行政院國家科學委員會專題研究計畫　成果報告

探討腫瘤化學治療時藥物作用與細胞凋亡信息傳遞間的關聯性

計畫類別：個別型計畫
計畫編號：NSC91-2314-B-040-009-
執行期間：91 年 08 月 01 日至 92 年 07 月 31 日
執行單位：中山醫學大學牙醫學系

計畫主持人：張育超

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 92 年 10 月 23 日

中文摘要

治療腫瘤就是殺傷和清除腫瘤細胞, 誘導腫瘤細胞凋亡是治療腫瘤的一條有效途徑, 臨床上使用放療及化療的作用機制之一就是引發瘤細胞凋亡。但是正常細胞亦會受到傷害，Matrix metalloproteinases (MMPs) 是一群天然的蛋白水解酵素, 其在正常生理狀態下可以分解細胞外基質與基底膜不同的組成, 於正常組織重建、血管生成、胚胎發育及病理狀態如癌症侵襲轉移、組織纖維化皆扮演著重要角色。本研究以以組織培養法，培養口腔癌細胞及正常口腔上皮細胞以 zymography 比較其分泌 MMPs 的型態；腫瘤的形成與 protein kinase C (PKC)之訊息傳遞有關，加入 protein kinase C (PKC) inhibitor (H7、staurosporine) 探討是否由此途徑影響 MMPs 分泌。結果發現口腔癌細胞及正常口腔上皮細胞皆會分泌 MMP-2 及 MMP-9，但口腔癌細胞分泌量較正常口腔上皮細胞高($P<0.05$)。加入無細胞毒性劑量 PKC inhibitor (H7、staurosporine)可明顯抑制口腔癌細胞分泌 MMP-2 及 MMP-9。PKC inhibitor 可能成為一種新的抗癌藥物。

Objective. The purpose of this study was to investigate gelatinaes (MMP-2 and –9) expression in oral squamous cell carcinoma (SCC) and further explore the potential mechanisms that may lead to inhibit gelatinases activity.

Study design. Thirty biopsy specimens of oral SCCs were examined by immunohistochemistry. Supernatants from primary cultures of human oral mucosal keratinocyte (OMK) and oral cancer-derived cells (KB and OC2) were analyzed using gelatin zymography. Furthermore, protein kinase C (PKC) inhibitors (H7 and staurosporine) were added to test how they can modulate the gelatinases production in human oral cancer cells.

Results. MMP-2 and –9 expression were significantly higher in oral SCCs and located in discreet clusters of tumor cells. OMK cultures, KB and OC2 were found to secrete and produce MMP-2 and –9. However, the amount of MMP-2 and –9 were highly elevated by two oral cancer cell lines as compared with OMK cultures ($P<0.05$). In

addition, PKC inhibitors were found to decrease MMP-2 and –9 activities in oral cancer cells ($P<0.05$).

Conclusion. Taken together, human oral SCCs produce MMP-2 and –9 in vivo and in vitro and the gelatinases activity are downregulated by PKC inhibitors in vitro. PKC inhibitors suppressing MMPs production and/or activity may therefore be valuable therapeutics in the pathogenesis of oral SCC, and might be proved clinically useful agents, in combination with standard treatment modalities, in the treatment of oral cancer patients.

Key words: Oral squamous cell carcinoma; Matrix metalloproteinases; Immunohistochemistry; Gelatin zymography; Protein kinase C inhibitor

INTRODUCTION

Matrix metalloproteinases (MMPs) are a family of at least 22 secreted and membrane-bound zinc- and calcium-dependent proteases, which function in the degradation, and remodeling of extracellular matrix (ECM) proteins. They are associated with normal tissue remodeling and pathological processes such as arthritis, wound healing, angiogenesis, inflammation, embryonic development, bone resorption, and tumor metastasis. [1-3]

The MMPs are produced by the cancer cells or through induction of surrounding stromal cells. The ability of both gelatinases (MMP-2 and -9) to degrade basement membrane type IV collagen appears especially important in cancer progression. [4-8] Over expression of one or both of the gelatinases may enable the cancer cells to cleave type IV collagen selectively and cross the basement membrane barrier to enter blood vessel during the early stages of metastasis. Previous studies have shown that MMP-2 and -9 have demonstrated a correlation with the invasive/metastatic phenotype of cancer cells. [9,10] However, little data have been systemically described the expression and activity of MMP-2 and –9 in oral squamous cell carcinoma (SCC) specimens as well as oral cancer cells. In addition, the potential inhibition of MMPs are still remained to be elucidated.

