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BETEL QUID CHEWING AND ORAL CANCER

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Abstract

Over the years, epidemiological research has uncovered the relationship between chewing of betel quid and oral cancer. The main ingredients used in the quid are betel nut, betel leaf, slaked lime and tobacco. The carcinogenicity of these ingredients has been investigated in experimental animals and laboratories. The results obtained are affirmative in some cases and not in others but it is highly probable that tobacco and betel nut are the source of carcinogens or their precursors. However, the carcinogens in the nut have not been unequivocally identified. Arecoline and arecaidine are suspected to be carcinogens. Nitrosamines formed from alkaloids in betel nut and tobacco during betel quid chewing may be implicated in the etiology of oral cancer. The information obtained from studies in humans, animals and laboratories is reviewed in this paper.

Introduction

The betel plant *(Areca catechu)* belongs to the same Order as pepper. The leaf which has a characteristic pungent taste, is usually chewed with one or more of the three ingredients, namely tobacco *(Nicatiana tabacum),* betel nut and slaked lime (calcium hydroxide). Crushed betel nut with or without tobacco is placed on a fresh betel leaf painted with slaked lime. The leaf is then folded into a "quid" and taken into the mouth where it is chewed or kept for a variable length of time. Betel nut is composed of 11.4-26.0% tannins, 0.15-0.67% alkaloids, 1.3-17.0 % fats, 47.2-84.5 % carbohydrates, 4.9-9.3 % proteins, 0.0185-0.05 % calcium, 0.13-2.35 % phosphorus and 1.5-11.6 % iron[1]. The nut has numerous pharmacological properties which are attributed to the major alkaloid, arecoline. Apart from arecoline, there are traces of other alkaloids such as arecaidine, guavacine and guavacoline. Betel nut has been used as an anthelmintic in humans and animals. It was considered so efficacious against tapeworms and round worms and so highly esteemed that it was used in British Pharmacopoea[2].

The chewing of betel quid is an ancient habit in many oriental countries such as India, Pakistan, Sri Lanka, Philippines, Malaysia, Thailand and Papua-New Guinea. The habit occupies the same behavior that tobacco smoking does in western countries. On a worldwide basis, it is expected that over 250 million individuals addict the betel chewing

habit[3]. Over the years the epidemiological approach has substantially contributed to our knowledge of a relationship between betel chewing and oral cancer. The pace of this research has recently quickened, particularly in efforts to identify carcinogens in betel quid. A substantial number of studies during the last two decades have been accumulated convincing circumstantial evidence to suggest the betel quid chewing as one of the major risk factors for oral cancer. Accordingly, this paper reviews the incidence and etiology of oral cancer, the role of betel quid chewing in the development of oral cancer and the studies on carcinogenicity of betel quid and its ingredients. Such considerations may contribute to future research of oral cancer.

Incidence and Etiology of Oral Cancer

Oral cancer is usually defined as neoplasm of the lip, tongue and intra-oral tissues including the oropharynx[4]. Although the disease has been reported in various age groups but it occurs frequently in patients over the age of 60 years[5]. Dramatically different patterns of incidence have been observed for the disease in different parts of the world. For example in some countries such as India and Sri Lanka oral cancer cases constituted almost 30 to 50 percent of all cancers in males whereas in most western countries the disease accounted for only 2 to 6 percent of all cases of cancer[6]. The high risk of oral cancer was also reported in some countries of South East Asia[7]. In Thailand, comparing with total cancer incidence, the rank of oral cancer was third in both sexes[8]. The incidence was 8 percent of all cancers.

Generally, incidence rates of oral cancer are higher in males than females[9]. The morbidity and mortality rates of the diseases vary with various countries, cultures within countries, geographic areas, occupations and ethnic background[10]. In addition to differences environmental exposure, variations in the incidence may be caused by differences in the methods of collecting data from country to country. Not all countries have cancer registries that require the reporting of cancer cases to a central agency. Instead, incomplete information may be obtained from hospitals, departments of pathology or death certificates. Such variations may influence the pattern of oral cancer being reported.

