Inhibition on JAK-STAT3 Signaling Transduction Cascade Is Taken by Bioactive Peptide Alpha-S2 Casein Protein from Goat Ethawah Breed Milk

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ABSTRACT

Background: RA is a systemic inflammatory disease that causes developing comorbidity conditions. This condition can cause by overproduction of pro-inflammatory cytokine. In a previous study, we have found bioactive peptide CSN1S2 from Ethawah goat milk for anti-inflammatory for repair the ileum destruction. However, the signaling transduction cascade of bioactive peptides inhibits inflammation still not clear yet. Therefore, we analyzed the signaling transduction cascade via JAK-STAT3 pathway by in vivo and in silico. Methods: The ileum was isolated DNA and amplification with specific primer. In vivo study was explained that the CSN1S2 protein act as an inhibitor of STAT3 that cause inflammation comorbidity condition on RA (3). According to Rohmah et al., 2015 (4), the RA condition was pro-inflammatory cytokine. In a previous study, we have found bioactive peptide CSN1S2 from Ethawah goat milk for anti-inflammatory for repair the ileum destruction (4) and reduce the pro-inflammatory cytokine in synovial rheumatoid arthritis rat (11). In vitro study shows that the CSN1S2 protein of goat milk can increase proliferation MC3T3E1 pre-osteoblast cells due to methylglyoxal exposure (12). Beside that, the modeling biological study was explained that the CSN1S2 protein act as an inhibitor of AGEs-RAGE interaction at cellular level (13). We predict that bioactive peptides may act as reducing agent of inflammation via JAK-STAT3 signal transduction cascade at the cellular level.

Key words: Goat milk Casein, Ileum, Inflammation, Rheumatoid Arthritis, STAT3.

1. INTRODUCTION

Rheumatoid arthritis (RA) is systemic inflammatory diseases characterized by inflammation at joint synovial (1). Several cases show that this disease can increase developing comorbidity conditions caused by high level inflammatory substance (2). In addition, the increasing self-reactive antibodies and pro-inflammatory T-lymphocytes was contributed the gut inflammation comorbidity condition on RA (3). According to Rohmah et al., 2015 (4), the RA condition was pro-inflammatory cytokine level increasing that influence of villi ileum destruction. However, the mechanisms of intestinal inflammation in RA are still unknown and need further research.

Signal transducer and activator of transcription 3 (STAT3) proteins is a transcription factor which plays a key regulatory in various cytokine-controlled cellular processes such as immune responses, differentiation, proliferation, and cell survival (5). The activation and expression of STAT3 on autoimmune disease has been well studied. The activated STAT3 was found in inflamed tissues histology also appeared the total STAT3 protein increasing compared with non-inflammatory control cell (6,7).

Functional food may provide nutritional benefits that have several active components (8). Goat milk is one of functional food has play important role for healthy, nutrition also therapeutic effects of neonates and adults. Goat milk was contrib-

ut for reduction gastrointestinal disorder and chronic disease risks (9). Recent study, we found the eight peptides of goat Ethawah breed milk CSN1S2 protein that suggest have multiple functions (10). In vivo study was explained that the CSN1S2 protein act as an anti-inflammatory agent that could repair the villi ileum destruction (4) and reduce the pro-inflammatory cytokine in synovial rheumatoid arthritis rat (11). In vitro study shows that the CSN1S2 protein of goat milk can increase proliferation MC3T3E1 pre-osteoblast cells due to methylglyoxal exposure (12). Beside that, the modeling biological study was explained that the CSN1S2 protein act as an inhibitor of AGEs-RAGE interaction at cellular level (13). We predict that bioactive peptides may act as reducing agent of inflammation on RA via STAT3 signaling transduction. Therefore, we analyzed the role of STAT3 mechanism and to design modeling structure the bioactive peptide against STAT3 that cause inflammation on ileum RA.

2. MATERIAL AND METHODS

2.1. Isolation of CSN1S2

Milk and yogurt was taken from Ethawah breed goat milk, at UPTD Indonesian local goat, and Singsosari, Malang.
tion of milk and yogurt goat CSN1S2 protein was performed according to the previous study (12) with some modifications.

2.2. Experimental Animals

The animal condition and handling was performed according to the previous study (11) with some modifications. All rats were grouped into normal rats group (N), normal rats group RA treated with milk CSN1S2 protein (NM), normal rats group RA treated with yogurt CSN1S2 protein (NY), CFA-induced rheumatoid arthritis rats group (RA), RA rats group treated with milk CSN1S2 protein (RAM) and RA rats group treated with yogurt CSN1S2 protein (RAY).

