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In vivo treatment with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) induces organ-specific alterations in *in vitro* repair of DNA pyridyloxobutylation

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ABSTRACT

To investigate the mechanisms responsible for inter-organ differences in susceptibility to 4-methylnitrosamino-1-(3-pyridyl)-1-butanone (NNK)-induced carcinogenesis, the objectives were to compare DNA repair activities of extracts from mouse lung and liver towards NNK-induced pyridyloxobutyl (POB) damage to plasmid DNA, and to determine if and the mechanism by which *in vivo* NNK treatment of mice alters DNA repair. Repair activity of POB adducts was three times greater in mouse liver than in mouse lung ($P < 0.05$). Repair activities of lung extracts from mice 4 or 24 h post-NNK treatment were 30–45% those of control ($P < 0.05$). Conversely, POB adduct repair was 2–3 times higher in liver extracts from NNK treated mice than in controls (4 h, 24 h, $P < 0.05$). NNK treatment also decreased incision of POB adducts by 92% (4 h, $P < 0.05$) in lung and increased incision by 169% (24 h, $P < 0.05$) in liver. NNK decreased immunoreactive levels of the incision protein RPA in lung ($P < 0.05$) 4 h post-treatment but increased immunoreactive lung RPA and XPB after 24 h ($P < 0.05$). In liver, levels of immunoreactive proteins, XPA, XPB and ERCC1 were increased after NNK treatment (24 h, $P < 0.05$). Binding of XPA and XPB from liver extracts to POB adducts increased following NNK treatment, while binding of XPA and XPB from lung decreased (4 h, 24 h). These results suggest that lower incision activity of nucleotide excision repair and NNK-mediated alterations in levels and activities of key incision proteins contribute to the relative susceptibility of mouse lung to NNK-induced carcinogenesis.

1. Introduction

NNK is a tobacco-specific nitrosamine (TSNA) that is present in substantial amounts in both unburned tobacco and tobacco smoke and is believed to play an important role in human tobacco-related cancers [1]. NNK is one of the most prevalent pulmonary carcinogens in tobacco smoke and is the most potent cancer-causing TSNA in all animal species tested [1]. NNK selectively induces lung adenocarcinomas in animals [1] and is believed to be a causal agent in the induction of lung adenocarcinoma in humans, which is now the leading form of human lung cancer [2,3].

To exert carcinogenicity, NNK is metabolically activated by α-carbon hydroxylation to form DNA-reactive species via two different pathways (Fig. 1). α-Methyl carbon hydroxylation generates 4-(hydroxymethylnitrosamino)-1-(3-pyridyl)-1-butanone (1), which decomposes to 4-oxo-4-(3-pyridyl)-1-butanediazohydroxide (2). This compound pyridyloxobutylates DNA. The compound 4-(acetoxymethylnitrosamino)-1-(3-pyridyl)-1-butanone (NNKOAc), a chemically activated form of NNK, is used experimentally because it forms pyridyloxobutyl (POB) adducts exclusively, analogous to those formed upon reaction of NNK with DNA [4,5]. α-Methylene carbon hydroxylation leads to formation of 4-hydroxy-4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (3), which decomposes to methanediazohydroxide (4), and methylates DNA.

NNK-induced DNA methylation occurs at the 7- and O^6-positions of guanine and at the O^4-position of thymine [6,7], while NNK-induced pyridyloxobutylation occurs at the N^7-, N^2- and O^6-positions of guanine and O^2 positions of cytosine and thymine [8–10]. Notably, formation of both POB and methyl adducts is believed to be important in the induction of carcinogenicity by NNK since less potent nitrosamines only either methylate or pyridyloxobutylate DNA [1].

Although bioactivation and formation of DNA adducts are critical determinants of mutagenicity, the maintenance of genetic integrity depends on the ability to repair damaged DNA. DNA methylation is repaired by O^6-alkylguanine-DNA alkyltransferases (AGT), while AGT [11] and nucleotide excision repair (NER) are involved in the repair of POB-DNA adducts [12].

The mechanisms underlying the inter-organ differences in susceptibility to the carcinogenicity of NNK have not been fully

Abbreviations: NNK, 4-methylnitrosamino-1-(3-pyridyl)-1-butanone; NNKOAc, 4-(acetoxymethylnitrosamino)-1-(3-pyridyl)-1-butanone; POB, 4-(3-pyridyl)-4-oxobut-1-yl; NER, nucleotide excision repair; GGR, global genome repair.

