Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 4 Issue 11 November 2022

Research Article

Effects of A1A1 and A2A2 Casein in Cow's Milk on Mice Intestine, Heart, and Spleen

Shikha Yadav[1], Bilal Ur Rehman[3], Mohammad Ahmad Khan[2] and Vijay Pal Singh[3]*

[1]*National Institute of Biologicals, Noida, India*

[2]*Department of Pharmacology, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, India*

[3]*CSIR-Institute of Genomics and Integrative Biology, New Delhi, India*

***Corresponding Author:** Vijay Pal Singh, CSIR-Institute of Genomics and Integrative Biology, New Delhi, India.

DOI: 10.31080/ASVS.2022.04.0539

Received: October 04, 2022

Published: October 18, 2022

Abstract

Milk is considered to be a complete dietary food that is consumed all over the world as a protein source. It has various proteins among which A1 and A2 variants of Beta-casein are the major varieties. Amidst them, A1 is believed to possess health issues. One of its metabolite Beta-CasoMorphin-7 (BCM7) is becoming a research point of interest among the scientific community. As the intestine is the site where maximum absorption of milk takes place, exploration of this intestinal tissue is a must. Along with it we also observed Heart and Spleen tissues for the effects of milk but there was no pathological change found in these organs. Histopathology is the key technique that has been used in our study to demonstrate the influence of A1 and A2 milk on the intestine. The goal of our study was to establish any possible relationship between the intestinal health of BALB/c mice and the consumption of milk containing A1A1 beta casein variants while no pathological changes were found in mice fed with the A2A2 variants of Beta casein. To our understanding, this is the opening study showing increased intestinal accessibility and villi atrophy in all the mice given with A1A1 milk which in turn may lead to nutrient deficiency and further health issues in these mice. In contrast, no pathological changes were found in the control mice and in those that were administered with the A2A2 Beta casein variant of cow milk.

Keywords: A1A1 and A2A2; Beta-Casomorphine7 (BCM7); Villous Atrophy; Intestinal Permeability

Introduction

Milk is a highly nutritious liquid that possesses almost all nutrients that our body needs like proteins, sugars, fibre, fat, vitamins, and minerals, and is a unique natural source that can be altered according to human health benefits [1]. Proteins in milk which is our molecule of exploration can be soluble as well as insoluble, soluble form is known as Whey proteins while the insoluble form is known as Casein having the ability to increase the absorption of Calcium, Phosphorus, and minerals. And this casein accounts for the highest portion of about 80% [2]. Caseins further consist of three forms Alpha, Beta, and Kappa caseins. Of all the total casein proteins, Beta casein accounts for approx. 35% in bovine milk [3]. The total tally of variants of Beta casein reaches 13 in bovine milk [3]. The variation in A1 and A2 beta caseins is because of a single nucleotide mutation at the 67th position where cytosine is replaced by adenine resulting in the substitution of proline with histidine, leading to the generation of the A1 form [4]. Crossbreeding among European and Indian cows, creating the genotypic involvement of A1 protein in the hybrid cows. In a survey of 2019, the total number of cattle appears to be 192.49 million, while the population of Exotic/Crossbred cattle in the country is reported to be 50.42 million [5]. Thus contributing to about 26.19% of the crossbreeds and hence an increase in the possibility of accumulation of A1 variant among Indian cows. Asian, Jersey, Guernsey, and African were the ones that produce A2 milk. Luckily, India has a considerably greater number of dairy cows and buffaloes that produce healthy A2 milk which is very much safe for consumption. The cattle and buffaloes of Indian origin have an A2 genotype of about 99 to 100%

and the A1 genotype is almost nil [6]. Hence it can be concluded that Indian cows produce A2 milk. Major varieties of cows that produce A1 enriched milk are Holsteins and Ayrshire, A1 beta-casein production among European cattle varies by breed and by country. In the parts of the world where European origin breeds predominate and especially Northern Europe the level of A1 milk is found high among cows [7]. The Holstein Friesian cows of North America and the North European region have a very high gene frequency that is more than 90% but in German Holstein Friesian cows the A2 gene frequency is about 97%. In other countries, the frequency range dips up to 40-65% of A1 in Holstein Friesian breed [6].

The histidine which is produced in A1 milk results in weaker interaction with isoleucine which is right next to it, thus making it more prone to cleavage by proteolytic enzymes resulting in the formation of Beta- casomorphins (BCMs) [2] an opioid receptor agonist that causes gastrointestinal complications. Both the common casein components have unrelatable actions, on one side A2 proves to be beneficial for human health while on the other hand, A1 proves to be a risk factor for various chronic clinical conditions like diabetes mellitus, ischemic heart disease, and autism [8]. The inflammation cascade in the gut is generally mediated by Th2 cytokines which include IL-4, IL-5, and IL-13, and these interleukins are associated with the promotion of the IgE [4]. And hence they are also responsible for the development of respiratory complications in mice as well as in humans. The intestine of mice comprises various villi that help absorb nutrients, and their pathology is disturbed by the consumption of A1A1 milk. In this study, we wanted to estimate the effect of A1A1 and A2A2 casein in cow milk on a mice model.

Materials and Methods

Cows genotyping

A1A1 and A2A2 beta-casein variants of cow milk were required for the study. Therefore, the dairy farm's Holstein Friesan and Hariana cows were genotyped to check for the A1 and A2 beta-casein alleles. Samples of blood were collected from cows, followed by isolation of Genomic DNA and establishing of purity of DNA by using suitable techniques [3].

