Inhibitory effect of kaempferol on skin fibrosis in systemic sclerosis by the suppression of oxidative stress

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Role of Oxidative stress in the pathogenesis of systemic sclerosis

Systemic sclerosis (SSc) is a connective tissue disorder characterized by the development of fibrosis in the skin and internal organs, vascular disorders, and autoimmunity.

The pathogenesis of SSc remains unknown....

It has been proposed that oxidative stress plays an important role in disease development.

- **Edaravone**, a free radical scavenger, has a significant inhibitory effect on fibrosis both in the bleomycin-induced SSc model and in tight skin mice. (Arthritis Rheum 2011;63:3086)
- **Selective NOX4 (major oxidative stress factor) inhibitor** exerted a highly effective anti-fibrotic effect in various animal models of tissue fibrosis. (Curr Rheumatol Rep 2015;17:473)

Kaempferol

Natural flavonol widely found in various vegetables, which has been reported to have excellent **anti-oxidant activity**.

kaempferol have a wide range of beneficial pharmacological activities.

Anti-oxidant defense against free radicals
(Free Radic Res 2002; 36; 1199)

• **Anti-cancer**
Inhibits cancer cell growth and angiogenesis and induces cancer cell apoptosis
(Cancer res. 2004; 64: 7936)

• **Cardioprotective**

• **Anti-inflammatory**
Inhibits NF-κB
(J. Cell Physiol 2010; 225: 472)

• **Anti-diabetic**

• **Anti-allergic**

• **Anti-bacterial**

Hypothesis

Kaempferol might improve skin fibrosis in SSc patients by reducing oxidative stress ?

Objective

To elucidate the effect of kaempferol on skin fibrosis, and the mechanism of the inhibitory action of fibrosis by kaempferol.

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The effect of kaempferol on bleomycin-induced skin fibrosis in mice

- Bleomycin induced dermal thickness inhibited by kaempferol.
- The collagen amount were increased by bleomycin and decreased by kaempferol treatment.
- The number of CD68⁺ macrophages and CD3⁺ T cells in the skin were increased by bleomycin and reduced by kaempferol treatment.

Kaempferol might have inhibitory regulation of skin fibrosis and inflammatory activity.

Investigating oxidative stress in bleomycin-induced skin fibrosis using OKD 48 transgenic mice

OKD 48 (Keap-1 dependent Oxidative stress Detector, NO-48) transgenic mice
OKD 48 mice have a transgene encoding a modified Nr1f2, which is an essential transcription factor for expression of anti-oxidative stress genes. (Oikawa D, et al. Sci Rep 2012)

Kaempferol reduced bleomycin-induced oxidative stress in vivo.

The effect of kaempferol on bleomycin-induced apoptosis in vivo

The number of apoptotic cells was increased by bleomycin but kaempferol treatment decreased.

Investigating the effect of kaempferol on H2O2-induced ROS accumulation in vitro

The ROS accumulation induced by bleomycin was inhibited by kaempferol at various concentrations.

kaempferol might reduce oxidative stress and oxidative stress-induced apoptosis in vivo and vitro.

Kaempferol is also the most potent ATP receptor (P2Y₂) antagonist

- ATP binds to purinergic receptors, such as P2X and P2Y receptors, and induce gene expression.
- Kaempferol inhibits the P2Y₂ receptor.

Next

We analyzed the effect of the blockade of ATP/purinergic receptor signal by Kaempferol on bleomycin-induced skin fibrosis.

Kaempferol might inhibit ATP-induced IL-6 production mediated by P2Y₂ receptor in vivo and vitro

The effect of kaempferol on ATP-induced IL-6 production and phosphorylation of p38 in vitro

- In SSc fibroblast, IL-6 mRNA levels were increased by ATP and Kempferol significantly decreased.
- The phosphorylation of p38 were decreased by Kaempferol.

Kaempferol might inhibited ATP-induced IL-6 production mediated via P2Y₂ receptor.

The model for the regulation of bleomycin-induced skin fibrosis by Kaempferol

- Oxidative stress induced by bleomycin injection increases collagen production from fibroblast.
- Bleomycin also induces extracellular ATP, and IL-6 is induced by ATP mediated by P2Y₂ receptor and activation of p38 signaling.
- IL-6 induces collagen I production from fibroblast.

- **Kaempferol** reduces oxidative stress and inhibits collagen production.
- **Kaempferol** also inhibits ATP-induced IL-6 production, leading to decrease of collagen production.

Conclusion

- **Kaempferol might improve bleomycin-induced fibrosis by reducing oxidative stress, inflammation, and oxidative cellular damage.**
- **Kaempferol can be an alternative treatment for skin sclerosis in patients with SSc.**