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Nicotine, cotinine, and β-nicotyrine inhibit NNK-induced DNA-strand break in the hepatic cell line HepaRG

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ABSTRACT

Recent *in vitro* work using purified enzymes demonstrated that nicotine and/or a nicotine metabolite could inhibit CYPs (CYP2A6, 2A13, 2E1) involved in the metabolism of the genotoxic tobacco nitrosamine NNK. This observation raises the possibility of nicotine interaction with the mechanism of NNK bioactivation. Therefore, we hypothesized that nicotine or a nicotine metabolite such as cotinine might contribute to the inhibition of NNK-induced DNA strand breaks by interfering with CYP enzymes. The effect of nicotine and cotinine on DNA strand breaks was evaluated using the COMET assay in CYP competent HepaRG cells incubated with bioactive CYP-dependent NNK and CYP-independent NNKOAc (4-(acetoxymethylnitrosoamino)-1-(3-pyridyl)-1-butanone). We report a dose-dependent reduction in DNA damage in hepatic-derived cell lines in the presence of nicotine and cotinine. Those results are discussed in the context of the *in vitro* model selected.

1. Introduction

The tobacco-specific nitrosamine, NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) (Fig. 1) originates from nicotine during the curing of tobacco (nitrosation of nicotine). NNK is a class 1 IARC carcinogen, indicating that it is a human carcinogen (Cogliano et al., 2004). NNK has been strongly linked to the aetiology of lung adenocarcinoma in humans, and in lung, liver, nasal, and pancreatic tumors in rodents (Hecht et al., 1994; Hecht, 1998, 1999, 2002; Hoffmann et al., 1996).

The metabolism of NNK follows two distinct routes, namely carbonyl reduction and α-hydroxylation, which occur simultaneously in the respiratory tract and the liver (Fig. 1). These reactions are catalyzed by a variety of phase I metabolic enzymes (Hecht et al., 1994; Hecht, 1998; Maser, 1998; Smith et al., 1992, 1995). Direct α-hydroxylation is one major bioactivation pathway for NNK, generating two DNA-reactive intermediates and their final products 4-oxo-4-(3-pyridyl) 1-butanol (HPB) (Fig. 1) and 4-oxo-4-(3-pyridyl) butanone (OPB) (Fig. 1) (Smith et al., 1999, 1992). Microsome-based experiments demonstrated that CYP1A2, 2A6, 2B6, 2D6, 2E1, 3A4 and 3A5 (Hecht, 1998; Jalas et al., 2005; Smith et al., 1992) are active towards NNK in liver. CYP1A2, CYP2B6, CYP3A4, CYP2A6, and CYP2E1 can bioactivate NNK and their relative abundance in the liver suggests that they could play a role in NNK α-hydroxylation in this tissue (Jalas et al., 2005). Due to the selective high level of mRNA expression of CYP2A13 in airway epithelium and favorable kinetics data this enzyme has been the focus of many studies aiming at characterising the bioactivation of NNK in the lung (He et al., 2004; Su et al., 2000). NNK carcinogenicity has been clearly established in systems using doses of a single chemical (Hecht, 1998). However, bioactivation of toxicants is ultimately the result of a complex series of chemical interactions with metabolic pathways. Interestingly recent publications have highlighted the complex and potentially diverging effects of such chemical interactions. *In vivo* work using A/J mice has suggested a protective effect of nicotine against metabolic activation of NNK (Brown et al., 1999), however the mechanism was not identified. In contrast, another *in vivo* study conducted with A/J mice and a K-ras mutant strain treated with NNK and nicotine concluded

Abbreviations: 8-MOP, 8-methoxypsoralen; dH_2O, deionosed water; HBECS, human bronchial epithelial cells; HPB, 4-oxo-4-(3-pyridyl) 1-butanol; HPLC, high pressure liquid chromatography; IARC, International Agency for the Research on Cancer; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNKOAc, 4-(acetoxymethylnitrosoamino)-1-(3-pyridyl)-1-butanone; OPB, 4-oxo-4-(3-pyridyl) butanone; MMS, methyl methanesulfonate; PSI, Pharmaceutical Industry Toxicology Special Interest Group; PPITC, 3-phenylpropyl isothiocyanate.

