Exploring the anti-inflammatory effects of spinach sterols based on network pharmacology

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Abstract. Aims To investigate the anti-inflammatory mechanism of action of spinach sterol (Spinasterol) by network pharmacology and molecular docking. METHODS: Firstly, the TCMSP database was used to find the targets of action of spinasterol, and then the pubchem database and UniProt database were used to analyze and predict the mechanism of action of the targets in spinasterol. Conclusion: Spinach sterols achieve anti-inflammatory effects through three mechanisms of action: modulation of immune molecular mediators, activation of immune cells and direct action on inflammatory cells.

Keywords: Cyber pharmacology; Spinach sterols; Anti-inflammatory; Molecular docking.

1. Introduction

Inflammation is a response of the body to pathogenic factors and their damaging effects, leading to cell degeneration, necrosis and abnormal metabolic functions. Currently, antibacterial and antiviral drugs are often used for clinical treatment, but these drugs are prone to resistance with long-term use. Therefore, there is an urgent need to screen for new anti-inflammatory drugs. [1][2]

Spinach sterols are in the steroid group phytosterin; phytosterol phytostearin class. and vitamin D family similar in structure. It is widely found in a variety of plants and is plant cell important component of the Often found in plant seeds or pollen together with oils and can also be the corresponding pau sterol glycosides.

Figure 1. Spinasterol spinach sterols

Network pharmacology is based on the similarity between drugs and medicines in terms of structure and efficacy, and takes into account the multiple interactions of target molecules and biological effect molecules in the organism. By constructing drug-drug, drug-target, target-disease and drug-disease networks, network pharmacology is combined with metabolic pathway networks to achieve the connection of active ingredient networks, pathological mechanism networks and endogenous metabolic networks in Chinese medicine, which provides new ideas and tools for the discovery of active ingredients, pharmacodynamic mechanisms and the development and optimization of formulations in modern Chinese medicine.

Previous studies have identified the anti-inflammatory effects of spinach sterols, which will be further analysed in this paper using network pharmacology.[2]
2. Database and software

Table 1. Databases and software used

| No | Name       | Website                                           | Uses                                           |
|----|------------|--------------------------------------------------|------------------------------------------------|
| 1  | PubChem    | https://pubchem.ncbi.nlm.nih.gov/                | Compound composition information gathering     |
| 2  | TCMSP      | http://ibts.hkbu.edu.hk/LSP/tcmsp.php             | Find substances and their targets of action in Zhi Mu |
| 3  | SwissTargetPrediction | http://www.swisstargetprediction.ch/ | Component target prediction                     |
| 4  | UniProt    | http://www.uniprot.org/                          | Screening for human origin genes, correction of standard gene names |

3. Target screening results

Through the traditional chinese medicine systems pharmacology database and analysis platform (TCMSP, http://tcmspw.com/tcmsp.php), the spinach Sterols were used for target search to obtain individual targets of action.

Table 2. Targets of action of spinach sterols

| Target name                                      | Source   |
|--------------------------------------------------|----------|
| Progesterone receptor                           | DrugBank |
| Mineralocorticoid receptor                      | DrugBank |
| Nuclear receptor coactivator 2                  | DrugBank |

4. Find biochemical responses related to inflammation based on target of action

The UniPort human target proteins and genes are then screened for each target.[5]

4.1 Progesterone receptor (Progesterone receptor)

Table 3. Progesterone receptor UniPort information for

| Entry     | Entry name     | Protein names     | Gene names       | Organism                | Length |
|-----------|----------------|-------------------|------------------|-------------------------|--------|
| P06401    | PRGR_HUMAN     | Progesterone receptor | PGR NR3C3       | Homo sapiens (Human)    | 933    |

Figure 2. Progesterone receptor

As can be seen from Table 3 Progesterone receptor is a protein of 933 units in length, corresponding to the gene name PGR NR3C3.

Progesterone receptor has a variety of identified functions, among which biochemical reactions related to anti-inflammatory effects include
4.1.1 Progesterone receptor Involved in the negative regulation of gene expression. (GO: 0010629)

Figure 3. GO: 0010629

GO: 0010629 is any process that reduces the frequency, rate or extent of gene expression. Gene expression is the process of converting the coding sequence of a gene into a mature gene product (protein or RNA).

The sub-biochemical reaction most closely associated with the anti-inflammatory effect during GO:0010629 is the negative regulation of molecular mediator production of the immune response (GO:0002701).