To investigate gelatinases expression in oral SCC, we design both in vivo and in vitro experiments. Immunohistochemistry and gelatin zymography were used to evaluate the expression and activity of gelatinases in oral SCCs. The potential to inhibit tumor cell invasion may reside in the ability to block the activation and/or synthesis of MMPs. Little is known about the regulation of MMPs synthesis and secretion in oral cancer cells. Protein kinase C (PKC) was originally described as an ubiquitous Ca^{2+}- and phospholipid-dependent protein kinase that plays an important

role in signal transduction related to cellular growth and differentiation, as well as tumor promotion. [11] Furthermore, the effects of PKC inhibitors (H7 and staurosporine) were added to test how they can modulate the MMPs production in human oral cancer cells.

MATERIALS AND METHODS

Tissue specimens

Formalin-fixed, paraffin-embedded specimens were obtained from 30 patients with oral SCCs from areca quid chewers. Diagnosis was based on the histological examination of hematoxylin and eosin stained slides. These patients were treated at the Department of Oral and Maxillofacial Surgery, Chung Shan Medical University Hospital, Taichung, Taiwan. Specimens were obtained from radical neck dissection surgery. In addition, normal tissues surrounding the lesions were also obtained and used as the controls.

Immunohistochemistry

Serial 5-μm thick sections from each specimen were stained with the monoclonal anti-MMP-2 and -9 antibody (1: 200 dilution; Santa Cruz Biotechnology, CA, USA) using a standard avidin-biotin-peroxidase complex method as described previously. [12] AEC (3-amino-9-ethylcarbazole) (DAKO, Carpinteria, USA) stain was then used as the substrate for localizing the antibody binding. The preparations were counterstained with hematoxylin, mounted with Permount and examined by light microscopy. To demonstrate the specificity of the staining, negative controls were included in which the primary antibody was replaced with phosphate-buffered saline.

Cell cultures

Cell lines derived from human oral squamous cell carcinomas, KB cell line ATCC CCL-17 (American Type Culture Collection, Rockville, MD) and OC2 cells, derived from buccal mucosa cancer, [13] were retrieved from frozen stock and cultured to confluence in Dulbecco's modified Eagle's medium (DMEM) (Gibco Laboratories ,Grand Island, NY) supplemented with 10 % fetal calf serum (FCS) (Gibco Laboratories ,Grand Island, NY).

Primary cultures of normal oral mucosal keratinocytes (OMK) were prepared from surplus human oral mucosal tissue discarded after third molar surgery with informed consent. The epithelium was carefully dissected from the underlying connective tissue and cut into small fragments. Fragments were plated in a tissues culture dish in modified keratinocytes growth medium (KGM) (Gibco Laboratories ,Grand Island, NY) supplemented with 10 % FCS. Any cultures showing evidence of fibroblasts contamination were discarded. After confluence, cells were detached using 0.25 % trypsin and 0.05M EDTA, passaged into DMEM supplemented with 10 % FCS, and used for experimented work.

Gelatin zymography

Confluent cells were trypsinized, counted, and plated at a concentration of 1×10^5 cells in 60 mm culture dish and allowed to achieve confluence. After overnight attachment, the conditioned medium samples were collected after 2 day culture period and stored at -20°C until assayed.

Gelatinase activity was assayed by zymography in 1.5 mm of 7.5 % sodium dodecyl sulfate (SDS)-polyacrylamide gel impregnated with 1 mg/ml of gelatin that had been labeled fluorescent with 2-methoxy-2,4-diphenyl-3[2H]furanone. [14,15] This method allows for visual monitoring of the lysis of gelatin under long-wave UV light during incubation. In this study a 2 µl sample was electrophoresed on a 10% SDS-polyacrylamide gel copolymerized with 2 % gelatin as substrate. After electrophoresis the gels were washed in 2.5 % Triton X-100 twice for 30 min to remove all SDS. The gels were then incubated in 50 mmol/L Tris (pH 7.5), 5 mmol/L $CaCl_2$, and 1 mmol/L $ZnCl_2$ at 37°C overnight. The gelatin cleavage rate was analyzed from the photographed gels with a densitometer (AlphaImager 2000; Alpha Innotech,

San Leandro, CA).