Epidemiological studies attempt to elicit possible causative factors for oral cancer. The etiology of the disease has been discussed extensively elsewhere[11,12]. There are many risk factors related to oral cancer, namely tobacco, alcohol, nutritional deficiencies, chronic candidosis, betel quid chewing and chronic oral irritaion. Tobacco is a traditionally high risk factor for oral cancer[13-15]. It has been, for many years, under suspicion as a carcinogen. Both volatile and non volatile carcinogens, N-nitrosamines, have been detected in tobacco and tobacco smoke[16,17]. Alcohol potentiated the effects of tobacco smoke (and perhaps other agents) on cancers of the mouth, pharynx, esophagus and larynx, and by including cirrhosis, it appeared to increase the risk of liver cancer[18-20]. Although it is difficult to estimate the contribution of alcohol to oral cancer risk, but in combination with tobacco smoking

it may account for 75 percent of oral cancer[21]. The mechanism(s) by which alcohol promotes carcinogenesis is not clear, but it may involve the promotion of nutritional deficiencies or some as yet unidentified cocarcinogens in alcoholic beverages.

The roles of dietary factors are also being regarded as of increasing importance in carcinogenesis. The close relationship between sideropenic dysphagia (Patterson-Kelly or Plummer-Vinson syndrome) caused by chronic iron deficiency and oral cancer has been demonstrated[22-24]. There is no doubt that iron is essential for overall integrity and health of the epithelia of the upper gastrointestinal tract and its importance may lie in its contribution to normal enzyme systems. One suggestion is that iron deficiency increases the susceptibility to carcinogens by increasing the permeability of oral mucosa[25,26]. In addition to dietary factors, chronic disease such as chronic candidosis may have a bearing on the occurrence of oral cancer[27]. Chronic hyperplastic candidosis itself was the cause of leukoplakia and as such might be regarded as having possible premalignant potential[28,29]. The development of malignancy in candidal leukoplakia was more frequent than in many other types of leukoplakia[30].

As mentioned previously, there is a relatively high frequency of oral cancer in oriental countries. Many investigators have associated the elevated risk of the disease to the widespread practice of betel quid chewing. The role of the quid in oral cancer will be discussed later. Other factors also mentioned as etiological agents are poor oral hygiene, faulty restoration, sharp edges of tooth and ill-fitting dentures[31-33]. However, it is extremely difficult to prove a causal relationship between chronic irritation and the development of oral cancer. The problem is that oral neglect is often part of the clinical picture of oral cancer. No experimental evidence exists to prove that poor oral hygiene *per se* has any role.

The Role of Betel Quid Chewing in the Development of Oral Cancer : Epidemiological Considerations

The high incidence of oral cancer in Central and South East Asia has for long been linked with the habit of betel nut chewing particularly when tobacco has been incorporated into the quid. It was shown that in India and Sri Lanka there was a low incidence(2.02 %) of oral cancer in betel nut chewers whereas an elevated incidence of disease(49.9 %) was demonstrated in patients who had habitually chewed both betel nut and tobacco[7]. In Thailand the relative risks of chewing betel quid with tobacco for oral cancer were 2.94 and 3.21 in males and females, respectively[34]. It is of special interest to note that in this study, the relative risk to cigarette smoking was low for oral cancer. The results were comparable to those in India and Sri Lanka. A study of 103 oral cancer patients in Taiwan, showed that 53.4 percent of all cases had the habit of betel quid chewing[35]. Among them, 36.3 percent chewed betel quid only, whereas 63.7 percent of them had one or more of other habits such as smoking and alcohol drinking. For the control group in the study, the percent distribution

of smoking, alcohol drinking and betel chewing was 42.5 %, 22.5 % and 0 % respectively. The difference between cancer and noncancer patients addicted to smoking and drinking was not significant but the difference in betel quid chewing was great. In reviewing the literature, Khadium[36] noted that the addition of tobacco to the betel quid increased the relative risk from 4 without tobacco, to as much as 29 with its addition.