Collection of milk samples

Cows possessing A1A1 and A2A2 Beta casein protein variants were milked weekly and the milk samples were boiled and stored at 4-7°C before being fed to the Balb/c mice [3].

Experimental design and procedure

Male mice of Balb/c strain which were 3-4 weeks old were chosen and kept in a controlled temperature range of 22°C ± 2°C, with humidity between 45-60%, and a light intensity of 300 lux. All animal study procedures had been endorsed by the IAEC (Institutional Animal Ethics Committee), constituted by CPCSEA (Committee for the Control and Supervision of Experiments on Animals). The mice were randomized into three groups of 10 mice each i.e., Control, A1A1, and A2A2 milk-fed groups which were kept in Individually Ventilated Polypropylene Cages (IVC). The control group was provided RO water while A1A1 and A2A2 groups were provided A1A1 and A2A2 beta-casein variants of cow milk by oral gavage of a quantity 10ml/kg for a duration of 36 weeks. A combination of anesthesia (Xylazine plus Thiopental sodium) as per body weight was used to sacrifice the mice after the milk consumption duration. The small intestine, Heart, and Spleen which were our target tissues were then dissected and collected in a 10% formalin buffered solution.

Results and Discussions

On histopathological examination of the tissues, desquamation and atrophy of villi are seen in intestinal tissues in the case of A1A1 milk-fed groups (Figure a) while the mice which were administered with A2A2 milk (Figure b) and control mice (Figure c) showed no sign of disrupted villi (Figure b) neither the control mice which were administered only with RO water show any kind of irregularity in intestinal sections (Figure c). Other than the intestine no other tissue neither Heart nor Spleen were found to be without any pathologically change. And as a result of the damage to the villi in A1A1 milk administered mice, there are chances of malabsorption of nutrients. Numerous studies indicate how important nutrients are for keeping intact the intestinal lining and epithelial barrier regulation, so improper nutrient absorption in these mice may produce alteration in the normal intestinal physiology [9]. Further, the tight junctions also get deformed thus altering the intestinal permeability [10]. This destruction can be reversible only if the damage occurs until the superficial layers of the intestine, our results suggest that the administration of the A1A1 beta-casein variant of cow milk for long duration results in pathological lesions in the intestine of mice.

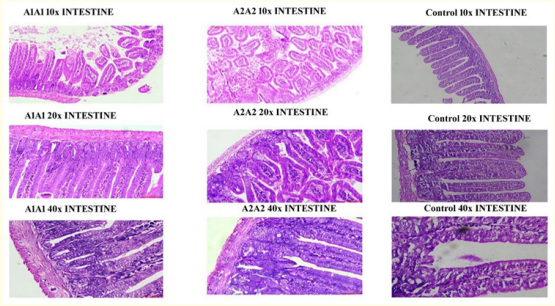

Figure 1: a) Intestine Histology with A1A1 administered milk. b) Histology of A2A2 administered mice stained with Periodic Acid Schiff 10x,20x,40x. c) Histology of intestinal section of Control mice.

Conclusions

We have concluded from our research that the administration of the A1A1 beta-casein variant of cow milk in mice model is resulting in pathological lesions only in the intestines, especially on the villi which were established with villous atrophy and altered intestinal permeability. With this evidence, we conclude that A1A1 milk may be responsible for nutrient malabsorption in the intestine. However, this is a preliminary study that would need further exploration in other animal models and humans.

Competing Interest

The authors proclaim that there is no conflicting situation.

Acknowledgment

The Authors acknowledge the help of Ms. Ritu Sharma in taking the images.

Bibliography

1. Sabikhi L. "Designer milk". *Advances in Food and Nutrition Research* 53 (2007): 161-198.
2. Yadav S., *et al.* "Oral Feeding of Cow Milk Containing A1 Variant of β Casein Induces Pulmonary Inflammation in Male Balb/c Mice". *Scientific Reports* 10.1 (2020): 1-8.
3. Yadav S., *et al.* "A1 β-Casein variant of cow milk exacerbates House Dust Mite induced Allergic airway disease in a murine model". *IJBPAS* 10.1 (2021): 326-345.
4. Ul Haq MR., *et al.* "Comparative evaluation of cow beta-casein variants (A1/A2) consumption on Th2-mediated inflammatory response in the mouse gut". *European Journal of Nutrition* 53.4 (2014): 1039-1049.
5. "Ministry of Fisheries, Animal Husbandry and Dairying". Census Cattle Buffalo 5 (2022): 57.
6. Behera R., *et al.* "A1 versus A2 Milk". *International Journal of Livestock Research* 8.4 (2018): 1-7.
7. Woodford K. "A1 Beta-casein, Type 1 Diabetes and Links to other Modern Illnesses". *Diabetes Research and Clinical Practice* 79 (2018): 1-16.
8. Guantario B., *et al.* "A Comprehensive Evaluation of the Impact of Bovine Milk Containing Different Beta-Casein Profiles on Gut Health of Ageing Mice". *Nutrients* 12.7 (2020): 2-19.
9. Murphy LY Wan., *et al.* "Influence of functional food components on gut health". *Critical Reviews in Food Science and Nutrition* 59.12 (2019): 1927-1936.
10. Suzuki T. "Regulation of intestinal epithelial permeability by tight junctions". *Cellular and Molecular Life Sciences* 70.4 (2013): 631-659.