Effect of PKC inhibitors on the gelatinases secretion by oral cancer cells

Gelatin zymography was used to evaluate the production and secretion by KB and OC2 cells as described above. For this study confluent cells were trypsinized, counted, and plated at a concentration of 1×10^5 cells in 60 mm culture dish and allowed to achieve confluence. Cells were cultured for 24 h, at which time the medium was changed to a medium containing 0.5 % FCS and appropriate concentrations of PKC inhibitors H7 and staurosporine (ST) (Sigma Chemical Co., St. Louis, MO). H7 were first dissolved in 100% ethanol and then diluted with the culture medium. Stock solutions of ST were prepared in dimethyl sulfoxide and stored at −20°C. The final concentration of each solvent in the medium did not exceed 0.25 % (vol/vol). At these concentrations the solvents used were not toxic to cells. The final concentrations of H7 used in this study were 2, 5, and 10 μM; and ST were 2, 5, and 10 nM, respectively. The conditioned medium samples were collected on days 1 and 2. Finally, cytosol fractions were also collected for this experiment according to our previous method. [16] Cell extracts were solubilized with SDS-solubilization buffer (5 mM EDTA, 1 mM $MgCl_2$, 50 mM Tris-HCl , pH 7.5 and 0.5 % Trition X-100, 2mM phenylmethysulfonyl fluoride, and 1mM N-ethylmaleimide) for 30 min on ice. Then, cell lysates were centrifuged at 12,000 g at 4°C and the protein concentrations determined with Bradford reagent using bovine serum albumin as standards. Triplicate separate experiments were performed throughout this study. The significance of the results obtained from control and treated groups was statistically analyzed by the paired Student *t*-test. A *p*-value of < 0.05 was considered to be statistically significant.

RESULTS

Immunoreactivity of MMP-2 was expressed in cancer nests (Fig. 1a), whereas only faint immunoreactivity was observed in normal epithelium. Immunolocalization of MMP-9 are the same pattern like MMP-2 expression (Fig. 1b).

The gelatin zymograms revealed that gelatinases secreted by human OMKs migrated at 72 kDa and represents pro MMP-2 and gelatinolytic bands were also observed at 92 kDa regions that correspond to pro MMP-9 (Fig. 2a). A similar observation was found for the conditioned medium from KB and OC2 cells (Fig. 2a). The quantitative measurement by the AlphaImager 2000 is shown in Fig.2b. The density of MMP-2 and –9 were significantly elevated as compared with normal OMK cultures ($P<0.05$). The levels of the MMP-2 increased about 6.0 and 7.2 fold to KB and OC2 cells, respectively, as with normal OMK cultures. The levels of the MMP-9 increased about 2.9 and 4.3 fold to KB and OC2 cells, respectively, as with normal OMK cultures.

To examine the effect of PKC inhibitors on the regulation of MMP-2 and -9 expression, KB and OC2 cells were treated with H7 and ST. The levels of gelatinase were measured by the gelatin zymography. The effects of PKC inhibitors on the gelatinases expression in KB and OC2 cells were similar. Therefore, we decided to show only the data of KB cells.

The conditioned medium samples from KB cells treated with ST and H7 showed the level of MMP-2 to be significantly inhibited during culture period. The quantitative measurement by the AlphaImager 2000 is shown in Fig.3. The inhibitory pattern was exhibited in a dose- and time-dependent manner ($P<0.05$). A similar observation was found for cytosol, that PKC inhibitors inhibited MMP-2 secretion and production and the inhibitory effect of ST was more significantly than that of H7.

The conditioned medium samples from KB cells treated with ST and H7 showed the level of MMP-9 to be significantly inhibited during culture period. The quantitative measurement by the AlphaImager 2000 is shown in Fig. 4. The inhibitory pattern was also shown in a dose- and time-dependent manner ($P<0.05$). A similar observation was found for cytosol, that PKC inhibitors inhibited MMP-9 secretion.

DISCUSSION

While previous studies have shown gelatinases expression in breast, prostate, colon, and uterine cancer. [4,6,8] This study demonstrates that MMP-2 and –9 are also highly expressed in human oral SCC. Similar results were found by Pickett et al. [17] and Hong et al. [18]. These imply that up-regulation of gelatinases expression may play an important role in the pathogenesis in oral SCC.

In Taiwan, there are two million people who have the areca quid chewing habit, [19] which has been suspected to elevate the incidence of oral submucous fibrosis; [20] about 80% of all oral cancer deaths are associated with this habit. [21] Abberrant tissue inflammation can promote the concurrence of cancers. [22] In addition, MMPs expression was found to be associated with many inflammatory processes. [23-25] Recently, areca nut extracts were found to up-regulate prostaglandin production, cyclooxygenase-2 expression in human oral kerationcytes [26] and buccal mucosa fibroblasts [27] in vitro. These might partially explain why areca quid chewing contributes to the pathogenesis of oral SCC by elevating gelatinases expression in Taiwan.

MMP-2 and –9 are of particular interest as they are synthesized by oral mucosal epithelial cells. [28-31] In this study, we demonstrated that normal oral keratinocytes and oral cancer cell lines produce MMP-2 and -9. MMPs are secreted higher by cancer cells than normal keratinocytes. The basement membrane proteins laminin and type IV collagen are MMP-2 and -9 principal substrates. As destruction of the basement membrane is a critical event in malignant transformation of oral epithelium. Thus, elevation of MMP-2 and –9 secretion and activity may play an important role in malignant transformation in epithelial cells.

