Inhibition of NF-κB Pathway as the Therapeutic Potential of Red Fruit (Pandanus conoideus Lam.) in the Treatment of Inflammatory Bowel Disease

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Abstract
NF-κB is a transcription factor that regulates the inflammatory response, immune system, cellular proliferation and apoptosis. The link between NF-κB and inflammation has been shown in some studies of various human diseases and animal models. The activation pathway of NF-κB has been identified; therefore, inhibition of NF-κB is considered to be an effective therapeutic alternative in inflammatory diseases and cancer. Ulcerative colitis (UC) is a form of inflammatory bowel disease (IBD) characterized by chronic inflammation, which is also a high predisposition to colorectal cancer. The activation of NF-κB within the colonic epithelial cells promotes inflammation in UC by increasing the production of pro-inflammatory cytokines, such as IL-1 and TNF-α. NF-κB also directly activates pro-survival pathway, which causes neo-plastic transformation, increases the expression of COX-2 induced by IL-1 and TNF-α, and promotes carcinogenesis. Currently, red fruit (Pandanus conoideus Lam.) has been considered to be an alternative therapy of IBD, and it is proposed that red fruit could reduce the inflammation by inhibiting the activity of NF-κB. Previous studies have proven that red fruit has the capacity to increase the proliferation of immune cells. Increased lymphocyte proliferation might induce the production of anti-inflammatory cytokines, such as IL-10 and IL-22, which subsequently inhibit the activation of NF-κB, COX-2, and the production of pro-inflammatory cytokines.

Keywords: NF-κB, red fruit, inflammation, ulcerative colitis.

Background
Nuclear factor κB (NF-κB) is a transcription factor that serves as an important regulator of the immune system, inflammatory response, cellular proliferation and apoptosis. The role of NF-κB in inflammation has been shown in some studies of various human diseases such as cancer, asthma, rheumatoid arthritis, atherosclerosis, and inflammatory bowel disease (IBD). NF-κB promotes inflammation by inducing transcription of pro-inflammatory cytokines, cytokine receptors, chemokines, adhesion molecules, and enzymes such as matrix metalloproteinases (MMPs), cyclooxygenase-2 (COX-2), and inducible nitric oxide (iNOS). The activation pathway of NF-κB and the factors regulating it have been recognized. For this reason, there is a considerable interest in inhibiting NF-κB activity for therapeutic purpose. However, the mechanisms responsible for the termination of NF-κB will not only affect the inflammatory response but will also interfere with the physiologic function of NF-κB, resulting in side effects. Nowadays, new regulators of NF-κB have been discovered,
providing more selective choices in inhibiting NF-kB activity.

The best example of inflammatory disease is ulcerative colitis (UC). This disease (UC) and Crohn disease (CD) have become the major forms of IBD. Mucosal biopsies from IBD patients show an increased activity of NF-kB.\(^3\) In IBD, besides promoting inflammation, NF-kB also has a potential role in developing carcinogenesis, which may result in colorectal cancer (CRC). Therefore, the inhibition of NF-kB may reduce inflammation and prevent carcinogenesis in IBD.

Red fruit (Pandanus conoideus Lam.) is a kind of fruit from Papua, Indonesia. Red fruit is a source of exogenous antioxidant, which contains a high amount of beta-carotene and tocopherol.\(^4\) In Papua, red fruit traditionally has been widely used to increase energy and immune system. Nowadays, red fruit is also used as anti-inflammatory and anticancer agents in several diseases. Recently, several studies about red fruit have been seeding a new insight in its potential therapeutic effect for UC in animal models, but the therapeutic mechanism is still unclear.\(^5,6\) It is proposed that red fruit could reduce the inflammation by inhibiting NF-kB activity.

