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REVIEW ARTICLE

THE ROLE OF VASPIN IN RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS

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ABSTRACT

Background: Adipo(cyto)kines are substances derived from adipose tissue that influence central processes in the body like inflammation, in addition to their effect on metabolism. Adipocytokines are implicated in inflammatory pathways that affect different types of cells in autoimmune disorders. Many rheumatic disorders, such as rheumatoid arthritis and psoriatic arthritis, are classified as autoimmune diseases. A chronic inflammatory environment develops as a result of autoimmune reactions, affecting the entire body, notably adipose tissue. Vaspin, an adipocytokine, is a visceral serpin protease inhibitor produced by subcutaneous adipose tissue, skeletal muscle, skin and stomach. **Objectives:** The present review summarizes the structure, mechanism of action and role of vaspin in the pathophysiology and development of rheumatoid arthritis and osteoarthritis. **Methodology:** The data was collected through the electronic search of several scientific sources including, Science Direct, PubMed, and Google scholar. **Results:** Because of changes in systemic vaspin levels, its potential role as a biomarker in the diagnosis of rheumatic diseases has been suggested. Furthermore, vaspin induction by adipose tissue may produce a compensatory response to obesity and its inflammatory consequences. Recent studies have shown that vaspin levels are lower in osteoarthritis and higher in rheumatoid arthritis and psoriatic arthritis compared to healthy control. Moreover, vaspin elevation may assist in predicting who is at risk of developing rheumatoid arthritis. **Conclusion:** Vaspin inhibitors can attenuate the pathological processes of rheumatoid arthritis and improve the outcome of treatment when used in combination with other inflammatory mediator inhibitors.

Keywords: Vaspin, Rheumatoid arthritis, Osteoarthritis, Adipose tissue.

INTRODUCTION

Musculoskeletal diseases affect the locomotor system, including bones, joints, muscles and other related tissues like ligaments and tendons, and are considered the second leading cause of disability in the world, depending on the World Health Organization (WHO) (1). Rheumatoid arthritis (RA) and osteoarthritis (OA) are two examples of musculoskeletal diseases that affect the joints. These two diseases are both inflammatory conditions but are different in their underlying pathogenic processes (2).

Osteoarthritis (OA) is the most common form of joint diseases, affecting about 18 % of people over the age of 60, however it may also affect young people, particularly juvenile athletes (3). It has been established that it is primarily caused by joint overload and biomechanical stress, however metabolic disorders and obesity are also important risk factors (4). The major tissues involved in the pathogenesis of osteoarthritis include subchondral bone, cartilage and synovium. Recently, proinflammatory mediators including reactive oxygen species, cytokines, matrix degrading enzymes and nitric oxide (NO) have been shown to play a key role in pathogenesis of OA (5).

Rheumatoid arthritis (RA) is a severe autoimmune disease, affecting about 1 % of people in the world. It is characterized by chronic diarthrodial joints inflammation, which leads to the destruction of bone and cartilage. RA is associated with physical function loss, poor quality of life and a significant prevalence of concomitant diseases (6). Rheumatoid arthritis occurs in people who have genetic susceptibility and with the help of environmental factors and epigenetic processes. This disease is heterogeneous with different underlying pathological processes and clinical manifestations (7).

It is critical to understand the processes that underlying both immune modulation and inflammation resolution in order to develop novel treatments for these two common rheumatic joint diseases. One way to explain this mechanism is to investigate the homeostasis of nervous, endocrine and immune system, which is critical for effective adaptation to stresses and maintaining homeostasis (8). This complex homeostasis and its regulation processes depend on the existence of co-mediators for example neurotransmitters, hormones, cytokines and their receptors. Therefore, immune and endocrine integrated circuits dysregulation has contributed to the development of severe metabolic disorders, including diabetes, metabolic syndrome and obesity (9). However, rheumatoid arthritis is one of the common diseases caused by dysregulation between these two systems (10). In this disease, reversal of abnormalities of cellular phenotypes has been demonstrated: for example, hormones/neurotransmitters might reprogramme synovial fibroblasts from RA patients, from a cartilage degrading phenotype to a regulating one (11).