The messenger-signaling pathways that effort MMPs gene expression are still

incompletely understood. PKC is a family of at least 12 isoenzymes that involved in the regulation of a diverse range of cell functions including cell proliferation and adhesion. [32] The high degree of conservation of PKC isoenzymes plays a specific role in the regulation of cell function. [33] The PKC signaling pathway is used by a wide variety of cell membrane receptors. H7 and ST are known to be pharmaceutical PKC inhibitors. [34,35] In this study both of them were found to decrease MMP-2 and –9 secretion in two oral cancer cell lines. Our data demonstrated that the regulation of gelatinases might be mediated via a PKC signaling pathway in oral cancer cells. In addition, H7 not only acts a PKC inhibitor but also a cAMP-dependent kinase inhibitor. [33] Thus, the role of cAMP-dependent kinase inhibitors on MMP-mediated tissue destruction is open to speculation.

Components of cell signal transduction pathways provide important targets for new anticancer drugs. Protein kinases are integral parts of the intracellular signaling process regulating actions including growth, differentiation, motility, and survival. PKC has a pivotal role in signal transduction and is a logical target for drug intervention. [36] It is a specific serine/threonine kinase regulating a variety of homeostatic processes, and it is important in tumor promotion. [37] Recently, PKC inhibitor staurosporine analogue CGP41251 has been used in colorectal cancer, adeno-carcinomas of unknown primary, breast cancer, and lung cancer in a phase I clinical trail. [38] In addition, PKC inhibitor 7-hydroxystaurosporine UCN-01 has been used in B cell lymphoma. [39] Thus, the role of PKC inhibitors in oral cancer is worthy of further investigations.

In this study, MMP-2 and –9 were found highly expressed in oral SCCs. Both normal and malignant oral epithelial cells produce MMP-2 and -9. However, supernatants from cancer cells demonstrated that MMP levels were greater than in normal oral

keratinocytes. Moreover, PKC inhibitors ST and H7 decreased MMP-2 and –9 activities in a dose- and time-dependent manner. These data indicate that pharmacological agents that target PKC signal transduction pathway in human oral cancer cells inhibit gelatinases expression, and such inhibition may contribute to the pathogenesis of oral SCC. PKC might be proved clinically useful agents, in combination with standard treatment modalities, in the treatment of oral cancer patients. However, more detailed studies should be undertaken to clarify the agents that can regulate other MMPs produced or secreted by oral cancer cells in vitro and in vivo.

REFERENCES

1. Davies B, Waxman J, Wasan H, Abel P, Williams G, Kransz T, et al. Levels of matrix metalloproteinases in bladder cancer correlate with tumor grade and invasion. Cancer Res 1993; 53: 5365-9.
2. Allan JA, Docherty AJ, Barker PJ, Huskisson NS, Reymoldes JJ, Murphy G. Binding of gelatinases A and B to type I collagen and other matrix components. Biochem J 1995; 309: 299-306.
3. Kinoh H, Sato H, Tsunezuka Y, Takino T, Kawashima A, Okada Y, et al. MT-MMP, the cell surface activator of proMMP-2 (progelatinase A), is expressed with its substrate in mouse tissue during embriogenesis. J Cell Sci 1996; 109: 953-9.
4. Stetler-Stevenson WG, Liotla la, Kleiner DE Jr. Extracellular matrix 6: role of matrix metalloproteinases in tumor invasion and metastasis. FASEB J 1993; 7: 1434-41.
5. MacDongall JR, Matrician LM. Contributions of tumor and stromal matrix metalloproteinases to tumor progression, invasion and metastasis. Cancer Meta

Rev 1995; 14: 351-62.

