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Noscapine recirculates enterohepatically and induces self-clearance

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ABSTRACT

Noscapine (Nos), an antitussive benzylisoquinoline opium alkaloid, is a non-toxic tubulin-binding agent currently in Phase II clinical trials for cancer chemotherapy. While preclinical studies have established its tumor-inhibitory properties in various cancers, poor absorptivity and rapid first-pass metabolism producing several uncharacterized metabolites for efficacy, present an impediment in translating its efficacy in humans. Here we report novel formulations of Nos in combination with dietary agents like capsaicin (Cap), piperine (Pip), eugenol (Eu) and curcumin (Cur) known for modulating Phase I and II drug metabolizing enzymes. *In vivo* pharmacokinetic (PK), organ toxicity evaluation of combinations, microsomal stability and *in vitro* cytochrome P450 (CYP) inhibition effects of Nos, Cap and Pip using human liver microsomes were performed. Single-dose PK screening of combinations revealed that the relative exposure of Nos (2 μg h/mL) was enhanced by 2-fold (4 μg h/mL) by Cap and Pip and their plasma concentration–time profiles showed multiple peaking phenomena for Nos indicating enterohepatic recirculation or differential absorption from intestine. CYP inhibition studies confirmed that Nos, Cap and Pip are not potent CYP inhibitors (IC_{50} > 1 μM). Repeated oral dosing of Nos, Nos + Cap and Nos + Pip showed lower exposure (C_{max} and AUC_{last}) of Nos on day 7 compared to day 1. Nos C_{max} decreased from 3087 ng/mL to 684 ng/mL and AUC_{last} from 1024 ng h/mL to 508 ng h/mL. In presence of Cap and Pip, the decrease in C_{max} and AUC_{last} of Nos was similar. This may be due to potential enzyme induction leading to rapid clearance of Nos as the trend was observed in Nos alone group also. The lack of effect on intrinsic clearance of Nos suggests that the potential drug biotransformation modulators employed in this study did not contribute toward increased exposure of Nos on repeated dosing. We envision that Nos-induced enzyme induction could alter the therapeutic efficacy of co-administered drugs, hence emphasizing the need for strategic evaluation of the metabolism of Nos to reap its maximum efficacy.

1. Introduction

Noscapine (Nos) is an antitussive agent that has been used for decades for its cough-suppressive action (Ke et al., 2000; Ye et al., 1998). Nos is a microtubule-modulating agent and was employed in Phase II clinical development for the therapy of multiple myeloma (Aneja et al., 2010). It is a weak base (pK_a 7.8) with poor oral bioavailability thus necessitating administration of relatively high doses (300–450 mg/kg body weight in preclinical models) for optimal therapeutic benefits (Aneja et al., 2007; Ke et al., 2000; Ye et al., 1998). The bioavailability of Nos is around 32% in mice and its rapid first-pass metabolism and shorter half-life (<2 h) due to extensive biotransformation can result in poor translation of its efficacy in humans (Aneja et al., 2007). This is in agreement with previous reports on preclinical pharmacokinetic (PK) parameters of Nos that suggest that this tubulin-binding agent undergoes a rapid metabolism as it peaks in blood as early as 5 min, followed by complete elimination within 4 h of oral administration (Aneja et al., 2007).

Abbreviations: ACN, Acetonitrile; Cap, capsaicin; Cur, curcumin; CYP, cytochrome P450; DLM, dog liver microsomes; DMSO, Dimethylsulfoxide; Eu, eugenol; IC_{50}, inhibitory concentration 50%; HLM, human liver microsomes; LC–MS/MS, liquid chromatography tandem mass spectrometry; LLOQ, lower limit of quantitation; MBS, microsomes buffer substrate mix; MLM, mouse liver microsomes; NADPH, β-Nicotinamide Adenine Dinucleotide 2′-Phosphate; NMP, N-methylpyrrolidone; Nos, Noscapine; PK, pharmacokinetics; RLM, rat liver microsomes.

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The metabolism of Nos in mice has been shown to result in the formation of at least 20 or more metabolites including a reactive intermediate that undergoes conjugation with glutathione (Fang et al., 2012). While Phase I metabolism of Nos has been shown to result in more than 10 metabolites, Phase II metabolism primarily produces at least 9 glucuronide conjugates (Fang et al., 2012). Several enzymes including cytochrome P450 (1A2, 2C8, 2C9, 2C19, 2D6 3A4, 3A5, UGTs (2B7, 1A1, 1A3, 1A9) and flavin monooxygenases (FMOs) have been shown to catalyze the biotransformation of Nos. It appears that Nos also inhibits/inactivates CYP enzymes resulting in drug–drug interactions when combined with other anti-cancer drugs such as doxorubicin (Fang et al., 2012). In a more recent study, Nos was shown to inhibit hydroxylation of (S)-warfarin by inhibiting CYP2C9 through formation of reactive metabolite (Zhang et al., 2013).

While most drugs typically undergo oxidation or hydrolysis during phase I metabolism, they conjugate to hydrophilic substances, like glucuronic acid, sulfate, and acetyl during phase II metabolism (Blaut et al., 2003; Cummings and Macfarlane, 1991, 1997). During phase I metabolism, various cytochrome P450 (CYP) enzymes play a crucial role in biotransformation of drug molecules. Glucuronidation, sulfation and acetylation are the most important phase II reactions, catalyzed by the uridine diphosphate glucuronosyl transferase (UGT), sulfotransferase (SULT) and N-acetyltransferase (NAT) enzyme. This process produces molecules that are highly hydrophilic and hence can be easily excreted. UGTs are a widely distributed superfamily of enzymes responsible for metabolism of endogenous substrates and xenobiotics to more polar, water-soluble conjugates for elimination (Jancova et al., 2010; Jansen et al., 1992). It is well known that many herbal/dietary agents can interact with drug metabolism processes and can inhibit drug metabolism (Zhang and Lim, 2008).