Interestingly, it has been shown that adipose tissue not only play a role in energy homeostasis, but also to function as an endocrine organ by producing a wide range of substances known as adipo(cyto)kines. Adipokines, chemokines, cytokines and complements are all factors secreted by adipose tissue (12, 13). Adipocytokines are biologically active proteins known as modulators for inflammatory and immunological responses, playing important roles in the management of rheumatic joint disorders at both the local and systemic levels. However, adipocytokines are not only synthesized by adipose tissue. Immune cells, synoviocytes and chondrocytes also produce these important mediators. Endocrine, paracrine and autocrine pathways presenting on target tissues and cells including bone, synovial membrane and cartilage have been identified in musculoskeletal diseases (14). Compared with healthy individuals, patients with RA revealed increased levels of adipocytokines in both synovial fluid and serum (15). Furthermore, patients with OA identified inflammatory profile in the infrapatellar fat pad (16). In conclusion, recent data suggests that adipocytokines have a role in both degenerative diseases and immune-mediated rheumatic disorders.

Rheumatoid arthritis

Rheumatoid Arthritis (RA) is a chronic and severe systemic autoimmune inflammatory disorder of unknown pathogenesis that primarily affects symmetrically the peripheral joints, resulting in progressive destruction and dysfunction of joints. A persistent inflammatory infiltrate in the synovial sublining layer is one of the features of RA, contributing to the generation of a micro-

environment with stromal cells that exhibit a hyperactivate phenotype, thus producing various tissue-damaging and pro-inflammatory mediators into the joint space (17). Although the pathogenesis of RA remains unknown, dysregulation of local and systemic immune system is thought to regulate the disease. Chronic synovitis is characterised by enhanced levels of proinflammatory cytokines (TNF-α, IL-6 and IL-1β) and the formation of osteoclasts with subsequent erosive damage to the joint. In addition to producing these major proinflammatory cytokines, RA presents with production of autoantibodies like rheumatoid factors (RFs) and anti-citrullined protein antibodies (ACPA). Interestingly, synovial macrophages and synovial fibroblasts play a critical role in the development of RA (7).

Synovial fibroblasts from patients with RA (RASF) specifically induce an auto-pathogenic phenotype, resulting in the ability to migrate and hyperproliferate, which contributes to synovial hyperplasia and spreads rheumatoid arthritis to other intact joints (18). Similarly, resident and RA synovial macrophages derived from monocyte, have a pro-inflammatory profile, correlated with activity of RASF disease. In the synovium of affected joints, the number of these cells has also been found to be linked with joint erosion and disease activity (19). Moreover, endothelial cells affected by synovitis are also known to include in chronic synovitis by immune cell recruitment and angiogenesis in RA (20).

Osteoarthritis

Osteoarthritis (OA) is the most common chronic form of arthritis, one of the main causes of psychological and physical impairment worldwide (21). OA is a complicated multifactorial disease involving biochemical, genetic, mechanical, metabolic and biological factors in which cell stress and degradation of extracellular matrix lead to a defect in joint tissue metabolism (22). While cartilage degradation is the most important factor in pathogenesis, OA damages the entire joint, leading to remodelling of the surrounding subchondral bone, synovitis and formation of osteophytes that can lead to pain, impaired joint function and disabilities in later stages (23).

Cartilage of OA is characterized by increased extracellular matrix (ECM) remodelling, angiogenesis and cartilage calcification (24). Chondrocytes contain receptors for interaction with inflammatory mediators, mechanical stress and components of the ECM such as products of cartilage degradation (25). Thus, an activated chondrocytes develop hypertrophic phenotype, proliferate and increase the production of inflammatory chemokines and cytokines, reactive oxygen species, matrix-degrading enzymes (matrix metalloproteinases and aggrecanases) and stress-induced intracellular signals. Furthermore, there was a decrease in the production of ECM protein. When the process of cartilage destruction is irreversible, aggrecan depletion and collagen type II degradation are key stages in this process (26).