6. Stetler-Stevenson WG. Type IV collagenases in tumor invasion and metastasis. Cancer Meta Rev 1990; 9: 289-303.
7. Liotta LA, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. Cell 1999; 64: 327-36.
8. Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, et al. Matrix metalloproteineases: a review. Crit Rev Oral Biol Med 1993; 4:197-250.
9. Kubota S, Mitsudom. T, Yamada Y Invasive human fibrosarcoma DNA mediated induction of a 92 kDa gelatinase/type IV collagenase lead to an invasive phenotype. Biochem Biophy Res Commun 1991; 181: 1539-47.
10. Poulsom R, Pignatell, M, Stetler-Stevenson WG, Liotla LA, Wright PA, Jeffrey RE, et al. Stromal expression of 72 kDa type IV collagenase (MMP-2) and TIMP-2 mRANs in colorectal neoplasia. Am J Pathol 1992; 141: 389-96.
11. Powis G, Albert DS. Inhibiting intracellular signaling as a strategy for cancer chemoprevention. Eur J Cancer 1994; 30A: 1138-44.
12. Chang YC, Tsai CH, Tai KW, Yang SH, Chou MY, Lii CK. Elevated vimentin expression in buccal mucosal fibroblasts by arecoline in vitro as a possible pathogenesis for oral submucous fibrosis. Oral Oncol 2002; 38: 425-30.
13. Wong DYK, Chang KW, Chen CF, Chang RCS. Characterization of two new cell lines derived from oral cavity human squamous cell carcinomas-OC1 and OC2. J Oral Maxillofac Surg 1990; 48: 385-90.
14. Heussen C, Dowdle EB. Electrophoretic analysis of plasminogen activators in polyacrylamide gels containing sodium dodecyl sulphate and copolymerized substrates. J Anal Chem 1980; 102: 196-202.

15. O'Grady RL, Nethery A, Hunter N. A fluorescent screening assay for collagenase using collagen labeled with L-methoxy-2,4-diphenyl-3(2H)furanone. Anal Biochem 1984; 140: 490-4.
16. Chang YC, Yang SF, Tai KW, Chou MY, Hsieh YS. Increased tissue inhibitor of metalloproteinase-1 expression and inhibition of gelatinase A activity in buccal mucosal fibroblasts by arecoline as possible mechanisms for oral submucous fibrosis. Oral Oncol 38: 195-200.
17. Pickett KL, Harber GJ, DeCarlo AA, Louis P, Shaneyfelt S, Windsor LJ, Bodden MK. 92K-GL (MMP-9) and 72K-GL (MMP-2) are produced in vivo by human oral squamous cell carcinomas and can enhance FIB-CL (MMP-1) activity in vitro. J Dent Res 1999; 78: 1354-61.
18. Hong SD, Hong SP, Lee JI, Lim CY. Expression of matrix metalloproteinases-2 and –9 in oral squamous cell carcinomas with regard to the metastic potential. Oral Oncol 2000; 36: 207-13.
19. Ko YC, Chiang TA, Chang SJ. Hsieh SF. Prevalence of betel quid chewing habit in Taiwan and related sociodemographic factors. J Oral Pathol Med 1992; 21: 261-4.
20. Shiau YY, Kwan HW. Submucous fibrosis in Taiwan. Oral Surg Oral Med Oral Pathol 1979; 47: 453-7.
21. Kwan HW. A statistical study on oral carcinomas in Taiwan with emphasis on the relationships with betel nut chewing: a preliminary report. J Formosa Med Assoc 1976; 75: 497-505.
22. Parsonnent J. Molecular mechanisms for inflammation promoted carcinogenesis of cancer-The sixteenth International Symposium of Sapporo Cancer Seminar. Cancer Res 1997; 57: 3620-4.

23. Chang YC, Yang SF, Hsieh YS. Regulation of matrix metalloproteinases-2 production by cytokines and pharmacological agents in human pulp cell cultures. J Endodon .2001; 27: 679-82.
24. Chang YC, Lai CC, Yang SF, Chan Y, Hsieh YS. Stimulation of matrix metalloproteinases by black-pigmented *Bacteroides* in human pulp and periodontal ligament cell cultures. J Endodon 2002; 28: 90-3.
25. Chang YC, Yang SF, Lai CC, Liu JY, Hsieh YS. Regulation of matrix metalloproteinases production by cytokines, pharmacological agents, and periodontal pathogens in human periodontal ligament fibroblast cultures. J Periodont Res 2002; 37: 196-203.
26. Jeng JH, Ho YS, Chan CP, Wang YJ, Hahn LJ, Lei D, Hsu CC, Chang MC. Areca nut extract up-regulates prostaglandin production, cyclooxygenase-2 mRNA and protein expression of human keratinocytes. Carcinogenesis 2000; 21: 1365-70.
27. Tsai CH, Chou MY, Chang YC. The upregulation of cyclooxygenase-2 expression in human buccal mucosal fibroblasts by arecoline: a possible role in the pathogenesis of oral submucous fibrosis. J Oral Pathol Med (In press).
28. Kylmaniemi M, Oikarinen A. Oikavinen K, Salo T. Effects of dexamethasone and cell proliferation on the expression of matrix metalloproteinases in human normal mucosal and malignant cells. J Dent Res 1996; 75: 919-26.
29. Makela M, Larjava H, Pirila E, Maisi P, Salo T, Sorsa T, Vitto VJ. Matrix metalloproteinases 2 (Gelatinase A) is related to migration of keratinocytes. Exp Cell Res 1999; 251: 67-78.
30. Bennett JH, Morgan MJ, Whawell SA, Atkim P, Roblim P, Furness J, Speight PM. Metalloproteinase expression in normal and malignant oral keratinocytes:

stimulation of MMP-2 and -9 by Scatter factor. Eur J Oral Sci 2000; 108: 281-91.

