Research Progress in Arecoline-induced Oral Submucous Fibrosis

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Abstract
Areca nut is a popular fruit, but is among the most common addictions following tobacco, alcohol and caffeine globally. Areca nut chewing is the major risk factor for OSF, which is a chronic, occult, and premalignant fibrotic disease. OSF is characterized by submucosal collagen accumulation and microvascular diseases, the primary microscopic manifestations of which include damaged vascular endothelial cells, increased vascular permeability, and decreased number of microvessels. As the basic structure of microvessels, endothelial cells paly an important role in the pathogenesis and progression of OSF. Arecoline is the main component of areca nut. This review summarized the machenism of arecoline-inducing OSF by acting on endothelial cells, mainly including that arecoline can promote endothelial cells inducing tissue fibrosis by acting on the vascular endothelial cells and is accomplished via multiple aspects such as inhibiting the proliferation and facilitating the apoptosis of endothelial cells, affecting their secretion of cytokines, or promoting the transformation process of EMT.

Keywords: Arecoline, OSF, Endothelial cells, Mechanism, TGF-β, EMT

Introduction

Areca nut is the dry and mature fruit of areca, predominantly planted in Taiwan, Hainan, and in other parts of China. Areca nut is regarded as a traditional ayurvedic medicine. It is used as an astringent, mouth freshener after meals; a taste enhancer, purgative and intoxicant; and for indigestion, impotence and gynecological problems, parasitic intestinal infection and for prevention of pregnancy-related morning sickness. Globally, areca nut is among the most common addictions following tobacco, alcohol and caffeine. Its chronic use contributes significantly to the high incidence of oral cancer in these countries.

Oral submucous fibrosis (OSF) is a chronic, occult, and premalignant fibrotic disease, characterized by submucosal collagen accumulation. The major clinical manifestations of OSF include irritative oral mucosal pain upon consumption of food, blanching and stiffing of the mucosa, appearance of palpable fibrotic bands, and progressive inability to open mouth as well as restricted movement. OSF is a common disease in India, Thailand, Taiwan, as well as in southern China, where the usage of areca nut is very popular. Its chronic use contributes significantly to the high incidence of oral cancer in these countries and areca nut chewing is an independent risk factor for OSF, which could increase the risk of OSF by 32 – 109.6 times [1]. The International Agency for Research on Cancer (IARC) has identified areca nut as a primary carcinogen as early as 2003 [2]. Areca nut seeds contain 0.3% - 0.6% of alkaloids, and especially Arecoline is one of the most important components [3]. The mechanism of arecoline-induced OSF is worth in-depth study.

OSF is characterized by submucosal collagen accumulation and microvascular diseases, the primary micro-

scopic manifestations of which include damaged vascular endothelial cells, increased vascular permeability, and decreased number of microvessels. As the basic structure of microvessels, endothelial cells paly an important role in the pathogenesis and progression of OSF. This review aimed to discuss the mechanism of arecoline-inducing OSF by acting on endothelial cells.

1 Cause the apoptosis of vascular endothelial cells

Yi *et al.* [4] discovered that in addition to apparent apoptosis under a fluorescence microscopevascular endothelial cells influenced by arecoline also demonstrated an increased Caspase-3 activity, the difference of which was significant, suggesting that arecoline could induce a Caspase-3 mediated apoptosis of vascular endothelial cells. In another study conducted by Yi *et al.* [5] arecoline solutions of various concentration gradients were used to perform in vitro pretreatment of human umbilical vein endothelial cells (HUVECs), and the results showed that arecoline could promote the apoptosis of HUVECs, and its effect depended on both the concentration and the intervention duration; thereby proposing an arecoline induced OSF by promoting the apoptosis of vascular endothelial cells. In a similar study by Wang *et al.* [6], the increasing arecoline concentration or intervention duration not only reduced the survival rate of HUVECs but eventually led to progressive apoptosis. Ullah M *et al.* [7] also verified the effects of arecoline in causing the cytotoxicity and apoptosis of vascular endothelial cells. Dai Z *et al.* [8] recently showed that by promoting theautophagy of HUVECs, arecoline could inhibit the proliferation and angiogenesis of vascular endothelial cells, thereby inducing OSF.

