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Areca alkaloids measured from buccal cells using DART-MS serve as accurate biomarkers for areca nut chewing

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Abstract

Background: Areca nut (AN) chewing is carcinogenic and biomarkers reflecting it are urgently needed to determine the effectiveness of emergent cessation programs. Buccal cells (BCs) may serve as an ideal matrix to measure such biomarkers, however, their utility for this purpose is unknown. Direct analysis in real time - mass spectrometry (DART-MS) is a sensitive technique that analyzes materials in the open air and requires minimal/no sample preparation. We utilized DART-MS to analyze BCs to test its usefulness in measuring areca alkaloids as biomarkers for AN chewing.

Methods: We applied DART-MS in positive-ion mode to quantitate over time human BCs: 1) exposed *ex vivo* to betel quid extracts (BQE) consisting of young AN, *Piper betle* L. leaf, slaked lime, and tobacco; and 2) obtained from 7 chewers before and after BQ chewing. Quantification was performed by normalizing DART-MS alkaloid signal intensities to cholesterol intensities.

Results: Signals for areca alkaloids arecoline and arecaidine-guvacoline were detected in BCs exposed *ex vivo* to BQE up to 7 days (the last day tested) after exposure and in BCs from chewers up to 3 days (the last day tested) post chewing.

Discussion: The presence of alkaloid signals in BQ-exposed BCs verified BCs as a valid matrix and DART-MS as a suitable technique to measure biomarkers for AN chewing and provided reliable information on AN chewing timing.

Conclusion: DART-MS analyses of BCs can be used to accurately determine areca alkaloids as AN chewing biomarkers up to 3 days post chewing and possibly longer.

Abstract

Using DART-MS in positive ion mode, we detected signals for areca alkaloids arecoline and arecaidine-guvacoline in buccal cells exposed *ex vivo* to betel quid extracts up to 7 days (the last day tested) after exposure and in buccal cells from areca nut chewers up to 3 days (the last day

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tested) post betel quid chewing, which verified buccal cells as a valid matrix and DART-MS as a suitable technique to measure biomarkers for areca nut chewing.

DART-MS spectra in positive ion mode of buccal cells collected from a non-areca nut chewer before ('BC alone') and 1 to 7 days following *ex-vivo* exposure to a betel quid extract for 1 hr ('BC+BQE'). Signals for arecoline (m/z 156.102; dotted circle) and arecaidine-guvacoline (m/z 142.087; solid circle) were absent in buccal cells before betel quid exposure and present after exposure with a decrease in intensity over time during the 7-day study period. a.u. = arbitrary units.

Keywords

areca nut; biomarkers; alkaloids; buccal cells; Direct Analysis in Real Time Mass Spectrometry

Introduction

Areca nut (AN) chewing is a culturally ingrained habit practiced by approximately 600 million people worldwide [1] including in Guam, where AN is chewed by approximately 11% of the population (2011–2015 prevalence) [2]. Betel quid (BQ) is a masticatory mixture composed of AN from the *Areca catechu* palm, betel leaf from the *Piper betle* L. vine, and slaked lime (calcium hydroxide) with tobacco and/or spice additions depending on ethnicity [1, 3].

BQ chewing with or without tobacco is associated with increased oral cancer risk with tumor formation typically occurring at the site of BQ - and particularly - slaked lime placement [4]. The presence of slaked lime during chewing drastically increases the oral cavity pH, which leads to inflammation and promotes the oxidation of BQ polyphenols with the concurrent generation of reactive oxygen species; these compounds in addition to areca alkaloid N-nitroso metabolites can promote carcinogenesis [1, 5]. Since buccal cells (BCs) are directly exposed to BQ during chewing and can be collected with minimal invasiveness, they may serve as an ideal matrix to measure biomarkers for AN chewing. However, the utility of BCs for this purpose remains to be determined.

Biomarkers for AN chewing are urgently needed to determine the effectiveness of emergent cessation programs such as the Betel Nut Intervention Trial ("BENIT" trial #NCT02942745), the first known randomized trial aimed at AN chewing cessation [6] that is currently being implemented in Guam and Saipan. Objective indicators such as measureable biomarkers can serve to validate, and perhaps avoid total reliance on self-reported exposure, which can be unreliable for addictive habits [7].

