Pediatr Pol 2018; 93 (3): 260–263
DOI: https://doi.org/10.5114/polp.2018.77440
Submitted: 16.04.2018, Accepted: 10.06.2018, Published: 20.07.2018

PEDIATRIA Polska
PISMO POLSKIEGO TOWARZYSTWA PEDIATRYCZNEGO
Polish Journal of PAEDIATRICS
OFFICIAL JOURNAL OF POLISH SOCIETY OF PAEDIATRICS

REVIEW PAPER

Stem cells in breast milk

Natalia Kuciel[1], Justyna Mazurek[1], Matylda Czosnykowska-Łukacka[2], Barbara Królak-Olejnik[2]

[1]Department and Division of Medical Rehabilitation, Wroclaw Medical University, Wroclaw, Poland
[2]Department and Clinic of Neonatology, Wroclaw Medical University, Wroclaw, Poland

ABSTRACT

Stem cells represent 10-15% of all cells that appear in breast milk. They participate in the mechanisms that control the homeostasis and processes self-healing of tissues in neonates. The first reports on the presence of stem cells in breast milk appeared in 2007. Stem cells exceed the barrier in the intestine, migrate to, and integrate with all body tissues and organs. The presence of the nestin marker may indicate that the stem cells in breast milk influence brain development. Populations of stem cells are extremely plastic and are able to develop into cells of all three embryonic layers (neurons, hepatocytes, pancreatic cells, osteoblasts, chondrocytes, heart cells, and adipocytes). Stem cells from breast milk may be used in regenerative medicine due to the huge potential of tissues from all three embryonic leaves. Stem cells from breast milk do not form tumours after implantation into animal tissues; moreover, their collection and transportation are non-invasive, safe, and ethical.

KEY WORDS:
stem cells, breast feeding, infant.

INTRODUCTION

The importance of breastfeeding from the very first moments after the birth of a baby has been emphasised for years by specialists in various fields of medicine. According to the Universal Declaration of Human Rights of 1948, the mother and child are of particular concern and attention. All children, including sick and preterm newborns, should have the same opportunities to achieve optimal health and development [1]. Care and awareness of the young mother's support is crucial for the success of lactation, which in the first weeks of childbirth may be associated with many difficulties and discourage the continuation of this way of the child's nutrition. Therefore, it is very important to make the parents aware of the importance of each millilitre of maternal milk for the health of both mother and child. The World Health Organisation (WHO) and UNICEF have developed a project based on Ten Steps to Successful Breastfeeding with the aim of harmonising practices related to the promotion of breastfeeding, including the education of health professionals, the education and support of patients, and emphasis on exclusive breastfeeding without supplementing with formula (formula only when medically justifiable) [2, 3]. If the mother is unable to breastfeed, especially the mother of premature babies, she can use a human milk bank, a method that is becoming more and more popular in Poland, Europe, and all around the world. Access to natural food is of paramount importance in decreasing neonatal mortality and incidence of necrotising enterocolitis (NEC) [4, 5].

The recommendations about breastfeeding of the WHO have remained unchanged for many years. It is recommended that breastfeed be continued exclusively until the end of the sixth month of life and then to continue breastfeeding for two years or longer [6]. Although both the government, the Ministry of Health, non-governmental organisations, numerous associations, support

ADDRESS FOR CORRESPONDENCE:
Natalia Kuciel, Department and Division of Medical Rehabilitation, Wroclaw Medical University, 213 Borowska St., 50-556 Wroclaw, Poland

groups, and hospital staff and lactation consultants are involved in promoting breastfeeding, the indicators for breastfeeding are not optimistic: in Poland only 46% of women are breastfeeding 6 weeks after delivery, 42% 2-6 months after delivery, and 11.9% 12 months. Only 50% of infants immediately after delivery are exclusively breastfed, and in the case of preterm newborns only about 60% are breastfed when leaving the hospital with I and II reference levels and even fewer (30%) on leaving the hospital with III reference level [7].

It is now known that breast milk is not only a source of food and bioactive compounds for the child [8]. To a large extent, breast milk determines the potential and activity of human metabolic processes [9]. It plays an important role in infection prevention and protection against microorganisms, and influences growth, development, metabolism, and colonisation of microflora in children's intestines [10]. Natural feeding includes prevention of infection and allergy in the child through cells and proteins present in breast milk, such as: antibodies (IgA, IgG), lymphocytes, monocytes, macrophages, lactalbumins, casein, lysozyme, lactoferrin, and many others [11-14]. Other bioactive components of milk are hormones (leptin, adiponectin, ghrelin, resistin) that support the proper functioning of central appetite control mechanisms, which not only affects feeding at a given time, but also protects against obesity and type I and type II diabetes in the future [15]. The benefit of natural feeding is also a lower incidence in the child or a milder course of such infections as respiratory tract, middle ear infection, sepsis, necrotizing enterocolitis (NEC), or bacterial meningitis [16, 17].

