嘉南藥理科技大學專題研究計畫成果報告

骨髓過氧化酵素的基因多型性與口腔癌及口腔黏膜下纖維化發生之研究

計畫類別：■個別型計畫　　□整合型計畫

計畫編號：CNPH92-10

執行期間：92 年 1 月 1 日至 92 年 12 月 31 日

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中華民國□93 年□2 月□27 日

一、中文摘要

在亞洲，口腔癌是常見的一種惡性腫瘤，在台灣更是男性癌症死亡率的第五位。咀嚼含菸葉的檳榔塊是造成口腔癌發生的主要原因，同時也與口腔下纖維化的發生有關。有許多研究證實咀嚼檳榔塊在口腔會產生活性氧化物。因為暴露在有毒物質下或因保護因子不夠，而導致過多活性氧化物的產生，可能會對細胞膜核甘酸及蛋白質等產生氧化性傷害。檳榔塊中的纖維在咀嚼的過程中，在口腔內膜造成的物理性創傷，可能會促使白血球細胞的侵潤，此時骨髓過氧化酵素會被釋放出，並產生活性氧化物。骨髓過氧化酵素基因在 $^{-463}$G→A 的取代，會造成轉錄的表現降低，因而酵素的表現及活性氧化物的產生也因而降低。因此本計劃的目的就是要來探討這三種酵素的基因多型性與口腔癌及口腔下纖維化之危險因子間的關係性。

關鍵詞：口腔癌; 口腔下纖維化; 檳榔塊; 骨髓過氧化酵素

Abstract

Oral squamous cell carcinoma (OSCC) is one of the most common malignant neoplasms in Asia countries, and is the fifth cause of male cancer mortality in Taiwan. Chewing betel quid (BQ) containing tobacco was found to contribute to the development of OSCC, and be causally linked to oral submucous fibrosis (OSF), a potentially malignant condition of the oral cavity. Previous studies found that chewing BQ generates reactive oxygen species (ROS) in oral cavity. The excessive formation of ROS may result from exposure to toxic agents and/or insufficiency of defense mechanisms, which might cause oxidative damage to the cellular membranes, DNA, and proteins. The fiber of BQ may cause physically injury of oral mucus membrane and neutrophils will be recruited and accumulate at the sites around oral mucosal lesions. Subsequently, myeloperoxidase (MPO) is released into the local environment and generate ROS. The $^{-463}$G→A substitution in the promoter region of the MPO gene has been associated with a decrease in transcriptional expression and thus reduced enzyme levels were available for the formation of ROS. **Therefore, the objective of our study is to investigate the association of MPO with BQ-related OSCC/OSF.**

Keywords: Oral squamous cell carcinoma; oral submucous fibrosis; betel quid; myeloperoxidase

I. Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common malignant neoplasms in Asia countries, and is the fifth cause of male cancer mortality in Taiwan (1). Chewing betel quid (BQ) containing tobacco was proposed as an important contributor to the development of OSCC (2). Although BQ in Taiwan does not contain tobacco, BQ chewing is highly associated with oral mucosal lesions (oral submucoous fibrosis, leukoplakia and OSCC) (3,4). Oral submucous fibrosis (OSF), a chronic disease of the oral cavity and oropharyngx, is characterized by fibrosis in the submucosa. OSF may be involved in progressive limitation of the mouth opening and the development of OSCC (5).

BQ is usually combined with areca nut, lime paste, betle leaf and tobacco. Recently, Nair *et al.* and we demonstrated that reactive oxygen species (ROS) is formed in the oral cavity while chewing BQ (6,7).

It has been proposed that ROS at low concentrations can induce signal transduction pathways and alter the expression of growth- and differentiation-related genes (8), but ROS at high concentrations have detrimental effects on cells. The excessive formation of ROS induced by exposure to toxic agents, such as tobacco and BQ, or insufficiency of defense mechanisms could cause oxidative damage to cellular membranes, DNA, and proteins. Moreover, ROS have been implicated in a broad variety of pathological processes ranging from atherosclerosis to carcinogenesis (9,10). Thus, chewing BQ might cause oxidative damage to buccal mucosa cells and then lead to or promote OSF/oral cancer formation.

Myeloperoxidase (MPO) is a metabolic/ oxidative enzyme located in the primary granules of neutrophils and monocytes/macrophages. The main function of MPO lies in the defense of the organism through production of HOCl, a powerful oxidant. The reaction products derived from the MPO-H_2O_2-Cl- system have a potent activity against a wide range of viruses, bacteria, fungi as well as some tumoricidal actions. Besides participating in the defense of the organism *via* the production of HOCl, MPO is released from neutrophils under inflammatory states. MPO and its reactive byproducts were found to induce oxidative stress and DNA strand breakage. Interactions between the neutrophil-derived reactive oxidants H_2O_2 and HOCl are probably involved in the etiology of inflammation-related cancer (11). The fiber of BQ may cause physically injury of oral mucus membrane and neutrophils will be recruited and accumulate at the sites of oral mucosal lesions. Subsequently, MPO may be released into the local environment and generate ROS.