In our previous pharmacokinetic study with AN chewers, we used high performance liquid chromatography (HPLC) with mass spectrometry (MS) to detect areca alkaloids in saliva and urine up to approximately 8 hours post chewing [8]. However, biomarkers that reflect AN chewing for lengthier time periods are needed for long-term verification in clinical and other cessation trials.

Direct analysis in real time (DART) is a sensitive ionization technique that allows MS-based analysis of materials in the open air at ambient conditions with minimal or no sample preparation [9], which circumvents the need for extractions and chromatographic separations thereby reducing analyte loss, analysis time, sample consumption, and assay turnaround time. DART-MS has been used to analyze various matrices for diverse applications such as drug screening, counterfeit verification, forensics, metabolomic fingerprinting, and chemical warfare [9–11]. However, we are unaware of DART-MS applications using human BCs particularly for the purpose of obtaining biomarkers for AN chewing.

In this pilot study, we determined the suitability of DART-MS to analyze areca alkaloids from BCs to obtain reliably measurable long-term biomarkers for the carcinogenic AN chewing habit.

Methods

Materials

Phosphate buffered saline (PBS) was purchased from Sigma Aldrich (St. Louis, MO) and slaked lime was obtained from Fisher Scientific (Hampton, NH).

Preparation of betel quid extract (BQE)

BQ material required for the *ex vivo* experiment (green mature AN, betel leaf, slaked lime, and tobacco) was obtained locally in Honolulu, Hawaii and kept at 4°C until use. The BQE was prepared by grinding one AN (husk included with ends removed and remaining nut diced with razor blade) with 2.0 g betel leaf (diced with a razor blade), 0.3 g tobacco (Largo brand) and 0.2 g slaked lime in 20 mL deionized water for 30 minutes using a mortar and pestle. The grinding of the BQ material was performed manually to mimic *in vivo* chewing. After grinding, the BQ solution was transferred to a 50 mL conical tube along with the 5 mL deionized water used to rise the mortar and pestle. The BQ solution was vortexed for 1 minute then transferred to a 0.22 µm vacuum filter unit and filtered overnight (~20 hours). The resulting BQE (~14 mL) was aliquoted into 1 mL volumes and stored at –80°C until use.

Ex vivo BC experiment

After consenting to this study healthy, non-AN chewing subjects self-collected BCs by gently brushing their inner cheeks up and down for 30 seconds with a soft bristle toothbrush. The toothbrush was vortex-mixed in 15 mL PBS to release the BC from the bristles then centrifuged at 700 x g for 5 minutes. The pelleted BCs were resuspended in 500 µL fresh PBS, briefly vortex-mixed, then aliquoted to: 1) one tube with 100 µL BCs only ('BC alone'); and 2) two tubes (duplicates) each with 100 µL BCs mixed with 100 µL BQE ('BC +BQE'). All tubes were incubated for 1 hour at 37°C with mild stirring then centrifuged at 700 x g for 5 minutes. The pelleted BCs were resuspended in 1 mL PBS then stirred for approximately 24 hours at 37°C followed by centrifugation a second time and resuspended in 500 µL PBS prior to DART-MS analysis. These constituted the 1-day BC+BQE samples. To obtain the 3-day BC+BQE samples, the 1-day samples were stirred for another 48 hours at 37°C then washed with PBS as before. This step was repeated accordingly to obtain the

5-day and 7-day BC+BQE samples. DART-MS analysis was performed each day including from the non-BQ exposed BC sample (BC alone). The institutional review board from the University of Hawaii (UH) approved this study and each participant signed an informed consent prior to study commencement.

***In vivo* BQ chewing study**

Non-smoking, self-reported AN chewing adult males (>18 yrs) were recruited in Guam through flyer advertisement and word of mouth. Interested participants were first screened in person to determine study eligibility. Information was obtained regarding age, ethnicity, health condition, and typical chewing habits. Participants with oral mucosal disease, periodontal disease, mouth sores, oral lesions or on medication were excluded and deemed ineligible. Participants were asked whether they were able to abstain from chewing BQ and any BQ related material including tobacco for at least 5 days prior to the study commencement ('washout' period) and up to 3 days post-BQ chewing; unwilling participants were excluded from joining the study. After screening, a total of 9 volunteers joined the BQ chewing study, 7 of which were assigned to chew a BQ once then abstain from any BQ consumption for 3 days followed by collection of BCs before and 1, 2, and 3 days post chewing. The remaining 2 volunteers were assigned as controls who did not chew BQ but provided BCs at the same time as the 7 chewers and were also instructed not to consume any BQ related material including tobacco for 3 days following baseline collection. The institutional review board from the University of Guam (UOG) approved this study and each participant signed an informed consent prior to study commencement.

