Nicotine, Cotinine or a cotinine metabolite inhibits NNK-induced DNA-strand break in metabolically competent hepatic cells

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Background: Nicotine is not considered to be genotoxic; however, nicotine has been reported to enhance tumor multiplicity in A/J mice treated with the tobacco nitrosamine NNK (4-(methylnitosamino)-1-(3-pyridyl)-1-butane) (Davis et al., 2009). In contrast, other in vitro studies with A/J mice concluded that nicotine had no influence on NNK-induced tumor multiplicity and progression (Murphy et al., 2011). Furthermore, a previous A/J mouse study has also suggested a protective effect of nicotine against metabolic activation of NNK (Brown et al., 1999). Recent in vivo work using purified enzymes demonstrated that nicotine or a nicotine metabolite could inhibit CYPs (CYP2A6, 2A13) involved in NNK bioactivation by a mechanism-based inhibition. Therefore, we hypothesized that nicotine or a nicotine metabolite such as cotinine might contribute to the inhibition of NNK-induced DNA strand breaks by inhibiting CYPs enzymes. The effect of nicotine or cotinine on DNA strand break were evaluated using the COMET assay in CYP competent HepaRG cells incubated with bioactive CYP-dependent NNK and CYP-independent NNKOAc (4-[(acetyl methyl nitrosamino)-1-(3-pyridyl)-1-butane]).

Methods: HepaRG, HBEcs, and BEAS-2B culture conditions, mRNA extraction, ORP-PCR and enzymatic probe assays have been described in Garcia-Canton et al., 2013 and Newland et al., 2011. The Alkaline COMET assay was based on the methods described by Tice et al. (Tice et al., 2000), Thorne et al. (Thorne et al., 2009) and the Comet assay interested group (http://www.cometassay.com) with slight modifications. Data were analysed by using the parametric statistical approach published by Bright et al. (Bright et al., 2011). The median by plate of the lognormal tail intensity were analysed in a mixed model in MinT lab software with treatment as fixed effect and run as random effect, differences between the treatments variances was specified. Post-hoc multiple comparisons were adjusted by Tukey’s.

Results: Hierarchical clustering representing the gene expression profiles of 39 selected metabolic genes tested in HBEcs (3 subjects) and HepaRG is shown in Figure 1. Columns represent individual samples and rows represent genes. Green, black, and red indicate high signal intensity, moderate to low signal intensity or no signal in normalized gene expression data (JGIC), respectively.

Figure 1

A comparison of comets 7 hesitation (Coun) activity in primary human bronchial epithelial cells (HBEcs) from 3 donors, BEAS-2B cells, and HepaRG cells is shown in Figure 2. B-methylsulfoxon (8-m) was used as an inhibitor of CYP2A6/CYP2A13. Results are presented as mean of three measurements ± standard deviation.

Figure 2

An individual value plot showing the COMET % tail intensity for each acquired nuclei following the incubation of HepaRG with NNKOAc (25 ¡M) for 3 hrs and HepaRG pre-incubated with cotinine, nicotine, and PPITC (10 ¡M) for 12 hours and NNKOAc (25 ¡M) added for the least 3 hours is shown in Figure 4. Mean value for each condition tested are shown in blue (+) and median values are shown in red (+). Pairwise comparison results (Tukey’s test) for significant differences between tested conditions at 95% confidence is shown in Table 3. N indicates the total number of slides counted from a minimum of 3 independent experiments with the overall total nuclei counted (approximately 100 per slide). The mean is the average of the median of the log transformed tail intensity calculated for each independent experiment. Means that do not share a letter are significantly different.

Figure 4

Conclusion: We have developed and applied a robust protocol to assess DNA damage by COMET in HepaRG cells, a cell line that shares greater metabolic and morphological similarities with normal human hepocytes. Using the COMET assay, we showed that nicotine, cotinine, or a metabolite of cotinine can protect against NNK-induced DNA damage in HepaRG by inhibiting metabolic enzymes, possibly of the CYP2A4 or CYP91A enzyme. Importantly, this and other researches highlight the current limitations of the classical paradigm of single toxicants assessment when a complex mixture such as tobacco smoke is considered. Single toxicant assessments do not take into account complex interactions between chemicals and biological systems.

Conflicts of interest: The authors report no conflict of interest. E. Minet, A. Banerjee, and O.M. Camacho and D. Waters were employees of British American Tobacco or contracted by British American Tobacco during the conduct of this study. Part of the work was contracted to Vivotecnia Research S.L., Santiago Grisolia 2, Tres Cantos, Madrid; Spain and Charles River, Trantum, Edinburgh, EH33 2NE, UK.

Table 1A

A summary of the inhibition kinetics reported in the literature for nicotine and the nicotine iminium ion metabolite. Table 1B presents the IC50 values we obtained following incubation of [H]3NNK with CYP2A13 bacitromines in the presence of nicotine, cotinine, and PPITC.

Table 1A

Inhibitor   Ki (¡M)   Kinact (min-1)   t½ (min)   References
CYP2A13   nicotine   17   0.1   7   He et al., 2004
         nic iminium   30   0.04   15.5   Von Weyman et al., 2013
CYP2A6   nicotine   21   0.021   33   He et al., 2005
         nic iminium   300   0.03   27   Von Weyman et al., 2013

Table 1B

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Ki (¡M)</th>
<th>Kinact (min-1)</th>
<th>t½ (min)</th>
<th>References</th>
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<tr>
<td>HPB</td>
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<td>Total CYP2A13 nicotin metabolism</td>
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Table 2

Table 3

Table 4

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