to use umbilical cord blood even if there is a greater HLA disparity.[4–6]

Pulkkinen *et al*[7] were the first to determine normal values for ASA and some steroid-sulfatases during intrauterine development. Previous results concerning the activity of α-glucosidase, mannosidase, fucosidase and ASA in chorionic villi did not show any correlation of enzyme activity with gestational age, except for α-glucosidase.[8] Recent clinical data indicate that an incomplete reconstitution of the enzyme activity following the engraftment of bone marrow cells from related donors who may carry the genetic defect (heterozygotes) results in a lesser improvement of the central nervous system status for diseases so treated, probably due to the inadequate corrective ability of the donor cells.[9] Therefore, the correct choice of donor is of particular importance. The use of umbilical cord blood for treatment of enzymatic defects seems to be a reliable alternative to bone marrow transplantation. However, our present knowledge regarding the levels of activity of lysosomal enzymes in cord blood is related to only one pioneering study showing that α-L-iduronidase, galactocerebrosidase and ASA

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levels in cord blood do not differ from adult levels.[10] Since no previous data exist regarding the levels of activity of ASA in umbilical cord blood related to gestational age and mode of delivery, we do not know if any cord blood sample, regardless of the gestational age and mode of delivery are equally effective in treating metachromatic leukodystrophy by transplantation. Therefore, the decision was made to investigate ASA activity in umbilical cord blood samples from preterm and full-term newborns, born by vaginal delivery and caesarean section.

Materials and methods

Patients

The study was previously approved by the Human Investigation Review Board, and blood samples were collected after informed consent had been obtained. According to the mode of delivery and gestational age four study groups were established: full-term normal spontaneous vaginal delivery (FT-NSVD, $n = 38$), full-term caesarean section (FT-CS, $n = 22$), preterm normal spontaneous vaginal delivery (PT-NSVD, $n = 26$) and preterm caesarean section (PT-CS, $n = 21$). In both the full-term and preterm category only elective caesarean section cases have been included. In each case venous blood was obtained at the time of delivery from the cord vein.

Both full-term and preterm healthy neonates irrespective of the mode of delivery were appropriate for gestational age, born to healthy mothers with negative medical and obstetrical history having a 5-min Apgar score $\geqslant 7$, at 38–42 and 34–37 weeks of gestation, respectively (mean gestational age $\pm$ s.d. was 39.1 $\pm$ 1.1 weeks for FT-NSVD, 39.3 $\pm$ 1.3 weeks for FT-CS, 35.5 $\pm$ 1.8 weeks for PT-NSVD and 35.2 $\pm$ 1.6 weeks for PT-CS). The entire patient population included in this study had negative anamnestic history in siblings, parents and grandparents for any inherited metabolic diseases.

Determination of ASA activity in cord blood samples

ASA activity was measured in leukocyte homogenates prepared from cord blood samples obtained at the time of delivery. Briefly, umbilical cord blood (10 ml) transported to the laboratory within 1 h from the time obtained, was subjected to Ficoll–Hypaque gradient centrifugation (Pharmacia, Piscataway, NJ, USA). After washing, the cell count was adjusted to 30×10^6 cells/ml in physiologic saline solution. The obtained cell suspension was then subjected to five freezing–thawing cycles in order to lyse the cells. The cellular debries were removed from the lysed leukocyte suspension by centrifugation at 8000 *g* for 10 min. The precleared leukocyte homogenate was further used for the determination of protein content by the method of Lowry *et al*[11] and for the direct measurement of ASA activity.

ASA was assayed by the method of Singh *et al*[12] All the reagents used in the assay were purchased from Sigma (Budapest, Hungary). The assay was performed using 20 mM nitrocatechol sulfate as substrate in 0.2 ml M-sodium acetate buffer (pH 4.9), also containing 0.5 M $Na_4P_2O_7$ and 1.7 M NaCl. To this, 200 μl of leukocyte homogenate was added and the mixture was incubated at 37°C for 4 h. The reaction was terminated by the addition of 100 μl 2.5 M NaOH and 100 μl 0.15 M EDTA. Liberated nitrocatechol was measured at 515 nm with nitrocatechol (20 μM) as standard, and ASA activity was expressed as nmoles nitrocatechol/h/mg protein. In the control samples leukocyte homogenate and substrate were incubated separately and mixed immediately prior to the addition of NaOH and EDTA.

Statistical analysis

Statistical analysis of the data was made by ANOVA. For significant ANOVA values groups were compared by Tukey's test for multiple comparisons with unequal sample size. A probability level of 0.05 was accepted as indicating significant differences.

Results

The mean values of ASA activity in cord blood leukocytes are indicated in Figure 1. No significant difference in ASA activity was detected in preterm *vs* full-term newborns (61.6 $\pm$ 41.4 for preterms and 67.6 $\pm$ 40.4 for full-terms; mean $\pm$ 1 s.d.) (Figure 1a). In FT-NSVD newborns the enzyme activity was 61.3 $\pm$ 35.2 (range 12.1–143). The mean ASA activity was slightly elevated in both the FT-CS and PT-CS category, without reaching statistical significance (87.5 $\pm$ 50.3, range 38–193 for FT-CS and 81.8 $\pm$ 39.6, range 43–159 for PT-CS). In contrast, PT-NSVD infants had a lower ASA activity (31.4 $\pm$ 22.2 range 9.6–68), which was not significantly different from the previous categories either, as shown by ANOVA (Figure 1b). The percentage of values below 30, which were considered as being indicative of a possible pseudo-deficiency was 18.4% for FT-NSVD, 0% for FT-CS, 15.4% for PT-NSVD and 4.7% for PT-CS, respectively. The frequency of possible pseudo-deficiency on the basis of enzyme activity level below 30 for the full-term population was 11.7% as well as for preterm newborns 10.6% (11.2% for the entire population).