In addition to the elements involved in building the child's immunity, there are also other cells in milk that are important for the creation of the child's tissues and organs: epithelial cells (50-90%), stem cells, fatty acids, monoglycerides, nucleic acids, and antioxidants [10, 11]. The cells can migrate from mother to child in two ways: during breastfeeding and by transplacental passage in pregnancy [18]. During intrauterine development, a number of cells migrate from mother to child, and the child's cells together with the multipotential cells migrate towards the mother and integrate with her tissues, such as blood, bones, joints, liver, heart, brain (foetal-maternal microchimerism) [11, 19].

STEM CELLS IN BREAST MILK

Stem cells represent 10-15% of all cells that appear in breast milk. They participate in mechanisms controlling homeostasis and self-healing processes of the organism [20]. The first reports on the presence of stem cells in breast milk appeared in 2007. Cregan *et al.* from the Australian Breastfeeding Association reported that mammary gland stem cells are present in breast milk [21]. CK5 cytokeratin marker, mature epithelial cell markers CK14, CK18, CK19, and nestin marker were used. CK5 (cytokeratin 5) is a marker of stem and progenitor cells in mammary gland [22]. CK14 and CK18 are markers of specific antigens found on surfaces of two different types of epithelial cells (myoepithelial and ductal cells) [9]. CK 19 is stained luminal epithelial cells [23]. Nestin is a well-known marker of multipotent stem cells, being a marker of bone and nervous system cells, pancreatic cells, and epithelial tissue. This protein appears during development of the central nervous system [24]. It was noted that because stem cells were previously identified and isolated from a non-lactating gland, they should therefore be present in breast milk [21].

Kaingade *et al.* noted the difference in quality between colostrum milk and mature milk among women who had given birth to a premature baby. In the breast milk of the mothers of premature infants, haematopoietic stem cells, mesenchymal stem cells, and immune cells have been detected. Such a composition may have a significant impact on the proper development of premature babies [25]. Interesting conclusions were reached by Brie *et al.*, who compared the phenotype and genotype of milk of women giving birth prematurely with milk of women who have a neonate born at term. Researchers found that: 1) stem cells were present in premature infant mother's milk; 2) the samples of breast milk from premature infants' mothers and neonates differed in expression of stem cell-specific markers; and 3) the percentage of expression of stem cell-specific markers differed when comparing samples of breast milk from premature infants and reported infants [26].

WHEN AND WHERE STEM CELLS ARE FORMED?

Maturation of the mammary gland is regulated by hormones: oestrogen, progesterone, and prolactin. The lactating breast consists of massive lobular epithelial tissue separated by fibrous stroma [27]. The stem cells in breast milk are mainly mesenchymal embryonic stem cells and luminal epithelial cells. The stem cells in the mammary gland are organised in the superficial layers of the secretionary ducts and occur already in the embryonic blastocyst stage [9]. Their organisation changes with the different phases of the nipple's development. During pregnancy the mammary gland proliferates and becomes a secretory organ [19]. The environment in the nipple, where stem cells are formed, changes during pregnancy, which affects progenitor cells. Its embryonic origin is the same as that of nervous system tissues [28]. The mammary gland is metabolically active not only during pregnancy and lactation [20]. After the lactating period, both the alveoli and ducts undergo degeneration in the process of massive apoptosis, which is stimulated by hormones [19]. Therefore, the extracellular matrix cooperating with steroid hormones influences the fate of the nipple cells, which are the precursors of stem cells [9]. In addition, Twigger *et al.* [29] noted that the nipple of mothers of

children born in time is more mature than that of mothers of premature infants, and the level of nestin marker in prematurely born mothers was lower.