**Inflammatory Bowel Disease (IBD)**

Inflammatory bowel disease (IBD) is a chronic inflammation caused by immune system dysregulation, genetic susceptibility, and stimulation by intestinal floras.\(^7\) IBD affects approximately 1.4 million people in the United States, with an estimate annual cost exceeding $2 billion.\(^3\) IBD mainly consists of two forms, Crohn disease (CD) and ulcerative colitis (UC).\(^8\)

UC is characterized by mucosal damage and ulceration, which begins in the rectum and spreads proximally. UC in animal models could be induced by an oral administration of dextran sulfate sodium (DSS), which may cause direct toxicity to the colonic epithelial cells, increase cell permeability and activate macrophages.\(^9\) Colitis in animal models induced by an administration of oral DSS has the same appearance as UC in humans.\(^10-12\) The administration of azoxymethane (AOM) combined with DSS to animal models will increase the incidence of colorectal cancer.\(^8,13\)

**The Role of NF-kB in IBD**

NF-kB activation within the colonic epithelial may increase the expression of pro-inflammatory cytokines, such as IL-1 and TNF-\(\alpha\). The expression of IL-1 will increase the proliferation of immune cells, which then produce IL-6, resulting in neo-plastic changes.\(^3,14\)

The increased expression of COX-2 is also found in colorectal cancer. COX-2 will convert arachidonic acid into prostaglandin (PGE\(_2\)), which promotes angiogenesis tumor. The administration of TNF-\(\alpha\) antagonist may reduce the expression of COX-2 in experimental animal models of colitis. On the contrary, the activation of NF-kB will increase the production of TNF-\(\alpha\), which will also increase the expression of COX-2.\(^15-18\)

IL-10 is an anti-inflammatory cytokine that suppresses the activation of NF-kB, which will inhibit the production of pro-inflammatory cytokines, such as IL-1 and IL-6.\(^19,20\) IL-22 is a super family member of IL-10, which is expressed by Th17 cell. IL-22
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plays an important role in inducing the expression of regulatory molecules, such as IL-10 and inflammatory molecules, such as IL-18. The administration of antibody anti-IL-22 may reduce the severity of colitis in experimental animal models.21
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Figure 1. The Role of NF-κB in Inflammatory Bowel Disease. Injury to the intestinal epithelium can result in DNA damage and altered gene expression and function. In addition, this is accompanied by activation of NF-κB within the epithelial cells, which promotes prosurvival pathways that are required for the initial growth of resulting neoplastic cells. NF-κB activation also promotes proinflammatory genes expression. TNF-α originating from the mucosa of possibly the epithelium itself participates in the activation of immune cells. Production of proinflammatory factors by the activated immune system participates in the ensuing inflammatory response but additionally plays a role in tumor growth by providing trophic signals to the early neoplastic lesions.

Red Fruit

Red fruit (Pandanus conoideus Lam.) is also known as buah merah, tawii, or sauk ekendi. The fruit, which is a source of exogenous antioxidant, contains carotenoid, 7,000 ppm of beta-carotene, 11,000 ppm of α-tocopherol, oleic acid, linoleic acid, decanoic acid, omega-3, and omega-9. The 7.000 ppm of beta-carotene and 11.000 ppm of tocopherol in red fruit are considered to be a high amount of antioxidant.

The antioxidant in red fruit plays an important role in the reduction of free radicals produced in inflammation. The antioxidant also increases splenocyte proliferation, antibody production, spleen and thymus sizes. The administration of beta-carotene 30 mg/day for 2-3 months will increase the number of immune cells, such as T-lymphocyte and natural killer (NK) cell. In addition, red fruit contains β-cryptoxanthin, which is used in lung cancer therapy.

Red Fruit as a Treatment of IBD

Several studies have showed that dysregulation of the immune system and
lymphocytes is important in the pathogenesis of UC induced by DSS. The pin point of the previous research indicates that the administration of red fruit 0.1 ml/day for 15 days will increase the proliferation of lymphocyte and decrease IFN-γ level in Listeria monocytogenes–inoculated mice. Moreover, the administration of red fruit 0.1 ml/day for 3 weeks will decrease the severity of colitis, which is indicated by decreased clinical scores and increased proliferation of lymphocyte in colitis-induced mice by DSS.