Interestingly, subchondral bone modification is associated with cartilage remodelling, hence play a major role in the progression of OA through the production of catabolic mediators which increase an abnormal chondrocyte metabolism (27). Therefore, the production of degradative and inflammatory mediators by joint cells leads to synovitis. Synovitis is accompanied by synovial macrophage and synovial fibroblasts proliferation, synovial hyperplasia and infiltration of immune cells. These cells produce inflammatory mediators such as interleukin-1β and tumour necrosis factor-α, in addition to other chemokines and cytokines that exacerbate inflammation (25). Moreover, synovial fibroblasts release enzymes that degrade the matrix, which also contributes to the degradation of ECM cartilage (28).

Vaspin and adipose tissue

Adipose tissue (AT) is the primary tissue that regulates energy homeostasis. Due to the release of a variety of bioactive chemicals, it also functions as an endocrine organ. Adipocytokines are chemicals produced by adipose tissue. These mediators involve hormones, complement factors, chemokines, cytokines and adipokines (29). Excess adipose tissue produces cytokines that influence the entire body, resulting in a low-level inflammation which has been found in obese people. Adipocytokines are mediators generated primarily in white adipose tissue by adipocytes. A significant number of physiologically active factors are secreted by adipose tissue. Several adipocytokines, for example adiponectin and leptin, are recognized as modulators of the immune system. Variations in serum levels of adipocytokines have been linked to a variety of chronic

inflammatory disorders (18, 30), and vaspin potentials will be discussed further. Accordingly, adipokines, including vaspin, have been studied in the context of degenerative and chronic inflammatory rheumatic diseases at the systemic and local levels in cells and tissues for many years. Moreover, anti- and pro-inflammatory properties of vaspin and other adipokines have been studied further. Interestingly, in recent years, it has become widely established that vaspin plays an important role in degenerative osteoarthritis and immune-mediated rheumatic disorder.

Vaspin

Vaspin (serpinA12) is an adipocytokine that belongs to the family of serpins, with targets in serine protease enzymes (kallikrein 7 and kallikrein 14) that are involved in skin desquamation (31). Consequently, the inhibition of kallikrein 7 is accelerated by vaspin and heparin interaction (31). Vaspin contains the characteristic domains typical of the original serpins structure, containing nine α-helices, three β-sheets and a flexible central reactive loop, with a protease-identification sequence at the top (32). Vaspin shows 40.5% homology to α1-antitrypsin in its amino acid sequence (33). Human, mouse and rat vaspins consist of 395, 394 and 392 amino acids, respectively. Moreover, the amino acids of mouse and human vaspin show 62.6% identity, whereas rat and human share 61.5% (34). Vaspin in human, rats and mice shows a peptide signal on the N terminal domain. Whereas in mice N terminal Edman sequence, the vaspin has been shown to start at the Leu-21 residue, and the G364–P381 residue is included in the reactive center loop of vaspin. The presence of a side chain containing an uncharged amino acid at position P14 inside the reactive center loop's hinge region indicates that vaspin is a serpin. In the reactive canter loop, the presence of cleavage in the sequence from methionine 378 (P1) to glutamate 379 (P10) has been experimentally established, which largely estimate the specificity of proteases. Arginine 302, permits vaspin identification by kallikrein 7, also regulates this protease specificity, while glutamate 379 (P1) is a key limiting factor for the blocking action of vaspin (35).

In human, the glycoprotein vaspin contains three glycosylation binding sites at the asparagine residue at the N-terminus. Two of these binding sites are adjacent to the RCL sequence and domains involved in conformational variations that regulate serpin-inhibitory action. Accordingly, vaspin glycosylation had no effect on the target protease's inhibition (36). The two glycosylation sites at the RCL are missing in rat and mouse vaspins, but an extra expected site is observed adjacent to helix-C bottom on the posterior side of vaspin (37). Following Western blot, vaspin (45 kDa) protein is present in human and mouse adipose tissue.

Vaspin is made up of 1242, 1245 and 1236 nucleotides in mice, human and rats, respectively. In human, vaspin is encoded by the gene SERPINA12, which is found on chromosome 14 (33). Interestingly, single nucleotide polymorphisms in the vaspin gene have been shown to affect serum levels of vaspin. The early stop codon in the rs61757459 polymorphism leads to a truncated protein which is hydrolysed due to mis-folding, which leads to a decrease in the circulating level of vaspin (38). On the other hand, by modulating vaspin transcriptional activity, the rs77060950 polymorphism, which was found in a Japanese population, resulted in considerably increased serum levels of vaspin (39).