31. Zinber BL, turner MA, Palefsky JM, Banda MJ, Kramex RH. Type I collagen degradation by invasive oral squamous cell carcinoma. Oral Oncol 2000; 36: 365-72.
32. Tang DG, Honn KV. Role of protein kinase C and phosphates in 12(s)-HETE-induced tumor cell cytoskeletal reorganization. Adv Exp Med Biol 1997; 400: 349-61.
33. Martiny-Baron G, Kazanietz MG, Mischak H, Blumberg PM, Kochs G, Hug H, Marme D, Schachtele C. Selective inhibition of protein kinase C isozymes by the indolocarbazole Go6976. J Biol Chem 1993; 268: 9194-7.
34. Kawamoto S, Hidaka H. 1-(5-Isoguinolinesulfonyl)-2-methylpiperazine (H-7) is a selective inhibitor of protein kinase C in rabbit platelets. Biochem Biophy Res Commun 1984; 125: 258-64.
35. Tamaoki T, Nomoto H, Takahashi I, Kato Y, Morimoto M, Tomita F. Staurosporine, a potent inhibitor of phospholipid/Ca dependent protein kinase. Biochem Biophy Res Commun 1986; 135: 397-402.
36. Caponigro F, French RC, Kaye SB. Protein kinase C: a worthwhile target for anticancer drugs? Anticancer Drugs 1997; 8: 26-33.
37. Nishizuka Y. The role of protein kinase C in cell surface signal transduction and tumor promotion. Nature 1984; 308: 693-8.
38. Thavasu T, Propper D, McDonald A, Dobbs N, Ganesan T, Talbot D, Braybrook J, Caponigro F, Hutchison C, Twelves C, Man A, Fabbro D, Harris A, Balkwill F. The protein kinase C inhibitor CGP41251 suppresses cytokine release and extracellular signal-regulated kinase 2 expression in cancer patients. Cancer Res 1999; 59: 3980-4.

39. Wilson WH, Sorbara L, Figg WD, Mont EK, Sausville E, Warren KE, Balis FM, Bauer K, Raffeld, Senderowicz AM, Monks A. Modulation of clinical drug resistance in a B cell lymphoma patient by the protein kinase inhibitor 7-hydroxystaurosporine: presentation of a novel therapeutic paradigm. Clin Cancer Res 2000; 6: 415-21.

Fig. 1 (a) Expression of MMP-2 in oral SCC. Immunoreactivity to MMP-2 was expressed in cancer nest. (200x) (b) Expression of MMP-9 in oral SCC. MMP-9 was expressed in perinuclear location in tumor cells. (400x)

