

S100B milk concentration in mammalian species

Fabio Galvano¹, Alessandro Frigiola², Luigi Gagliardi³, Sabina Ciotti³, Matteo Bognanno⁴, Anna Maria Iacopino⁴, Francesco Nigro⁵, Gabriella Lucia Tina⁵, Daniela Cavallaro⁵, Michele Mussap⁶, Andrea Piva⁷, Ester Grilli⁷, Fabrizio Michetti⁸ and Diego Gazzolo^{9,10}

¹Department of Biochemistry, Medical Chemistry and Molecular Biology, University of Catania, Italy, ²Perinatal Research Laboratory IRCSS San Donato Milanese, Milan 20100, Italy, ³Division of Pediatrics and Neonatology, "Versilia" Hospital, Lido di Camaiore, Italy, ⁴STAF Department, Mediterranean University of Reggio Calabria, Italy, ⁵Department of Maternal, Fetal and Neonatal Health, "G. Garibaldi" Hospital, Catania, Italy, ⁶Biochemical and Clinical Laboratory, "S. Martino" Hospital, Genoa, Italy, ⁷DIMORFIPA, University of Bologna, Italy, ⁸Institute of Anatomy and Cell Biology, Catholic University, Rome, Italy, ⁹Neonatal Intensive Care Unit "Cesare Arrigo" Children Hospital, Alessandria, Italy; ¹⁰Department of Pediatrics, "G. Gaslini Children's University" Hospital, Genoa

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1. ABSTRACT

S100B is a neurotrophic protein detectable in biological fluids and in human milk. Since there are several maternal-neonatal conditions requiring the administration of animal milks the aim of the present study was to quantify S100B in milk from different mammalian species and to compare protein's concentration among human and mammalian milks. We assessed S100B concentrations in donkey (n=12), goat (n=15) sheep (n=15), commercially available cow (n=8) and human (n=15) milk samples. S100B measurements were performed using an immunoluminometric assay. S100B concentration in human milk (10.41 ± 4.2 microg/L) was higher ($P < 0.001$) than mammalian milks. Of note, S100B concentration in cow milk (3.13 ± 0.56 microg/L) was higher ($P < 0.01$) than that showed in donkey (1.17 ± 0.26 microg/L), sheep (0.25 ± 0.11 microg/L) and goat (0.26 ± 0.11 microg/L). S100B in donkey milk was higher ($P < 0.01$) than sheep and goat samples whilst protein's concentration did not differ between goat and sheep. The present study suggests the opportunity of S100B addition to animal milk intended for infant feeding.

2. INTRODUCTION

S100B is an acidic calcium-binding protein mainly concentrated in the central nervous system, that can acts as a cytokine with a neurotrophic effect at physiological concentrations (1, 2). Among different biological fluids in which the protein has been detected (3), its presence in milk, at higher concentrations than that observed in other biological fluids (i.e. cord blood, peripheral blood, urine, cerebrospinal and amniotic fluid) appears particularly interesting (4). In this respect, human breast milk is known to contain a variety of substances that may actively influence the growth and development of the infant, including hormones, growth factors and cytokines (5, 6) through which biochemical communication between mother and child is established (7). Furthermore, the presence of S100B in milk is in agreement with a previous observations on the presence of calcium-binding proteins (e.g. alpha-lactalbumin, calmodulin, osteocalcin) (7, 8, 9) in a biological fluid where calcium is known to be abundant. Human milk should be the exclusive milk used in infants feeding: however, under different conditions such as maternal-neonatal diseases (10, 11) other milks such as

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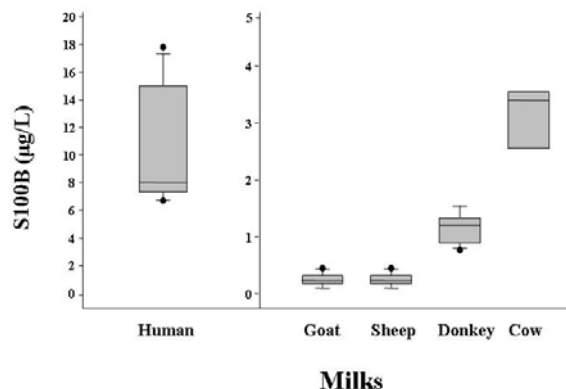


