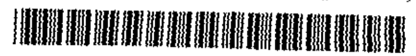

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行政院國家科學委員會專題研究計畫成果報告

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一、中文摘要

嚼食檳榔是造成口腔黏膜下纖維化的主要原因，其會造成黏膜上皮萎縮與過量的膠原蛋白堆積在結締組織層中。檳榔素是檳榔中含量最多的植物鹼，其亦被認為與口腔黏膜下纖維化關係密切，以往有關此類研究多從膠原蛋白著手，鮮少有研究探討檳榔成分對金屬蛋白脢與組織蛋白抑制脢的影響。本研究以組織培養法，培養人類頰黏膜纖維母細胞與口腔黏膜下纖維化纖維母細胞，結果發現口腔黏膜下纖維化纖維母細胞有較高 TIMP-1 的表達，檳榔素 40 μg/ml 以下會提高正常頰黏膜纖維母細胞 TIMP-1 的表現，酵素電泳拓蹼分析發現檳榔素 80 μg/ml 以上會抑制正常頰黏膜纖維母細胞 MMP-2 的表現，從本研究結果發現檳榔可能透過此一機轉造成正常黏膜轉變成黏膜下纖維化症。

關鍵詞：檳榔素；口腔黏膜下纖維化症；頰纖維母細胞；細胞骨架

Abstract

Oral submucous fibrosis (OSF) is a pre-malignant fibrotic lesion of the mouth in areca quid chewers. It is probably a consequence of disturbances in the hemeostatic equilibrium between synthesis and degradation of extracellular matrix molecules (ECM). To date, there has been little research about the role of tissue inhibitors of metalloproteinase (TIMPs) and matrix metalloproteinases (MMPs) in the pathogenesis of OSF. In the present study, we examined the activity of TIMPs from cells cultured from OSF and normal buccal mucosa. OSF specimens were found to have higher TIMP-1 expression than normal buccal mucosal fibroblasts (BMFs) by Western blots. To verify whether arecoline, a major areca nut alkaloid, could affect TIMP or MMP production by human BMFs, Western blots and gelatine zymography were used. Arecoline was found to elevate TIMP-1 expression at the concentration level under 20 μg/ml in a dose-dependent manner. The amount of TIMP-1 was about 2.7 fold at a concentration level of 10 μg/ml compared with control. From gelatin zymograms, the main gelatinolytic proteinase secreted by the human BMFs was MMP-2, and only minimal amounts of MMP-9 could be detectable from zymogram. In addition, arecoline was found to inhibit MMP-2 secretion and

production at the concentration level of 40 μg/ml. The gelatinolytic activity of MMP-2 was about 54 % at a concentration level of 80 μg/ml compared with control. Taken together, it was found that arecoline acted as not only an inhibitor on gelatinolytic activity of MMP-2, but also a stimulator for TIMP-1 activity. These synergistic effects may contribute to the ECM components accumulation in the areca quid associated OSF.

Keywords：Arecoline; Buccal mucosal fibroblasts; Oral submucous fibrosis; Tissue inhibitors of metalloproteinases; Matrix metalloproteinases

二、緣由與目的

Oral submucous fibrosis (OSF), which is regarded as a precancerous condition (Pindborg *et al.*, 1984), is characterized by juxta-epithelial inflammatory reaction followed by fibro-elastic change in the lamina properia and epithelial atrophy. This leads to restricted oral opening, causing trimus and inability to eat (Pindborg and Sirsat, 1966). The fibro-elastic changes are almost entirely due to abnormal accumulation of collagen fiber in the subepithelial layers (Canniff *et al.*, 1986; Van Wyk *et al.*, 1990), resulting in dense fibrous bands in the mouth.

Epidemiological studies have shown that the habit of areca quid chewing is one of the most important etiologic factors in the pathogenesis of OSF (Sinor *et al.*, 1990; Maher *et al.*, 1994). The etiology involved in the pathogenesis of OSF is believed to be multifactors, such as autoimmunity, nutritional deficiency states and even genetic susceptibility (Mutri *et al.*, 1995).

The biochemical events involved in the development of the areca quid associated OSF are not well understood at present. It is probably a consequence of disturbances in the homeostatic equilibrium between synthesis and degradation of extracellular matrix molecules or interference in the fibroblast proliferation rates. Herein, we hypothesized that OSF is caused by increased or altered *de nove* synthesis and deposition of ECM and/or altered fibrolysis, which, if unbalanced, may result in fibrosis during areca quid chewing. To further elucidate the pathobiological effects of areca quid chewing on human buccal mucosa, Western blot for TIMP activity and gelatin zymography were used to evaluate arecoline on human primary BMFs cultures.

三、結果與討論

The excessive collagen deposition associated with connective tissue disorders, including fibrosis and drug-induced tissue overgrowth may be explained by several mechanisms. First, there may be accelerated production of collagen by fibroblasts which exceeds the rate of degradation (Overall 1991,

Milani et al. 1994). Second, increased collagen deposition might result from increased formation of type IV collagen which is resistant to fibroblast collagenases (Benyon et al. 1996). In the present study, we provide evidence that the TIMP-1 level in OSF is higher than normal buccal mucosa. Arecoline could elevated TIMP-1 expression as well as inhibit the gelatinolytic activity of MMP-2 in human BMFs. Taken together, the data presented here suggest that the stimulate effect of TIMP-1 and inhibitory effect of gelatinase A by arecoline may contribute to the ECM components accumulation in oral mucosa. These effects might act as a synergistic effect on the pathogenesis of OSF. Further work is required to establish the exact role of areca quid as well as other compounds in the pathogenesis of OSF. Such studies would give insights about the biochemical mechanisms underlying other drug-induced tissue overgrowth.