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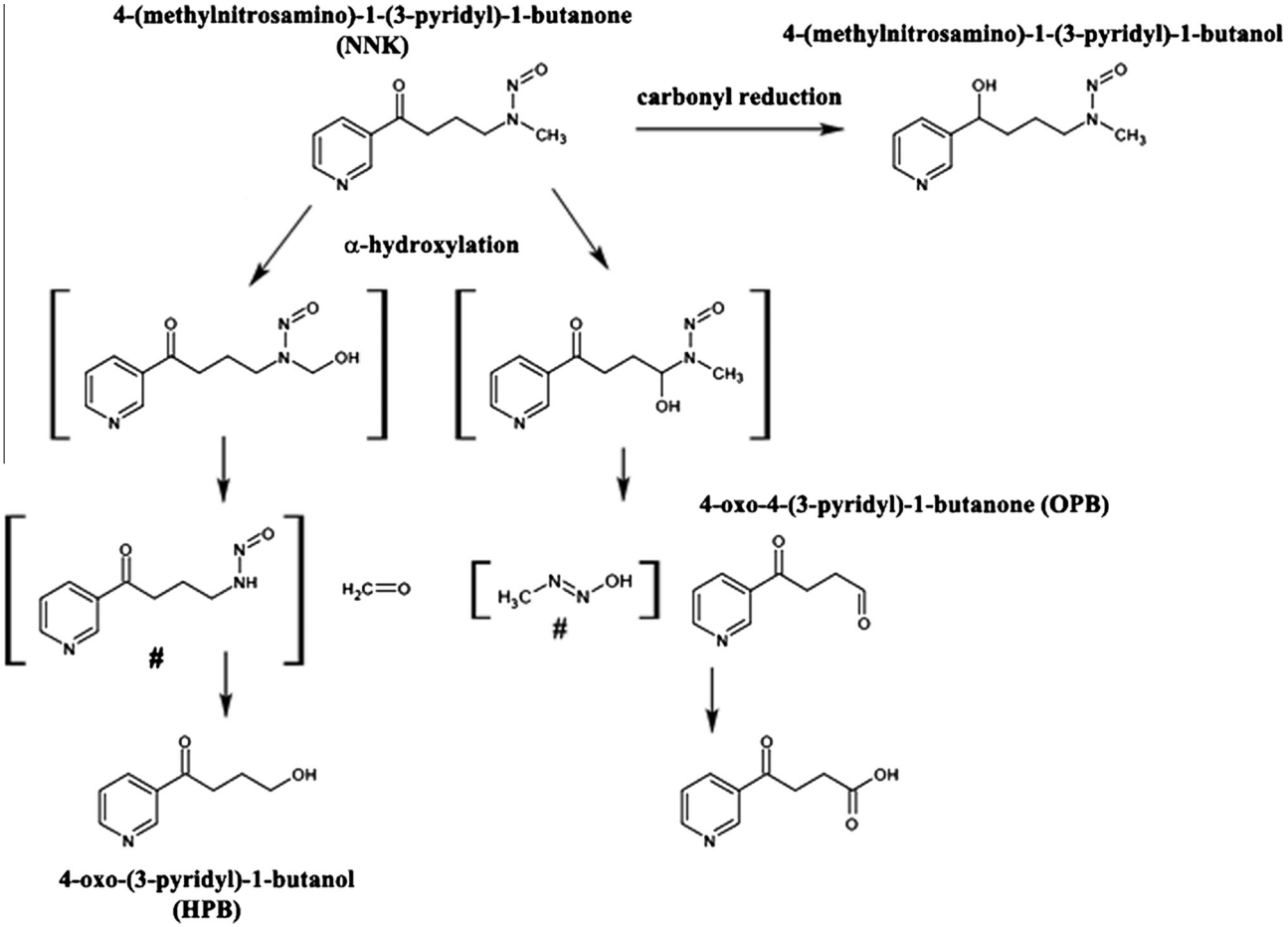

