行政院國家科學委員會專題研究計畫成果報告

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中文摘要

咀嚼檳榔塊是口腔癌發生的主要危險因子，最近的研究顯示咀嚼檳榔塊所釋放之活性氧化物是口腔癌發生的一個貢獻因子，然而台灣地區所食用的檳榔塊與其他國家所食用的組成有所不同，因此我們計畫研究台灣本土的檳榔塊是否也會產生活性氧化物。我們讓志願者咀嚼不同組成的檳榔塊，並同時加入苯氨基丙酸以測量唾液中氫氧自由基將其氧化成臨-及間-酪氨酸的濃度，結果發現咀嚼檳榔塊者比咀嚼口香糖之對照組來的高，另外咀嚼含荖花之檳榔塊的組別其間酪氨酸的量也較含荖葉之檳榔塊的組別來的高，此研究成果證實咀嚼檳榔塊確實會在唾液中釋放出活性氧化物。

關鍵詞：檳榔嚼塊、活性氧化物、臨-及間-酪胺酸

Abstract

Chewing betel quid (BQ) has been implicated as a major factor for the development of oral squamous cell carcinoma (OSCC). Recent studies have suggested that BQ-generated reactive oxygen species (ROS) as one of the contributing factors for oral carcinogenesis. However, the BQ used in Taiwan is different from that used in other countries. This study is designed to test whether ROS is generated and consequent effects in locally prepared BQ *in vivo*. We measured the hydroxyl radical formation, as represented by the presence of *o*- and *m*-tyrosine in saliva from volunteers who chewed BQ containing 20 mg phenylalanine. Their saliva contained significantly higher amounts ($p<0.05$) of *o*- and *m*-tyrosine than that in the controls. In addition, chewing BQ containing *Piper betle* inflorescence generated higher amounts of *m*-tyrosine, but not *o*-tyrosine, in saliva than chewing BQ containing betle leaf. The above findings demonstrated that ROS such as hydroxyl radical is formed in human oral cavity during BQ chewing.

Keywords: Betel quid, Reactive oxygen species, *o*- and *m*-Tyrosine

Introduction

Chewing betel quid (BQ) containing tobacco has contributed to the development of oral squamous cell carcinoma (OSCC) (1). OSCC is one of the most common malignant neoplasms in Asia countries, and now is the fifth cause of male cancer mortality in Taiwan (2). The number of BQ chewers was estimated at two million among the 20 million inhabitants in Taiwan (3). However, the OSCC incidence is low in Taiwan compared to other BQ chewing countries (4). The reason for this discrepancy has remained elusive.

BQ basically is a combination of areca nut, lime paste, betle leaf and tobacco. Recent studies have pointed out that areca nut extract (ANE) and lime (pH 9.5) generated reactive oxygen species (ROS) and induced oxidative DNA damage *in vitro* (5-7). We have demonstrated that ANE also induced oxidative DNA damage in cultured CHO-K1 cells (8). Recently, Nair et al., (9) further confirmed the formation of hydroxyl radical in the oral cavity by using phenylalanine as a trapping agent. All these evidence suggest that chewing BQ generates

ROS in oral cavity, and consequently might cause oxidative damage in DNA of buccal mucosa cells. This oxidative DNA damage may then lead to or promote oral cancer formation.

It is well known that the preparation of BQ varies in different geographical locations. The BQ chewed in Taiwan contains tender areca with husk instead of the ripe and dried areca nut without husk, the kernel of areca nut, used in other countries. In Taiwan, *Piper betle* inflorescence, sometimes substituted by betle leaf, is often added to BQ, which is not used elsewhere except in Papua New Guinea and Gum (10). In addition, tobacco is not included in the locally prepared BQ. By analyzing 8-OH-dG formation, we have shown that the ANE prepared from tender areca nut generated less oxidative DNA damage in CHO-K1 cells as compared with ANE prepared from ripe areca nut (8). The reason behind this differential oxidative DNA damaging effect between these two kings of ANE has not been elucidated. *Piper betle* inflorescence contains high level of hydroxychavicol (11), which is potent in inducing oxidative DNA damage *in vitro* and in culture cells (12,13). Whether chewing BQ containing tender areca nut and *Piper betle* inflorescence can generate ROS have not been documented.

In this study, we have tested the ROS generating potential in saliva from volunteers chewing different types of local BQ by measuring the formation of *o*- and *m*-tyrosine from L-phenylalanine.

Results

The currently employed method using HPLC equipped with fluorescence detector easily separates the *o*-, *m*- and *p*-tyrosine (Figure 1A). No *o*- and *m*-tyrosine was detected in saliva from subjects who chewed gum and 20 mg phenylalanine (Figure 1D). However, high concentration of *p*-tyrosine and small amount of phenylalanine and *o*- and *m*-tyrosine were detected in human saliva from subject who chewed BQ containing *Piper betle inflorescence* but without phenylalanine for 15 min (Figure 1C). On the other hand, saliva samples taken from subject who chewed BQ containing phenylalanine showed the elevated amount of *o- and m*-tyrosine in saliva (Figure 1B). Total amounts of *o*- and *m*-tyrosine detected in saliva from subjects who chewed BQ with phenylalanine (n=9) were significantly higher ($p<0.05$) than those of controls who chewed BQ without phenylalanine (n=4) (Figure 2). The ROS generating capacity in saliva between two types of locally chewed BQ, which contains fresh areca nut, lime paste, *Piper betle* inflorescence or betle leaf, was also tested. Chewing BQ containing betle leaf generated less ($p<0.05$) *m*-tyrosine as compared to chewing BQ *Piper betle inflorescence*. However, the *o*-tyrosine concentration in saliva was not different following chewing this two types of BQ.

