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Evaluation of hepatic metabolism and pharmacokinetics of ibuprofen in rats under chronic hypobaric hypoxia for targeted therapy at high altitude

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ABSTRACT

With studies indicative of altered drug metabolism and pharmacokinetics (DMPK) under high altitude (HA)-induced hypobaric hypoxia, consideration of better therapeutic approaches has continuously been aimed in research for HA related illness management. DMPK of drugs like ibuprofen may get affected under hypoxia which establishes the requirement of different therapeutic dose regimen to ensure safe and effective therapy at HA. This study examined the effects of the chronic hypobaric hypoxia (CHH) on hepatic DMPK of ibuprofen in rats. Experimental animals were exposed to simulated altitude of 7620 m (~25,000 ft) for CHH exposure (7 or 14 days) in decompression chamber and administered with ibuprofen (80 mg/kg, body weight, p.o.). Results demonstrated that CHH significantly altered PK variables of ibuprofen and activities of both phase-I and II hepatic metabolic enzymes as compared to the animals under normoxic conditions. Hepatic histopathological observations also revealed marked alterations. Increase in pro-inflammatory cytokines/chemokines viz. IL-1β, IL-2, IFN-γ, TNF-α exhibited close relevance with diminished CYP2C9 expression under CHH. Moreover, the down-regulated CYP2C9 level further supported the underlying mechanism for reduced metabolism of ibuprofen and as a result, increased retention of parent drug in the system. Increased mean retention time, Vd, *T½* of ibuprofen, and decreased AUC, C_{max} and clearance during CHH further strengthened the present findings. In conclusion, CHH exposure significantly affects hepatic DMPK of ibuprofen, which may further influence the usual therapeutic dose-regimen. Further, there is requirement of human studies to evaluate their susceptibility toward hypobaric hypoxia.

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1. Introduction

An intricate sequence of pathophysiological effects arises in human body following exposure to high altitude (HA). At HA, the first and foremost environmental stress i.e., hypobaric hypoxia exerts impact on the human body, causing a wide range of physiological illnesses such as acute mountain sickness (AMS), high altitude cerebral edema (HACE) and high altitude pulmonary edema (HAPE) [1]. Hypobaric hypoxia affects the normal physiology and functions of vital organs like lungs, brain, heart and liver due to subnormal oxygen concentration in the cells [2]. Liver regulates various biochemical pathways including the metabolism of endogenous, exogenous compounds and their detoxification. Hepatic metabolism is a complex physiological process aiming at converting the parent lipophilic drug to a more polar metabolite. Oxygen plays a crucial role of substrate for drug oxidations as a terminal electron acceptor in the mitochondria that regulates other processes dependent upon the cellular redox state and synthesis of essential high energy co-factors and reducing equivalents (ATP and NAD) required for drug transport and conjugation pathways. The enzymes of these metabolic pathways possess different affinities for oxygen, thus their functional sensitivity to the severity of hypoxia also differs. Therefore, for regulation of these metabolic pathways, liver requires more oxygen than other tissues and is more prone to hypoxia-induced oxidative stress [3].

Previous studies have reported alterations in metabolism of drugs under hypoxia [4,5]. Various studies on drug metabolism (DM) and pharmacokinetics (PK) of common drugs like car-

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bonic anhydrase inhibitors (acetazolamide), anti-pyretic and anti-analgesic (acetaminophen), corticosteroids (dexamethasone, prednisolone), non-steroidal anti-inflammatory drugs (NSAIDs; ibuprofen), furosemide and lithium etc., have demonstrated alterations in oxidative as well as conjugation pathways along with elimination of these drugs [6–8]. Therefore, it is crucial to understand the requirement of safe therapy under HA-induced hypoxia for treating various HA-related maladies. For the use of NSAIDs as prophylactic and therapeutic intervention under HA-induced headache, the focus has been continuously aimed on their safe use, effectiveness and absorption, distribution, metabolism and elimination (ADME) properties. Various studies have been conducted earlier for studying usefulness and efficacy of ibuprofen for treatment of HA-induced headache [9,10]. Previously, our study reported that acute hypobaric hypoxia (24 h) exposure significantly altered DMPK of ibuprofen [7]. However, it necessitates further investigation to evaluate the effect of chronic hypobaric hypoxia (CHH) on DMPK of ibuprofen, for safe and effective administration as a therapeutic intervention.

CYP2C9 (isoform of CYP450) is best known for its significant role in metabolism of ibuprofen. In conditions associated with oxygen tension, the activity and expression of various CYP proteins get affected which implies that their expression and/or activity may be implicated in hypoxic conditions. Earlier studies have revealed altered expression of isoforms of CYP450 such as CYP1A1, CYP1A2, CYP2B6, CYP2C9 and CYP2C19, CYP2E1 under hypoxic conditions [11,12]. However, there is no conclusive evidence to show the role of CHH in affecting hepatic metabolism and CYP2C9 activity and expression. Hence, the aim of the present study was to investigate the effect of CHH stress on hepatic DM, PK of ibuprofen and protein expression levels of CYP2C9 and inflammatory cytokines/chemokines, if any. Furthermore, the probable associated mechanism involved in regulation of activity and expression of CYP-mediated DM of ibuprofen under CHH has also been determined. Thus, the present work has therapeutic implications in case of any altered dose-regimen of ibuprofen under CHH conditions.