Figure 1. S100B concentration in milk of mammalian species. The lower and upper bars represent the 10th and 90th centiles respectively and interquartile range indicated by the box, the median value being the horizontal line in the box. S100B protein milk concentrations in human milk were significantly higher ($P<0.001$, for all) than in all animal milks. S100B cow milks concentration were significantly higher ($P<0.01$, for both) than sheep, goat and donkey milk concentrations. Donkey milk contained higher ($P<0.01$, for both) S100B concentrations than those of sheep and goat milks. No significant differences ($P>0.05$) in protein's concentrations were observed between goat and sheep milks.

bovine-derived milk formulae, and milks from other animal species can be administered. In this respect, it is noteworthy that data on S100B milk concentrations in different animal species are to date lacking.

The aim of the present study was to investigate whether milk samples obtained from animal species (i.e. cow, donkey, goat and sheep) contain S100B and to compare their concentrations with those detected in human milk.

3. MATERIALS AND METHODS

3.1. Milk samples

We collected mature milk samples obtained, at the same postnatal day of lactation, from donkey ($n=12$), goat ($n=15$) and sheep ($n=15$) bred in local farms and in commercially available cow milk from different manufacturers ($n=8$) and stored for S100B measurement. As control we used human mature milk ($n=15$), obtained from donors. Mature human milk was classified according to Playford and co-workers (12). Human donors had a physiological pregnancy (parity 1-2), delivered vaginally between 37 and 42 wks' gestation (mean 39 wks) and none of them smoked tobacco. Exclusion criteria were: multiple pregnancies, gestational hypertension, diabetes and infections, fever, chromosomal abnormalities, metabolic diseases, diseases of the breast, diseases of the central nervous system, malnutrition, maternal allergy. *The Local Ethics Committee approved the study protocol and the donors gave informed consent.*

3.2. S100B measurements

Milk samples were immediately centrifuged at 900g for 10 min, and the supernatants stored at -70°C

before measurement. The S100B protein concentration was measured in all samples, using a commercially available immunoluminometric assay (Lia-mat Sangtec 100, AB Sangtec Medical, Bromma, Sweden). This assay is specific for the β subunit of the protein. Each measurement was performed in duplicate according to the manufacturer's recommendations and the averages were reported. The limit of sensitivity of the assay was 0.02 microg/L. The S100B levels in the milk are expressed as mean \pm SD.

3.3. Validation of immunoassay for the study of different milks

Because milk is known to contain soluble receptors and other proteins that can bind to other moieties, different milk samples were divided in two equal parts. The first aliquot was not treated, while a known quantity of the human protein (4 microg) was added to the second aliquot. The quantities of the protein in both aliquots were then determined using the immunoassay. The percentage of recovery of the added protein was calculated by subtracting the amount in the first aliquot from the quantity in the second and dividing the difference by the amount of the protein that was added to the second aliquot. The percentages of recovery from the different examined milks were greater than 90% as follows: human mature (92%); cow (93%); donkey (95%); goat (96%); sheep (93%).

3.4 Statistical analysis

S100B concentrations in milk are expressed as mean values \pm SD. Statistical analysis was performed using the Kruskal-Wallis one-way ANOVA and multiple means comparison by Dunn's test. Statistical significance was set at $P<0.05$.

4. RESULTS

S100B protein was detectable in all analyzed milk samples and data are reported in Figure 1. Mean S100B protein milk concentrations in human milk (10.41 ± 4.2 microg/L) were significantly higher ($P<0.001$, for all) than those detected in the other mammalian milks. Of note, among mammalian species, S100B cow milk (3.13 ± 0.56 microg/L) concentration was significantly higher ($P<0.01$, for both) than sheep (0.25 ± 0.11 microg/L), goat (0.26 ± 0.11 microg/L) and donkey (1.17 ± 0.26 microg/L) milk concentrations. Donkey milk showed higher ($P<0.01$, for both) S100B concentrations than those obtained from sheep and goat samples. No significant differences ($P>0.05$) in protein's concentrations were observed between goat and sheep milks.

5. DISCUSSION

The present study provides evidence that a brain constituent with a neurotrophic effect, namely S100B protein, is detectable in samples obtained from various mammalian species although at lower concentration than human milk. Furthermore, when protein's concentration was compared among various mammalian species, it appeared that S100B was significantly higher in cow milk, than in other animal species as well as donkey milk contained a higher protein's concentration than goat and

sheep. No significant differences in goat and sheep S100B milk concentrations have been shown.

