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Review Article

Cystatin C: Its role in pathogenesis of OSMF☆

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ABSTRACT

Oral Submucous Fibrosis (OSF) is a chronic disorder characterized by fibrosis of the mucosa lining the upper digestive tract involving the oral cavity, oro- and hypopharynx and the upper third of the oesophagus. The alkaloids from areca nut are the most important chemical constituents biologically, in producing this lesion. These chemicals appear to interfere with the molecular processes of deposition and/or degradation of extracellular matrix molecules such as collagen. Increased collagen synthesis or reduced collagen degradation have been considered as a possible mechanism in the development of the disease. Increased and continuous deposition of extracellular matrix may also take place as a result of disruption of the equilibrium between matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMP). Arecoline a product of areca nut was found to elevate Cystatin C mRNA (CST3) and protein expression in a dose-dependent manner. Cystatin C expression was significantly higher in OSF specimens and expressed mainly by fibroblasts, endothelial cells, and inflammatory cells. Cross-links between the molecules are essential for the tensile strength of collagen fibres. These areas are resistant to attack by collagenases but can be attacked by a number of other serine and cysteine proteinases. CST3 encoding a cysteine proteinase inhibitor might contribute to the stabilization of collagen fibrils in OSMF. Treatment directed against Cystatin C may serve as a novel treatment for submucous fibrosis and also in preventing its transformation into malignancy.

1. Introduction

Oral submucous fibrosis was first described by Schwartz in 1952 as a fibrosing condition that occurred in five Indian women in Kenya and he called it as Atrophica idiopathic (tropica) mucosa oris'. Oral submucous fibrosis is well established in medical literature since the time of Sushrutha, a renowned Indian physician, who lived in 2500–3000 BC and described a condition resembling OSF which he referred to as 'Vidari'. Similar conditions were found in betel nut (BN) chewers in early texts dating back to 1908. The submucous fibrosis is characterized by juxta-epithelial inflammatory reaction followed by chronic change in the fibro-elasticity of the

☆ Cystatin C expression was significantly higher in oral submucous fibrosis specimens and expressed mainly by fibroblasts, endothelial cells, and inflammatory cells.

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lamina propria and epithelial atrophy. This resulted in burning sensation in the oral cavity, blanching, and stiffening of oral mucosa and oropharynx, resulting in restricted mouth opening, limited food consumption which further resulted in difficulty in maintaining oral health, and impairing the ability to speak. The signs and symptoms depend on the evolution of lesions and number of affected sites. The malignant transformation rate of OSF has been reported to be around 7.6% over a 17-year period.[1]

2. Areca nut or betel nut

The constituents of BN include crude fiber, carbohydrates, fats, polyphenols, alkaloids, tannins, proteins and water. Trace amounts of fluorine, sapogenins (glycosidic derivatives of steroids and triterpenoids) and free amino acids have also been reported in some forms. The active components of dry and wet forms of BN, which produce BN associated effects, are primarily the alkaloids, polyphenols, and tannins. Prolonged as well as excessive usage of BN has been reported to exert significantly adverse effects on human health. There is enough evidence to suggest that BN products, even without tobacco, are associated with increased risk for the development of oral malignancy, such as oral squamous cell carcinoma (OSCC). A vast majority of BN users show pre-cancerous clinical conditions, such as oral leukoplakia (OL) as well as its variant, oral erythroplakia or oral submucous fibrosis (OSF) among others. The risk is reported to be higher for paan masala chewers. The popularity of BN mixtures like paan masala, gutkha and mawa has resulted in an epidemic of OSF, particularly among young individuals in India and South east Asia.[1]

Paan masala is basically a preparation of areca nut, catechu, cardamom, lime and a number of natural and artificial perfuming and flavoring materials. Gutkha is a variant of paan masala, in which in addition to these ingredients flavored chewing tobacco is added. Both products are often sweetened to enhance the taste. Paan masala chewing was found to have the highest risk for developing OSF.[2]

Among the chemical constituents, alkaloids from areca nut are the most important, biologically, whilst tannin may have a synergistic role. These chemicals appear to interfere with the molecular processes of deposition and/or degradation of extracellular matrix (ECM) molecules such as collagen. In vitro studies on human fibroblasts using areca extracts or chemically purified arecoline support the theory of fibroblastic proliferation and increased collagen formation that is also demonstrable histologically in human OSF tissues.[3]

Etiology of submucous fibrosis was found to be multifactorial. Consumption of chillies, nutritional deficiency, tobacco, genetic susceptibility, immunologic basis, and areca nut chewing were all said to play a role in formation of OSF. It is apparent that fibrosis and hyalinization of connective tissue account for most of the clinical features encountered in this condition. Moreover, substantial amount of research on elucidating the etiology and pathogenesis appear to have been focused on changes in the extracellular matrix. It is logical to hypothesize that the increased collagen synthesis or reduced collagen degradation as possible mechanisms in the development of the disease. There are numerous biological pathways involved in the above processes and, it is likely that the normal regulatory mechanisms are either down-regulated or upregulated at different stages of the disease. Not a single case of OSF was found without any chewing habits in a study conducted by Shah et al[1] The involvement of HLA and genetic predisposition has been reported, but specific haplotypes have not been determined. Nutritional deficiencies may not play a primary role but it could synergize the symptomatology by contributing to epithelial atrophy.[4]

3. Molecular basis for the changes caused by areca nut

From a clinico-pathologic point of view, fibrosis may be considered as a somewhat irreversible state of tissue alteration, during which resolution of the healing process fails to occur. Increasingly, it has become appreciated that certain of these actions of ECM derive from its ability to sequester and modulate the activity of specific growth factors (Nathan and Sporn, 1991). Of all of the growth factors, none has been found to have the diversity of effects on ECM ascribed to transforming growth factor-β (TGF-β). This peptide plays a critical role not only in synthesis and degradation of ECM but also in response of cells to ECM; moreover, specific components of the ECM, in turn, can both deliver TGF-β and regulate its activity. TGF-β was also produced during wound healing.[2] During tumorigenesis, however, the prevailing model suggests a process whereby pre-cancerous epithelial cells acquire multiple genetic mutations and the associated stroma becomes “activated” commonly expressing myofibroblastic markers and hence the reduced extensibility of the oral mucosa. The characteristics of an activated carcinoma-associated fibroblast are not completely understood. Such cells are presumed to express α smooth muscle actin, ECM proteins, and growth factors that act in an autocrine and paracrine fashion to potentiate and support the survival of a tumor.[5] In addition to anti-inflammatory properties, TGF-β promotes fibrosis as part of altered tissue repair. For example, in pulmonary fibrosis, TGF-β is found in the lung and the elevation in levels of TGF-β correlates with the extent of fibrosis. Thus, defining the specific mechanisms regulating the production and activation of TGF-β may have therapeutic opportunities to help patients with fibrotic diseases. It is held that the hypersensitivity to local irritants results in persistent mucosal inflammation which acts as the initiating factor for a protracted and defective inflammatory-reparative response, culminating in fibrotic healing. Thus understanding the molecular regulation of TGF-β activation and recognition may provide opportunity to intercede on this process, being a significant mediator of tissue repair. TGF-β signaling pathway has been considered both as a tumor suppressor pathway and a promoter of tumor progression and invasion.[5]

Growth factors like TGF-α, INF-α are also stimulated and over a period of time, due to persistent areca nut chewing habit, chronic inflammation sets in at the site. Initial irritation leads to further atrophy and ulceration of the mucosa. Cytokines like interlukin-6 (IL-6), tumor necrosis factor (TNF),