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Extraction and Metabolism of NNK in the Isolated Perfused Lung System

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Purpose

To validate the use of the recirculating isolated perfused lung system for studying the lung tissue retention of NNK and its metabolites.

Introduction

4-(Methylnitrosamine)-1-(3-pyridyl)-1-butanone (NNK) is formed by nitrosation of nicotine during the smoking and curing processes of tobacco[1]. NNK is one of the most abundant and potent carcinogens found in cigarette smoke, and requires metabolic activation to elicit its carcinogenic effects[2,3]. NNK and its major metabolite, 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanol (NNAL), can be metabolized by α-hydroxylation pathways to form keto alcohol, keto acid, hydroxy acid, and diol (Figure 1). The α-hydroxylation metabolic pathways of NNK and NNAL have been shown to result in the formation of DNA adducts. However, NNK and NNAL can also be metabolized to their respective *N*-oxide metabolites, which are considered detoxification pathways for the elimination of NNK and NNAL[3].

NNK induces pulmonary tumors in rodents regardless of the route of administration[3]. Doses as low as 8.7 μmol/kg have been shown to induce lung tumors, whereas doses of 3 mmol/kg or higher are required to induce the formation of liver tumors[5]. Thus it appears that the lung tissue is selectively sensitive to the carcinogenic effects of NNK. It has been previously reported that the liver clearance (6.9 ± 1.6 ml/min) of NNK is greater than lung clearance (2.1 ± 0.5 ml/min) in rats, and each organ produces a different metabolic profile[6]. Since *in vivo* studies of investigating the carcinogenicity effects of NNK on the lung are complicated by liver metabolism, it would be of interest to use the isolated lung perfusion system to investigate NNK metabolism and the retention of its metabolites in the lung.

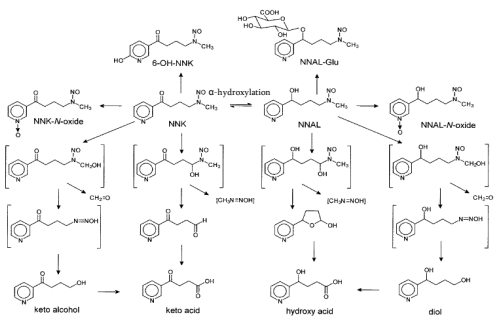
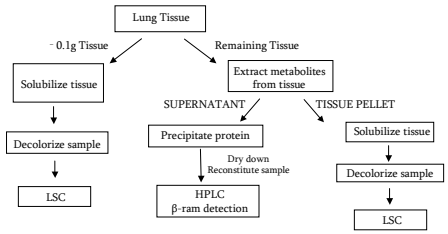


Figure 1: Metabolic scheme of NNK[4]

Tissue Sample Analysis



Methods

- Anesthetized rats (Male Fisher 344 rats, 315 ± 15 g) with pentobarbital sodium (60 mg/kg ip)
- Cannulated trachea (control lung inflation) and pulmonary artery
- Excised lungs from chest cavity and rinsed of blood
- Lungs inflated at constant pressure (4 cm H_2O) and perfused with 50 ml of Ringers buffer (pH 7.4) at 8 ml/min
- Perfusate oxygenated with 95% O_2 and 5% CO_2, and maintained at 37˚C
- 50 μCi bolus dose of H^3-NNK administered to perfusate reservoir
- Perfusate samples drawn at 1, 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, and 120 minutes
- Following perfusion the lungs were rinsed and flash frozen with liquid nitrogen

Results

Table 1: Metabolites extracted from the tissue (n=4) and perfusate (n=6) following a 120 min perfusion with 50 μCi of ^{3}H-NNK in an isolated lung system. The metabolites are expressed as the mean percent (± standard deviation) of total radioactivity in the sample.

	Tissue [% of total radioactivity]	Perfusate [% of total radioactivity]
Hydroxy Acid	9 ± 0.5 %	< LOD*
Keto Acid	21 ± 4 %	12 ± 4 %
NNAL-*N*-Oxide	24 ± 3 %	5.5 ± 1 %
Diol	7 ± 0.5 %	2 ± 0.7 %
NNK-*N*-Oxide	10 ± 5 %	48.5 ± 7 %
Keto Alcohol	3 ± 0.8 %	14 ± 4 %
NNAL	5 ± 2 %	5 ± 1 %
NNK	6.5 ± 2 %	4.5 ± 4 %

* Below the limit of detection

Table 2: Estimated pharmacokinetic parameters for NNK in an isolated lung system expressed as mean ± standard deviation (n=5).

Clearance (ml/min)	1.63 ± 0.4
Extraction Ratio	0.21 ± 0.05
Terminal Elimination Half-Life (min)	24.8 ± 8.2

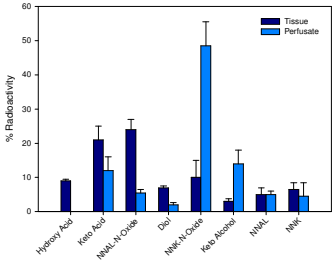
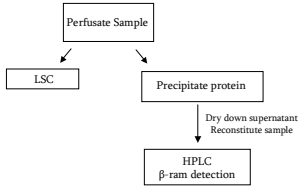


Figure 2: NNK and metabolites in the tissue and perfusate following a 120 min perfusion with 50 μCi of ^{3}H-NNK. The metabolites are expressed as the mean percent (± standard deviation) of total radioactivity in the sample.

Perfusate Sample Analysis



Conclusions

The perfusate metabolites measured in this study are similar to those previously reported[6]. However, our ability to measure tissue metabolites indicates that perfusate data alone will not give an accurate reflection of metabolic profile and retention by the lung tissue.

(S)-NNAL has been reported to be as carcinogenic as NNK and we hypothesize that its selective retention in the lung tissue may be the cause[7]. This study demonstrates the feasibility of the use of the isolated perfused lung system to evaluate formation and retention of NNK and NNAL metabolites in the lung.

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