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Original article

The effects of metronidazole on Cytochrome P450 Activity and Expression in rats after acute exposure to high altitude of 4300 m

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ABSTRACT

Background: The purpose is to observe the changes of CYP450 enzyme activity expression as well as the physiological and pathological states of Wistar rats given metronidazole drug intervention after acute exposure to high altitude of 4300 m.
Methods and results: Thirty healthy adult male Wistar rats of average weight 200 ± 20 g were randomly assigned into three groups of 10 rats per group as follows: Group A (55 m), Group B (4300 m) and Group C (metronidazole intervention, 4300 m). After three days, the main blood gas levels were detected and the liver tissue pathological slices were observed. The enzymatic activity of CYP1A2 and 3A1 as well as the total protein, total CYP450, cytochrome b5 and the protein expression of CYP450 isoforms CYP1A2 and 3A1 were detected. Compared with Group A and Group B, the expression of total CYP450 and CYP1A2 was significantly reduced ($P < 0.01$or $P < 0.05$), the enzymatic activity of CYP1A2 and CYP3A1 were significantly reduced ($P < 0.05$, $P < 0.01$), respectively and CYP3A1 had no significant changes ($P > 0.05$). Compared with Group B and Group C the enzymatic activity of CYP1A2 had no significant changes ($P > 0.050$), the enzymatic activity of CYP3A1 was significantly reduced ($P < 0.01$). The total cytochrome p450, CYP1A2 and cytochrome b_5 were significantly reduced ($P < 0.01$ and $P < 0.05$), respectively and CYP3A1 had no significant changes ($P > 0.05$). Pathological observation showed that rats in Group A were normal with no significant pathological changes in their livers; rats in Group B suffered from liver injuries and edema with a few of them caught inflammatory cell infiltration; rats in Group C caught liver cell edema, inflammatory diseases and lobular vena cava expansion.
Conclusions: Acute exposure to high altitude CYP450 isoform expression and the enzymatic activity were significantly reduced, both the physiology and pathology of rats were substantially impaired, and which maybe resulted from the hypoxia and drug intervention interrelated effected in plateau filed test.
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1. Introduction

Lack of oxygen in the high altitude environment with hypobaric hypoxia can lead to a series of compensatory changes in the organizational structure and morphology of the organism as well as the physiological and biochemical indicators. Hypoxia is the main factor that affects life activities in high altitude. Low partial pressure of oxygen decreases the oxygen in trachea and alveolar, which leads to hypoxia in tissues and organs of the organism [10]. Drug metabolism by cytochrome P450 enzymes is an oxygen-dependent process. Interindividual differences in P450 isozyme activity are caused by genetic polymorphism as well as by specific xenobiotic induction or inhibition [6]. The activities of CYP enzymes could be altered by many drugs, which causes drug-drug interactions [22]. It has been reported that acute hypoxia down-regulates multiple CYP isoforms, such as CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2E1 and CYP2J2, although it up-regulates CYP3A6 [9,8,25,26]. The studies conducted earlier have provided the basis for the conclusion that drug metabolism and pharmacokinetics are modified under hypoxia [2,1,25,26,11].

Metronidazole is a substituted imidazole antibiotic that is widely used to treat anaerobic bacterial infection caused by Helicobacter pylori, and protozoal infections. Metronidazole is reported to have several clinically significant drug interactions due to the inhibition of oxidative drug metabolism, and these reports have described elevated concentrations of CYP3A4 substrates (e.g.