2. Materials and methods

2.1. Experimental animals

Experiments were conducted on adult male Sprague-Dawley rats (200 ± 20 g; animal colony of DIPAS, Delhi). The normoxic animals were maintained under controlled environment at the institute's animal house at temperature of 25 ± 1° C, humidity 55 ± 5% and 12 h: 12 h light: dark cycle with free access to food and water. The study was approved by the Institute's Animal Ethical Committee (IAEC-02/DIPAS/2013), and experiments were performed in accordance with the national guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) of the Government of India.

2.2. Experimental design for PK under CHH

A total of 24 rats were randomized into four groups (six animals in each) and treated with various regimes: group I and III remained at sea level atmospheric pressure within the same laboratory conditions; experimental rats of group II and IV were exposed to CHH for duration of 7 and 14 days respectively in decompression chamber (Seven Star, Delhi, India) at a simulated altitude of 7620 m above sea level (~25000 ft, 41329.9 Pa/310 mm Hg), as described earlier [7]. The decompression chamber was maintained at 28 ± 2 °C, 55 ± 5% humidity and air flow 9–10 l/min during the exposure to prevent accumulation of exhaled gases. Single dose of ibuprofen (80 mg/kg body weight, p.o.) prepared in 2% methylcellulose vehicle was administered immediately after 7 or 14 days CHH exposure to all normoxic and hypoxic groups, as described earlier [7]. Ibuprofen was administered after stipulated exposure duration because pressure in the decompression chamber cannot be released repeatedly during hypobaric hypoxia exposure. The blood samples were collected through orbital sinus at 0 min time point for pre-dose and at 0.25, 0.5, 1, 2, 3, 4 and 6 h for post-dose sample for each hypoxia exposure period. The blood samples were immediately centrifuged at 419 g for 10 min and the plasma was separated and stored at −80 °C for further analysis by liquid chromatography–mass spectrometry (LC–MS/MS).

2.2.1. Evaluation of PK variables by LC–MS/MS

The plasma concentration of ibuprofen was analyzed using LC–MS/MS bioanalytical method, carried out as described previously [13]. The LC chromatographic separation system (Thermo Scientific, Waltham, MA, USA) consists of a quaternary pump (Agilent 1100, Agilent Technologies, Santa Clara, CA, USA), an online solvent degasser and an auto-sampler. An Applied Biosystems/MSD Sciex API 4000 triple quadrupole mass spectrometer (MDS Sciex, CA, USA) equipped with ESI source was used for mass spectrometric analysis and detection. Instrument control and data acquisition were carried out with Applied Biosystems/MSD Sciex Analyst software (version1.4). The LC–MS/MS analysis was performed at room temperature using a Chromolith Flash RP-18 endcapped column (25 mm × 4.6 mm) (Merck Life Science Pvt., Ltd., Mumbai, Maharashtra, India). Acetonitrile/water and methanol/water mixtures were initially evaluated for use as mobile phase. Analytes were eluted with mobile phase-A, containing water with 0.3% formic acid and, mobile phase-B containing acetonitrile with 0.3% formic acid (35:65, v/v) pumped at a flow rate of 500 μl/min.

2.2.2. Sample preparation

20 μl of plasma sample was mixed with 180 μl of extraction solvent containing 70% acetonitrile with 0.1% formic acid and 250 ng/ml sulphadimithoxine and vortexed for 1 min. The mixture was centrifuged for 10 min at 10,000 rpm and the resulting supernatant was collected and subjected for LC–MS/MS analysis. The concentration data obtained was analyzed through WinNonlin pharmacokinetic software Version 5.1, Scientific Consultants, MD, USA for further evaluation of PK variables viz. elimination half-life ($T½$), mean residence time (MRT), clearance (Cl) and volume of distribution (Vd), C_{max}, T_{max}, AUC (obs) 0-t etc.

2.3. Experimental design for hepatic drug metabolism under CHH

In another set of experiment, forty animals were randomly allocated into four groups. Animals of the group I and III remained at sea level atmospheric pressure within the same laboratory conditions and were administered with ibuprofen (80 mg/kg body weight, p.o.) daily for duration of 7 and 14 days respectively. Animals of group II and IV were exposed in decompression chamber for duration of 7 and 14 days respectively and were also administered with ibuprofen (80 mg/kg body weight, p.o.) daily for stipulated period of hypoxia exposure.

2.3.1. Hepatic drug metabolizing (phase I and II) enzyme activities

After CHH exposure (7 or 14 days), all the rats in the normoxic and hypoxic groups were sacrificed and microsome were prepared using differential centrifugation. The microsomal pellets obtained were re-suspended in 0.1 M potassium phosphate buffer (pH 7.4) containing 0.5 mM EDTA and 20% (w/v) glycerol for evaluating activities of hepatic metabolizing enzymes (phase I and II) [7]. Total CYP450 content was estimated in microsomes [14]. Microsomal NADPH Cyt c reductase assay was carried out as

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