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Hepatic metabolism of ibuprofen in rats under acute hypobaric hypoxia

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ABSTRACT

Hypobaric hypoxia induced at high altitude causes a subnormal oxygen concentration in cells which affects the drug metabolic and pharmacokinetic (PK) capacity of the body. The metabolism and PK of drugs like ibuprofen may be impaired under hypoxia and may require a different than usual therapeutic dose regimen to ensure safe therapy. The present investigation was undertaken to evaluate the effect of acute hypobaric hypoxia (AHH) on hepatic metabolism and PK of ibuprofen in rats. Animals were exposed to simulated altitude of 7620 m (~25,000 ft) for AHH exposure (6 and 24 h) in a decompression chamber and were administrated with single dose of ibuprofen (80 mg/kg body weight, p.o.). The results showed that GST activity was significantly reduced at 6 h (15%) and 24 h (23%) ($p < 0.05$) in hypoxic group as compared to normoxic. A significant increase by 20–24% ($p < 0.05$) in AST level was observed after AHH exposure. LDH activity also exhibited significant increase ($p < 0.05$) after 24 h of AHH. A significant down-regulated CYP2C9 level and mild histopathological changes were observed after 24 h of AHH. Furthermore, PK variables viz. elimination half-life ($T½$) and mean residence time (MRT) of ibuprofen exhibited significant increase by 42% and 51% ($p < 0.05$) respectively after 24 h of AHH. Thus, results suggest that AHH exposure of 24 h significantly affects phase II conjugation pathway, CYP2C9 level, AST level, liver histology and PK parameters. This asserts that AHH can impair disposition of ibuprofen however, it requires further investigation under chronic hypobaric hypoxic conditions.

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1. Introduction

On induction to high altitude (HA) human body encounters the first environmental stress i.e. hypobaric hypoxia which causes many pathophysiological effects to the individuals during their initial days of induction and also following prolonged residency at HA. The normal physiological mechanism and metabolism in an individual is hampered under hypoxia (Cymerman and Rock, 2009). At HA, the low barometric pressure of the atmosphere results in diminished alveolar oxygen tension and as a result, arterial partial pressure of oxygen (pO_2) drops, in turn decreasing the oxygen saturation.

The functions and physiology of vital organs like liver, brain, heart and lungs get affected due to hypoxia interceded at HA due to subnormal oxygen concentration in cells (Hoppeler and Vogt, 2001; Muhlinga et al., 2006; Nakanishi et al., 1995; Pagani et al., 2000; Sulkowska, 1997). Liver is responsible for regulation of a wide variety of biochemical pathways including the metabolism of endogenous and exogenous compounds and detoxification. For regulation of these metabolic processes liver requires more oxygen than other tissues and is more prone to hypoxia mediated oxidative stress (Savransky et al., 2007; Berendsohn, 1962). The major pathways of hepatic drug metabolism are dependent on oxygen. Oxidases and oxygenases are responsible for altered hypoxic functions due to deficient metabolic activities. As these enzymes have different affinities for O_2, it follows that their functional sensitivity to the severity of hypoxia also differs. Phase I hepatic enzymes usually converts the parent lipophilic drug to a more polar metabolite by introducing or unmasking a functional group. However in phase II metabolism, the hydroxylated or other compounds produced in phase I are conjugated with glucuronic acid, sulphate, acetate, glutathione or certain amino acids or by methylation, etc. Phase II enzyme systems are indirectly dependent on oxygen for the generation of essential co-factors, such as NAD and ATP (Guengerich, 1991). The cellular oxygen deficiency caused due to hypoxia at HA perturbs cellular metabolism.

The studies conducted earlier have provided the basis that drug metabolism and PK get affected under hypoxia (Costa, 1990; Jones et al., 1989; Jürgens et al., 2002; Shan et al., 1992; Woodrooffe et al., 1995). The metabolism and PK studies on various classes of drugs including corticosteroids, carbonic anhydrase inhibitors, antipyretic and anti-analgesic drugs like acetaminophen,

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acetazolamide, prednisolone, furosemide and lithium have supported that hypoxia causes alterations in metabolism of drug (Arancibia et al., 2003, 2004, 2005; Aw et al., 1991; Ritschel et al., 1998). Though there has been continued interest in the safety and absorption, distribution, metabolism and elimination (ADME) properties w.r.t. non-steroidal anti-inflammatory drugs (NSAIDs) for its implication as prophylactic and therapeutic intervention, still there is paucity of literature regarding the effect of hypoxia on NSAIDs. Therefore, we have selected ibuprofen as a candidate drug of this class.

NSAIDs play crucial role as therapeutic agents for the treatment of pain and inflammatory diseases. Ibuprofen [2-(4-isobutylphenyl)-propionic acid], a member of NSAID family, is a widely used and well-tolerated analgesic (Davies, 1998). Ibuprofen is a known non-selective cyclooxygenase (COX) inhibitor, inhibiting both COX-1 and COX-2 forms. However its analgesic, antipyretic and anti-inflammatory effects are principally due to COX-2 inhibition (Neupert et al., 1997). Ibuprofen is metabolized to form carboxy and hydroxy ibuprofen, as well as an acyl glucuronide, which are excreted in urine (Mills et al., 1973).