2 Promoting the inflammatory cytokine secretion of vascular endothelial cells

2.1 nuclear factor-kappa β (NF- κβ)

Nuclear factor-kappa β (NF- κβ) is a nuclear transcription factor, that exists in multiple cells. It plays a vital role in regulating the immune and inflammatory responses as well as the apoptosis of cells. NF- κβwas significantly expressed in the endothelial and inflammatory cells of OSF patients, which is barely expressed in normal human buccal mucosal fibroblasts. However, areca nut chewing can activate NF- κβ and consequently induce inflammation and further induce OSF [9]. Furthermore, Su *et al.* [10] discovered that arecoline not only facilitated the morphology transformation of fibroblasts in the lamina propria of normal oral mucosa to muscle cells but also induced oral mucosal epithelial cells secrete inflammatory factors such as NF- κβ, thereby promoting the activation of oral mucosal fibroblasts. In another study by Li *et al.* [11], arecoline was shown to induce the expression of connective tissue growth factor (CTGF) in fibroblasts, the effect of which was positively correlated with arecoline concentration and intervention duration; the expression of CTGF was also related to the activation of signaling pathways such as NF- κβ, JNK, p38, and MAPK.

2.2 Correlation with angiotensin-II (Ang-II)

Angiotensin-II (Ang-II) is an important pro-inflammatory cytokine and a primary biologically active effector produced by RAS. As a vasoconstrictor factor, it is mainly involved in physiological processes including blood vessel contraction, positive inotropic action, stimulation of aldosterone secretion, and promotion of the release of catecholamines from sympathetic nerve endings, thereby playing an essential role in maintaining blood pressure, ensuring circulating blood volume and regulating water and electrolyte balance. It has been well acknowledged that Ang-II is not only a vasoactive substance but also a multi-effect growth factor, which contributes significantly to tissue repair and fibrosis and can affect the proliferation, differentiation, and apoptosis of cells. Existing studies have verified the important effects of Ang-II, and its receptor angiotensin II type 1 receptor (AT1R) in tissue and organ fibrosis. Different mechanism of Ang-II-AT1R complex has been proposed and included: ① directly promote the secretory effect of extracellular matrix (ECM); ② positive regulate the secretion of the transforming growth factor β (TGF- β) of interstitial fibroblasts; and ③ induce tissue inflammation and recruit the infiltration of inflammatory cells such as eosinophils [12]. Luo *et al.* found that arecoline could induce the expressions of Ang-II and AT1R proteins in HaCaT cells, which predominantly occurs on cells with morphological changes, thereby speculating the role of Ang-II and AT1 in the process of arecoline-induced OSF [13]. In the in vitro HUVECs intervened by arecoline, Wang *et al.* [14] found consistent increases in the expressions of Ang-II, TGF-β1, and α-smooth muscle actin (α-SMA) proteins, suggesting that arecoline could induce the expression of Ang-II in HUVECs and demonstrated a positive correlation with Ang-II and TGF-β1. The results showed that under the intervention of arecoline, the increased expression of TGF-β1 in HUVECs regulated by Ang-II further influenced the progression of OSF. Similarly, Zhou *et al.* [15] discovered that arecoline could induce the expression of α-SMA in HUVECs, and additionally, the expression level of which was increased with the concentration of arecoline. Alternatively, as an AT1R antagonist of the Ang-II pathway, Losartan can inhibit the expression of α-SMA in HUVECs under the intervention of arecoline. Since the expression of the protein is negatively correlated with the concentration of Losartan, it can, therefore, inhibit the progression of OSF. This result implies the involvement of Ang-II in arecoline-induced OSF. Yon *et al*. [16] found that arecoline could induce the synthesis and migration of collagen through the Smad pathway, the process of which could be activated by NLPR3 inflammasome. By inhibit-

ing Ang-II, Ang-(1-7) altered the angiotensin-converting enzyme 2 (ACE2) to Ang-(1-7) axis (ACE2 / Ang-(1-7)), which led to the activations of reactive oxygen species (ROS) and NLRP3 induced by RAS imbalance. The resultant decreased capacities of collagen synthesis and migration subsequently inhibited the process of arecoline-induced OSF, suggesting the participation of Ang-(1-7) in the process.