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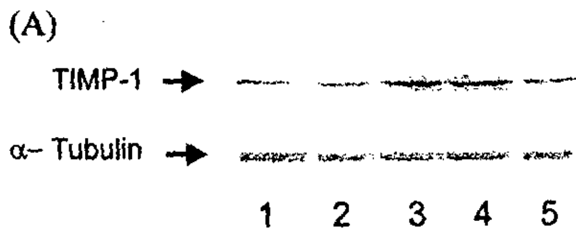


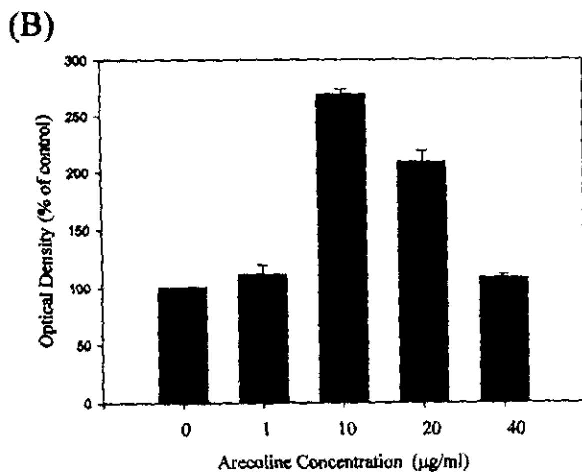


Fig. 2. (A) Elevated TIMP-1 expression by buccal mucosal fibroblasts (BMF) with arecoline. Lanes 1–5 represent control, arecoline concentration 1,10, 20, and 40 μg/ml, respectively. (B) Densitometric analysis of the tissue inhibitors of metalloproteinase (TIMP)-1 bands obtained by Western blots were calculated from their protein activity. Optical density values represent the means of six different BMF±standard deviations.

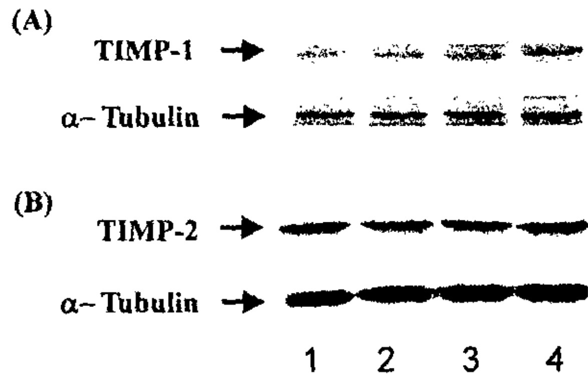
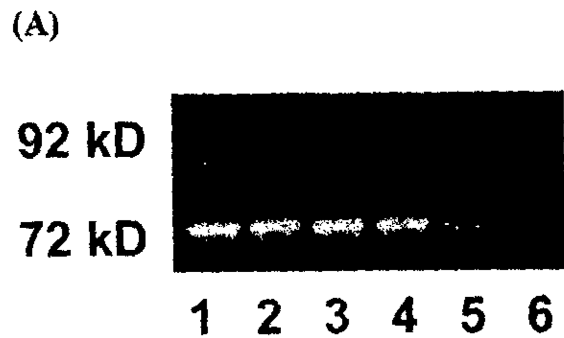


Fig. 1. (A) Comparison of the tissue inhibitors of metalloproteinase (TIMP)-1 protein expression from BMFs and OSFs using Western blot assay. Lanes 1 and 2 represent BMFs. Lanes 3 and 4 represent OSFs. OSF specimens were found to have higher TIMP-1 expression than buccal mucosal fibroblasts (BMFs). (B) Comparison of the TIMP-2 protein expression from BMFs and OSFs using Western blot assay. However, no difference in the TIMP-2 level was noted between the oral submucous fibrosis (OSF) and BMFs.



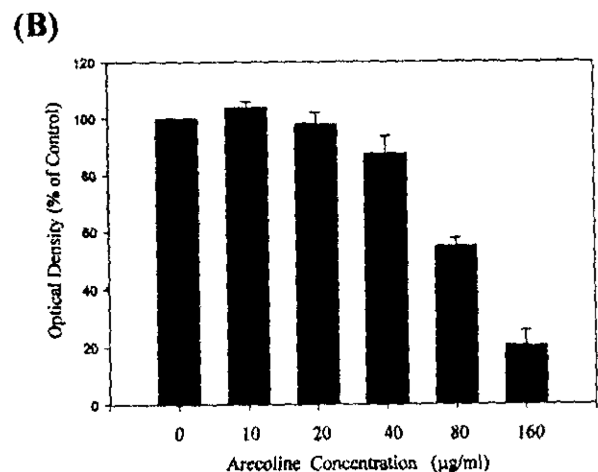


Fig. 3. (A) Gelatin zymogram of conditioned medium from primary human buccal mucosal fibroblasts (BMFs) treated with different arecoline concentrations during 48 h. The 72 kDa band correspons to matrix metalloproteinases (MMP)-2 (Gelatinase A) and was inhibited by the addition of arecoline in a dose-response manner. Lanes 1–6 represent control, arecoline concentration 1,10, 20, 40, and 80 μg/ml, respectively. (B) Levels of MMP-2 from conditioned medium were calculated from their gelatinolytic activity, as measured by Alpha-Imager 2000. Optical density values represent the means of six different BMF±standard deviations.