2.3. DNA Isolation

Ileum samples from rat were isolated of DNA based on Starke et al., 2014 (14) with some modifications. Quality and quantity of DNA were measured by using NanoDrop spectrophotometer and 1% agarose gel electrophoresis then visualized with BioRad Gel Documentation.

2.4. DNA amplification

Blood DNA was amplified by primer STAT3-F-1873 & STAT3-R-2330. Our primer was designed specifically of STAT3 gene. PCR program: hot start 94°C for 1 min, denaturation 94°C for 30s, annealing 54°C for 30 s, and extension 72°C for 45 s (35 cycles), and then post extension 72°C for 7 min. PCR products were measured qualitatively by using 2% agarose gel electrophoresis. PCR products were sequenced by same primer to identified STAT3 gene.

2.5. DNA sequencing

Amplification product was purified based on Greco et al., 2014 (15) with some modifications. The sequencing was performed by The ABI 3730xl DNA Sequencer (Koeln, Germany) using Sanger sequencing method. The sequences were alignment by ClustalX software.

2.6. STAT3 protein peptide sequence retrieval

The protein sequences of NSTAT3, NSSTAT3, NYSTAT3, RASTAT3, RASSTAT3, and RAYSTAT3 was taken from DNA sequencing. The DNA sequence was translated into protein using Bioedit v.7.2.5. The peptide sequence fragments of caprine milk CSN1S2 protein was isolated and identified by MALDI-TOF(10).

2.7. Protein modeling 3D-structure Preparation

Modeling 3D-structure of PepT1; NSTAT3, NSSTAT3, NYSTAT3, RASTAT3, RASSTAT3, and RAYSTAT3 and peptide sequence fragments of caprine milk CSN1S2 protein were predicted by SWISS-MODEL web server by homology modeling method (16,17,18,19).

2.8. Docking of Bioactive peptide–Protein interaction and their Visualization

To analyze the virtual interaction among PePT1 and peptide segment fragment of caprine milk CSN1S2 protein; NSTAT3, NSSTAT3, NYSTAT3, RASTAT3, RASSTAT3, RAYSTAT3 and peptide sequence fragments using Cluspro 2.0 (20, 21, 22) and Patchdock (23, 24). Interaction visualization among them was showed off by Pymol and Discovery Studio 4.0 as proper.

2.9. Analysis for Binding interaction and Binding Energy

The type of binding among receptor, protein, peptide and other ligand was identified using Cluspro 2.0 (20, 21, 22) and Patchdock such as amino acids residue; atoms belong to
the protein and ligand and also type of hydrogen bonds, van der Waals contacts and covalent bonds. The binding energy of their interaction was calculated by Cluspro 2.0.

2.10. Ethics

This study has been evaluated and approved by the research ethics committee of Faculty of Sciences, Universitas Brawijaya, Malang, East Java, Indonesia (Registration number, KEP-90-UB).

3. RESULT

3.1. STAT3 gene analysis

The SH-2 and transactivation domain of STAT3 protein is an activation region to phosphorylation when interact with another protein. To identify any mutation in this region, we were observing the nucleic acid sequences of STAT3 gene (480bp) on N, NS, RA, and RAS (Figure 1a). The alignment of sequencing product was shown that normal (N) group, normal treating-CSN1S2 milk (NS) have similarity compared with STAT3 gene NM_012747.21 from Genbank (Figure 1b). Otherwise, RA group had transversion mutation that the purine change into pyrimidine (2111G into 2111C, 2112T into 2112A, 2113C into 2113G, 2114T into 2114A, 2115G into 2115C, 2116T into 2116A, 2117A into 2117T, 2118G into 2118C, 2119A into 2119T, 2120A into 2120T). This transversion mutation induced the amino acid residues in normal (Leu into Asp, Asp into Ile, Asn into Gln) also changed into Asp-Ile-Phe. Interestingly, we found that the STAT3 gene was appearance normally of the Rat group after inducing with caprine milk CSN1S2 protein, the nucleic acid sequence mutation (2111G-C-A-G-A-C-A-T-C-T-T-C-T-T(2120)) reverses into the normal nucleic acid sequence (2111G-T-C-T-G-T-A-G-A-A(2120)). The STAT3 protein also displayed the reversibility of amino acid sequence residue (Leu into Asp, Asp into Ile, Asn into Phe) (Figure 1B).