Discussion

The formation of *o*- and *m*-tyrosine from phenylalanine has been reported in the presence of hydroxyl radical generating systems (14,15), and this reason may serve as a good marker of hydroxyl radical-induced damages *in vivo* (9,16). Using this system, the present study has demonstrated that chewing BQ containing tender areca nut, lime paste and *Piper betle inflorescence* or betle leaf generates elevated ($p<0.05$) amount of hydroxyl radical in human oral cavity as compare to controls (Figure 2). This result corresponded well with the *in vitro* studies, which indicated that tender ANE and lime (pH>9.5) generate ROS and induce 8-OH-dG *in vitro* and in CHO-K1 cells. This reason may result from the auto-oxidation of polyphenols in ANE and consequently the generation of hydrogen peroxide, which then lead to oxidative damage through iron catalyzed Fenton reaction (8,9).

Piper betle inflorescence is a unique additive to the locally used BQ, and it contains high concentrations of safrole and hydroxychavicol, respectively. Betle leaf, a substitute for *Piper betle* inflorescence, contains small amount of hydroxychavicol but without safrole. Safrole is a documented rodent hepatocarcinogen, and has been shown to induce oxidative DNA damage in the liver of rats (17).

Hydroxychavicol is the major safrole urinary metabolite in human (18), and exhibits dose-dependent suppression of 7,12-dimethyl benzathracene-induced mutagenesis *in vitro* (19) and methyl-(acetoxymethyl)-nitrosamine -induced hamster oral carcinogenesis (20). On the other hand, we have shown that hydroxychavicol has potent oxidative damaging potential in an *in vitro* test system and in cultured cells (13,21).

In conclusion, this study demonstrated that hydroxyl radical is formed in the oral cavity while chewing differently prepared BQ by measuring *o*- and *p*-tyrosine formation from phenylalanine as a trapping agent. The results also suggest that areca nut, the major component of BQ, is responsible for ROS generation.

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Fig. 1 HPLC chromatograms of *p-, m-,* and *o*-tyrosine standards (A); saliva sample from subjects who chewed BQ containing *Piper betle* inflorescence and 20 mg phenylalanine (B); saliva sample from subjects who chewed similarly prepared BQ without phenylalanine (C); saliva sample from subjects who chewed gum with 20 mg phenylalanine (D).

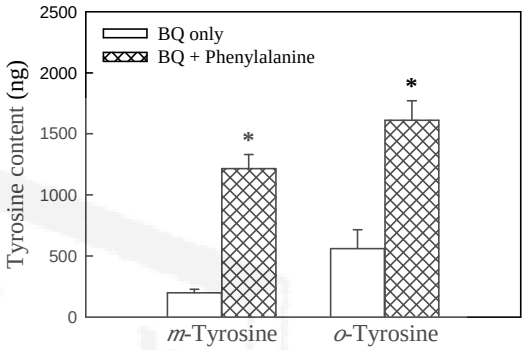
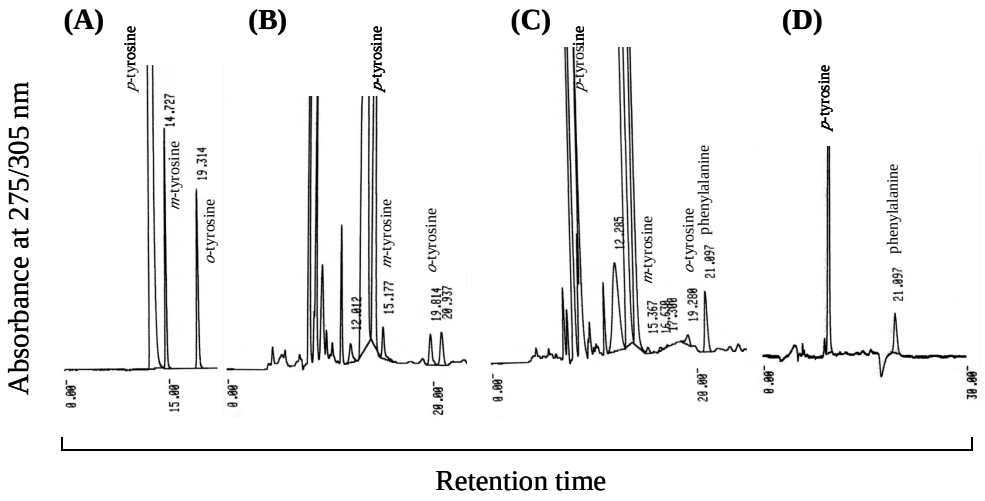


Fig. 2 Total *m-* and *o*-tyrosine content detected in saliva from subjects who chewed BQ containing *Piper betle* inflorescence with (n=9) or without phenylalanine (n=4). *p<0.05 by Student's t-test.



行政院國家科學委員會補助專題研究計畫成果報告

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