TOBACCO SMOKE XENOBIOTIC COMPOUND APPEARANCE IN MOTHERS' MILK AFTER INVOLUNTARY SMOKE EXPOSURES I. NICOTINE AND COTININE

(Nicotine; cotinine; breast milk)

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SUMMARY

In the development of the extraction procedure for the analysis of nicotine and cotinine from a single breast milk sample, nicotine and cotinine were detected in 3 of 10 nonsmoking mothers' milk samples. Interviews of these women revealed the presence of nonsmoking husbands and households but of tobacco smoke exposures during the working day. Clinically these levels may be considered inconsequential in regard to the threat to the mother and nursing infant but may be important in studies designed to monitor tobacco smoke xenobiotic compound appearance in breast milk.

INTRODUCTION

In previous studies nicotine in breast milk of smoking mothers has been measured [1, 2]. Perlman et al. [2] demonstrated a rough correlation between the number of cigarettes smoked and the concentration of nicotine in residual milk. Ferguson [3] used gas chromatography to measure nicotine concentrations in the milk of smoking mothers ranging from 20 to 512 parts per billion (average 91 ppb). Since then several investigations have led to the increased sensitivity of detection and measurement of nicotine and its major metabolite, cotinine, in the plasma, urine, and breast fluid of smoking mothers [4–5]. Kogan et al. [7] recently reported a method for the simultaneous determination of nicotine and cotinine in plasma. While the analytical techniques used are adequate, we have found that the complexity of the breast milk composition requires a modification of the extraction procedure to allow for the analysis of both nicotine and cotinine in breast milk.

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MATERIALS AND METHODS

Nicotine and isoquinoline were purchased from Aldrich Chemical Co., Milwaukee, WI, vacuum-distilled and checked for purity by gas chromatography before use. Lidocaine HCl was obtained from Astra Pharmaceutical Products, Worcester, MA, recrystallized from other products and checked by gas chromatography before use. Cotinine was prepared from nicotine [8] and confirmed by IR, GC-MS and GLC. Pesticide-grade solvents purchased from Baker Chemical Co., Phillipsburg, N.J., were used without further purification except for butyl acetate which was triple-distilled in glass immediately before each use.

Breast milk samples (30 ml) were hand-expressed into provided screw-topped glass vials, labelled and stored frozen by each mother at various times. Milk samples were thawed and triplicate, 3-ml, aliquots taken for analysis. Samples were adjusted to pH 11 by addition of 5 M NaOH. Internal standard (aqueous isoquinoline) and diethyl ether (3 ml) were added to each sample. After vortex agitation for 2 min samples were centrifuged and the organic layer removed to a second test tube. The aqueous layer retained for cotinine analysis. 100 µl of 5 M HCl were added to the organic layer and vortexed. The organic layer was reduced by evaporation under a stream of nitrogen at room temperature. After vortexing and centrifuging, the aqueous layer was removed and washed with diethyl ether. Remaining ether was blown off with nitrogen and 400 µl of 5 M NaOH were added. To this, 50 µl of freshly distilled butyl acetate were added, then vortexed and centrifuged. An aliquot (3 µl) of the butyl acetate layer was injected onto the gas chromatograph.

The excess ether was evaporated from the original aqueous layer under a stream of nitrogen in preparation for the cotinine analysis. To this aqueous layer 5 ml of dichloromethane and the internal standard (aqueous lidocaine, 100 µl) were added. This was gently shaken for 5 min and then centrifuged. The organic layer was transferred to a second tube and evaporated to 500 µl. This was washed with two 500 µl aliquots of NaOH. The organic layer was then transferred to another tube and evaporated to dryness. 25 µl of acetone were added, vortexed and a 3 µl aliquot was injected onto the gas chromatograph.