3.2. Virtual docking PepT1 and peptide sequence fragment of caprine milk CSN1S2 protein interaction

The possibility interactions of PepT1 and peptide sequence fragments of caprine milk CSN1S2 protein were shown in Figure 2. The sequence fragment can interact with PepT1 just only three fragments. These fragments are PepT1-41-NMAIHPR-47; PepT1-182-KISQYYQK-189 and PepT1-214-TNAIPYVR-221. Total energy binding was shown in Table 1. The energy binding between PepT1 and caprine milk CSN1S2 peptide fragment

Table 1. Interaction and total energy binding of PepT1 and caprine milk CSN1S2 peptide fragment.

| No. | Interaction Point | Interaction Donor | Acceptor Atom | Type | Chemistry Bond | Energy binding |
|-----|------------------|------------------|--------------|------|----------------|---------------|
| 1   | THR116-GLN355    | THR116:H         | GLN355:O     | Hydrogen Bond | Hydrogen Bond | -630.20 kJ/mol |
| 2   | THR440-ARG447    | THR440:H         | ARG447:O     | Hydrogen Bond | Hydrogen Bond | -640.20 kJ/mol |
|     | ASN576-THR574    | ASN576:H         | THR574:O     | Hydrogen Bond | Hydrogen Bond | -650.20 kJ/mol |
|     | VAL581-THR580    | VAL581:H         | THR580:O     | Hydrogen Bond | Hydrogen Bond | -660.20 kJ/mol |
|     | ASN582-THR580    | ASN582:H         | THR580:O     | Hydrogen Bond | Hydrogen Bond | -670.20 kJ/mol |
|     | ILE183-ASP645    | ILE183:H         | ASP645:O     | Hydrogen Bond | Hydrogen Bond | -680.20 kJ/mol |
|     | LYS182-ASP645    | LYS182:H         | ASP645:O     | Hydrogen Bond | Hydrogen Bond | -690.20 kJ/mol |
|     | GLN188-SER114    | GLN188:H         | SER114:O     | Hydrogen Bond | Hydrogen Bond | -700.20 kJ/mol |

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ACTA INFORM MED. 2015 AUG 23(4): 233-238 / ORIGINAL PAPER
Inhibition on JAK-STAT3 Signaling Transduction Cascade Is Taken by Bioactive Peptide Alpha-S2 Casein Protein

| No. | Interaction                  | Point Interaction | Donor Atom | Acceptor Atom | Type       | Chemistry | Bond          | Energy binding |
|-----|------------------------------|-------------------|------------|---------------|------------|-----------|---------------|----------------|
| 1   | LEU 757- SER 755             | LEU 757 N         | SER 755 O  | Hydrogen Bond | Hydrogen Bond |
|     | SER 186- GLU 744             | SER 186 N         | GLU 744 O  | Hydrogen Bond | Hydrogen Bond |
|     | SER 186- GLU 744             | SER 186 O         | GLU 744 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- PRO 272             | GLN 185 N         | PRO 272 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- PRO 272             | GLN 185 O         | PRO 272 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- XYS 182             | GLN 185 N         | XYS 182 O  | Hydrogen Bond | Hydrogen Bond |
|     | TRV 116- XYS 182             | TRV 116 N         | XYS 182 O  | Hydrogen Bond | Hydrogen Bond |
|     | TRV 116- XYS 182             | TRV 116 N         | XYS 182 O  | Hydrogen Bond | Hydrogen Bond |
|     | XYS 110- TRV 116             | XYS 110 N         | TRV 116 O  | Hydrogen Bond | Hydrogen Bond |
|     | XYS 110- TRV 116             | XYS 110 N         | TRV 116 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- ALA 279             | GLN 185 N         | ALA 279 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- ALA 279             | GLN 185 O         | ALA 279 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- ALA 279             | GLN 185 N         | ALA 279 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- ALA 279             | GLN 185 O         | ALA 279 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- ALA 279             | GLN 185 N         | ALA 279 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- ALA 279             | GLN 185 O         | ALA 279 O  | Hydrogen Bond | Hydrogen Bond |
|     | LEU 757- SER 755             | LEU 757 N         | SER 755 O  | Hydrogen Bond | Hydrogen Bond |
|     | SER 186- GLU 744             | SER 186 N         | GLU 744 O  | Hydrogen Bond | Hydrogen Bond |
|     | SER 186- GLU 744             | SER 186 O         | GLU 744 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- PRO 272             | GLN 185 N         | PRO 272 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- PRO 272             | GLN 185 O         | PRO 272 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- XYS 182             | GLN 185 N         | XYS 182 O  | Hydrogen Bond | Hydrogen Bond |
|     | TRV 116- XYS 182             | TRV 116 N         | XYS 182 O  | Hydrogen Bond | Hydrogen Bond |
|     | TRV 116- XYS 182             | TRV 116 N         | XYS 182 O  | Hydrogen Bond | Hydrogen Bond |
|     | XYS 110- TRV 116             | XYS 110 N         | TRV 116 O  | Hydrogen Bond | Hydrogen Bond |
|     | XYS 110- TRV 116             | XYS 110 N         | TRV 116 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- ALA 279             | GLN 185 N         | ALA 279 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- ALA 279             | GLN 185 O         | ALA 279 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- ALA 279             | GLN 185 N         | ALA 279 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- ALA 279             | GLN 185 O         | ALA 279 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- ALA 279             | GLN 185 N         | ALA 279 O  | Hydrogen Bond | Hydrogen Bond |
|     | GLN 185- ALA 279             | GLN 185 O         | ALA 279 O  | Hydrogen Bond | Hydrogen Bond |