Vaspin expression was detected in subcutaneous and visceral adipose tissue, ovaries, hypothalamus, cerebrospinal fluid, stomach, placenta, pancreas, liver and skin (40, 41). Therefore, the tissue determines the level of expression of vaspin. Accordingly, vaspin is expressed in the liver, skin and brain of mice, with modest expression in adipose tissue. Interestingly, vaspin is not expressed in the muscles and kidney (42). Serum levels of vaspin vary as well, ranging in human between 0.18-1.55 ng/mL (43), whereas it was 1 ng/mL in pigs (40). Compared to control, the underweight children had significantly reduced vaspin levels, although vaspin gene expression increased with increased body mass (44).

Many vaspin regulators have been described in the literature. Serum concentration and visceral adipose tissue expression reduced with weight loss, whereas increased with leptin elevation, obesity and insulin resistance in humans and rats (45). After eating a high fat diet, vaspin was shown to be elevated in the brown adipose tissue and liver (42). Accordingly, previous results showed that vaspin acts as a compensatory molecule in

insulin resistance and obesity (46). Furthermore, it was discovered that vaspin interacts with a variety of hormones. In Otsuka Long Evans Tokushima Fatty rat, insulin sensitizer metformin enhanced mRNA of vaspin protein in the gonadal adipose tissue and insulin raised levels of adipose tissue vaspin (33). However, in polycystic ovary syndrome patients, metformin reduced serum levels of vaspin (47). Moreover, sexual dimorphism was detected in vaspin levels, with women having significantly increased vaspin levels compared to males, suggesting that steroid hormones influence vaspin expression (48). The fact that serum levels of vaspin were higher among women who used oral contraceptives confirms these findings (49). Moreover, a higher serum level of vaspin in girls was detected in children, and this was associated with the puberty period (50). Interestingly, vaspin concentrations vary throughout the day, with levels being higher before meals than after meals. The maximum quantity was seen at evening, which is equivalent to insulin level (51).

Mechanism of action

Vaspin links to a member 5 of the heat shock protein family A protein, which is a binding immunoglobulin or 78 kDa glucose regulated protein present on the cell surface. This protein is released to the plasma membrane from the endoplasmic reticulum (ER) (52), and contains 3 domains. These domains are a substrate binding domain (20 kDa) at C terminal (carboxyl), an ATP binding domain (44 kDa) at N terminal (amino) and a 10-kDa domain in the C terminal tail of unknown function (53). The glucose-regulated protein 78-kDa (GRP78) has 60 % homology with the heat shock protein 70 (HSP70) family, with the SBD and ABD domains conserved, and its protein has 655 amino acids in mice and 654 in human (54).

