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Betel Quid and Oral Cancer: A Review

Steven Thomas and John Kearsley

INTRODUCTION

INITIAL REPORTS suggesting a relationship between betel quid chewing and oral cancer appeared early this century in India [1–4]. Fells [3] in 1908 observed that almost every Indian in Southern India, regardless of social class or sex, chewed a mixture of areca nut, betel leaf, tobacco and slaked lime (calcium hydroxide) and commented that "the favourite spot for the cancer to originate is just the spot where the quid lies". Davis [5] described precisely the components of the quid in Filipino chewers, remarking on the inclusion of tobacco in the quid and the importance of slaked lime in the development of "Buyo" cheek cancer. Orr [6] considered that both the tobacco type and the source of slaked lime were important aetiological factors in Southern India. Much attention has since focused on the role of tobacco in the betel quid as a cause of oral cancer.

The term "betel" can be confusing since it refers to a combination of ingredients in the form of a quid. Principal constituents of the quid are the fruit of the 'betel-nut palm' *Areca catechu* Linn., the leaf, steam or inflorescence of *Piper betle* Linn. and calcium hydroxide. In India, tobacco is frequently added to the quid as shredded sun-dried tobacco leaves or stems, together with catechu (a resin from the *Acacia catechu* or *Acacia suma*) and a variety of spices such as cardomon, cloves, sandalwood, camphor, nutmeg, mace, peppermint and an extract of the flower of *Pandanus ordoratissimum* [7, 8].

The habit of betel quid chewing is widespread and its use has been documented from the East African coast to Eastern Melanesia and throughout India and South East Asia. The belief that betel quid chewing originated in an area of West Malaysia [9] is supported by botanical evidence that *Areca catechu* and *Piper betle* are native to this region. Furthermore, the history of betel quid chewing in the Malay area appears to have been lost in antiquity [10]. Betel chewing probably spread to Southern India in a period of trade and early missionary activity following the Indian colonisation of the Malay area and South East Asia [9]. About 50 species of *Areca* palms are found from India to the Solomon Islands; 10 species are found in Papua New Guinea (PNG) alone [11] and Theodoratus [9] has suggested that the *Areca catechu* may be native to Ceylon (Sri Lanka), Southern India and Melanesia.

BETEL QUID AND ITS CONSUMPTION

Betel quids have been classified as either wrapped or unwrapped [9]. In most of Asia the leaf of the male vine is used to produce wrapped quids which contain peeled areca nut, lime and often tobacco with other ingredients, although in India, Pindborg *et al.* [12] described 38 different combinations of areca nut and tobacco usage. In contrast, the quid in Melanesia is always unwrapped. In PNG the quid is formed by chewing the kernel of the areca nut, and by adding slaked lime using the inflorescence of the betel pepper vine moistened with saliva, dipped in lime and then bitten off at the corner of the mouth. Tobacco is never added.

The areca nuts are consumed at different stages of maturity; for instance, young green nuts are favoured in Taiwan [13], in parts of Melanesia and in the Assam province of North East India [14]. More mature dry or cured nuts are used in many other parts of India and Sri Lanka [14]. In PNG, nuts are used at all stages of their maturity, although young nuts and nuts of intermediate maturity are preferred. Comprehensive reviews of the chemical composition of the areca nut have been reported [15]. Raghavan and Baruah [16] have analysed the Indian species of the areca nut and Farnworth [17] the PNG species. Alkaloids are recognised as the active ingredients of the areca nut; six were reported by Arjungi [15]. The alkaloid and tannin content of betel nuts varies with maturity, as does the appearance and taste. The tannin content is highest in young areca nuts and decreases substantially with increasing maturity [16] and arecoline content has also been reported to differ between areca nut types [18]. Variation in the polyphenol content of the nut may be relevant to carcinogenesis [16, 19, 20].

Slaked lime is usually derived from heating shells, coral or limestone to 1000°C. At this temperature carbon dioxide is lost and calcium oxide results. When water is added to the calcium oxide, an exothermic reaction accompanied by physical expansion causes the material to break down to a fine white powder of predominantly slaked lime. As slaked lime readily absorbs moisture and will react with carbon dioxide, the powder is therefore stored in airtight containers in PNG. In contrast, the slaked lime in Asia is frequently mixed with water to form a paste.