Table 2. Interaction and total energy binding of caprine CSN1S2 peptide and STAT3 protein.
3.3. Virtual docking peptide sequence fragment of caprine milk CSN1S2 protein and STAT3 interaction

The interaction of peptide sequence fragments of caprine milk CSN1S2 protein that enters to the small intestine and STAT3 protein was identified on Figure 3 and Table 2. The fragment which interacts with STAT3 is only peptide fragment number 182-KISQYYQK-189. The energy binding of NSTAT3- f182-KISQYYQK-189 and NSSTAT3- f182-KISQYYQK-189 were Σ = -184.07 kJ/mol. Whereas, the energy binding of RASTAT3 was Σ = -402.43 kJ/mol and RASSTAT3 have the lowest energy binding was Σ = -407.09 kJ/mol. The point interactions of NSTAT3 and f182-KISQYYQK-189 are SER184- GLU744; GLN185- PRO727; GLN185- PRO728; TYR187- GLN733; GLN188- ALA729; LYS189-SER755. Whereas the interaction between NS-STAT3 amino acid and f182-KISQYYQK-189 are SER184-GLU744; GLN185- PRO727; GLN185- PRO728; TYR187-GLN733; GLN188- ALA729 and LYS189-SER755. Different point interaction of RASTAT3 amino acid and f182-KISQYYQK-189 are ALA743-GLN185; LEU744-GLN182; GLN747- TYR186; GLN747- GLN188; GLN747- LYS189; GLN748- GLN185; GLN748- GLN188; GLN185- LYS742; GLN185- ALA743; TYR186- GLN747; GLN187- GLU749; LYS189- GLN747; LYS189- LEU744; LYS189-SER745. Conversely, when f182-KISQYYQK-189 interact with RASTAT3, the interaction at the CYS726- GLN185; ALA729- SER184; GLN747- GLN188; SER751- GLN731; GLN185- PRO727; GLN185- PRO728; TYR186- SER753; CYS726-GLN185; ALA729- SER184; GLN747- GLN188; GLN185- PRO727; GLN185- PRO728; TYR186- SER753; GLN188- ALA729; LYS189- SER755.

4. DISCUSSION

This study revealed that STAT3 gene transversion mutation (G into C: A into T) on ileum RA model show that inflammatory may lead to frameshift mutation on STAT protein (Leu into Asp, Asp into Ile, Asn into Phe). Conversely, the treating group CSN1S2 milk shows the reverse of STAT3 gene sequence became normally as proper. STAT3 protein is the treating group CSN1S2 milk shows the reverse of STAT3 transactivation activity to decline the STAT3 nuclear localization. This study predicted that the fragment 182-KISQYYQK-189 may indicate have influence different affinity when binding at STAT3 amino acid residues, it was Σ = -402.43 kJ/mol. The energy binding of f182-KISQYYQK-189 and RA-STAT3 amino acid residues is elevated into Σ = -407.09 kJ/mol. The lower negative energy shows the strong binding of ligand-receptor. CSN1S2 peptide may indicate have influence different affinity when binding at STAT3 on RA ileum.

According to Szlag et al., 2015 (32), STAT3 can be activated by tyrosine phosphorylation of Jak-tyrosine kinase family in response to variety cytokines and growth factors. Here we report that bioactive peptide f182-KISQYYQK-189 can bind at 722-755 at transactivation domain. The increase of STAT3 transactivation activity can increase nuclear localization. This study revealed that the CSN1S2 may be able to influence the transactivation activity to decline the STAT3 nuclear localization.

5. CONCLUSION

This study predicted that the fragment 182-KISQYYQK-189 of caprine milk CSN1S2 protein may act as an agent anti-inflammatory via JAK-STAT3 signal transduction cascade at the cellular level.

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CONFLICT OF INTEREST: NONE DECLARED

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Inhibition on JAK-STAT3 Signaling Transduction Cascade Is Taken by Bioactive Peptide Alpha-S2 Casein Protein

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