On the day of the study, participants refrained from eating or drinking for 30 minutes prior to BQ chewing and, if food was consumed, brushed their teeth no less than 10 minutes before donating baseline (and consecutive) samples. Following the baseline BC collection, the participants were provided with one BQ to chew for the time period typical to their chewing habit. Subsequent BCs samples were collected at 1, 2, and 3 days post chewing. 500 μ L of the BC suspensions from each timed collection was stored immediately in PBS at -80°C for DART-MS analysis while the remaining volume was frozen in a cryoprotectant mixture for cytometric measurements. After all collections from all participants were collected and frozen, samples were shipped on dry ice to the UH Cancer Center for DART-MS analyses.

The BQ consisted of a young AN wrapped in a betel leaf with slaked lime and in some cases tobacco and was provided by the UOG personnel. Participants were asked to have the BQ touch their entire mouth equally during chewing. After their BQ chewing episode and up to 3 days post chewing, the participants were allowed to eat and drink ad libitum but asked to refrain from consumption of any BQ related ingredients –namely AN, betel leaf, slaked lime, or tobacco until after the last (3 days post-BQ chewing) sample was collected. The BQ material was obtained in Guam at the beginning of the study and kept cool until use.

Prior to the DART-MS analysis, each BC sample was thawed then centrifuged at $600 \times g$ for 2 minutes, after which the supernatant was removed and saved in a separate tube. 100 μ L of ice cold PBS was added to the cell pellet and 3 μ L of each sample was placed on a DART QuickStrip (IonSense LLC, Saugus, MA). Six replicates were prepared for each *ex vivo*

sample and 3–6 replicates were prepared for each of the timed collection samples from the *in vivo* chewing study; replicate averages were used for interpretation and analysis.

MS instrumentation

Mass spectra were acquired with an atmospheric pressure ionization time-of-flight mass spectrometer (AccuTOF-DART 4G, JEOL USA, Inc., Saugas, MA) equipped with a DART ion source (Model SVP, IonSense LLC, Saugas, MA), placed 1 cm away from the sampling orifice. The instrument has a resolving power of 10,000 (FWHM definition) at m/z 500. Voltage settings and acquisition parameters for positive-ion mode are as follows: the RF ion guide voltage was set at 700 V and the detector voltage set at 2200 V. The atmospheric pressure ionization interface potentials were as follows: orifice 1 = 30 V, orifice 2 = 5 V, ring lens = 5 V. Mass spectra were stored at a rate of one spectrum per second with an acquired m/z range of 100 – 1000. The DART interface was operated in positive-ion mode using helium gas with the gas heater set to 300°C. The glow discharge needle potential was set to 3.5 kV. Electrode 1 was set to 150 V and electrode 2 (grid) was set to 250 V.

During analysis of the *ex vivo* BC sample replicates, a QuickStrip was held manually in the ion source at a distance of 0.5 cm from the inlet until the sample solvent was completely evaporated (approximately 5–6 seconds per sample). During analysis of the *in vivo* chewing BC sample replicates, a linear rail system was used to hold, position, and move replicates uniformly in the ion source at a distance of 0.5 cm from the inlet. Relative quantification was performed by integrating the area beneath the signals corresponding to arecoline ($[M+H]^+ = m/z$ 156.102; $C_8H_{13}NO_2$) and arecaine-guvacoline ($[M+H]^+ = m/z$ 142.087; $C_7H_{11}NO_2$). For the *ex vivo* BC samples, relative quantification was expressed as arbitrary kilo units (kU). For the *in vivo* BC samples, signals were further normalized to the signal corresponding to cholesterol with loss of water ($[M+H-H_2O]^+ = m/z$ 369.352). DART MS signal intensity can vary depending on placement position in the ion source. Cholesterol is a component of the cell membrane and serves as an internal standard for cell quantity and sample position within the ion source relative to the ionizing gas stream.

Calibration for exact mass measurements was accomplished by acquiring a mass spectrum of polyethylene glycol (average molecular weight 600) as an external reference standard in every data file. Analysis was done with JEOL MassCenter software (version 1.3.0.1). Accurate mass measures and isotope pattern matching by MassMountaineer (FarHawk Marketing Services, Ontario, CA) were used to support elemental composition assignments.