There are clearly many differences in the composition of betel quids and the way in which they are chewed; these factors, which may well be related to oral carcinogenesis, require more precise definition.

EPIDEMIOLOGICAL STUDIES OF BETEL QUID CHEWING AND ORAL CANCER

For nearly a century descriptive epidemiological studies have suggested a link between betel quid chewing and oral cancer [1–4]. Orr [6] implicated several aspects of the quid as being potentially carcinogenic. Oral cancer was more common in the coastal areas than in highland tea estates; coastal people preferred fresh green nuts and used lime manufactured from shells, whereas the highland populations preferred dried areca nut and lime made from limestone. More irritant types of tobacco were chewed in areas where oral cancer was common.

Correspondence to S. Thomas, The Epidemiology Unit, The Queensland Institute of Medical Research, 300 Herston Road, Brisbane, Queensland 4029; and J. Kearsley is the Director of Radiation Oncology, St George Hospital, Belgrave St., Kogarah, New South Wales 2217, Australia.
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In West Malaysia, Chin and Lee [21] observed that tobacco was commonly included in the betel quid by ethnic Indians but never by indigenous Malays, a practice which may partly explain the high relative frequency of oral cavity cancer reported among ethnic Indian males (14.9%) compared with indigenous Malays (2.3%) and ethnic Chinese (1.8%) [22]. Similarly, cancers of the mouth in Pakistan are particularly common in the southern centres of Karachi and Hyderabad where chewing betel quid with tobacco is a common practice [23]. However, in descriptive studies such as these other ethnic or genetic factors cannot be excluded.

Since Orr's innovative study [6], which included a comparison of 100 oral cancer cases with 100 controls, 16 further case–control studies of betel quid chewing and oral cancer have been undertaken, only three of which examined non-Indian populations. Both tobacco smoking and the incorporation of tobacco in the quid independently increased the risk of oral cancer in chewers of betel quids, whereas for chewers of betel quid without tobacco, the risk was increased significantly in one study only. That there has been considerable variation in the estimates of risk among published studies most likely reflects several biases including subject selection, inadequate definition of carcinogenic exposure and tumour site, and an inability to control effectively for confounding variables. The variation is reduced among studies in which these factors have been defined.

Comparison between clearly defined betel quid chewing among smokers and non-smokers is only possible from seven reported studies [24–30] of which only two studies examined all combinations of smoking (S) and chewing betel quid with (BQ+T) and without tobacco (BQ). The probability values and point estimates of odds ratios (OR) which follow have been calculated from the published crude data in these studies, heterogeneity among odds ratios was assessed using the Breslow–Day test [31]. When compared with non-(BQ+T) chewers and non-smokers, chewers of BQ+T had a significantly increased risk in each of the seven studies, point estimates ranging from 4.26 to 13.67. For subjects who also smoked, the risk increased further in five of the studies, although this increase was statistically significant in only one ($P=0.045$) [29]. The data of Jafarey and Zaidi [29] suggest a significantly increased risk for BQ [OR 3.57, 95% Confidence limits (CL) 2.36, 5.27]. This risk increased further if tobacco was chewed in the quid (OR 13.67 95% CL 10.68, 17.82) or smoked (OR 21.02 95% CL 15.86, 28.39). The risk of oral cancer on exposure to BQ+T also varies at different intra oral sites; the point estimate for tongue and floor of mouth cancers was lower than cancers of the gingiva, lip or buccal mucosa ($P=0.043$). There was no equivalent variation in the risks at the same sites for smoking "bidi" cigarettes, manufactured from tobacco dust wrapped in temburni leaf ($P=0.934$) [25–27]. A large cohort recruited in India between 1966 and 1969 [32] has shown essentially similar results to the case control studies. No cases were found among subjects who neither chewed nor smoked, and the risk among BQ+T chewers was not increased further by smoking (relative risk 1.2, 95% CL 0.3, 3.8).

The role of alcohol has been investigated in six case-control studies in which exposure to betel quid chewing has been documented. A synergistic increase in risk of oral cancer has been shown among people who smoke, chew betel quid and consume alcohol [33]. Although other studies are less conclusive, alcohol not emerging as a strong risk factor for oral cancer, this is thought to be due to underestimates of the exposure because of stigma associated with alcohol consumption in India [25, 26].