The increased proliferation of lymphocytes might increase the production of IL-10 and IL-22, which may inhibit the activation of NF-κB and the production of IL-1 and COX-2, and finally reduce the inflammation. The production of IL-10 and IL-22 is also expected to increase mucus production in colonic epithelial, which will prevent the development of chronic inflammation towards cancer. The suppression of NF-κB activation is marked by the decreasing expression of IκB protein.

**Activation of NF-κB**

The mammalian NF-κB family consists of p50 (NF-κB1), p52 (NF-κB2), p65 (RELA), RELB, and REL (cRel), which all share an amino terminal REL homology domain (RHD). Moreover, the RHD mediates the DNA binding, dimerization, and nuclear transport of the NF-κB proteins. Most members of this family can homodimerize as well as form heterodimers with each other. On the other hand, NF-κB family members REL, RELB, and p65 contain a transactivation domain (TAD), which is necessary for gene induction. By contrast, p50 and p52, which are derived from the inactive precursor p105 and p100 respectively, lack of TAD. Therefore, in order to promote transcription, p50 and p52 have to form heterodimers with other TAD-containing proteins, because as homodimers, p50 and p52 will repress transcription when bound to κB-site. The most prevalent activated form of NF-κB is a heterodimer, which consists of a p50 or p52 subunit and p65.

In unstimulated cells, NF-κB is sequestered in the cytoplasm by an inhibitor of NF-κB (IκB) proteins, which comprises three functional groups: (1) the typical IκB proteins IκBα, IκBβ, and IκBε; (2) precursors proteins p100 and p105; and (3) atypical IκB proteins IκBζ, BCL-3 (B-cell Lymphoma 3) and IκBNS.

Following cell stimulation, IκB undergoes degradation by IκB Kinase (IKK) complex, which consists of two homologous kinase subunits IKKα, and IKKβ, and a regulatory subunit IKKγ (NEMO). After the degradation of IκB, free dimers of NF-κB translocate to the nucleus and bind to target genes, the transcription of which is regulated through transcriptional co-activators and co-repressors.
Figure 2. Activation of NF-κB. An inflammatory stimulus (TNF-α, IL-1, LPS, free radicals, etc) activates signal transduction pathways that induce the activation of the IKK complex. This results in the phosphorylation of IκB proteins and consequently their degradation by the proteasome. Released NF-κB dimers translocate to the nucleus and bind the κB sites in the promoters or enhancers of target genes, which leads to their transcription.¹

Possible Mechanisms of Inhibition of NF-κB using Red Fruit as IBD Treatment

Figure 3. Proposed Possible mechanisms of inhibition of NF-κB using Red Fruit. a | Red fruit may increase the proliferation of the immune cells, which increases the production on antiinflammatory cytokines IL-10 and/or IL-22. IL-10 and IL-22 then inhibit the activity of proinflammatory cytokines, TNF-α and IL-1. Inhibition of TNF-α and IL-1 inhibits the activation of NF-κB and expression of COX-2, preventing neoplastic transformation of colonic epithelium. b | Red fruit may directly inhibit the expression of COX-2. c | Red fruit may directly inhibit the activation of NF-κB. d | Red fruit may prevent DNA damage and altered gene expression and function in colonic epithelium. RF: Red Fruit.
Conclusion

Red Fruit (*Pandanus conoideus* Lam.) could be used as antiinflammatory and anticancer agents in inflammatory bowel disease, particularly in ulcerative colitis. The antioxidant in red fruit performs its function either by (1) directly inhibiting the activation of NF-κB, (2) preventing DNA damage and altering gene(s) expression, (3) directly inhibiting expression of COX-2, or (4) increasing the production of anti-inflammatory cytokines, such as IL-10 and IL-22, which suppress the activity of proinflammatory cytokines, such as TNF-α and IL-1.

Suggestion

Further research should be addressed to investigate and clarify this potential use of red fruit in inhibiting ulcerative colitis by repressing NF-κB activity.

References

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