STEM CELL POTENTIAL

After the first scientific reports on the presence of stem cells in breast milk, the research was focused on the penetration of cells into the child's blood and on their differentiation. The stem cells cross the intestinal barrier and migrate with blood to all tissues of the body and internal organs. Abd Allah *et al.* [13] isolated the stem cells of nursing rabbits. Using a fluorescence microscope, the presence of stem cells in samples of tissues prepared from various organs of young rabbits was proven: liver, muscle, heart, and duodenum. Previously, Hassiotou *et al.* [30] studied the distribution of stem cells in the body of the fed mouse. At that time a genetically modified mouse was created with gene coding cells that shone red in fluorescent light. Red cells penetrated with breast milk into the baby and were observed in tissues such as blood, brain, thymus, pancreas, liver, spleen, and kidneys. Hassiotou *et al.* [30] demonstrated that in adult mice, mother's milk stem cells were still circulating three weeks after the birth and were identified in the abdominal, thymus, and liver walls of the young fed mice. On the basis of these reports it can be concluded that stem cells survive in the child's digestive system, penetrate into the bloodstream, just like leukocytes [31], and then integrate with the organs of the child. The presence of the nestin marker may also indicate that breast milk stem cells exceed the brain-blood barrier, which confirms the thesis that mother's milk has a significant impact on brain development [8]. Stem cell populations are extremely plastic and can be transformed into cells of all three germ layers: ectoderms, endoderms, and mesoderms (neurons, hepatocytes, pancreatic cells, hearts, osteoblasts, chondrocytes, adipocytes) [30]. These cells may also differentiate into hormones or proteins (including insulin, albumin). Two types of stem cells have been proven: 1) embryonic stem cells that have an extraordinary ability to differentiate, and 2) mature stem cells whose task is to produce new stem cells [32]. It is also known that stem cells in breast milk have the potential to differentiate into two main lines of epithelial cells (myoepithelial and luminal cells) [31].

THE USE OF BREAST MILK STEM CELLS

Breast milk stem cells can be used in regenerative medicine due to their huge potential for tissue formation from all three germ layers [32]. Unlike embryonic stem cells, multipotent cells from breast milk do not form tumours after implantation into animal tissues. Importantly, they are obtained and transported in a non-invasive, safe, and ethical manner [13, 16, 30]. The discovery of stem cells in breast milk is an extremely important, even revolutionary event, both in the field of interest in regenerative medicine and the promotion of breastfeeding, because it is extremely important in the context of conveying information about breastfeeding to future mothers. Stem cells may be used as a tool to treat some of the infant diseases [33]. However, after the first research, further questions arise that challenge researchers in order to fully understand the phenomenon of these cells and their role and potential for differentiation. Questions remain whether stem cells change during lactation, whether the caesarean section affects their development and differentiation, and whether they affect the health of pregnant woman. It is important to define their role for the health potential of children, but also of adults, and for the prevention of chronic and autoimmune diseases [9]. It is now known that the milk of the mothers who give birth on time contains stem cells, and their expression varies between women. The subject will therefore be a great challenge for researchers in the coming years.

CONCLUSIONS

Breast milk, known as a biologically active, living tissue, should be a natural, optimal method of feeding infants. It contains thousands of active components, which have a non-food value and cannot be produced by technological processes. The large number of breast milk ingredients and their multifunctionality are the subject of intensive research. There is also no doubt that breast milk, especially for premature babies, is treated as a medicine. Since breast milk is a rich, easily accessible, and non-invasive reservoir of pluripotent stem cells, it is becoming an excellent candidate for use in cell therapy and regenerative medicine. More research and data based on the Evidence Based Medicine principles are likely to expand the use of breast milk, especially in the care and prevention of infants.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Arnold LD. Global health policies that support the use of banked donor human milk: a human rights issue. Int Breastfeed J 2006; 1: 26.
2. www.who.int/nutrition/bfhi/ten-steps/en/
3. http://www.gov.pl/zdrowie/10-krokow-do-udanego-karmienia-piersia
4. Meier P, Patel A, Esquerra-Zwiers A. Donor Human Milk Update: Evidence, Mechanisms and Priorities for Research and Practice. J Pediatr 2017; 18: 15-21.
5. Adhisivam B, Vishnu-Bhat B, Banupriya N, et al. Impact of human milk banking on neonatal mortality, necrotizing enterocolitis, and exclusive breastfeeding - experience from a tertiary care teaching hospital, south India. J Matern Fetal Neonatal Med 2017; 1: 1-4.