From all the above information, it seems therefore that it is virtually difficult to exclude the contribution of tobacco to the carcinogenicity of betel quid. There remains, however, some disagreement as to whether it is, in fact, the tobacco component of the quid which imparts its observed carcinogenic capacity. Alkinson *et al.*[37], however, suggested that the slaked lime may be the possible carcinogen in betel quid. This possibility is supported by the observation of elevated oral cancer incidence related to endemic chewing habits in Malaysia[38] and Papua-New Guinea[39] where it is customary to use the betel nut with the lime, but without tobacco. In contrast, the incidence was low in Afghanistan and Nigeria where tobacco was chewed without the lime[7]. Although there is no definite evidence that betel nut has a direct carcinogenic action, it is believed that chronic irritation and continued friction of the cheek against the betel quid and the sharp edges of the abraded teeth (caused by the chewing habit) may cause traumatic ulcer, leukoplakia and even malignant transformation. The changes that occur in the oral mucosa as a result of betel quid chewing in individuals who have or have not yet developed carcinomas and the incidence of such changes have been studied in different parts of the world where the habit is widespread[40-42] The results may be a reflection of the differences in betel quid composition and also the difficulty of catagorizing such changes.

At the present time, the exact carcinogenic agent of the betel quid has not been defined. Nevertheless, the high risk of oral cancer among betel quid chewers implies that the quid may contain suspected carcinogen(s). However, the additional risk of the above mentioned factors should not be overlooked. Additional research is needed to assess the risk of oral cancer and the various types of betel quid and the critical dose and duration of chewing. With an improvement in the methodology of assessing the exposure variables, investigators should be able to clarify their relationship with oral cancer.

In Vitro and In Vivo Studies on Carcinogenicity of Betel Quid and its Ingredients

Many genotoxic assays have been used to evaluate the toxic effect of betel quid in humans. Studies in which small numbers of betel quid chewers and non-chewers were compared have shown an increased occurrence of micronuclei in exfoliated cells of the oral mucosa and a higher frequency of sister-chromatid exchanges in peripheral lymphocytes from chewers[43,44]. Moreover, the saliva of betel quid and tobacco chewers has been shown to cause chromosomal aberration(chromatid break and chromatid exchange) in Chinese hamster ovary (CHO) cells[45]. Compounds that have been detected or may be present in

the saliva of betel quid chewers include arecoline[46], arecaidine[47], eugenol[48] and anatabine[49], all of which have been shown to exert genotoxic effects. Although the genotoxicity tests do not directly demonstrate the carcinogenic property of betel quid but a fairly good correlation between the tests and carcinogenicity in rodents[50] implies that the quid has some carcinogenic potency. A longer duration of betel chewing may increase a chance of neoplastic development in the oral cavity. In addition to genotoxic assays, considerable efforts have gone to determining N-nitroso compounds, potent carcinogens, in the saliva of betel chewers. Some betel nut-specific N-nitroso compounds such as N-nitrosoguvacoline(NG) have been reported in the saliva of betel chewers. Moreover, nicotine, conitine and tobacco specific nitrosamines were also detected in the saliva of chewers of betel nut with tobacco and in that of chewers of tobacco[51,52]. The results suggested that N-nitroso compounds could be formed under the condition of betel quid chewing. Direct proof that such nitrosation reactions can occur was provided by Wenke and Hoffmann[53]. The authors reported that N-nitrosoguvacoline(NG), 3-(methyl nitrosamino) propionitrile (MNPN) and 3-(methyl nitrosoamino) propionaldehyde (MNPA) could be formed by N-nitrosation of arecoline, the major alkaloid in betel nut. Formation of NG occurred probably by oxidative clevage of the N-methyl bond, similar to the reaction which other cyclic tertiary amines such as N-methyl-piperidine, N-methylpyrrolidine or nicotine underwent with nitrite. In case of MNPN, it was suggested that the cyano group of MNPN did not stem from the thiocyanate ion because MNPN could be formed in the absence of the ion. The mechanism of this reaction is still unknown. A large excess of $NaNO_2$, pH 3-4 and high reaction temperature favored formation of NG relative to MNPN and MNPA. The amounts of MNPN and MNPA were increased when the reactions were performed under milder conditions (pH 4-5, only a slight excess of $NaNO_2$). In neutral solution, only NG and MNPA could be detected, however, at slightly acidic conditions (pH 6) all three nitrosamines were present. A reaction time of at least several hours was needed to obtain detectable amounts of all three nitrosamines. Longer reaction times led to increased yields of the agents.