Fig. 1. Simplified representation of NNK metabolism detailing the α-hydroxylation route. DNA reactive intermediates are marked by a '#' symbol.

that nicotine had no influence on lung tumor multiplicity, size, and progression (Maier et al., 2011; Murphy et al., 2011). Yet, a number of publications using *in vitro* systems have reported that nicotine or a nicotine metabolite were inhibitors of diverse CYPs involved in NNK bioactivation including CYP2A6, 2A13, and 2E1. More specifically, *in vitro* work using purified enzymes have demonstrated that nicotine or a nicotine metabolite could protect against CYP2A13/2A6-dependent NNK bioactivation (Bao et al., 2005; von Weymarn et al., 2006). Further investigation identified the nicotine $\Delta^{5'(1')}$ iminium ion as a mechanism based inhibitor of CYP2A6 and CYP2A13 (von Weymarn et al., 2012). β-nicotyrine, a nicotine related alkaloid, has also been shown to inhibit CYP2A6 and CYP2A13 *in vitro* (Denton et al., 2004; Kramlinger et al., 2012). CYP2E1 which has been shown to activate nitrosamines in human and rat liver (Yamazaki et al., 1992) is also inhibited by nicotine and cotinine, albeit modestly (Van Vleet et al., 2001). Interestingly cotinine is an uncompetitive inhibitor of CYP2E1 which indicates that nicotine or a nicotine metabolite does not necessarily have to be an enzyme substrate to exert an inhibitory activity (Van Vleet et al., 2001). In the context of tobacco-smoke chemical interactions and toxicological assessment, the nicotine-dependent inhibition of CYPs raises an interesting and potentially controversial question regarding the possibility that nicotine or nicotine metabolites could protect against some form of NNK-induced DNA damage. Therefore we hypothesized that nicotine or cotinine might inhibit CYP-dependent NNK-induced DNA single and double-strand breaks in metabolically competent cells. The effect of nicotine, cotinine and an isothiocyanate control on DNA damage was compared using the COMET assay in metabolically active HepaRG cells incubated with bioactive CYP-independent NNKOAc and bioactive CYP-dependent NNK. The results are presented and discussed in the context of recent *in vitro* and *in vivo* data looking at the complex interaction of nicotine and nitrosamines.

2. Materials and methods

2.1. Chemicals and reagents

NNKOAc [(Acetoxymethyl)nitrosamino]-1-(3-pyridyl)-1-butanone] (NNKOAc; CAS No. 127686-49-1) and 3-Phenylpropyl isothiocyanate (PPITC; CAS No. 2627-27-2) were obtained from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany). Cotinine (CAS No. 486-56-6), Methyl methanesulfonate (MMS; CAS No. 66-27-3) and all other reagents required for the COMET assay were purchased from Sigma Aldrich (Madrid, Spain). Pooled lung mRNA (5 subjects) was supplied by BioChain® (Newark, USA).

2.2. Cell line and culture conditions

Human hepatic CYP-competent HepaRG® cells were obtained from Merck Millipore (catalog number: MMHPR116). Seven days prior to treatment, cells were seeded on BioCoat™ Collagen I 24-well plates (Becton Dickinson, S.A.) at 0.48×10^6 viable cells/well and maintained according to the manufacturer's instructions. HBEC cells at passage 1 from three non-smoker male donors were obtained from Lonza (Lonza Group Ltd., Switzerland) (Donor 1 (D1): 44 years old; donor 2 (D2): 39 years old, donor 3 (D3):