MPO was also documented to activate specific procarcinogens, including benzo[*a*]pyrene intermediates, 4-aminobiphenyl, and the arylamines (12-14). Recently, MPO was found to be involved in non-infectious diseases like atherosclerosis, cancer and promyelocytic leukemia, as well as in neurodegenerative diseases, including Alzheimer's disease and multiple sclerosis (11). MPO is linked to these pathological states through its strong oxidative activity and/or its polymorphism characterized by differential expression of the protein.

Austin *et al.* reported a G→A transition in the promoter region at position –463 relative to exon 1 (15). This polymorphism lies within an *Alu* repeat containing a composite *SP1*-thyroid hormone-retinoic acid hormone response element. This single base-pair change alters the 4-bp invariant GCGG sequence of the *Sp-1* transcription factor consensus-binding site and thus results in reduced gene expression *in vitro* (16). Nevertheless, the effects on *in vivo* expression levels are remaining established. This G→A polymorphism in the 5' untranslated region of the MPO gene may lead to a reduced expression of MPO and thereby resulting in decreased bioactivation of carcinogen. Thus, individuals with one or more copies of the A-allele may be afforded a protection due to the decreased expression of MPO, the reduced formation of ROS, and the decreased metabolic activation of procarcinogens (such as benzo[*a*]pyrene in tobacco). The polymorphism of MPO was found to be involved in numerous diseases, such as acute promyelocytic leukemia and cystic fibrosis (16,17). On the other hand, individuals with a low activity allele (A allele) have

subsequently been found to be at a decreased risk of lung cancer (18). On the basis of this observation and the implication of MPO in the formation of ROS or in the metabolic activation of aromatic organic compounds, polymorphic MPO may also act as a susceptibility factor for those malignant diseases that are etiologically associated with BQ/tobacco-related OSCC. **Therefore, the objective of our study is to investigate the relationship of the MPO $^{-463}$G/A mutation with OSCC or OSF.**

II. Material and Methods

1. Study population and DNA isolation

Patients with diagnosed and histologically confirmed OSCC or OSF were recruited at the Department of Dentistry of Veterans General Hospital-Taipei, according to a protocol approved by the committee for the conduct of human research. Cancer-free control subjects were frequency-matched to case subjects on age, sex derived from questionnaires. These healthy controls did not have benign or neoplastic conditions. After informed consent, each subject donated 30 ml of blood and completed a questionnaire. History of BQ chewing, cigarette smoking, alcohol consumption were carefully recorded. DNA was purified from peripheral blood lymphocytes by standard SDS/proteinase K treatment and phenol/chloroform extraction. All isolated DNA samples were stored at –20 ^{o}C and aliquots of DNA for immediate analysis were stored at 4 ^{o}C.

2. Genotyping of MPO polymorphism

The PCR-RFLP-based assay described by London *et al.* was used to characterize the wild-type (G) and variant (A) MPO alleles at position –463. Using a Perkin-Elmer Corp. 9600 thermocycler, PCR products were generated using 300 ng of genomic DNA as a template and the forward primer 5'-CCGTATAGGCACAC-AATGGTGAG-3' and reverse primer 5'-GCAATGGTTCAAGCGATTC TTC-3'. The PCR reaction was carried out in a 30- l reaction with a final concentration of 50 M deoxynucleotide triphosphates (dNTP), 1.5 M $MgCl_2$, 0.1 M for each primer, and 1 unit of Taq polymerase (Perkin-Elmer, Norwalk, CT). After an initial denaturation at 94 ^{o}C for 1 min, 56 ^{o}C for 1 min, and 72 ^{o}C for 1 min, with a final extension at 72 ^{o}C for 7 min. The PCR products were then digested with 5 units of *Aci*I (New England Biolabs, Beverly, MA) overnight at 37 ^{o}C and separated on a 2.5% agarose gel containing 0.5 g/ml ethidium bromide. The G→A substitution at position –463 leads to a loss of the *Aci*I restriction site within the 350-bp amplification fragment that is used to distinguish the two alleles. In addition, an invariant *Aci*I restriction site present in both alleles yields a 61-bp fragment that serves as an internal control (18).

III. Results & Conclusions

	Case	G/G type	G/A type	A/A type
Control	156	126 (80.77%)	28 (17.95%)	2 (1.28%)
OSCC	68	47 (69.11%)	17 (25.0%)	4 (5.89%)
OSF	60	51 (85%)	8 (13.33%)	1 (1.67%)

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