A study by Shivapurkar *et al.*[54] indicated nitrite levels in saliva of individuals in the Bombay area were higher than in those living in western countries. Users of betel nut without tobacco showed an increase of salivary nitrite during chewing in contrast to those who chewed betel nut product with tobacco who reflected even lower nitrite levels in saliva than nonchewers. It was also indicated in a limited number of cases that salivary thiocyanate remained rather constant (0.2-0.5 M) during chewing while the pH changed from slightly acidic to neutral. These conditions would facilitate the formation of nitrosamines from arecoline during betel quid chewing. Although recent evidence suggests that nitrosamines can be formed in the oral cavity but carcinogenic action of the agents in oral cavity has not been investigated. Lijinsky and Taylor reported NG to be noncarcinogenic in Sprague-Dawley rats when given at 0.88 mM in drinking water five days weekly for 50 weeks[55]

MNPA has so far not been tested for carcinogenic activity. MNPN was genotoxic in the hepatocyte primary culture DNA repair test[56]. Subcutaneous injection of 1.1 mM of MNPN in 60 subdoses could induce tumors in all of 15 male and of the 15 female F 344 rats within 24 weeks[57]. Twenty-six of the rats had tumors in at least two different organs. Twenty-seven rats had esophageal tumors, 21 had nasal tumors, 11 had tongue tumors and two animals had either pharyngeal carcinomas or papillomas of the forestomach. The different types of tumors found in the MNPN treated animals resembled those found in F 344 rats treated with methylvinylnitrosamine, a possible metabolite of MNPN[58].

In addition to forming nitroso compounds, arecoline could be metabolized to arecaidine in rats[59]. Both alkaloids were derivatives of 1,2,5,6, tetra-hydro pyridine, i.e. they all contained a Δ^3-ethylenic bond. The bond could react with N-acetyl-L-cysteine and with glutathione in neutral aqueous solution at 37° C. The addition of thiol groups of glutathione across the Δ^3-ethylenic bond of arecoline was apparently not enzyme-catalyzed because various liver preparations did not increase the rate of the reaction at pH 6.8 and 37 ° C. The mechanism of the addition of thiol to the Δ^3-ethylenic bond of arecoline was complex. It might be a nucleophilic addition involving attack by thiol on an activated ethylenic bond. The reaction occurred maximally at neutral pH and decreased rapidly with increasing acidity. At neutral pH, the reaction was bimolecular and second order when the reactants were in approximately equimolar concentrations and pseudounimolar first-order when arecoline was in large excess. Arecaidine could also react with the both thiol-containing substances but much more slowly. The finding suggested that in the betel quid chewing condition, arecoline might react with the methyl ester group of cysteine and be converted into arecaidine by hydrolysis with lime. The reactions of arecoline and arecaidine with thiol groups indicated that they were biological alkylating agents. Such a property was a feature of many chemical carcinogens either with or without metabolic activation[60]. Using cellular transformation techniques based on the changes of baby hamster kidney cells due to carcinogens, arecoline and arecaidine gave an almost identical positive responses[61]. A very good correlation between cellular transformation and carcinogenicity[62] supports the notion that betel quid contains carcinogens or their precursors. Other studies have emphasized a relationship between mutagenicity and carcinogenicity of the quid. Shirname *et al.*[63] demonstrated the mutagenicity activity of betel quid and its ingredients by using *Samonella typhimurium* tester strains TA 100, TA 1535, TA 98 and TA 1538, both in the presence and absence of S-9 mixture. Aqueous extracts of betel quid, betel quid with tobacco and betel nut were mutagenic in strain TA 100 whereas that of betel leaf was not mutagenic in any of the four strains. Arecoline and arecaidine were also mutagenic in all four tester strains. Arecoline had a greater mutagenic potential than arecaidine. Feeding Swiss mice with the above constitutents showed that betel nut and betel quid produced lung tumors in 47% and 26% of the treated animals, respectively. However, when betel nut was fed with betel leaf, tumorigenicity was lowered to 38%. Betel leaf

alone was not tumorigenic. The rich ascorbic acid content of this leaf was suggested to be responsible for the antitumorigenicity. Ascorbic acid could inhibit the formation of nitrosamine from betel quid ingredients by reacting with nitrite to form non-nitrosating species[64].