Elevated S100B levels in mature human milk, as compared to other milks and biological fluids (3, 4), is not a surprising finding and confirms previous observations (13) supporting the notion on the presence of calcium binding proteins (e.g. alpha-lactalbumin, calmodulin, osteocalcin) in milk where calcium is known to be abundant (7, 8, 9). Data offer additional evidence that S100B plays a neurotrophic role as a cytokine (2), and corroborate the exclusive role of breast milk in stimulating brain maturation. In this respect, it has been reported that the brain stems of both term and preterm infants, fed on breast milk, mature faster than those of infants fed on formula milk (5, 6). This is thought to be related to the different compositions of breast milk and other milks (14, 15).

In the present study we also found that protein's concentration in milk can vary accordingly to different animal species. The finding constitutes, to our best knowledge, the first observation on the quantitative assessment of S100B in various mammalian milks. There is, in this regard, a single observation of D'Auria and co-workers (16) showing the presence of S100B protein, in a wide range of proteins' patterns by using a proteomic approach, in different mammalian milks.

S100B milk presence in various animals species admitted into the study warrants consideration: i) results on recovery procedure suggest that S100B levels detected in donkey, goat and sheep correspond to the immunoreactivity observed using immunoluminometric assay as previously reported in human and cow milks (3, 13) ii) reports on S100B aminoacid sequence comparison among different animal species showed no significant differences in the epitope region (corresponding to the binding site of the specific S100B antibody), used for immunoluminometric assay, suggesting no effects on the accuracy in the quantitative protein's measurement; iii) proteomic evaluation in milk of different mammalian species showed that animal which are phylogenetically related have quite similar milk protein expression (16); taken together, the present findings show that the S100B detected in milk is likely a S100 brain-derived isoform and that its neurotrophic action can reasonably vary in a concentration-related manner. Furthermore, there are a series of evidences that corroborate protein's trophic role. In particular S100B: i) stimulates neurite outgrowth (17) triggering a cascade of events involving nuclear translocation of NF- κ B, up-regulation of Bcl-2 in target neurons and finally the binding of the protein to RAGE (Receptor for Advanced Glycation End Products) (18, 19); ii) enhances neurons' survival during development (through a RAGE-mediated effect and the activation of the Cdc42/Rac signalling pathway) (20) and after injury (intraventricular S100B infusion enhances hippocampal neurogenesis in rats) (21); iii) prevents motor neuron degeneration in newborn rats after sciatic nerve section (22), iv) is involved in the mechanisms modulating learning and memory (23, 24). All together, the present findings can support the notion on S100B eventful addition

in mammalian species opening-up speculations about new infants' feeding strategies.

The possibility that S100B milk differences, among various mammals species, could be at least in part related with procedures (i.e. pasteurisation) routinely employed in the commercial preparation (25, 26) has to be taken into account. Of note, although S100B is known to be stable, in biological fluids such as blood and urine, at different freezing degree (27) and at room temperature up to 120-hours (28), to date, there are no data confirming this stability at high temperature (about 70°C) occurring during pasteurisation. Therefore, further studies aimed at investigating S100B modifications during commercial preparation of milk are needed in order to offer conclusive information on the fate of the protein during these processes. Another explanation resides in the possibility that milk proteins occur in heterogeneous populations of isoforms which are subjected to post-translational modifications (16).

Finally, it has been widely reported that milks from various mammalian species can be suitable as a substitute in feeding those infants who are complicated by allergic reactions. This holds for milk-formulae such as those based on soy and cow milk, albeit proteins' extensive hydrolysis (16, 29). On this light, it can be speculated that donkey milk, because of its higher S100B content among mammalian milks other than cow, may be a suitable feeding choice when substitution of human and cow milk is needed.

In conclusion, the present study, reporting the pattern of S100B concentration in mammalian milks, offers matter of speculation on the possibility that S100B protein, due to its neurotrophic properties, could be suggested for eventful supplementation to other mammalian species.

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Send correspondence to: Diego Gazzolo, Neonatal Intensive Care Unit “Cesare Arrigo” Children Hospital, Alessandria, Italy, Tel: 39-0131-207241, Fax: 39-010-3741592- E-mail: dgazzolo@hotmail.com

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