HSPA5, the gene that encodes GRP78 in humans, was discovered on chromosome no.9 and consists of 4532 nucleotides organized into 8 exons (55). The thyroid, testes, spleen, ovaries, placenta, endometrium and brain all express GRP78 mRNA in human. GRP78 expression was found in the brain, thymus, testes, spleen, ovaries and placenta of mice with the gene located on chromosome 2. GRP78 is located on chromosome 1 in pigs and is expressed in the adipose tissue, spleen and ovaries, whereas it is found on chromosome 3 in rats and is expressed in the thymus, testes, spleen and brain (54). Small domains in the hydrophobic portion of the functional GRP 78 gene are largely preserved with heat shock protein 70 from Escherichia coli, yeast, Xenopus, Drosophila and humans (55). The intracellular protein (GRP 78) was expressed in the endoplasmic reticulum lumen and on cell surface, suggesting that it may function as a receptor (56). GRP78 expression may be controlled by hormones such as follicle-stimulating hormone upregulates it in cows, whereas prostaglandin PGF2α upregulates it in rat ovaries (57). GRP78 expression in neurons was also induced by leptin (58). The primary role of GRP78 is to transport polypeptides across the endoplasmic reticulum membrane, regulate the process of calcium efflux from the endoplasmic reticulum to mitochondria and maintain intracellular calcium homeostasis. On cell surface, its primary function is to proliferate cell and promote survival (59). In the female reproductive system, GRP78 protein also has a role in the physiology and pathophysiology of preimplantation, embryo, uterus, oviduct, corpus luteum and follicular development (60). On the cell surface, GRP78 protein inhibits endothelial cell angiogenesis and is a prominent autoantigen in ovarian cancer (61). GRP78 significantly decreased serum levels of insulin and was involved in adipose tissues glucose homeostasis and adipogenesis in eIF2- mutant mice that acquired a reduced metabolism with obesity when following a high-fat food (62). Differences in the activation or expression of GRP78, a key regulator in the endoplasmic reticulum, have been linked to neurodegenerative diseases, cardiovascular disease and cancer (54). GRP78 overexpression enhances the invasion, migration and proliferation of pancreatic cancer cells, in addition to the proportion of cells in the S-phase of the cell cycle (63). Additionally, previous research has shown that vaspin can bind to GRP78 and transduce intracellular signalling through several kinase pathways. Vaspin can bind to GRP78 through the helical domain at the N terminus, not the RCL region, as Nakatsuka et al. (52) discovered. However, the binding site of GRP78 and the method of signal transduction into the cell are unclear, and it is thought to be dependent on the high affinity of GRP78 and negatively charged phospholipids at the cell

membrane (64). Accordingly, Vaspin can bind to phospholipids, which are important in membrane trafficking (65). Furthermore, a positive interaction of vaspin with Protein kinase-B (66) and adenosine monophosphate (AMP) activated protein kinase phosphorylation was shown in a previous study (52).

In pancreatic islets, vaspin raised the protein level of AKT phosphorylation, but negatively affects nuclear factor-kappa B (67). Vaspin may reduce cytokine-induced adhesion molecule gene expression by blocking nuclear factor-kappa B after activation of AMP activated protein kinase (68). Vaspin activates Janus kinase (STAT3) in the vascular endothelial cells, which enhances the activity of nitric oxide synthase (69). Vaspin controls ovarian function in porcine by stimulating the GRP78 and mitogen-activated kinase (MAP3/1) pathways (70). In human, vaspin may protect osteoblasts from apoptosis by activating the MAPK/p38 pathway (71). In aortic endothelial cells, vaspin was shown to be colocalized with liver DnaJ-like protein (MTJ1) and voltage dependent anion channel, in addition to GRP78. In hepatocytes, interactions between Vaspin and the GRP78/MTJ1 complex result in intracellular signalling, which leads to activation of AKT and AMP-activated protein kinase. However, Vaspin inhibits the rise of intracellular calcium levels in endothelial cells by blocking the interaction between Kringel5 and GRP78/voltage dependent anion channel complex (37). The relationship between vaspin and GRP78, in addition to several kinase pathways, has been suggested to play a crucial role in cell function regulation.

Vaspin in rheumatic diseases

The potential activity of vaspin in rheumatic joint diseases, rheumatic arthritis and osteoarthritis, has yet to be completely investigated. Vaspin was found in cartilage, osteophytes and synovium in patients with osteoarthritis having joint surgery. In vitro, vaspin affected chondrocytes and bone cells, it protected human osteoblasts from apoptosis (71) and suppressed osteoclastogenesis in bone marrow-derived cells, RAW264.7 murine macrophage cell line and MC3T3-E1 pre-osteoblast cell line (72). In the RAW264.7 murine macrophage cell, it has been found that vaspin inhibits RANKL induced expression of matrix metalloproteinases-9 (MMP-9) and cathepsin K. Moreover, vaspin has been shown to inhibit receptor-activator of nuclear factor kappa B ligand RANKL-induced osteoclast synthesis, reduce human osteoblast apoptosis, and thus regulate the osteoblast development of the MC3T3-E1 pre-osteoblast cell line (73). Furthermore, vaspin also inhibited the production of the pro-inflammatory and catabolic mediator induced by leptin and IL-1 in rat or murine chondrocytes, respectively (74). Another in vitro study by Wang et al. 2020 showed that vaspin exerts a protective activity against bone loss caused by a high-fat diet and promotes osteoblastic development via activating the SmadRunx2-signaling pathway (75). These are the potential pathological mechanisms by which vaspin may contribute to arthritic disorders.