In fact, there have been several reports which demonstrated carcinogenicity of betel quid in experimental animals. Painting extracts of betel quid with tobacco to the ears of mice for 2 years could develop a low incidence of papilloma and squamous cell carcinoma at the treated sites[65]. Similar changes were also observed in the hamster buccal pouches treated with extracts of betel nut alone or in combination with tobacco[66,67]. On subcutaneous administration of betel nut extract, 60 percent of Swiss mice developed fibrosarcomas at the site of injection[68]. In addition when a mixed dimethyl sulfoxide (DMSO) extract of tobacco and betel nut was used, skin papilloma and epidermoid carcinoma could develop in some animals. The findings were later confirmed by Shivapurkar *et al.*[69]. Subcutaneous injection of polyphenol fractions of betel nut could induce fibrosarcomas in treated Swiss mice whereas administration of betel nut with betel leaf showed total protection to tumor development. In contrast, some parallel experiments could not produce any tumors in rats fed with betel nut[70] and in hamster cheek pouches painted with betel quid ingredients[71,72]. The reason for the conflicting findings is not known. These observations, however, imply that betel nut has some carcinogenic potency which is expressed only under suitable experimental conditions. This suggestion has been supported by the experiments of Rao[73] who treated hamster buccal pouches with benzo(a)pyrene and betel quid ingredients for 10 days and found complete or partial suppression of tumor production depending on the dose of benzo(a)pyrene. Alternatively, when the carcinogen included with tobacco or betel nut was given for 6 months, there was a considerable increase in squamous cell carcinomas. However, it became apparent that betel nut was not equally effective in potentiation of carcinogenesis by all carcinogens. The simultaneous feeding with 1,4-dinitrosopiperazine and saccharin-coated betel nut for 40 weeks showed no potentiation of carcinogencity of 1,4-dinitroso-piperazine. The carcinogen alone could induce more forestomach tumors than with the coated nut[74]. In such studies, mice fed with betel nut could also develop tumors.

As mentioned above, nutritional status may play an important role in oral cancer. The effects of vitamin deficiencies on carcinogenesis of betel quid have been studied in experimental animals and also in humans. A high incidence of focal epithelial hyperplasia and papilloma was observed in the upper digestive tract (tongue, buccal, oral mucosa, esophagus and forestomach) of rats given a vitamin A-deficient diet mixed with betel nut and calcium hydroxide[75]. This is supported by the report of Stich *et al.* who found that supplementation of a diet with retinol and beta-carotene could reduce micronucleus formation in the oral mucosa of betel chewers[76]. The relationship of vitamin A, beta-carotene and other retinoids to neoplasia has been recently reviewed in a number of publications[77,78]. Vitamin A is necessary for the normal differentiation of tissues. Since cancer can be viewed as a disease of

arrested cellular differentiation, vitamin A is proposed to induce normal development in premalignant cells and prevent the progression into cancer. However, the exact biochemical mechanism(s) by which the vitamin is involved in oral carcinogenesis is unknown. In general, a model for transformation from the normal to the malignant phenotype is the two-step model of carcinogenesis[79] This approach divides the malignant process into initiation and promotion. Initiation is the irreversible genetic alteration of the target cell by a carcinogen. Promotion results in the phenotypic expression of that genetic alteration. Initiators and promotors may be identical, may vary in dose or frequency of contact with the target cell or may be entirely different entities. The provitamin, beta-carotene (but not the retinoid) may function directly to block initiation by blocking the effects of single oxygen, a potentially carcinogenic free radical. The role of retinoids in this model of carcinogenesis appears to be that of a late-stage antipromotor. In the production of skin cancer in mice, retinoic acid inhibited the induction of the enzyme ornithine decarboxylase, an enzyme inducible by some tumor promotors, but not by initiators[80]. Alternatively, it has been hypothesized that retinoids may directly compete with promoting agents for control of cellular differentiation[77]. A third possibility is that retinoids inhibit transforming growth factors, polypeptides capable of inducing neoplastic growth in normal cells. Retinoids have been shown to block the effects of a sarcoma-transforming growth factor *in vitro,* and possibly may accomplish this activity by blocking the function of oncogene[81].

