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

Sara J. Matthews, Stephen S. Hecht, Helen M. Picton, Ming Ye, Steven G. Carmella, Susan Shires, Christopher P. Wild,¹ and Alastair W. M. Hay

Molecular Epidemiology Unit, Epidemiology and Health Services Research, School of Medicine [S. J. M., S. S., C. P. W., A. W. M. H.] and Department of Obstetrics and Gynaecology [H. M. P.], University of Leeds, United Kingdom and University of Minnesota Cancer Center, Minneapolis, Minnesota 55455 [S. S. H., M. Y., S. G. C.]

Introduction

The TSNA² 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 with glutathione 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 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 rpm², 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

Received 10/5/01; revised 10/5/01; accepted 12/20/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹To whom requests for reprints should be addressed, at Molecular Epidemiology Unit, Epidemiology and Health Services Research, School of Medicine, University of Leeds, Leeds LS2 9JT, United Kingdom. Phone: (0044)(113)2336602; Fax: (0044)(113)2336603; E-mail: c.wild@leeds.ac.uk.

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

Downloaded from cebp.aacrjournals.org on October 13, 2017. © 2002 American Association for Cancer Research.
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.

References


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

<table>
<thead>
<tr>
<th></th>
<th>Smokers (n = 12)</th>
<th>Nonsmokers (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Follicular fluid</td>
<td>67–360</td>
<td>138</td>
</tr>
<tr>
<td>Saliva</td>
<td>168–690</td>
<td>278</td>
</tr>
<tr>
<td>Saliva (partner)</td>
<td>144–526</td>
<td>284</td>
</tr>
</tbody>
</table>

* Eight additional nonsmokers had nondetectable cotinine in saliva and follicular fluid.
No Association between Smoking and the Presence of Tobacco-specific Nitrosamine Metabolites in Ovarian Follicular Fluid

Sara J. Matthews, Stephen S. Hecht, Helen M. Picton, et al.


Updated version  Access the most recent version of this article at: http://cebp.aacrjournals.org/content/11/3/321

Cited articles  This article cites 6 articles, 1 of which you can access for free at: http://cebp.aacrjournals.org/content/11/3/321.full#ref-list-1

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.