Thus co-administration of CYP and UGT substrates and/or modulators with drugs is likely to be beneficial for improving pharmacokinetic properties and maintaining sustained drug levels. Although a majority of known CYP/UGT modulators are pharmaceutical compounds, it is noteworthy that several food components and natural products are also substrates or inhibitors of CYP and UGT enzymes (Scheepens et al., 2010; Zhang, 2001). A wide range of studies have reported that a number of flavonoids, including quercetin, tannic acid, benzoin gum, capsaicin, dihydrocapsaicin, eugenol, gallocatechingallate, geraniol, menthol, menthyl acetate, naringenin, all spice berry oil, N-vanillylnonanamide, clove bud oil, peppermint oil, silibinin, piperine, silymarin, epigallocatechingallate and curcumin, inhibit the metabolism of various drugs by interacting with one or more CYP/UGT enzymes (Benet and Wacher, 2003; Gerk et al., 2014; Scheepens et al., 2010). As a result, an inhibitor helps in increasing and maintaining the concentration of a therapeutic drug. For example, co-administration of curcumin with pioglitazone significantly decreased the metabolism of the latter, in normal and diabetic rats (Prasad Neerati and Kanwar, 2012). Similarly, capsaicin and ellagic acid strongly inhibit the rat CYP 2A2, 3A1, 2C11, 2B1, 2B2 and 2C6 (Zhang et al., 1993). Hence, it is important to identify a compound or different classes of compounds that are capable of enhancing bioavailability by inhibiting CYP/UGT enzymes at the intestine and liver and hence reducing the first pass effect.

In the current study, we investigated whether co-administration of Nos with putative CYP/UGT modulating herbal/dietary agents such as capsaicin (Cap), piperine (Pip), eugenol (Eu) and curcumin (Cur) would alter the pharmacokinetics of Nos. We rationalized that these dietary agents may influence the bioavailability of Nos as they might inhibit its extensive biotransformation and increase the overall exposure. Thus the overarching aim of the study was to examine if Nos's rapid first-pass metabolism could be slowed down or inhibited to improve its overall pharmacokinetic (PK) parameters.

2. Materials and methods

2.1. Chemicals and reagents

Noscapine (Nos), capsaicin (Cap), piperine (Pip), eugenol (Eu), curcumin (Cur), polyethylene glycol 300 (PEG 300), N-methylpyrrolidone (NMP) and β-glucuronidase were purchased from Sigma (St. Louis, MO). Acetonitrile (ACN), and methanol (MeOH) were obtained from Fisher Scientific (Pittsburgh, PA). Liver microsomes from male CD-1 mouse, Sprague–Dawley rat, and Beagle dog, and mixed gender human (pool of 50) were procured from XenoTechLLC (Kansas, USA; protein content: 20 mg/mL). Standard substrates and inhibitors used for CYP inhibition assay were procured from Sigma (Bengaluru, India). All the stable labeled internal standard(s) (IS) used for analyzing the CYP inhibition samples were from Toronto Research Chemicals, Canada. NADPH, formic acid, ammonium formate, sodium dihydrogen phosphate and disodium hydrogen phosphate, and dimethyl sulfoxide (DMSO) and were purchased from Sigma (Bengaluru, India). 96-well plates of 1 mL capacity were purchased from Axygen Scientific, USA. Milli-Q® water was used for preparation of buffer (Millipore Corporation). All other reagents used in the assay were of analytical grade.

2.2. Formulation recipe of Nos and various dietary ingredients for in vivo PK studies

Oral bioavailability of Nos is reported to be 30% in mouse and humans. In order to rule out the possibility of Nos's limited solubility being a rate-limiting step in determining its bioavailability in presence of dietary agents, water-soluble co-solvents like N-methyl-2-pyrrolidone (NMP) and polyethylene glycol (PEG300) were used. The main objective was to produce a homogenous solution for dosing. These excipients are considered as safe in rodent pharmacokinetic and toxicokinetic studies. Required amounts of Nos, Cap, Pip, Eu and Cur were weighed and triturated with 10% v/v NMP and 30% v/v PEG 300. The volume was made up with Milli-Q water, vortex mixed and sonicated for 5 min. The dose volume used for dosing was 10 mL/kg.

2.3. Pharmacokinetic studies of Nos and dietary ingredients

Pharmacokinetic studies were performed in male CD-1 mice (NCI, Frederick) following a single oral (PO) administration of Nos, Nos + Cap, Nos + Eu, Nos + Pip and Nos + Cur (at 50 mg/kg Nos and 5 mg/kg dietary ingredients). A clinical study was conducted for Nos, where Nos was given at 50 mg/kg t.i.d. (Olsson et al., 1986). Furthermore, another clinical study involving a dose of 300 mg Nos was conducted by Karlsson et al. Applying a scaling factor of 12.3 and considering 70 kg as body weight, the mouse equivalent dose was determined to be 50 mg/kg (Karlsson et al., 1990). Based on these studies, we employed a dose of 50 mg/kg bw Nos in our study. To avoid masking the effect of Nos, Cap, Eu, Pip and Cur were administered at 5 mg/kg bw, as these modulators are known to act locally at the intestine. Formulations were prepared freshly before dosing. They were assessed for accuracy by LC/MS/MS and were within 20% of nominal concentration on Day 1 and 7. All animals of 8–12 weeks age and 30–40 g body weight were acclimatized for 3 days before dosing. Feed and water was provided *ad libitum* throughout the study period. Animals were marked and housed (three per cage) in polypropylene cages and