Statistics

Paired t-tests were used to compare means to baseline levels using Excel version 14.7.1 (Microsoft). Level of significance was set at 0.05.

Results

Analyses of BCs after *ex vivo* exposure to BQE

DART-MS analysis of *ex vivo* samples in positive-ion mode showed no signals for any areca alkaloids in the non-BQE exposed sample (BC alone, figure 1). In contrast, analysis of areca

alkaloid standards and BCs after BQE exposure (BC+BQE) revealed distinct signals corresponding to well established areca alkaloids, namely arecoline ($[M+H]^+ = m/z$ 156.102) and arecaidine-guvacoline ($[M+H]^+ = m/z$ 142.087) (figure 1). The latter pair is isobaric and, therefore, cannot be further resolved by MS alone. The semi-quantitative kU signal intensity decreased for the measured alkaloids from 1 to 7 days (the last day tested) following BQE exposure (figure 1). The PBS wash (BC supernatant) was also analyzed up to 3 days following BQE exposure and showed the same signal pattern but at much lower intensity (data not shown).

Analyses of BCs from subjects chewing a BQ

Analysis of BCs collected *in vivo* with toothbrush scrapings from 7 male AN chewers (age >18 years, average chew time 10.9 minutes), revealed the presence of arecoline and arecaidine-guvacoline signals up to 3 days post BQ chewing (figure 2), the last day tested, thereby confirming our *ex vivo* findings. Arecoline and arecaidine-guvacoline signals were detected at baseline for all 7 chewers, however, normalized mean arecoline levels from the chewers were 124% higher at 1 day post chewing ($p=0.004$; paired t-test) and 23% lower at 2 and 3 days post chewing ($p=0.484$; paired t-test) compared to baseline (figure 2). No significant changes over time were observed for normalized mean arecaidine-guvacoline signals (figure 2). Controls ($n=2$) had baseline levels very similar to those of the chewers but showed no significant changes over time in either of the two alkaloid signals (data not shown).

Discussion

Arecoline, arecaidine, guvacoline, and guvacine are areca alkaloids specific to ANs and, therefore, serve as excellent biomarkers for AN chewing. We previously measured these alkaloids in saliva and urine from chewers after AN chewing using HPLC with high-resolution accurate-mass MS [8, 12]. The appearance pattern of the alkaloids in saliva paralleled that excreted in urine and peaked approximately 2 hours post chewing. However, alkaloid levels in both matrices returned to baseline after approximately 8 hours, making them useful only as short-term biomarkers.

DART-MS was more sensitive than our preliminary attempts using spectroscopy, fluorescence detection, and HPLC with ultraviolet detection, which could not detect areca alkaloids in BCs beyond 8–10 hours post-chewing (data not shown). Notably, DART-MS analysis of BCs showed signals up to 7 days (the last day tested in this study) after *ex vivo* BQ exposure (figure 1) and up to 3 days (the last day tested in this study) after subjects chewed a BQ (figure 2). In addition, analyses of BC using DART-MS was much more efficient than analysis using our previous attempts because sample workup was circumvented. This resulted in reduced sample consumption, much faster analysis time (approximately 5–6 seconds per sample), and a much higher throughput than our (or any) HPLC MS-based method.

We chose BCs as our study matrix due to their direct exposure to BQ during chewing. Our results confirm that BCs are excellently suited to measure areca alkaloids as biomarkers of AN chewing and may be a better option than using saliva or urine. It is unknown why

guvacine was not detected in BCs using our DART-MS technique. One possibility is that the carboxylic acids arecaidine and guvacine are not bound to BCs to the same extent (or at all) as their respective methyl esters arecoline and guvacoline. If so, the unbound carboxylic acids would escape the analysis entirely or after a 1 day period, the first analyzed timed collection following BQ exposure in this study. Following this rationale, the signal we detected at m/z 142.087 would then be due solely to guvacoline. In our previous study [8], guvacine and arecaidine displayed the same appearance pattern in saliva than guvacoline and arecoline – peaking in concentration 2 hours post chewing before returning to baseline by 8 hours. If the pharmacokinetics of areca alkaloids in BCs mirror that of saliva, it is possible that our first post-chewing analyzed timed collection at 1 day post chewing did not capture the short-term guvacine (and arecaidine) appearance in BCs. Since the aim of our current study was to look for biomarkers that were detectable longer than 8–10 hours post chewing (i.e. longer than our previous pharmacokinetic study and preliminary methods), we did not analyze BCs collected prior to 1 day post chewing. Future studies analyzing several timed sample collections within the first hour after BQ exposure and using stable isotopes of arecaidine and guvacine as internal standards will help to shed light on this issue.