6. http://www.who.int/maternal_child_adolescent/topics/newborn/nutrition/breastfeeding/en/
7. Karmienie Piersią w Polsce, Raport 2015.
8. Faa G, Fanos V, Puddu M, et al. Breast milk stem cells: four questions looking for an answer. J Ped Neonat Ind Med 2016; 5: e050203.
9. Reali A, Puddu M, Pintus M, et al. Multipotent stem cells of mother's milk. J Pediatr Neonat Individual Med 2016; 5: e050103.
10. Riskin A, Almog M, Peri R, et al. Changes in immunomodulatory constituents of human milk in response to active infection in the nursing infants. Pediatr Res 2012; 71: 220-225.
11. Sharp JA, Leferve Ch, Watt A. Analysis of human breast milk cells: gene expression profiles during pregnancy, lactation, involution and mastitic infection. Funct Integr Gen 2016; 16: 297-321.
12. Laskowska J, Książyk J. Aktualne wytyczne dotyczące karmienia piersią. Pediatr Med Rodz 2011; 7: 110-114.
13. Abd Allah SH, Shalaby SM, El-Shal AS, et al. Breast Milk MSCs: An Explanation of Tissue Growth and MAturation of Offspring. JUBMB Life 2016; 68: 935-942.
14. Witkowska-Zimny M, Ewa Kaminska-El-Hassan. Cells of human breast milk. Cell Mol Biol Lett 2017; 22: 11.
15. Savino F, Liguori S, Fissore M, et al. Breast milk hormones and their protective effect on obesity. Int J Ped Endocrinol 2009; 2009: 327505.
16. Hassiotou F, Hartmann P. At the Dawn of a New Discovery: The Potential of Breast Milk Stem Cells. Adv Nutr 2014; 5: 770-778.
17. Szajewska H, Horwath A, Rybak A, et al. Karmienie piersią. Stanowisko Polskiego Towarzystwa Gastroenterologii, Hepatologii i Żywienia Dzieci. Standardy Medyczne/Pediatria 2016; 13: 9-24.
18. Zhan L, Yoshimura Y, Huang Y, et al. Two independent pathways of maternal cell transmission to offspring: through placenta during pregnancy and by breast-feeding after birth. Immunology 2000; 101: 570-580.
19. Sani M, Hosseini SM, Salmannejad M. Origins of the breast milk-derived cells; an endeavor to find the cel sources. Cell Biol Int 2015; 39: 611-618.
20. Pichiri G, Lanzano D, Pivon M, et al. Human breast milk stem cells: a new challenge for perinatologists. J Pediatr Neonat Individual Med 2016; 5: e050120.
21. Cregan MD, Fan Y, Appelbee A, et al. Identification of nestin-positive putative mammary stem cells in human breastmilk. Cell Tissue Res 2007; 329: 129-136.
22. Corr BR, Finlay-Schultz J, Rosen RB, et al. Cytokeratin 5-positive cells represent a therapy resistant subpopulation in epithelial ovarian cancer. Int J Gynecol Cancer 2005; 25: 1565-1573.
23. Clarke R, Spence K, Anderson E, et al. A putative human breast stem cells population is enriched for steroid receptor positive cells. Dev Biol 2005; 277: 443-456.
24. Suzuki S, Namiki J, Shibata S, et al. The neural stem/progenitor cel marke nestin is expressed in proliferative endothelial cells, but not in mature vasculature. J Histochem Cytochem 2010; 58: 721-720.
25. Kaingade P, Somasundaram I, Sharma A, et al. Cellular Components, Including Stem-Like Cells, of Preterm Mother's Mature Milk as Compared with Those in Her Colostrum: A Pilot Study. Breastfeed Med 2017; 12: 446-449.
26. Briere CE, Jensen T, McGrath JM, et al. Stem-Like Cell Characteristics from Breast Milk of Mothers with Preterm Infants as Compared to Mothers with Term Infants. Breastfeed Med 2017; 12: 174-179.
27. Hassiotou F, Geddes D. Anatomy of the human mammary gland: current status of knowledge. Clin Anat 2013; 26: 29-48.
28. Hosseini S, Talaei-Khozani T, Sani M. Differentiation of Human Breast Milk Stem Cells to Neural Stem Cells and Neurons. Neurol Res Int 2014; 2014: 807896.
29. Twigger AJ, Hepworth AR, Lai CT, et al. Gene expression in breastmilk cells is associated with maternal and infant characteristics. Sci Rep 2015; 5: 12933.
30. Hassiotou F, Beltran A, Stuebe A, et al. Breastmilk Is a Novel Source of Stem Cells with Multilineage Differentiation Potential. Stem Cells 2012; 30: 2164-2174.
31. Hassiotou F, Hepworth AR, Metzger P, et al. Maternal and infant infections stimulate a rapid leukocyte response in breastmilk. Clin Transl Immunol. 2013; 2: e3.
32. Briere CE, Jensen T, Matsen A, et al. Breast Milk Stem Cells: Current Science and Implications for Preterm Infants. Adv Neonat Care 2016; 16: 410-419.
33. Bode L, McGuire M, Rodriguez JM, et al. It's alive: microbes and cells in human milk and their potential benefits to mother and infant. Adv Nutr 2014; 5: 571-573.