Differences in direct pharmacological effects and antioxidative properties of mature breast milk and an infant formula

S.D. Spasić[1], N. Lugonja[1], O. Laugier[1], Z. Oreščanin-Dušić[1], A. Nikolić-Kokić[2], I. Spasojević[3], M.M.Vrvić[4]

[1]Department of Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, Belgrade, Serbia
[2]Department of Physiology, Institute for Biological Research, University of Belgrade, Bulevar Despota Stefana 142, Belgrade, Serbia
[3]Institute for Multidisciplinary Research, University of Belgrade
[4]Faculty of Chemistry, University of Belgrade, Studentski trg 16, Belgrade, Serbia University of Belgrade

Summary

Early onset and exclusive breastfeeding provides a significant health benefit to infants in comparison to infant formulas. There is groving evidence that presence of some specific protein components in mothers milk are responsible for observed significant health benefit in infants feed with mothers milk. The aim of this paper was to compare mature breast milk and a standard infant formula by examining their effects on smooth muscle contraction and their antioxidative properties. Electron paramagnetic resonance (EPR) spin-trapping spectroscopy was used to compare the antioxidative capacities of breast milk (obtained on the 9th week of lactation) with a commercial infant formula against hydroxyl radical production in the Fenton reaction. The activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and their vitamin C and sulfhydryl group (–SH) contents were determined in the milks. Pharmacological research was performed on the isolated rat uterus. In contrast to the infant formula, breast milk exerted a relaxing effect on isolated non-vascular smooth muscle. Using EPR and the Fenton reaction as a radical-generating system, we showed that breast milk possesses a three-fold higher antioxidative activity against the hydroxyl radical compared to the infant formula. In both samples, generation of the hydroxyl radical (•OH) led to the production of carbon-centered radicals. The ascorbyl radical was detected in breast milk but not in the infant formula. Human milk has direct pharmacological effects and provides better antioxidant protection than the infant formula due to the presence of specific protein components such as human SOD.

Introduction

Positive correlation between breastfeeding and the reduction of certain health risks is well established. The general recommendation is to breastfeed newborns for 6 months exclusively, and then to introduce complementary foods and continue breastfeeding. Namely, breastfeeding is associated with the reduction of the risk for acute otitis media, non-specific gastroenteritis, severe lower respiratory tract infections, atopic dermatitis, asthma (in toddlers), obesity, type 1 and 2 diabetes, childhood leukaemia, sudden infant death syndrome (SIDS), and necrotizing enterocolitis. There is no correlation between breastfeeding and cognitive performance in term infants, while the relationship between breastfeeding and cardiovascular diseases is still not clear. The presence of specific protein components in human breast milk may be responsible for significant health benefits observed in breastfed infants. A "shotgun" proteomics study examined the differences in the on-host defence-related proteins of human and bovine milk identifying 268 and 269 proteins, respectively. Significant quantitative differences between the human and bovine milk were observed for 33 proteins. Human breast milk contains enzymes with antioxidative activities. Catabolic Cu, Zn superoxide dismutase (CuZnSOD) and mitochondrial MnSOD are present in human milk, both in the colostrum and in the mature milk. It is important to note that the total concentration of SOD in human milk is 5 times lower than in human plasma, but 2-2.3 times higher in comparison to bovine milk. Significant changes in SOD activity at different phases of human lactation were observed, being highest during the third week of lactation. Human breast milk is rich in other antioxidative enzymes, such as glutathione peroxidase (GSH-Px).

In this study we compared the direct pharmacological effects of breast milk and infant formulas on non-vascular smooth muscles. The isolated uterus is very suitable for this type of study because of the presence of complex signal transduction systems, high levels of membrane receptors and the redox sensitivity of uterine smooth muscles. We also measured the activities of key antioxidant enzymes (SOD and GSH-Px) and sulfhydryl groups (-SH) and performed an electron paramagnetic resonance (EPR) spin-trapping study to test the capabilities of breast milk and infant formulas to suppress free radical production in the Fenton system.