Conflicting studies on the potential anti-inflammatory activity of vaspin, resulting from investigation of various disorders, for example rheumatoid arthritis, juvenile idiopathic arthritis, osteoarthritis, psoriatic arthritis and ankylosing spondylitis. Vaspin involvement in skeletal muscle inflammation have been demonstrated (76) and serum levels of vaspin have been associated with inflammation in rheumatoid arthritis and clinically manifest rheumatoid arthritis development after follow-up (77). Vaspin transgenic mice revealed an improvement in markers related to metabolism and inflammation, resulting in increased glucose tolerance, resistance to obesity caused by a high-fat diet, and reduced levels of systemic IL-6 (78). Vaspin's cellular effect has also been investigated, especially with regard to metabolism. Glucose homeostasis and adipocyte differentiation are modulated by vaspin (79). In human, the pro-inflammatory phenotype of macrophages was reduced by vaspin in the coronary atherosclerotic plaques (80). Conflicting results have been found on serum levels of vaspin in osteoarthritis, rheumatoid arthritis and other rheumatic disorders. Compared with the healthy control, patients with osteoarthritis showed reduced serum vaspin levels (81). Conversely, psoriatic arthritis and rheumatoid arthritis patients revealed higher serum vaspin levels compared to the healthy control (82). Furthermore, serum vaspin levels and its gene expression levels were higher in patients with RA and was linked with laboratory and clinical characteristics of RA (83). Interestingly, in rheumatoid arthritis patients, short term

treatment with high doses of glucocorticoids supresses inflammation and thus increases serum vaspin levels, which may be associated with inflammation, but are unlikely to be causative (84). On the other hand, vaspin levels in synovial fluid were significantly higher in rheumatoid arthritis patients than in those with osteoarthritis, and vaspin was positively associated with the activity score of 28-joint disease in the rheumatoid arthritis patients. In the same study, no correlation was shown between serum levels of vaspin and C-reactive protein or leukocyte count in patients with rheumatoid arthritis (85). Interestingly, no correlations between serum vaspin and the inflammatory markers (ESR or CRP) was investigated in this study. On the other hand, no statistically significant variations in serum vaspin levels were found in children with juvenile idiopathic arthritis and active joints compared with those without active joints, and no correlation was revealed between serum levels of vaspin and the existence or the number of active joints (86). Moreover, reduced levels of vaspin were associated with endothelial dysfunction in ankylosing spondylitis patients (87). However, there are few research investigating the role of vaspin in rheumatic diseases.

CONCLUSION

The most significant lines of experimental and clinical evidence regarding the relationship between vaspin and the molecular and cellular pathogenic aspects of RA and OA were discussed in this review. However, because of the pleiotropic and immunomodulatory effects of vaspin at both systemic and local levels, studying its role in the development and exaggeration of RA and OA is complex with conflicting findings. Interestingly, the relationship between rheumatic disorder progression/activity and vaspin levels in the synovial fluid may disappear when serum levels are considered. Accordingly, a significant correlation was shown between the progression of arthritis in people at risk for RA and serum levels of vaspin, indicating the complexity of evaluating its potential as a diagnostic biomarker (77). In this regard, the increased levels of vaspin in the synovium are likely to be due to the activation of joint-resident cells affected by rheumatic disorders, proposing that antagonizing local vaspin may be a potential approach for developing novel treatment techniques. However, further study is needed to specify the role of the vaspin in rheumatic diseases, as well as to determine the possibility of using vaspin as a potential prognostic or diagnostic biomarker for RA and OA.

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Authors' contributions

ZHF and JAM contributed to study concept, study design and data collection. ZHF and JAM contributed in data analysis and interpretation. ZHF, BA and MHA did the literature review and critically reviewed the manuscript. All the authors contributed equally and approved the final manuscript.

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Conflict of interest

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