Figure 4. GO:0002701

GO:0002701 A process that negatively regulates a variety of lysozyme and immune cells, resulting in the cessation, prevention or reduction of the frequency, rate or extent of production of molecular mediators of the immune response.[3]

The suppression of this response increases the frequency of immune response factor production, which in turn improves immunity to achieve an anti-inflammatory result.
4.1.2 Progesterone receptor Involved in the positive regulation of gene expression (GO: 0010628)

**Figure 5. GO: 0010628**

GO: 0010628 is any process that increases the frequency, rate or extent of gene expression. Gene expression is the process of converting the coding sequence of a gene into a mature gene product (protein or RNA).

The sub-biochemical reaction most closely associated with the anti-inflammatory effect in the GO:0010628 process is the positive regulation of the production of molecular mediators of the immune response (GO:0002702).

**Figure 6. GO:0002702**

GO:0002702 is any process that activates or increases the frequency, rate, or degree of production of molecular mediators of the immune response.[3] In this reaction it is possible to upregulate the production of a variety of immune response factors, thereby enhancing immunity to achieve an anti-inflammatory effect.
4.2 Mineralocorticoid receptor (Salicorticoid receptor)

**Table 4.** Mineralocorticoid receptor UniPort information for

| Entry  | Entry names | Protein names            | Gene names          | Organism            | Length |
|--------|-------------|--------------------------|---------------------|---------------------|--------|
| P08235 | MCR_HUMAN   | Mineralocorticoid receptor | NR3C2 MCR, MLR      | Homo sapiens (Human) | 984    |

**Figure 7.** Mineralocorticoid receptor

As can be seen from Table 4, Mineralocorticoid receptor is a protein of 984 units in length, corresponding to the gene name NR3C2 MCR, MLR.

Mineralocorticoid receptor There are a variety of identified functions, among which biochemical responses related to anti-inflammatory effects include

3.2.1 Mineralocorticoid receptor Involved in the positive regulation of NIK/NF-kappaB signalling (GO: 1901224).

**Figure 8.** GO: 1901224

GO: 1901224 is any process that activates or increases the frequency, rate or range of the NIK/NF-kappaB signal.

The GO:1901224 process contains a variety of biochemical reactions that are closely linked to the anti-inflammatory effect.

3.2.1.1 Positive regulation of multiple immune factors (including but not limited to interleukin-8, interleukin-6[3], interleukin-1, interleukin-12, interferon alpha, interferon beta).

3.2.1.2 Positive regulation of cytokine production that acts directly on the inflammatory response (GO.0050729, GO: 1900017).
GO.0050729 For any process that activates or increases the frequency, rate, or degree of an inflammatory response.

Figure 9. GO.0050729

GO: 1900017 For any process that activates or increases the frequency, rate or degree of production of cytokines involved in an inflammatory response.

Figure 10. GO: 1900017

3.2.1.3 Activation of multiple immune cells (GO: 0002281).
GO: 0002281 Process for the activation of macrophages involved in the immune response.
Figure 11. GO: 0002281

4.3 Nuclear receptor coactivator 2 (Nuclear receptor coactivator 2)

Table 5. Nuclear receptor coactivator 2 UniPort information

| Entry  | Entry name   | Protein names                  | Gene names              | Organism         | Length |
|--------|--------------|--------------------------------|-------------------------|------------------|--------|
| Q15596 | NCOA2_HUMAN  | Nuclear receptor coactivator 2 | NCOA2 BHLHE75, SRC2, TIF2 | Homo sapiens (Human) | 1,464  |

Figure 12. Nuclear receptor coactivator 2

Because the protein is not significantly associated with inflammation, it will not be discussed.

5. Predictive analysis of the anti-inflammatory mechanism of action of spinach sterols

As can be seen from the above diagram, the targets of action of spinach sterols related to anti-inflammation fall into three main categories.

The first type of mechanism of action is to regulate immune molecular mediators to promote the production of immune cells and improve immunity to achieve anti-inflammatory effects. Represented by GO:0002702 by activating or increasing the frequency, rate or extent of production of molecular mediators of the immune response. It leads to an increase in a variety of immune response factors, which in turn improves immunity to achieve an anti-inflammatory effect.

The second type of mechanism of action is the activation of immune cells and the activation of more immune cells.
This is an anti-inflammatory agent that works by directly activating a specific immune cell.

The third category is the direct action on inflammatory cells to achieve an anti-inflammatory effect. For example, a spinach sterol can significantly inhibit the synthesis or release of inflammatory mediators such as PGE2 and bradykinin, and has an inflammatory effect on inflammatory mediators such as PGE2, bradykinin, histamine, and 5-HT.[4]

As GO: 1900017, the anti-inflammatory effect is achieved by directly reducing the frequency, effect, and degree of inflammation.

In summary, the results of this study provide further support for previous studies and provide some reference for future clinical studies, and it is also hoped that subsequent studies will make up for the shortcomings of this study and produce more valuable results.

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