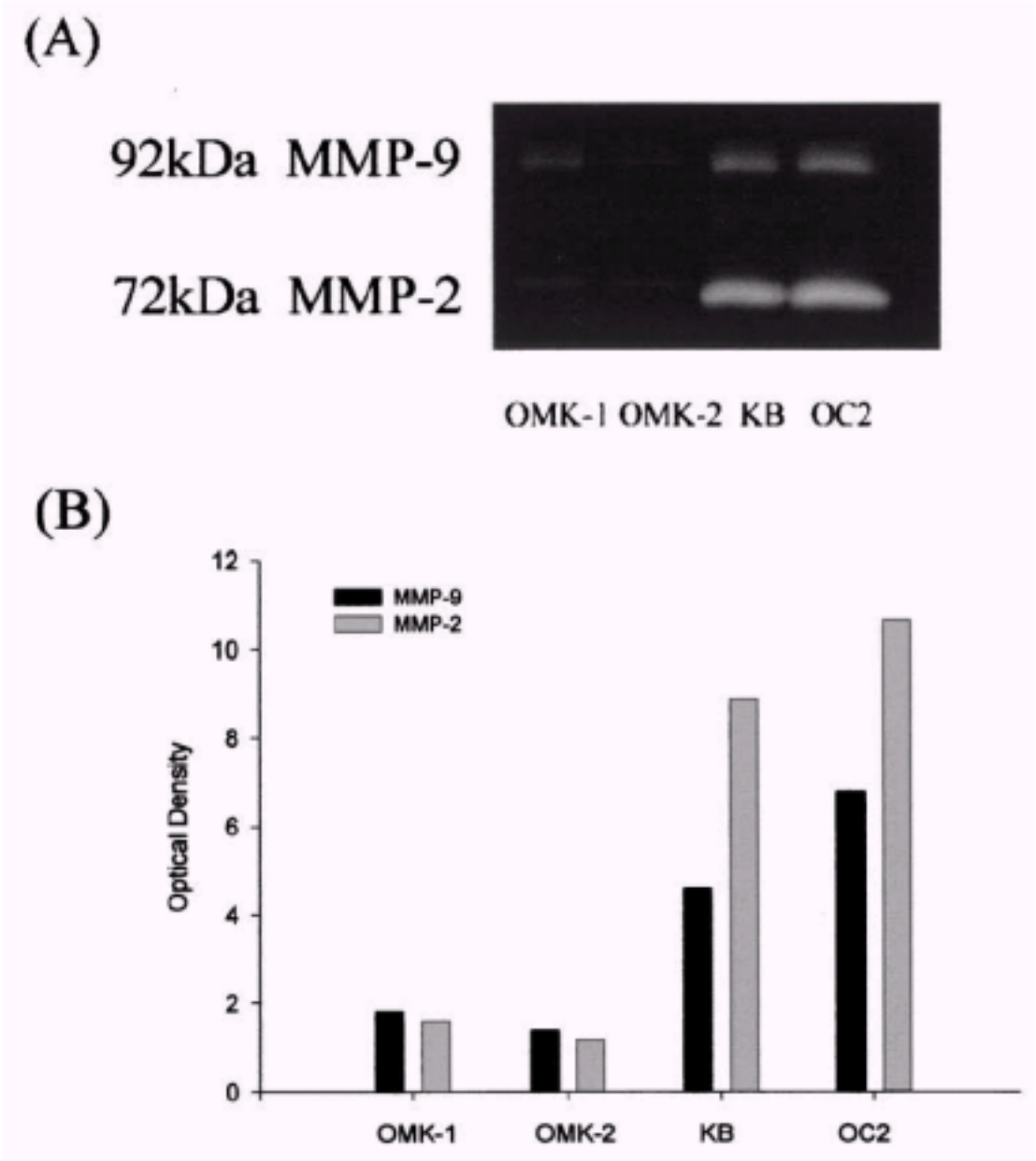

Fig. 2 (a) Gelatin zymogram of MMPs secreted by oral epithelial cells. Gelatinolytic activity and molecular weight positions of MMP-2 (72kDa) and –9 (92kDa) activity are indicated. (b) The optical density values of MMP -2 and –9 were

calculated from their gelatinolytic activity by AlphaImager 2000.

