10

行政院國家科學委員會補助專題研究計畫成果報告

※※※※※※※※※※※※※※※※※※※※※※※※

※ 檳榔素對人類頰黏膜纖維母細胞 ※

※ 細胞骨架蛋白影響之研究 ※

※※※※※※※※※※※※※※※※※※※※※※※※

計畫類別：☑個別型計畫　□整合型計畫

計畫編號：NSC 89-2314-B-040-015

執行時間：民國 88 年 8 月 1 日至民國 89 年 7 月 31 日

計畫主持人：張育超

共同主持人：周明勇

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：中山醫學院牙醫系

中華民國 89 年 10 月 18 日

行政院國家科學委員會專題研究計畫成果報告

計畫編號：NSC89-2314-B-040-015
執行期限：88 年 8 月 1 日至 89 年 7 月 31 日
主持人：張育超 中山醫學院牙醫學系
共同主持人：周明勇 中山醫學院牙醫學系
共同主持人：李宗貴 中山醫學院營養科學研究所

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細胞骨架蛋白的影響。本研究以組織培養法，培養人類頰黏膜纖維母細胞探討檳榔素對其細胞骨架蛋白的影響，結果發現檳榔素會提高 57KDa 蛋白的表現，此一現象隨著藥物計量增加而升高，在 arecoline 100 μg/ml 時約為對照組的 15 倍左右，且與從口腔黏膜下纖維化培養出的纖維母細胞有相同劑量的 57KDa 蛋白表現，經由西方墨點法發現此一 57KDa 蛋白為 Vimentin，另外從病理切片以免疫組織化學染色法亦發現 Vimentin 在纖維化較正常組織有明顯的表現。從本研究結果發現此一 57KDa 細胞骨架蛋白 Vimentin 的表現，可能與正常黏膜轉變成黏膜下纖維化關係密切。

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

Areca quid chewing has a strong correlation with oral submucous fibrosis in Taiwan. The cytotoxicity of arecoline, a major areca nut alkaloid, on human oral fibroblasts has been extensively studied. To date there has been little research into the possible effects of arecoline on cytoskeleton components. In this study, in addition to cytotoxicity assay, we especially pay attention to the effect of arecoline on vimentin, an intermediate filament, expression of human buccal mucosal fibroblasts exposure to various levels of arecoline (0 to 200 μg /ml) for 48 hour. At a concentration higher than 50 μg/ml, arecoline was cytotoxic to cultured fibroblasts and the cytotoxicity was dose-dependent ($p<0.05$). From the result of SDS-polyacrylamide gel electrophoresis, arecoline was found to elevate 57 KDa cytoskeletal protein level in a dose-dependent manner. By immunoblotting assay, evidence indicated this 57 KDa cytoskeletal protein was vimentin. The increased of vimentin with arecoline was correspondent to that obtained in fibroblasts cultured from oral submucous fibrosis (OSF) patients. Immunohistochemical assay also revealed that vimentin expression was much higher in OSF specimen than in

normal buccal mucosa. These results probably advance our understanding the possible pathogenesis how normal buccal mucosa transform to submucous fibrosis because of areca quid chewing.

Keywords：arecoline; oral submucous fibrosis; buccal fibroblasts; cytoskeleton

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).Our recent study have shown that arecoline act as not only a cytotoxic agent, but also a stimulator for double stranded nucleic acid synthesis on human buccal mucosa fibroblasts (Chang *et al.*, 1998a). Arecoline also induced cell morphology change in human oral fibroblasts (Chang *et al.*, 1998a, 1998b, and 1999). The change on cell morphology implicates the possible effects of arecoline on the disturbance of cytoskeleton, which leads to interfere the cell mitosis as well as the intracellular transport mechanism.

To further elucidate the pathobiological effects of areca quid chewing on human buccal mucosa, we treated mucosal cells with the major areca nut alkaloid, arecoline, to exam whether the cytoskeletal proteins were influenced.

三、結果與討論

Arecoline has been reported as one of the causing factors for chromosome aberrations and increasing sister chromatid exchanges in mouse bone marrow cells (Panigraphi 1982; Panigraphi 1983). Recently, it was also shown to induce unscheduled DNA synthesis in Hep 2 cells derived from human larynx carcinoma (Sharan and Wary 1992) and increase the frequency of Chinese hamster ovary cells with micronuclei *in vitro* (Lee *et al.* 1996). In this study, we found arecoline, at a concentration of 50 μg/ml or higher, was cytotoxic to buccal fibroblasts by using LDH assay (Fig. 1). Similar results were found by human buccal fibroblasts *in vitro* (Jeng *et al.* 1994; Van Wyk *et al.* 1995; Chang *et al.* 1998). They have clearly shown the cytotoxicity of arecoline.