3 Inducing OSF via endothelial to mesenchymal transition (EMT)

EMT refers to the process during which endothelial cells differentiate into mesenchymal cells under the influence of certain factors. During the process of tissue fibrosis, endothelial cells are separated from the inner vessel wall and acquire a mesenchymal phenotype. Characterized by reduced cell adhesion, the endothelial cells lose their specific antigenic markers such as vascular endothelial-cadherin (VE-cadherin) and platelet-endothelial cell adhesion molecule, transform into mesenchymal or fibroblast-like cells, and acquire the surface antigens of interstitial cells such as α-SMA and collagen [17], hence completing the process of tissue fibrosis. Multiple studies have verified the participation of EMT in the fibrosis of organs and tissues. Abnormalities in vascular endothelial cells will lead to the same in tissue repair [18].

Arecoline causes EMT mainly via the following mechanism: ① Induce the activation of TGF-β1. Tgf1 and TGF-β / Smad signaling pathway play important roles during the pathogenesis of EMT [20]. Hsieh *et al.* [21] found that arecoline could induce the activation of TGF-β1 through activating mitochondrial reactive oxygen, which further triggered the increased expressions of connective tissue growth factor (CCN2) and early growth response protein 1 (Egr-1) in human oral mucosa, thereby promoting OSF. Fang *et al.* [22] suggested that the newly-discovered g lcRNA LINC00974 played an important role in arecoline-induced OSF, as inhibiting the expression of LINC00974 could substantially decrease the expressions of α-SMA and α-1 type I collagen and fibronectin, as well as reduce the expression level of TGF-β and the phosphorylation level of Smad 2. This result indicated that arecoline induced OSF through the activation of LINC00974-mediated TGF-β signaling pathway. ② Ang-II can stimulate angiotensin I receptors and ROS and lead to the activation of TGF-β, which then actives fibrotic gene programs though Smad or non-smad dependence, thereby promoting fibroblast proliferation, leukocyte infiltration, matrix degradation, collagen deposition, and myofibroblast transdifferentiation. Pretreating HUVECs with arecoline in vitro increases the expressions of Ang-II, TGF-β1, and α-SMA. The result indicated that the increased expression of TGF-β1 in HUVECs under the intervention of arecoline regulated by Ang-II further influenced the progression of OSF [6,15]. ③ Arecoline promotes the upregulation of matrix metalloproteinase-9 (MMP9) by upregulating the expression of transcription factors such as NF-κβ, AP-1, and Ets-1 in HaCaT epithelial cells. The upregulated MMP3 then serves as one of the key enzymes for the degradation of type IV collagen, a central component of the basement membrane and promotes the progression of EMT [22]. ④ Acting on HUVECs: a study by Ma *et al.* [23] suggested that when arecoline was adopted to HUVECs, with the extension of action time, the levels of N-cadherin, Vimentin, α-SMA, and MMPs were considerably upregulated, the level of epithelial marker E-cadherin was substantially down-regulated, while the level of mesenchymal markers were increased. Therefore, they concluded that arecoline could stimulate the microfilament polymerization of HUVECs and cause EMT and collagen accumulation, thereby inducing submucosal fibrosis. Similarly, Zhou *et al.* [24] found that arecoline could stimulate the transformation of HUVECs into myofibroblasts by expressing α-SMA, which indicated that arecoline could induced the EndMT of HUVECs. Wang *et al.* [25] discovered the high expression of nuclear receptor coactivator 7 (NCOA7) in arecoline-stimulated HUVECs and the level related with arecoline concentration. Transfecting NCOA7 siRNA for 48 h could further improve the ability of arecoline induced EMT and upregulate the expression of α-SMA while downregulate the expression of E-cadherin.