A similar observation was made for vitamin B. In this case, deficiency of vitamin B complex also enhanced tumorigenesis of betel quid ingredients in Swiss mice[82]. Tumors were observed in 39 percent of vitamin B complex-deficient mice receiving arecoline in combination with KNO_3 and lime while tumors could be developed in only 6 percent of the corresponding group of mice kept on a normal diet. Arecoline tumorigenicity in females was evident only in vitamin B-deficient animals. Little is known about the mechanism(s) by which the deficiency of vitamin B enhances oral carcinogenesis. Vitamin B deficiency had some effect on metabolism of carcinogens especially nitrosamines. In the study of carcinogenicity of dimethyl nitrosamine (DMN), the activity of the microsomal enzyme, DMN-demethylase I was increased in riboflavin deficient rats whereas DMN-demethylase II was unaffected[83]. The level of DNA damage by DMN was also enhanced in riboflavin deficient rats. The level of 0^6-methylguanine was slightly higher in the treated group. In contrast to riboflavin, thiamine deficiency caused an increased DMN-demethylase II but not DMN-demethylase I. However, the damage of DNA due to DMN was also observed in thiamine-deficient animals. For this reason, it is possible that vitamin deficiency somehow alters the metabolism of betel quid ingredients in the body which may in turn, increase the susceptibility of the host to carcinogenic effect of the quid.

Concluding comments

According to the evidence obtained from studies in humans, animals and *in vitro*

studies, it is likely that betel quid has a carcinogenic property. However, it has been difficult to pinpoint a single carcinogen in this mixture. Substantial studies have provided evidence that alkaloids especially arecoline and arecaidine as well as nitrosamines, which can be generated within oral cavity during chewing betel nut alone or in combination with tobacco, may be implicated in the development of oral cancer. It has been shown by a number of investigators that the metabolism of carcinogens in oral tissues may be an important determinant in the induction of oral cancer[84-86] Thus chemical carcinogens in betel quid must be metabolized to a common electronic form, i.e. reactive electrophiles, before they can react with cellular components and act as carcinogens. However, at the present time, evidence for this idea has not been obtained. More study is required to elucidate the nature of carcinogens in betel quid. The molecular mechanisms involved in both the initiation and promotion stages are not yet evident in oral carcinogenesis. Additional research on the carcinogenicity of betel quid are required in order to clarify the role of the betel chewing habit in the etiology of oral cancer. Nevertheless, present knowledge of betel quid suffices to warrant precautions towards its toxic effect. Messages on health education to prevent oral cancer should therefore be directed against betel quid chewing and smoking habits.

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บทคัดย่อ

ผลวิจัยทางวิทยาการระบาด แสดงให้เห็นว่าความชุก (prevalence) ของโรคมะเร็งในช่องปากมีสูงในกลุ่มคนที่เคี้ยวหมาก จึงทำให้มีความเชื่อว่าหมากและส่วนผสมอื่น ๆ ที่ใช้ร่วมในการเคี้ยวหมาก อันได้แก่ ใบพลู ยาเส้น และปูน น่าที่จะมีส่วนเกี่ยวข้องกับการเกิดโรคดังกล่าว อย่างไรก็ตามผลการศึกษาในสัตว์ทดลองและในห้องปฏิบัติการยังมิอาจระบุได้ว่าสารใดในหมากที่เป็นสารก่อมะเร็ง หรือสารเริ่มต้นการก่อมะเร็ง แต่เชื่อว่า arecoline และ arecaidine ซึ่งเป็นสารประเภทอัลคาลอยด์ (alkaloid) ในหมากน่าจะเป็นสารก่อให้เกิดมะเร็งในช่องปาก โดยพบว่าอัลคาลอยด์ในหมากและยาเส้น สามารถเปลี่ยนเป็นไนโตรซามีน (nitrosamines) อันเป็นสารก่อมะเร็งได้ในขณะเคี้ยวหมาก บทความนี้ผู้เขียนได้ทำการรวบรวมผลงานวิจัยที่เกี่ยวกับความเป็นพิษของหมากอันได้จากการศึกษาในคน สัตว์ทดลองและห้องปฏิบัติการ