CYP2C9 is a major cytochrome P450 isoform, which is responsible for metabolic clearance of a wide variety of therapeutic agents, including NSAIDs. It plays a major role (>70%) in the oxidative metabolism of ibuprofen (Hamman et al., 1997; Leemann et al., 1993; McGinnity et al., 2000). In conditions related to oxygen tension the expression and activity of several CYP proteins get affected which suggests that their expression and/or activity may be implicated in hypoxic conditions. Previous in vitro and ex vivo studies have reported decreased activity and expression of CYP450 isoforms i.e. CYP1A1, CYP1A2, CYP2B6, CYP2C9 and CYP2C19, CYP2E1 under hypoxic conditions (Fradette et al., 2002, 2007; Fradette and Du Souich, 2004; Michaelis et al., 2005). Along with effect of hypoxia on drug metabolism, hepatic damage is also caused under hypoxia. Previous studies have also demonstrated liver damage under chronic intermittent hypoxia (Feng et al., 2011; Savransky et al., 2007, 2009).

As a result of altered drug metabolism and PK, safe drug dosage regime may differ under HA induced hypoxia which establishes the requirement for safe and effective treatment of HA related maladies. Despite a number of studies conducted at HA evident for usefulness of ibuprofen for treatment of HA induced headache (Berghold, 2000; Broome et al., 1994; Harris et al., 2003; Gertsch et al., 2010), there is paucity of literature on ibuprofen w.r.t. its altered metabolism and PK as well as therapeutic dosage under hypoxia.

Hence, the present study was undertaken with the aim to investigate the effect of AHH stress on alteration of hepatic metabolism and PK of ibuprofen, if any. For further understanding the potential effect of hypoxia on hepatic metabolism extensive analysis of several detoxification enzymes (phase I and II), liver pathology, CYP2C9 protein level and PK was carried out using ibuprofen as a candidate drug.

2. Materials and methods

2.1. Chemicals

Chemicals including ibuprofen, horse heart cytochrome c, mefenamic acid, UDP used in the study were procured from Sigma–Aldrich Chemical Co., St. Louis, USA. Rabbit polyclonal anti-CYP2C9 antibody was purchased from Abcam, MA, USA and all other chemicals of high analytical grade used in the experiments were obtained from S.D. Fine chemical, and SISCO research laboratories, India. HPLC grade acetonitrile, methanol, ortho-phosphoric acid (OPA) and water were purchased from Merck, India.

2.2. Experimental animals

Male Sprague-Dawley rats (180 ± 20 g), from the animal colony of the Defence Institute of Physiology and Allied Sciences (DIPAS), Delhi were used for this study. The animals were maintained under controlled environment at the Institute's animal house at 25 ± 1 °C and 12-h light–dark cycle and had food and water ad libitum. The experiments were performed in accordance with the regulations specified by the Institute's Animal Ethical Committee and conform to the national guidelines on the care and use of laboratory animals, India.

2.3. Induction to hypobaric hypoxia and experimental design

Experimental animals were randomly divided into four groups of six animals each. Animals of the group I and III remained at sea level atmospheric pressure within the same laboratory conditions and were administered with single dose of ibuprofen (80 mg/kg body weight, p.o.). Animals of the group II and IV were exposed to AHH for a duration of 6 and 24 h respectively in decompression chamber (Seven Star, Delhi, India) at a simulated altitude of 7620 m (~25,000 ft). The animals of hypoxia group II and IV were also administered with single dose of with ibuprofen (80 mg/kg body weight, p.o.), immediately prior to hypoxia exposure. The hypobaric hypoxia decompression chamber was maintained at 28 ± 2 °C temperature, 55 ± 5% humidity and air flow 9–10 lit/min during the exposure to prevent accumulation of exhaled gases.

2.4. Evaluation of phase I and II drug metabolizing enzymes

On completion of the stipulated period of hypoxia exposure (6 or 24 h), the rats of both normoxic and hypoxic groups treated with ibuprofen were sacrificed by cervical dislocation. The liver was perfused with cold saline, excised and weighed. One portion of liver was used to prepare a 10% liver homogenate (w/v) in (0.15 M KCl + 5 mM Sod. EDTA) buffer for lactate dehydrogenase (LDH), alanine tranaminases (ALT) and aspartate transferases (AST) assays. Another portion of each liver was homogenized in 0.1 M potassium phosphate buffer (pH 7.4) for microsome preparation. Microsomal fractions were isolated with differential centrifugation method (Omura and Sato, 1964). Briefly, liver homogenate (prepared in 0.1 M potassium phosphate buffer pH 7.4) was centrifuged at 800 × *g* in refrigerated centrifuge for 10 min (to remove nuclei and cell debris). The post-nuclear supernatant was collected in separate tubes and centrifuged at 12,000 × *g* in refrigerated centrifuge for 10 min (to remove mitochondria). Post-mitochondrial supernatant was centrifuged at 1,05,000 × *g* in refrigerated ultracentrifuge for 1 h and 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 further analysis of hepatic metabolizing enzyme (phase I and II) activities.

2.4.1. Total cytochrome P450 (CYP 450) content

Total CYP 450 content in microsomes was determined by carbon monooxide (CO)-difference spectrophotometry of dithionite-reduced samples, using a molar extinction coefficient of 91 cm^{-1} mM^{-1} as method described by Omura and Satto method (1962). Results were expressed in *n* moles/mg microsomal protein.

2.4.2. NADPH cyt c reductase assay

The assay of NADPH cyt c reductase was carried out by the method as described previously by Phillip and Langdon (1962) with some modifications. 0.50 mM horse heart cyt c (prepared in 10 mM potassium phosphate buffer) and 0.30 mM potassium phosphate buffer were mixed with microsomal suspension and NADPH was added to initiate reaction. Then rate of reaction was

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