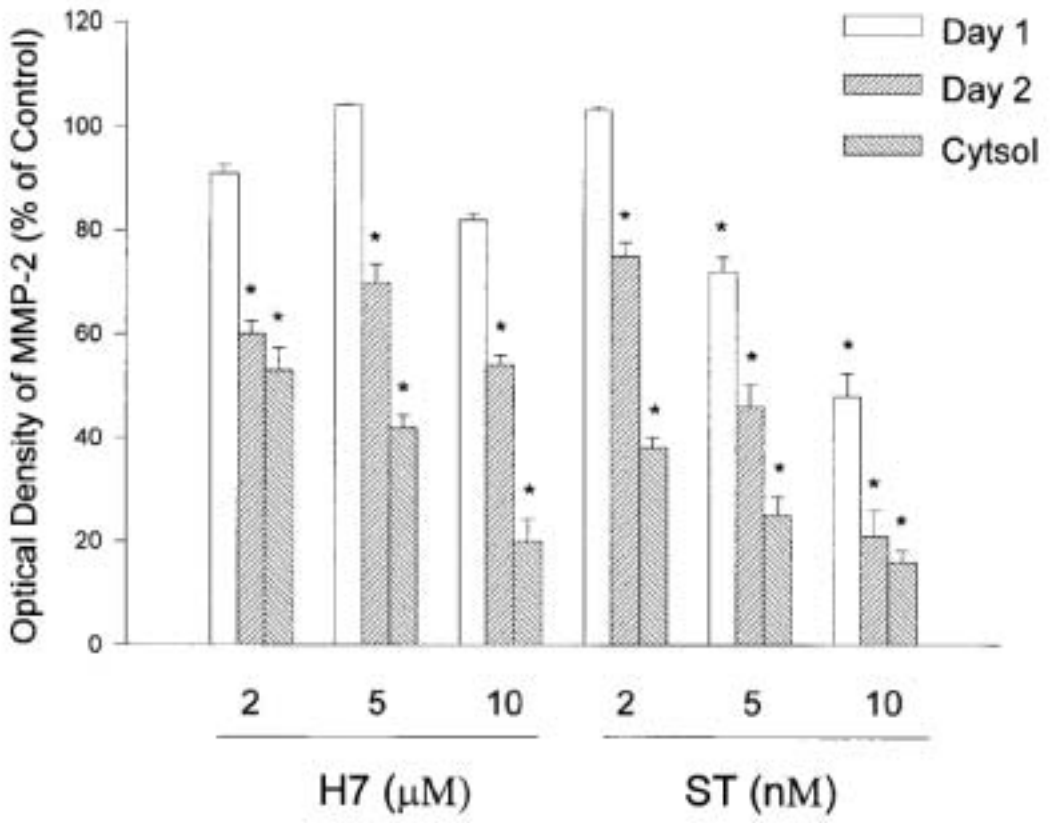

Fig. 3 Levels of MMP-2 from conditioned medium and cytosol from KB cells treated with ST or H7 were calculated from their gelatinolytic activity, as measured by AlphaImager 2000. Days 1 and 2 represent conditioned medium collected in day 1and day 2, respectively. Cytosol represents cell extract collected at day 2. Values are means and standard deviations of optical density from triplicate independent experiments. * denotes significant differences from control values with $P<0.05$.

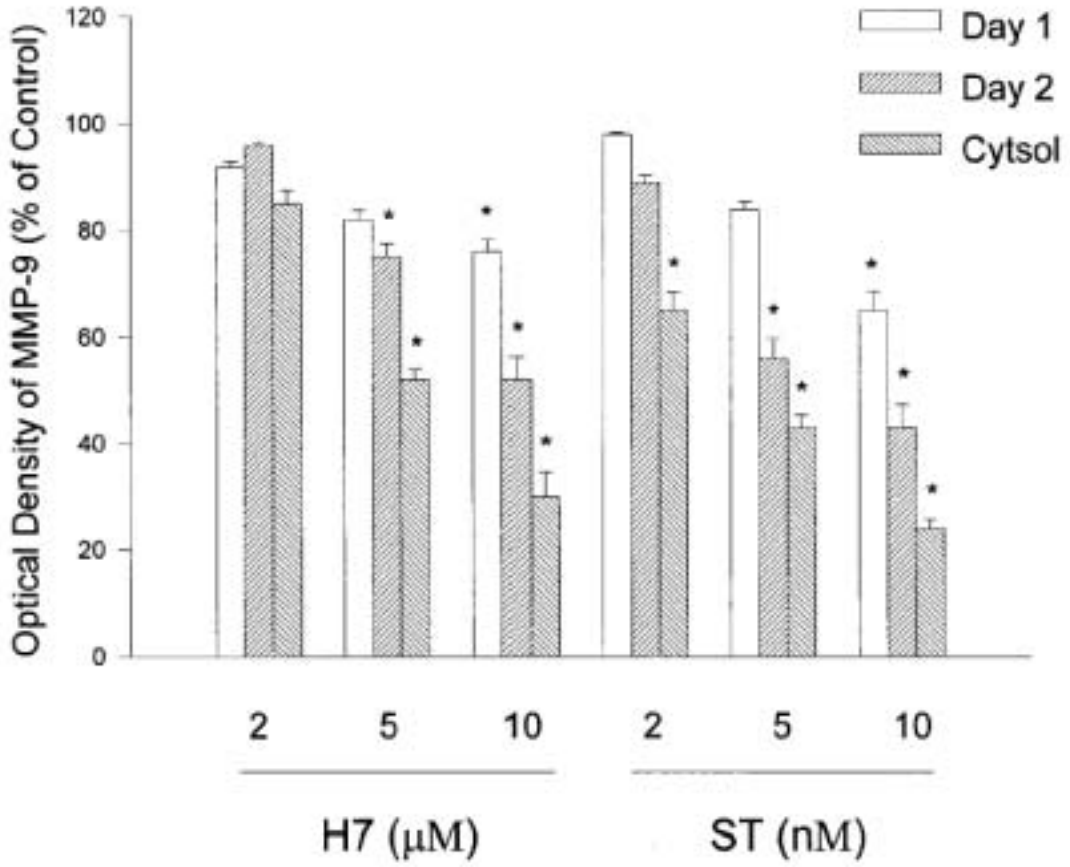

Fig. 4 Levels of MMP-9 from conditioned medium and cytosol from KB cells treated with ST or H7 were calculated from their gelatinolytic activity, as measured by AlphaImager 2000. Days 1 and 2 represent conditioned medium collected in day 1and day 2, respectively. Cytosol represents cell extract collected at day 2. Values are means and standard deviations of optical density from triplicate independent experiments. * denotes significant differences from control values with $P<0.05$.