Discussion

This is the first study determining the values of ASA in four different newborn categories. Prior to this study it has been shown that ASA activity of umbilical cord blood is equivalent to the activity of normal adults.[10] Metachromatic leukodystrophy is an important pediatric disease which is inherited in an autosomal recessive manner. Its incidence is particularly high in the North American and Eastern European population and represents an important burden for both the patient's parents and the health care providers. Until the advent of the very promising new modality of treatment which is represented by the transplantation of bone marrow-derived cells thought to be able to overcome the genetic defect, the traditional cure for this disease had

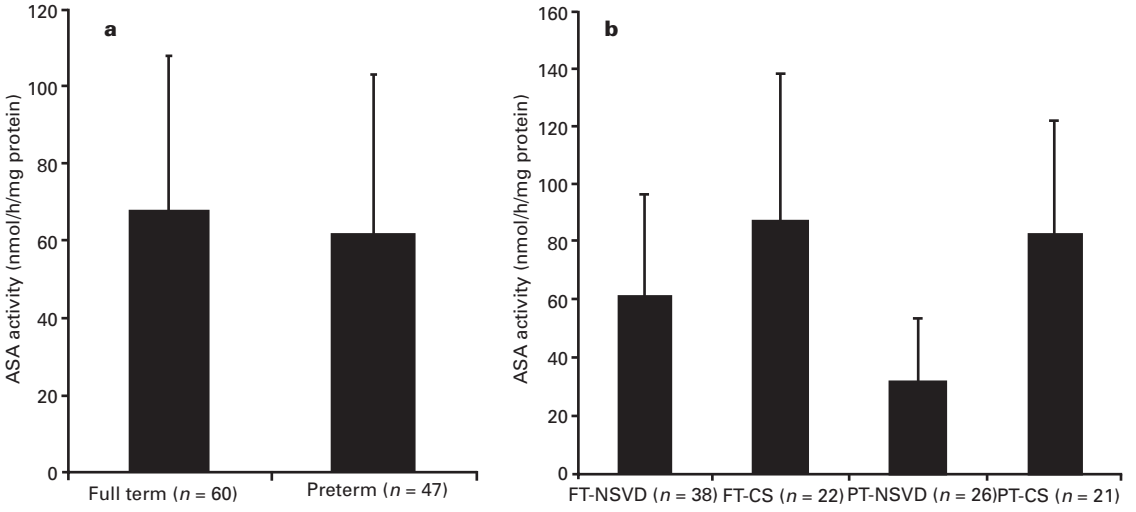

Figure 1 Arylsulfatase A (ASA) activity in human umbilical cord blood leukocytes. The bars represent the mean ± 1 s.d. values. (**a**) Comparison between average enzyme activity for full-term and preterm healthy neonates. (**b**) Comparison between average enzyme activities in umbilical cord blood samples obtained from full-term normal spontaneous vaginal delivery (FT-NSVD), full-term caesarean section (FT-CS), preterm normal spontaneous vaginal delivery (PT-NSVD) and preterm caesarean section (PT-CS) neonates (*n*, number of samples).

very poor results. However, even this new treatment modality was overshadowed by difficulties because of availability of suitable donors, which is significantly restricted due to HLA disparity. While in other cases the best donor is a close relative of the patient, in the case of genetic disorders such as metachromatic leukodystrophy the use of cells obtained from related donors is not recommended because even if the donor is clinically healthy it cannot be ruled out that the related donor is a heterozygote carrier of the genetic defect. This incovenience became evident after incomplete restoration of the enzyme activity resulting in a lessened improvement of the central nervous system status in patients so treated was reported.[9] Therefore, one possible alternative to bone marrow transplantation would be the engraftment of umbilical cord blood. In favor of this treatment modality is the fact that umbilical cord blood is one of the most readily available sources of hematopoietic stem cells. Moreover, after umbilical cord blood transplantation, even in the case of a greater HLA disparity, the incidence of graft-versus-host disease is lower. Thus, the only limitation to the use of umbilical cord blood for the treatment of metachromatic leukodystrophy seems to be related to the possibility that the newborn from whom the cord blood had been obtained might be a carrier of the genetic defect as well. Another possible limitation to qualify as a donor is the high incidence of pseudodeficiency of the enzyme in the general population, which could be as high as 10–15%.[13] The very recent data reported by de Gasperi and co-workers[10] concerning the ASA activity in umbilical cord blood refer to a random neonate population, which certainly increases the value of the reported data as reference values for the enzyme activity. However, our knowledge about the ASA activity in different newborn categories remains insufficient. The variation of enzyme activities with gestational age is not an uncommon phenomenon, raising the possibility that ASA activity could be lower in prematures than in full-term newborns. In today's HMO-oriented cost-effective health care system the use of easy to obtain donor cells with maximal therapeutic potential and with minimal costs is highly recommended. Therefore, the study of the ASA activity in different newborn categories in order to verify the potential corrective ability of the cells to be engrafted and to establish the reference values of ASA activiy as a function of gestational age and mode of delivery is of paramount importance. Our data clearly demonstrate that the activity of ASA is not different in any of the categories studied. Moreover, our data are comparable to those reported by de Gasperi *et al*.[10] As a result, every normal newborn's umbilical cord blood could qualify equally as an effective donor cell source for the treatment of metachromatic leukodystrophy. However, before engrafting umbilical cord cells, the determination of the enzyme activity by a qualified laboratory in order to exclude any possible pseudodeficiencies is recommended.

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