So, what are we to make of the published literature? Firstly, chewing tobacco in the betel quid is clearly an important risk factor. Secondly, smoking tobacco is a major determinate of risk for oral cancer among chewers of betel quid, in particular when tobacco is not included in the quid. The role of betel quid without tobacco in non-smokers remains uncertain; however, further epidemiological studies in carefully selected populations to address this issue appear to be warranted.

CARCINOGENICITY OF BETEL QUIDS

There is strong (and increasing) epidemiological support for the association between betel quids which contain tobacco and the risk of oral cancer. Animal evidence for the carcinogenicity of betel quids has been demonstrated in several studies. Muir and Kirk [34] painted an aqueous extract of betel quid containing tobacco on the ears of Swiss mice, and produced squamous cell carcinomas (SCC) in two of 12 animals. Dimethyl sulphoxide (DMSO) extracts of areca nut only, tobacco only, and areca nut plus tobacco were painted on Syrian hamster cheek pouches by Suri *et al.* [35]. Eight out of 21 animals developed local SCC and 19 out of 21 formed leukoplakia when treated with areca nut extract; SCC resulted in 16 out of 21 animals and leukoplakia in 18 out of 21 animals treated with areca nut and tobacco extract, significantly more SCC than in the areca nut extract group ($P=0.013$); no carcinomas but 8 leukoplakias developed in 12 animals treated with tobacco extract. There was no significant difference in the incidence of leukoplakia between the groups ($P=0.196$). Ranadive *et al.* [36] used the hamster cheek pouch to test a variety of betel quid ingredients and found that tobacco enhanced the carcinogenicity of areca nut extract from 14 to 22% of exposed animals.

MUTAGENIC AND CYTOTOXIC EFFECTS

Although epidemiological studies have clearly associated BQ+T with an increased risk of oral cancer (*vide supra*), the causative agents have not been unequivocally identified. A great deal of research has, however, focused on the mutagenic and cytotoxic effects of four areca nut alkaloids (arecoline, guvacoline, guvacine and arecadine) and several tobacco specific nitroso compounds (TSNA). Nair *et al.* [37] have demonstrated that nitrosation of both TSNA and areca-specific alkaloids at pH 7.4 can occur in the mouths of betel quid chewers.

The mutagenicity of the components of the betel quid has been assessed using a variety of assays [38–42]. Studies in which betel quid chewers and non-chewers were compared, have demonstrated an increased occurrence of micronuclei in exfoliated oral mucosal cells [43, 44] and a higher frequency of sister chromatid exchanges in peripheral blood lymphocytes in betel quid chewers [45, 46]. Sudquist *et al.* [47] have demonstrated that an aqueous extract of areca nut, and in particular the N-nitroso compound, 3-(N-nitroso methylamino) propioaldehyde, are highly cytotoxic and genotoxic to human buccal epithelial cells, whereas no single areca nut alkaloid was shown to have such effects.

Of the TSNA, the compounds NNK and NNN are the only carcinogens that have been shown to induce oral malignancy in

experimental animals. NNK and NNN are the most potent carcinogens present in the tobacco used in betel quids and saliva of BQ+T chewers. Thus TSNA, known to be a potent animal carcinogen, may act either alone or in combination with areca-specific nitrosamines and other quid ingredients to exert potent genetic and cytotoxic effects on mucosal cells [48]. However, the evidence for carcinogenicity of particular areca nut components remains contradictory and incomplete.

Piper betle leaf extract appears to be non-mutagenic and may suppress mutagenicity. Benzo(a)pyrene tumorigenesis in hamster buccal pouches was inhibited by betel leaf extract in both short and long term assays [49]. Both the Ames Salmonella/microsome assay and the micronucleus assay suggest that betel leaf extract inhibits the mutagenic effects of TSNA [50, 51]. Hydroxychavicol, a phenolic compound isolated from the *Piper betle* leaf, was shown to inhibit nitrosation *in vitro* [52], the inhibition is dose-dependent and may result from the scavenging of nitrite ions, thus rendering them unavailable for nitrosation. Animal experiments have recently shown tumour incidence was reduced and latency and regression increased in hamsters treated with Piper betle leaf extract or its constituent micronutrients [53].

DOES CELLULAR PROLIFERATION PLAY A ROLE?