Methods

Pharmacological research was performed on the isolated rat uterus obtained from virgin female Wistar rats (200-250g; 3 months of age). All protocols for handling experimental animals were approved by the local ethics committee for animal experimentation which strictly follows international regulations. The rats were kept at 22 ºC, housed three per cage and fed *ad libitum*. Rat uteri were isolated during the oestrous phase of the oestrous cycle. The oestrous phase was determined by daily vaginal ravage. All rats were killed by cervical dislocation. The uterine horns were rapidly excised and carefully cleaned of surrounding connective tissue and mounted vertically in a 10 ml volume organ bath containing De Jalon's solution (NaCl 154 mM, KCl 5.6 mM, $CaCl_2$ x $2H_2O$ 0.41 mM, $NaHCO_3$ 5.9 mM and glucose 2.8 mM), under 1 g tension. The bath was maintained at 37 °C and aerated with 95% oxygen and 5% carbon dioxide. After an equilibration period (about 30 min) when the uteri established stable calcium ion-induced contractions, single doses of either breast milk or the infant formula (500µl) were applied. Myometrial tension was recorded isometrically with a TSZ-04-E isolated organ bath transducer (Experimetria, Budapest; Hungary).Results were tested by one-way ANOVA and post hoc compared by Tukey's HSD t-test.

Results

Statistically significant increased total SOD was observed in the breast milk in comparison to infant formula. The content of -SH groups was statistically significant decreased in infant formula in comparison to the breast milk. In contrast to the infant formula, breast milk exerted a relaxing effect on isolated non-vascular smooth muscle (Table 1). Using EPR and the Fenton reaction as a radical-generating system, we showed that breast milk possesses a three-fold higher antioxidative activity against the hydroxyl radical compared to the infant formula. In both samples, generation of the hydroxyl radical (•OH) led to the production of carbon-centered radicals. The ascorbyl radical was detected in breast milk but not in the infant formula (Figure 2). Human milk has direct pharmacological effects and provides better antioxidant protection than the infant formula due to the presence of specific protein components such as human SOD (Figure1).

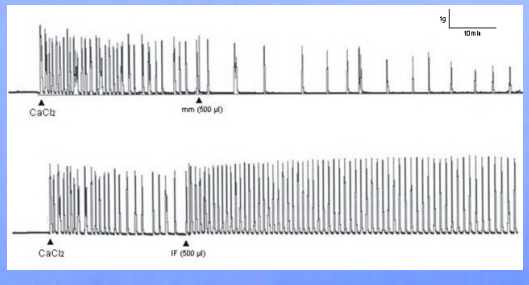

Figure 1. The effect of ions contained in breast milk (500 µL) and the examined infant formula (500 µL) on Ca^{2+}-induced uterine contractions. Results obtained from five separate experiments are presented. As shown in Table 1, breast milk had significantly higher activities of SOD and GSH-Px, and contained higher concentrations of free thiol groups than the infant formula.

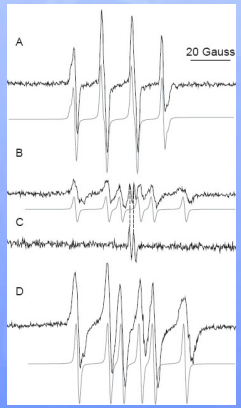

Figure 2. Panel A: Fenton reaction in PBS (pH 7.4), Fe^{2+} (0.2 mM) + H_2O_2 (1 mM). Gray – spectral simulation of the BMPO adduct with a •OH radical (BMPO/OH). Panel B: The Fenton reaction in breast milk. Gray – spectral simulation of the BMPO adduct with the carbon-centered radical (BMPO/C). Panel C: The Fenton reaction in breast milk without a spin trap. Characteristic profiles of the ascorbyl radical are present (connected with the signal of Asc radical in Panel B). Panel D: The Fenton reaction in the infant formula (Impamil). Gray – BMPO/C.

Samples	breast milk	Infant formula
SOD U/g proteins	5.00 ± 2.3***	1.56 ± 0.56
-SH (µ M/L)	35.6±2.4**	23.32 ± 3.1
GSHPx (µmol NADPH/min/ proteins)	9.72± 2.16***	1.58 ± 0.75
Proteins (g/L)	12.02±0.3	15.58 ± 0.2
Vit C (mg/L)	44 ± 5	32 ± 8

Table 1. The activities of the antioxidant defense system enzymes in breast milk and in the infant formula. The results are expressed as the mean ± SD. *** p<0.005; ** p<0.01.

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