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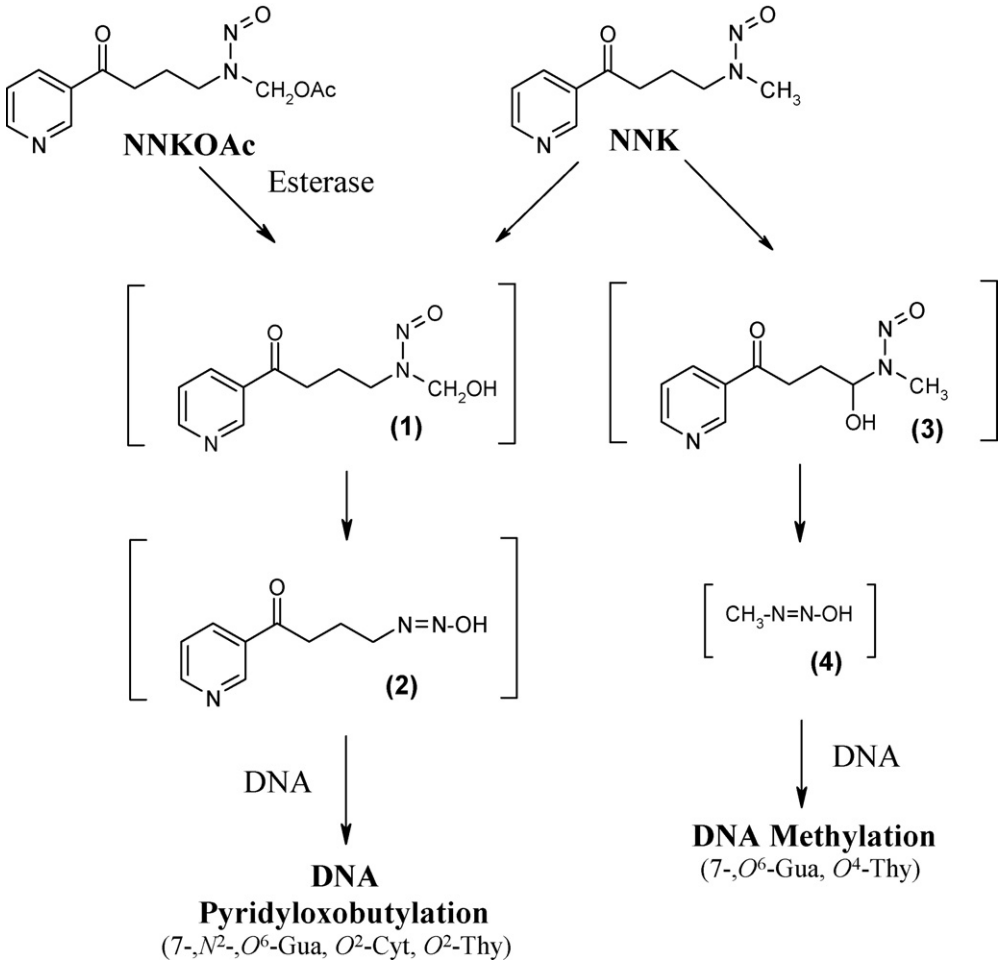

Fig. 1. Pathways of NNK and 4-(acetoxymethylnitrosamino)-1-(-pyridyl)-1-butanone (NNKOAc) bioactivation and DNA adduct formation.

characterized. As anticipated, the extent of both metabolic activation and detoxification of NNK in the lung and liver can affect tumourigenicity [1,13]. In addition, DNA repair capacity of the tissue likely contributes to the organoselectivity of NNK [14]. Inter-organ differences exist in AGT activity, with higher activity occurring in liver relative to lung. Furthermore, NNK alters activity in an organ-specific manner, significantly decreasing the activity of this repair protein in lung but not in liver [14]. However, it is not known if inter-organ differences also exist in NER and whether NNK treatment can alter the activity of this pathway. Moreover, determining the roles of these factors in POB adduct repair is of importance since POB adducts contribute to NNK-induced tumourigenicity in rodents [14–16] and are likely involved in tobacco-induced cancers in human smokers [17].

NER can be divided into two subpathways; global genome repair (GGR) which is involved in the removal of lesions from non-transcribed regions of the genome and the non-transcribed strand of transcribed genes, and transcription-coupled repair (TCR) which removes RNA-polymerase-blocking lesions from transcribed strands of active genes [18]. Both subpathways are mediated by the sequential assembly of repair proteins at the site of DNA damage and involve multiple steps including DNA lesion recognition, incision and excision of the damaged DNA strand, and resynthesis and ligation of DNA [19]. Although the two subpathways employ a common set of proteins for the incision/excision and DNA resynthesis steps, they differ in their mode of damage recognition. In GGR, DNA lesion recognition is based upon the structural distortion and/or chemical alteration of DNA and is carried out by XPC-hHR23B [20], while in TCR, the repair process is initiated when RNA polymerase II is blocked at the DNA lesion site [18]. Once the damage has been identified and verified (by XPC-hHR23B, XPA, and RPA), the damaged site is unwound by helicases (3′ to 5′ helicase XPB and 5′ to 3′ helicase XPD of the TFIIH complex) before the damaged single-stranded DNA fragment is excised by two endonucleases (XPG and XPF-ERCC1). Oligonucleotide excision is followed by repair synthesis and DNA ligation which is mediated by DNA polymerases δ or ε, proliferating cell nuclear antigen, replication factor C and RPA and DNA ligase I, respectively [18].

The GGR process can be reproduced in an *in vitro* assay [21,22], in which repair activity of cell-free nuclear protein extracts prepared from whole tissue is measured by the extent of DNA repair synthesis in damaged plasmid DNA. Repair of plasmid DNA resembles genomic repair *in vivo* [23] and defective repair occurs in extracts from cells from individuals with the NER-deficiency disease, xeroderma pigmentosum (XP) [21,24].

In the present study, we found that NER activity in mouse lung and liver extracts correlates with organ-specific susceptibility to NNK-induced carcinogenesis. We also found that *in vivo* treatment of mice with NNK alters *in vitro* repair of NNK-induced DNA adducts, and we examined the mechanism by which these alterations in repair may occur.

2. Methods

2.1. Animal treatments

Female A/J mice, aged 9–10 weeks (Taconic Labs, Hudson, NY) were housed with a 12-h light/dark cycle and provided food and water *ad libitum*. A/J mice were chosen for this study since the genetics and processes involved in lung tumourigenesis