In this study, we noted that arecoline significantly increased a 57 kDa insoluble cytoskeleton protein expression in human normal buccal mucosal fibroblasts in a dose-dependent manner. This protein, however, was highly expressed in the fibroblasts from OSF patients. Immunoblotting assay revealed that this cytoskeleton protein is vimentin, one of the intermediate filaments (Fig. 2). Vimentin is a member of type III intermediate filament and it is primarily expressed in cells of mesenchymal origin, but not in normal epithelial cells (Funchs and Weber 1994). However, it is also present in transformed cell lines and tumors (Tsarfaty *et al.*, 1994; Chen *et al.*, 1996: Atula *et al.*, 1997). In this study, we first report that vimentin expression highly elevated in normal human buccal mucosal fibroblasts effected by arecoline and correspondent to that obtained in fibroblasts cultured from OSF patients.

From immuohistochemtistry, homogenous and intensive staining for vimentin was subepithelially and in the deeper layers of the connective tissue stroma from OSF specimens (Fig. 3). In addition, vimentin was found to be highly expression in OSF specimens as compared to that in normal buccal mucosa. Vimentin forms a highly insoluble network providing cell shape and integrity (Funchs and Weber 1994). It is significant that protein expression around fibrotic areas. Numerous speculations have been put forward to explain the pathogenesis of OSF, many of which are related to the areca nut and its components. Elevated vimentin protein expression might be a new approach in the pathogenesis of OSF patients who chew the areca quid.

However, further researches are required, including detection of vimentin gene trancripts, whether OSF is caused solely by increased or altered *de novo* synthesis and deposition of vimentin by areca nut constitutes.

五、參考文獻

Chang YC, Tai KW, Cheng MH, Chou LSS, Chou MY (1998a). Cytotoxic and non-genotoxic effects of arecoline on human buccal fibroblasts *in vitro*. *J Oral Pathol Med* 27: 68-72.

Chang YC, Cheng MH, Huang MF, Liao PH, Chou LSS, Chou MY (1998b). Effects of betel nut alkaloids on human gingival fibroblasts *in vitro*. *Chin Dent J* 17: 23-29.

Chang YC, Tai KW, Lii CK, Chou MY (1999). Cytopathologic effects of arecoline on human gingival fibroblasts *in vitro*. *Clin Oral Invest* 3: 25-29.

Caniff JP, Harvey W, Harris M (1986). Oral submucous fibrosis: its pathogenesis and management. *Br Dent J* 160: 429-434.

Fuchs E, Weber K (1994). Intermediate filaments: structure, dynamics, function and disease. *Annu Rev Biochem* 63:345-382.

Jeng JH, Kuo ML, Hahn LJ, Kuo MYP (1994). Genotoxic and non-genotoxic effects of betel quid ingredients on oral mucosal fibroblasts *in vitro*. *J Dent Res* 75: 1043-9.

Lee CH, Lin RH, Liu SH, Lin SY (1996). Mutual interactions among ingredients of betel quid in inducing genotoxicity on Chinese hamster ovary cells. *Mutat Res* 367: 99-104.

Maher R, Lee AJ, Warnakulasuriya KKAS, Lewis JA, Johnson NW (1994). Role of areca nut in the causation of oral submucous fibrosis: a case-control study in Pakistan. *J Oral Pathol Med* 23: 65-69.

Mutri PR, Bhonsle RB, Gupta PC, Daftary DK, Pindborg JJ, Mehta FS (1995). Etiology of oral submucous fibrosis with special reference to the role of areca nut chewing. *J Oral Pathol Med* 24: 145-152.

Pagan R, Martin I, Alonso A, Llobera M, Vilaro S (1996). Vimentin filaments follow the preexisting cytokeratin network during epithelial-mesenchymal transition of cultured neonatal rat hepatocytes. *Exp Cell Res* 222: 333-344.

Panigraphi GB, Rao AR (1982). Chromosome-breaking ability of arecoline, a major betel nut alkaloid, in mouse marrow cells *in vivo*. *Mutat Res* 103: 197-204.

Panigraphi GB, Rao AR (1983). Influence of caffeine on arecoline-induced SCE in mouse bone-marrow cells *in vivo*. *Mutat Res* 122: 347-53.

Pindorg JJ, Sirsat SM (1966). Oral submucous fibrosis. *Oral Med Oral Surg Oral Pathol* 22: 764-769.