4 Discussion

The above literature review has shown that as the primary factor of OSF, endothelial cells, make a substantial contribution to the induction of tissue fibrosis (Figure 1). Arecoline can further promote this role by acting on the vascular endothelial cells and is accomplished via multiple aspects such as inhibiting the proliferation and facilitating the apoptosis of endothelial cells, affecting their secretion of cytokines, or promoting the transformation process of EMT. These findings suggest that the mechanism of arecoline-induced OSF is complicated, where multiple mechanisms are involved that all play a part in the induction of OSF.

In recent years, the number of people chewing areco nuts, and the geographic scope they are in has been steadily increasing. Moreover, as patients with OSF tend to be younger, the malignant rate and severity of OSF will gradually rise over time [26]. Therefore, in-depth studies on the toxicology of areco nut are compulsory to improve people's awareness of its hazards. Following the summary of the mechanism of arecoline-induced OSF, we aim to conduct further clinical research to investigate the mechanism of arecoline-induced OSF in the real world, thereby fully preparing for the subsequent prevention and treatment of OSF.

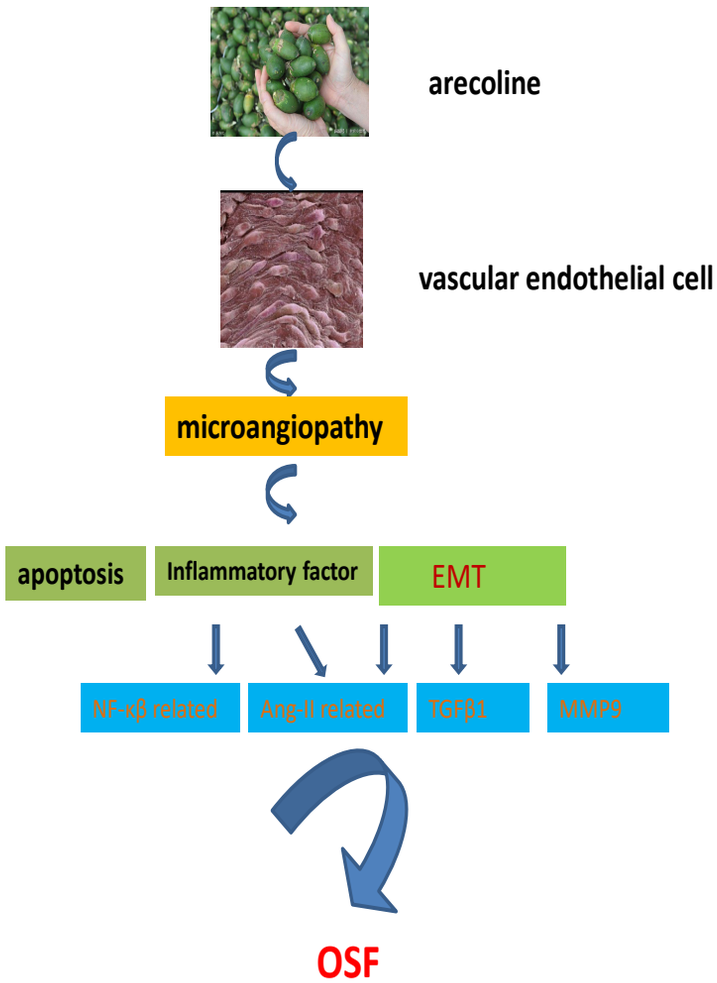

Figure 1: Mechanism of arecoline in inducing OSF by acting on endothelial cells.

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Competing interests

The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at https:// www.tmrjournals.com/ fthc

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