Null Results in Brief

No Association between Smoking and the Presence of Tobacco-specific Nitrosamine Metabolites in Ovarian Follicular Fluid

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Introduction

The TSNA[2] NNK is a rodent carcinogen likely to play an important role in smoking-induced lung cancer (1). Metabolism of NNK can proceed directly via cytochrome P450 activation to α-hydroxyNNK or formation of NNAL and subsequent α-hydroxylation to α-hydroxyNNAL, resulting in formation of promutagenic DNA adducts. NNAL can be detoxified by conjugation to NNAL-Gluc. NNAL and NNAL-Gluc in body fluids are accurate biomarkers of tobacco smoke carcinogen exposure in humans (1). NNK is found in cervical mucus of women who smoke, possibly contributing to risk of developing cervical carcinoma (2), and smoking reduces pregnancy rates in women having assisted reproduction. We tested the hypothesis that NNK metabolites are elevated in the follicular fluid of smokers. If so, DNA damage may occur, possibly resulting in germ-line mutations in oocytes, increased risk of ovarian carcinogenesis, or poor outcomes during IVF.

Materials and Methods

"Clean catch" (minimal blood contamination) specimens of follicular fluid were collected from women undergoing transvaginal oocyte recovery as part of an IVF program. Samples were centrifuged (1200 rpm[2], 10 minutes, 4°C) to remove cellular debris, cryopreserved, and stored at −70°C until analysis. Smoking status was assessed by questionnaire and by high-performance liquid chromatography measurement of salivary cotinine in women and their male partners and follicular fluid cotinine (3). Twenty-two follicular fluid samples (12 smokers and 10 nonsmokers) blinded as to cotinine status were shipped on dry ice from Leeds, United Kingdom to the University of Minnesota Cancer Centre for analysis of NNAL and NNAL-Gluc by gas chromatography with nitrosamine-selective detection (limit of detection, ~9 fmol/ml in 5 ml of follicular fluid) (4). The hospital ethics committee approved the research protocol, and all subjects gave written informed consent.

Statistical Analysis. The study was conducted to test whether NNAL and NNAL-Gluc could be identified in the follicular fluid. These metabolites are specific to tobacco-exposed individuals. In the study of Lackmann *et al.*, (4) the mean level of NNAL plus NNAL-Gluc in urine from newborn children whose mothers smoked was 0.062 pmol/ml (95% confidence interval 0.035–0.11), and a nominal mean value of half the detection limit (0.01 pmol/ml) was assigned to the children of nonsmokers. Assuming similar data in the current study, the numbers of subjects used would detect a significant difference between the groups ($P < 0.01$) with a power of 90%.

Results

Patients in the smoking group reported smoking ≤20 cigarettes/day (mean 10 cigarettes/day). Cotinine was detected in the saliva and follicular fluid of all apart from one self-reported smoker (Table 1). There was a strong correlation at the individual level between the two measures ($r = 0.88$; $n = 22$; $P < 0.01$). Overall, concentrations of cotinine in follicular fluid were about half those seen in saliva. Two patients in the self-reported nonsmoking group also had significant amounts of cotinine, in the same range as the smokers. NNAL was detected at a low level (0.049 pmol/ml) in only 1 of 22 follicular fluid samples, and this was from a nonsmoker, negative for cotinine analysis.

Study Limitations. The study has two principal limitations: (*a*) it involves relatively small numbers of women, however, these women were selected as heavy smokers (confirmed by cotinine data) undergoing IVF and represent a group which are now relatively rare and, therefore, difficult to recruit, and (*b*) the method of detection of NNAL and NNAL-Gluc may not be sensitive enough for follicular fluid analysis; however, one sample did contain detectable levels, and the method has been shown to be sensitive enough to measure these metabolites even in the urine of children exposed transplacentally to NNK (4).

Discussion

This study demonstrates that levels of the NNK metabolites are likely to be low in the mature Graafian follicles of smokers. The 1 patient with a detectable level of NNAL was a self-reported nonsmoker. However, the cotinine assay used may not detect low levels of exposure to passive smoking, and indeed, earlier studies have detected some subjects with detectable urinary NNAL-Gluc in the absence of cotinine (4). Animal studies have suggested that the presence of tobacco-related polycyclic aromatic hydrocarbons is associated with oocyte maturation defects (5), but no studies have investigated the presence of TSNA in the human ovary. The absence of NNK metabolites from the ovary, in the presence of cotinine, may reflect low

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[2] The abbreviations used are: TSNA, tobacco-specific *N*-nitrosamine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNAL-Gluc, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol glucuronide; IVF, *in vitro* fertilization.

Table 1 Cotinine levels (ng/ml) in smokers and nonsmokers[a]

	Smokers ($n = 12$)		Nonsmokers ($n = 2$)
	Range	Mean	
Follicular fluid	67–360	138	67 and 204
Saliva	168–690	278	260 and 374
Saliva (partner)	144–526	284	144 and 354

[a] Eight additional nonsmokers had nondetectable cotinine in saliva and follicular fluid.

uptake or limited metabolism of TSNA in this organ. Despite the results in the current study, it should be remembered that other tobacco carcinogens may be activated to DNA-damaging metabolites in follicles and that polycyclic aromatic hydrocarbon exposure causes progressive oocyte destruction and subsequent ovarian tumorigenesis in animal models (6) consistent with lower conception rates per assisted reproduction cycle in smokers than nonsmokers.

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