In the current study, all baseline samples of chewers had detectable signals for arecoline and arecaidine-guvacoline despite our protocol that asked for a 5-day washout before study commencement. This may indicate that our study washout period was not long enough and/or our study participants did not fully comply with the washout. However, the significant ($p=0.004$) increase in mean arecoline levels at 1 day post chewing compared to baseline indicates that our washout was at least long enough to detect the expected increase in alkaloid levels following BQ exposure. Further studies with a larger sample size, a much longer washout period, and longer post-chewing collection periods will help to draw more definitive conclusions on the ultimate applicability of areca alkaloid analyses as AN chewing biomarkers.

BQ chewing is dose-dependently associated with the incidence of oral cancer [1, 13] and estimates suggest that AN chewing may be responsible for up to 50% of oral cancer in some countries [14]. The BENIT is the first trial aimed AN cessation trial in the western Pacific region and was recently implemented in Guam and Saipan [6] to address the disproportionally high extent of AN chewing and the associated high oral cancer rates among minority Pacific Islander populations. This trial is the first of its kind and, therefore, requires measureable indicators to examine its success and efficacy of achieving its overall goal of preventing oral cancers resulting from AN and BQ chewing. Our findings will be most helpful in these efforts.

To our knowledge, this is the first report utilizing DART-MS to detect areca alkaloids in BCs from individuals after AN chewing. An inherent limitation of DART-MS analysis is poor signal reproducibility. This is typically due to manual sample placement in the ionization gas stream during analysis, which was performed for the *ex vivo* BC experiments. However, this issue can be resolved with the use of an automated position device (e.g. linear rail system). This device serves to optimally position samples between the ionization region and the mass spectrometer inlet and move them uniformly to facilitate rapid and consistent assessments

for quantitative analysis. This device was successfully applied when analyzing BC samples from our *in vivo* BQ chewing study.

Signal quantification was performed in our study by integrating the area under the arecoline ($[M+H]^+ = m/z$ 156.102) and arecaidine-guvacoline ($[M+H]^+ = m/z$ 142.087) signals then normalizing to the signal corresponding to cholesterol ($[M+H-H_2O]^+ = m/z$ 369.352). Cholesterol is a component of all cell membranes and in our case served as a standard for cell quantity and sample position within the ion source relative to the ionizing gas stream. Since we were interested in alkaloid signals relative to baseline, relative quantitation was a conceivable and convenient choice for our study purposes. We considered other means to adjust for the cell number including optical density readings of serial BC dilutions performed in PBS using a Shimadzu UV/VIS-1800 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD) and a Versamax microplate reader (Molecular Devices, San Jose, CA). These readings showed extremely high linearity when plotted against absorbances between 400–800 nm and optimally at 600 nm ($r^2 > 0.991$). However, when final data were adjusted for optical density readings, the overall results were not different to the final data adjusted for cholesterol.

A limitation of using single MS detection is that the accurate masses of 156.102 and 142.087 alone are insufficient to definitely assign these signals to arecoline and arecaidine-guvacoline.

However, as the two signals occurred concurrently in all BC samples exclusively after AN exposure *ex vivo* or *in vivo* it is very likely that our tentative assignments to arecoline and arecaidine-guvacoline are correct. Furthermore, recent measurements of hair samples using DART-MSMS in MRM mode revealed the presence of arecoline and arecaidine but absence of guvacoline in AN chewers and absence of any AN alkaloid in non-chewers. Tandem MS particularly in MRM mode is a more specific technique than accurate-mass MS and allows for a more definitive molecular assignment of signals because the precursor ions (156.102 and 142.087) provide different fragmented product ions, which can provide better structural information for the compound(s) of interest. Detection of alkaloids in BCs using DART-MSMS are needed to confirm our findings in hair and these experiments with more chewers are planned in the near future.

