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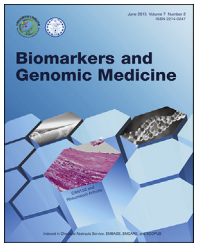

ORIGINAL ARTICLE

The anti-osteoporosis effects of CSN1S2 protein of goat milk and yoghurt on a complete Freund's adjuvant-induced rheumatoid arthritis model in rats

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Abstract This study aimed to evaluate the anti-osteoporosis effect of CSN1S2 protein from goat milk and yoghurt on a complete Freund's adjuvant (CFA)-induced rheumatoid arthritis (RA) model in rats. Twenty-four rats were randomly divided into six groups: control (untreated) group (C), group treated with CSN1S2 (CM) protein from goat milk, group treated with CSN1S2 protein from goat yoghurt (CY), RA group, RA group treated with CSN1S2 protein from goat milk (RAM), and RA group treated with goat yoghurt (RAY). Mineral elements and mesostructure were analyzed using X-ray fluorescence and scanning electron microscopy. Bone histomorphometry was analyzed using the BoneJ software. One way analysis of variance and Tukey post hoc tests were used to analyze and compare the means of all variables between groups. The phosphorus levels were not significantly different between treatment groups relative to the control group ($p > 0.05$), but were significantly higher in the CM and RAY groups relative to that observed in the CY group ($p < 0.05$). CSN1S2 protein of goat milk repaired the collagen structure in the femur trabecular bone. The trabecular thickness and volume were significantly lower in CM and CY groups relative to the control group ($p < 0.05$). The trabecular volume also decreased significantly in the CM group relative to the control group ($p < 0.05$). The trabecular thickness was significantly lower in the CY group relative to the CM group ($p < 0.05$), but the

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trabecular separation and trabecular volume were significantly greater ($p < 0.05$). The trabecular volumes were significantly elevated in the RAM and RAY groups relative to the CM group ($p < 0.05$). The trabecular thickness was significantly higher in the RAY group relative to the CM and CY groups ($p < 0.05$). CSN1S2 protein of goat milk is better than goat yoghurt in repairing femur crystallization and mesostructure in CFA-induced rheumatoid arthritis in rats.

Introduction

Rheumatoid arthritis (RhA) is a chronic inflammatory disease with multiple risk factors. The pathomechanism of RhA is associated with chronic soft-tissue inflammation often followed by bone and cartilage destruction.[1] Bone complications are the major extra-articular complications of RhA and can be described as three different forms: periarticular bone loss, adjacent to the inflamed and swelling joints; bone erosions, which share common mechanisms with the periarticular osteopenia; and systemic osteoporosis.[2] Studies indicated that there is higher frequency of osteoporosis and reduced bone mass in RhA patients, in which the largest effect is found on the hip, as compared to healthy controls.[3–6] There is also a two-fold increase in osteoporosis in female RhA patients ranging in age from 20 years to 70 years,[7] with osteoporosis being one of the determinants of fracture risk. RhA patients have an increased risk of fractures of the hip, vertebrae, and pelvis.[7–9] Studies also suggested that women with early-onset RhA have significantly lower femoral neck and whole-body bone-mineral density (BMD), but have similar lumbar spine BMD as compared with controls. The frequency of bone loss at all sites is significantly greater in women with RhA than that of the controls.[10]

Nutritional disorders affect the pathological condition and long-term outcome of physical functions in RhA. Many studies have reported that malnutrition is a well-recognized symptom associated with RhA.[11] The values of nutritional markers, such as albumin, are low in patients with RhA, owing to malnutrition and excessive protein catabolism triggered by high levels of inflammatory substances.[12] Therefore, the nutritional status may be deeply involved in RhA outcome or symptoms.

Bioactive peptides from milk proteins were included within the primary sequence of native proteins that are hydrolyzed by digestive enzymes and then cleaved and modified to produce an active product as a fragment of specific proteins. Bioactive peptides play important roles as signaling molecules in physiological functions and pathogenesis-related chronic diseases, such as hypertension, diabetes, cancer, and osteoporosis, as well as immunomodulatory, anti-hypertension,[13,14] anti-inflammation,[15] anti-oxidative,[16] and anti-glycosylation[17] molecules. Milk proteins are major suppliers of amino acids and micronutrients in young mammals or humans. Milk is also rich in biologically active peptides necessary for health. Bioactive peptides are products of enzymatic fermentation that is an important aspect of dietary proteins and offer adequate nutritional effects. Many researchers have reported that peptide-derived milk fermentation products, such as yogurt, cheese, or other fermented-foods, display more beneficial biological functions related to cell signaling pathways as compared to peptides from fresh milk.[13,14,18,19] However, the bioactive peptides from fresh milk and yogurt that regulate pathways associated with osteoporosis and RhA remain unclear.

Food-derived bioactive peptides represent a source of health enhancing components that may be incorporated into functional foods and/or used as nutraceuticals.[20] Moreover, milk protein-derived bioactive peptides are health-enhancing components that can be used to reduce the risk of disease or to enhance certain physiological functions.[21] Whole casein and electrophoretic-casein fractions have been shown to exhibit different biological activities, such as immunomodulation.[22,23] Our recent study reported that goat milk has the alpha-S2 casein (CSN1S2), a protein with a molecular weight of 36 kDa, which is not present in bovine milk. We have also determined that this protein contains eight peptides analyzed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry[24]; however, there is no study to evaluate the ability of CSN1S2 protein isolated from goat milk and yoghurt to inhibit femur osteoporosis in RhA. Therefore, this study aimed to evaluate the anti-osteoporosis properties of CSN1S2 protein from goat milk or yoghurt in RhA-associated osteoporosis.

Materials and Methods

Animals

Twenty-four 12-week old adult male Wistar rats, weighing 150—200 g, were acclimatized for 1 week to the laboratory conditions prior to experimental manipulation. These animals were purchased form Integrated Research and Testing Laboratory, Gadjah Mada University, Yogyakarta, Indonesia. The animals were exposed to a 12-hour light and 12-hour dark cycle at room temperature of 24°C. They had free access to a standard laboratory diet and water *ad libitum*. The animals were randomly divided into six groups ($n = 4$ each): control (untreated) group (C), group receiving CSN1S2 protein from goat milk (CM), group receiving CSN1S2 protein from goat yoghurt (CY), RhA group (RA), RhA group receiving CSN1S2 from goat milk (RAM), and RhA group receiving CSN1S2 from goat yoghurt (RAY).