Many important risk factors for human cancer strongly affect cell division [54]. In the multistage/multifactor hypotheses of cancer causation shifts towards malignancy are fixed at the time of cell replication and the likelihood of neoplastic change is increased if the probability of mutagenesis or cell replication is increased [54, 55]. Animal experiments suggest that slaked lime induces cellular proliferation; moderate to severe hyperplasia has resulted when slaked lime was painted on the palate and buccal mucosa of Wistar rats [56] and after repeated applications of aqueous slaked lime to the cheek pouch of Syrian hamsters [57]. No carcinomas were demonstrated in either study [56, 57]. If lime was acting as a non-genotoxic agent, the induced cellular proliferation may have been insufficient to produce detectable tumours within the lifetime of the experimental animals [58]. However, in the case of betel chewing, it is probable that other genotoxic exposures may initiate carcinogenesis. The array of carcinogens in the betel quid and in tobacco is well known and the repeated application of lime may cause continuous rapid cellular turnover in response to tissue inflammation and necrosis, thus increasing the likelihood of hereditable mutations being transmitted to daughter cells prior to complete DNA repair [59].

GENERATION OF REACTIVE OXYGEN SPECIES

Reactive oxygen species (ROS) produce *in vitro* from polyphenolic betel quid ingredients and lime may induce oxidative and chromosomal damage linked to various aspects of oral carcinogenesis [19, 60, 61]. In a comprehensive review of the role of ROS in carcinogenesis, Cerutti [62] provided evidence that active oxygen plays an important role in tumour promotion. This view is supported by the inhibition of known tumour promoters in the presence of ROS scavengers [63]. Stich and Anders [61] proposed that if ROS have a role in tumour promotion the mucosal cells of betel quid chewers would require initiation, possibly by tobacco specific nitrosamines. Nair *et al* [60] examined the formation of H_2O_2 and its resulting radicals (ROS) from areca nut catechu and tobacco in the presence of lime or alkaline pH. The formation of ROS was pH dependent in that they found no response below pH 9.5 and release of ROS was enhanced by $Fe^{2+} > Fe^{3+} > Cu^{2+}$ but Mn^{2+} was inhibitory as were higher concentrations of Mg^{2+}.

However, ROS are extremely short-lived [61] and only the more robust ROS may gain access to the susceptible basal cells but this would be facilitated by ulceration at the site of lime and betel quid application, which would expose the proliferative layer [57]. The balance between production of ROS and various protective mechanisms could be upset by a rapid increase in ROS production and the mucosal ulceration which occurs in chewers of betel quid [61, 65].

Finally, there is presumptive evidence for oxygen radical involvement in oral carcinogenesis following trials of antioxidants such as β-carotene [66]. Supplementation of the diet of Filipino betel chewers with retinol and β-carotene was associated with a 3-fold decrease in the mean proportion of cells with micronuclei [67]. Remission of oral leukoplakias and inhibition of new leukoplakia, have been reported following dietary supplements of β-carotene and β-carotene plus retinol [68].

UNRESOLVED ISSUES IN THE AETIOPATHOGENESIS OF BETEL QUID-RELATED ORAL CANCER

Given the enormous impact of oral cancer on health care in Asia and Melanesia, it is clear that further basic research together with more refined epidemiological studies are mandatory. Any model which proposes to explain the aetiopathogenesis of betel quid-related oral cancer may well raise as many questions as it seeks to answer. Although betel quid with tobacco undoubtedly contain many carcinogens capable of initiating the cancer process, the role for instance, of areca nut or slaked lime still remains unresolved. Their effect could be mediated by direct genotoxic effects or by in the case of lime, by secondary cellular proliferation. Similarly, tobacco smoking probably plays an important initiating role, and drinking alcohol may act synergistically with betel quids +/− tobacco to increase further the risk of oral cancer through tumour promotion. But is there a greater risk of oral cancer for people who are exposed to tobacco smoke, alcohol and betel quid compared to smoking and alcohol alone? And, to what extent can pre-neoplastic changes be reversed or modified by dietary β-carotene? An understanding of these and many other questions in the genesis of betel quid-related oral cancer are clearly important if modification of cancer risk behaviour is to be most effective and incur the minimum disruption to traditional customs of those most at risk.

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