Samples were injected onto a Varian 3700 gas chromatograph equipped with a thermionic specific detector (N,P-sensitive). A silanized 2-m glass column packed with 80–100 mesh Chromosorb W coated 10% (w/w) with Apiezon L and 10% KOH was used. This column has been proven to be stable with constant use in other laboratories. The instrument settings were as follows: column temperature, 180°C (220°C, cotinine); injection port, 220°C; detector temperature, 250°C; carrier gas (helium) flow rate, 20 ml/min (30 ml/min, cotinine); air flow rate, 175 ml/min; hydrogen flow rate, 25 ml/min. Using these conditions the retention times were: nicotine, 380; isoquinoline, 340; cotinine, 320; lidocaine, 550 s. Standard curves were obtained by adding known amounts of nicotine and cotinine to the biological fluid of interest and carrying each through the analytical procedure. The extraction
schemes produced no interfering peaks at the above retention times. Standard curves were prepared by spiking blank (determined previously to be nicotine/cotinine-free) samples with nicotine concentrations of 1, 5, 10, 20, 50, 100 ng/ml and cotinine concentrations of 5, 10, 50, 100 and 300 ng/ml. Samples were then analyzed as previously outlined and results reported as peak area ratios vs added concentrations.

RESULTS AND DISCUSSIONS

The extraction efficiency and recovery approached that reported by Feyerabend and Russel [9] with 95% recovery for nicotine and 93% for cotinine, the reproducibility was found to be ±2% at the higher concentrations (nicotine 100 ng/ml; cotinine 300 ng/ml) and ±5% at the lower concentrations (nicotine 1 ng/ml; cotinine 5 ng/ml). Storage of one spiked sample at −4°C showed no apparent change in nicotine or cotinine over 3 months. Fig. 1,a and b shows the detector response vs. time for injections of cotinine extractions of smokers’ and nonsmokers’ breast milks. Fig. 1,c and d shows detector response vs time for injections of cotinine extractions of smokers’ and non smokers’ breast milks. Nicotine (20–150 ng/ml) and cotinine (50–300 ng/ml) were identified in the milks of all three smoking mothers tested. Nicotine concentrations are in the range reported by others [3,9]. Concentrations of cotinine found reflect plasma [10] and breast fluid [5] concentrations. The nicotine to cotinine ratios in plasma and breast fluid are similar to those.

![Fig. 1. Chromatograms of nicotine and cotinine extractions for (a) and (c) smoking mothers’ milk (b) and (d) nonsmoking mothers milk, (e) and (f) passively exposed nonsmokers’ breast milk (N), nicotine; (C), Cotinine; (I), isoquinoline; (L), lidocaine.](image-url)
found for breast milk suggesting little metabolism of nicotine to cotinine in breast milk. The concentration variation within mothers appeared to be as great as the variation between mothers. No effort was made to control smoking, feeding or sampling at this stage of the experiment.

Fig. 1,e and f show the appearance of nicotine and cotinine in samples of nonsmokers' milk. Of 10 nonsmoking mothers tested, 3 mothers had detectable levels of nicotine (1–7 ng/ml) and cotinine (2–10 ng/ml) in their milk samples. Interviews with these nonsmoking mothers revealed that they had nonsmoking husbands and households but they were passively exposed to tobacco smoke during the day at work. These small amounts of nicotine and cotinine decreased to unmeasurable levels (nicotine <0.1 ng/ml; cotinine <1 ng/ml) during the course of the weekend.

In a report of the Surgeon General, 'Smoking and Health' [11] there are many references which indicate the difficulty to establish levels of smoke exposure in individuals. Our data indicate that, if the pharmacokinetic parameters for nicotine disposition in an individual are known, the appearance and disappearance of nicotine and cotinine within that individual may be an accurate indicator of smoking exposures. Although less than that of smokers, levels of nicotine (0.9 ng/ml) and carbon monoxide (1.6–2.1% COHb) were measured in the plasma of nonsmokers [11]. While the levels of these compounds found in nonsmoking mothers here may not represent a clinical threat to the mother or nursing infant, these data indicate that any study designed to measure tobacco smoke xenobiotic appearance in the breast milk must include the possibility of involuntary exposures.

REFERENCES