Conclusion

To our knowledge, this is the first report utilizing DART-MS to detect areca alkaloids in BCs of chewers after AN chewing. DART-MS detection of areca alkaloids arecoline and arecaidine-guvacoline (m/z 156.102 and m/z 142.087) in positive ionization mode from alkaloid standards and BCs exposed to BQE *ex vivo* and BCs exposed to BQ *in vivo* verified the utility of DART-MS for our purposes. This technique can be applied to verify intervention efficacy in cessation programs such as the BENIT and replace, supplement, or validate the use of subjective self-reports to achieve the program's overall goal of preventing oral cancers resulting from AN chewing.

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References

1. IARC, Betel-quid and areca-nut chewing and some areca-nut derived nitrosamines. IARC Monogr Eval Carcinog Risks Hum, 2004 85: p. 1–334. [PubMed: 15635762]
2. Paulino YC, et al., Epidemiology of areca (betel) nut use in the mariana islands: Findings from the University of Guam/University of Hawai'i cancer center partnership program. Cancer Epidemiol, 2017 50(Pt B): p. 241–246. [PubMed: 29120831]
3. Paulino Y, Areca (Betel) Nut Chewing Practices in Micronesian Populations. Hawaii J Public Health, 2011 3(1): p. 19–29. [PubMed: 25678943]
4. Thomas SJ and MacLennan R, Slaked lime and betel nut cancer in Papua New Guinea. Lancet, 1992 340(8819): p. 577–8. [PubMed: 1355157]
5. Nair UJ, et al., Role of lime in the generation of reactive oxygen species from betel-quid ingredients. Environ Health Perspect, 1992 98: p. 203–5. [PubMed: 1486850]
6. Moss J, et al., Developing a Betel Quid Cessation Program on the Island of Guam. Pac Asia Inq, 2015 6(1): p. 144–150. [PubMed: 27057560]
7. Patrick DL, et al., The validity of self-reported smoking: a review and meta-analysis. Am J Public Health, 1994 84(7): p. 1086–93. [PubMed: 8017530]
8. Franke AA, Li X, and Lai JF, Pilot study of the pharmacokinetics of betel nut and betel quid biomarkers in saliva, urine, and hair of betel consumers. Drug Test Anal, 2016.
9. Cody RB, Laramée JA, and Durst HD, Versatile new ion source for the analysis of materials in open air under ambient conditions. Anal Chem, 2005 77(8): p. 2297–302. [PubMed: 15828760]
10. Chernetsova ES, Morlock GE, and Revelsky IA, DART mass spectrometry and its applications in chemical analysis. Russian Chem Rev, 2011 80(3): p. 235.
11. Gross JH, Direct analysis in real time—a critical review on DART-MS. Anal Bioanal Chem, 2014 406(1): p. 63–80. [PubMed: 24036523]
12. Franke AA, et al., Composition of betel specific chemicals in saliva during betel chewing for the identification of biomarkers. Food Chem Toxicol, 2015 80: p. 241–246. [PubMed: 25797484]
13. Nair U, Bartsch H, and Nair J, Alert for an epidemic of oral cancer due to use of the betel quid substitutes gutkha and pan masala: a review of agents and causative mechanisms. Mutagenesis, 2004 19(4): p. 251–62. [PubMed: 15215323]
14. Mehrtash H, et al., Defining a global research and policy agenda for betel quid and areca nut. Lancet Oncol, 2017 18(12): p. e767–e775. [PubMed: 29208442]

Figure 1. DART-MS spectra in positive ion mode of buccal cells collected from a non-areca nut chewer before ('BC alone') and 1 to 7 days following *ex-vivo* exposure to a betel quid extract for 1 hr ('BC+BQE').

Chemical structures for arecoline and arecaidine-guvacoline and their respective m/z are shown (top). Signals observed for arecoline (m/z 156.102; dotted circle) and arecaidine-guvacoline (m/z 142.087; solid circle) decreased in intensity over time from 200 kilo units (kU) to 19 kU and from 100 kU to 4 kU, respectively, during the 7-day study period. These signals were absent in buccal cells before betel quid extract exposure (BC alone). a.u. = arbitrary units.

Figure 2. Averaged DART-MS signal intensities for arecoline (m/z 156.102) and arecaidine-guvacoline (m/z 142.087) normalized to cholesterol intensity (m/z 369.352) from buccal cells over time of 7 individuals who chewed one betel quid at baseline then abstained from chewing for 3 days. Error bars indicate SEM. Significantly different from baseline **p<0.005 by paired t-test.