Pindorg JJ, Murti PR, Bhonsle RB, Gupta PC, Daftary DK, Mehta FS (1984). Oral submucous fibrosis as a precancerous condition. *Scand J Dent Res* 92: 224-229.

Sharan RN, Wary KK (1992). Study of unscheduled DNA synthesis following exposure of human cells to arecoline and extracts of betel nut *in vitro*. *Mutat Res* 278: 271-276.

Sinor PN, Gupta PC, Mutri PR, Bhonsle RB, Daftary DK, Mehta FS, *et al.* (1990) A case-control study of oral submucous fibrosis with special reference to the etiology role of areca nut. *J Oral Pathol Med* 19: 94-98.

Van Wyk CW, Seedat HA, Phillips VM (1990). Collagen in submucous fibrosis: an electron-microscopic study. *J Oral Pathol Med* 19:182-187.

Van Wyk CW, Olivier A, Grobler-Rabie AF (1995). Growth of oral and skin fibroblasts from patients with oral submucous fibrosis. *J Oral Pathol Med* 24: 349-53.

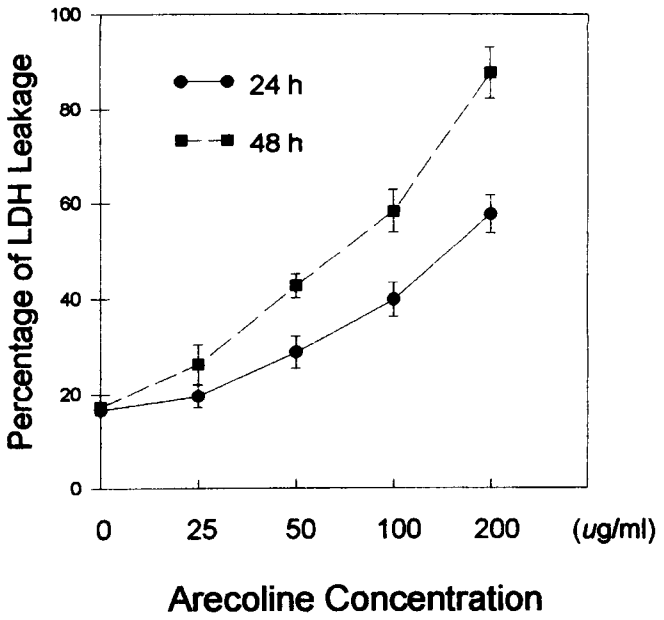

Fig.1 Cellular toxicity measured by LDH assay .

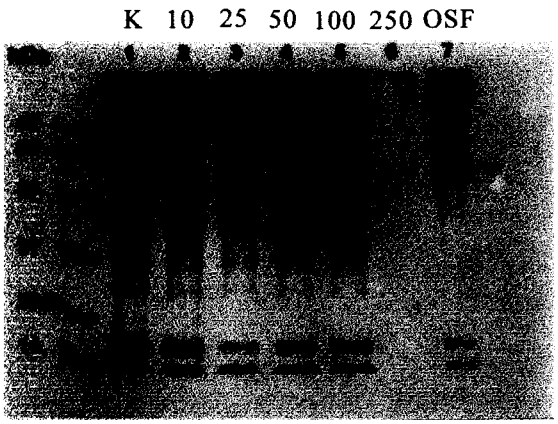

Fig.2 Cytoskeletal fraction analyzed by SDS-PAGE .

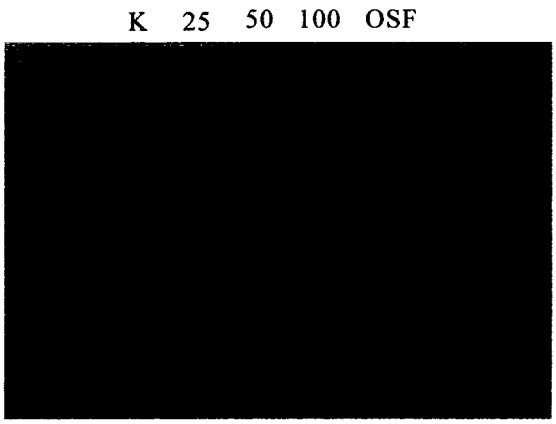

Fig.3 Immunoblotting of cytoskeletal component with anti-vimentin antibody

Fig.4a Vimentin was labeled in the collagen fiber slightly in the connective tissue within normal buccal mucosa.

Fig.4b Homogenous and intensive staining for vimentin was subepithelially and in the deeper layers of the connective tissue stroma in OSF specimens.