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Tacrolimus, carbamazepine, and quinidine) during metronidazole treatment. Data on the effects of metronidazole on specific pathways of drug metabolism are limited, and several of the reported drug interactions are based on single case reports [33,5,14]. One potential explanation to resolve the apparent discrepancy between the controlled studies and case reports regarding the effect of metronidazole on metabolism of CYP3A substrates is that the case reports all included patients who were acutely ill. It is increasingly well appreciated that acute illness and the associated acute inflammatory response can result in a downregulation of drug metabolism by CYP enzymes; this is often referred to as a drug-cytokine interaction [35,17]. Based on the above evidence, several groups of investigators reported that metronidazole is not an inhibitor of CYP3A4 [39,12,37]. But, drug-interaction screening software programs routinely warn of drug interactions between metronidazole and CYP3A substrates [34]. Inflammation is the defense reaction of stress, and high altitude can lead to large outbreak of cytokines such as IFN-γ, IL-1β, IL-2, IL-4, IL-5 and IL-6 [30] and generate erythropoietin [19]. These cytokines may lead to down-regulation of CYP enzymes in drug metabolism. Earlier studies of our team have shown that the different parameters of drug metabolism in rats at different altitude, we noted that the elimination rates of propranolol and metoprolol were lowered in high altitude compared with that in plain areas [41,42]. So we will investigate the effects of metronidazole on CYP1A2 and CYP3A1 activity and expressions in rats under hypoxic conditions in this study.

CYP1A2 is another important metabolizing enzyme in the liver, comprising approximately 13% of all CYP protein. There are over 100 substrates reported for CYP1A2 including many clinically important drugs, procarcinogens, and endogenous substrates. In addition, Cyb5 donates electrons into the cytochrome P450 system, as well as causing allosteric modification of P450 isozymes, resulting in either increased or reduced drug metabolism [13]. The active changes are closely related to drug metabolism, so the effect of acute exposure to high altitude on CYP1A2 and CYb5′s expression were investigated in our study, respectively.

Till date, there is little information about the effects of metronidazole on protein binding of hypoxia and activity of CYP450. On the basis of these considerations, the current study was carried out to elucidate the effects of the activity and expression of CYP450, and changes of pathological and physiological indicators in rats under hypoxia with the metronidazole intervention. We designed our experiment to compare the control of 55 m with the hypoxia of 4300 m to research whether hypoxia affected the CYP450, and compare the hypoxia with the hypoxia plus metronidazole to find how metronidazole affected the CYP450 after acute exposure to high altitude of 4300 m. This study may provide a basis and new ideas for clinical pharmacy at high altitude, for improving clinical medication and avoiding reactions.

2. Materials and methods

2.1. Chemical and reagents

Metronidazole powder was purchased from JinHuaHuiXing Pharmaceutical Co., Ltd (Shanxi, China, Certification number 120708). Anti rat CYP3A1 antibody was acquired from Abcam (Abcam, MA, USA), Anti rat CYP1A2 antibody and anti rat β-actin antibody was acquired from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Secondary antibodies were obtained from ZSGB-BIO (Beijing China). The P450-GloTMCYP1A2 (Luciferin-1A2) and P450-GloTMCYP3A4 Assay (Luciferin-IPA) kits as well as NADPH were obtained from Promega (Madison, WI, USA). Rat IL-1β, IL-2, IL-6 and IFN-γ ELISA kits were acquired from Boster Biological Technology., LTD (Wuhan, China). All other chemicals were of the highest quality available from commercial sources.

2.2. Animals and experimental design

Healthy adult Wistar rats aged 7–8 weeks, weighing 200 ± 20 g, were obtained from the ShangHai SLAC Laboratory Animal CO.LTD (certification number was 2007000524909), and were randomly assigned into three groups according to their weight, respectively. Group A (plain area, 55 m above sea level), Group B (high altitude, 4300 m above sea level) and Group C (high altitude, metronidazole intervention, 4300 m above sea level) and with each group containing 10 animals.

Group A animals were housed in a cage at 23–25 °C and allowed free access to regular rodent diet and water. After the 1-week acclimatization period, the rats were used for experiments and all efforts were made to minimize any animal suffering. Groups B and C were transported by airplane to Lanzhou (1500 m above sea level). The next day 8:00 a.m. the animals were transported to Maduo County, in Northwest China's Qinghai Province, which has an altitude of 4300 m. Transport by land used with the air conditioning truck, all the animals were fed Teklad Certified Rodent Diet and jelly for water replacement in the process of air transport, while clean tap water was given in the process of van transport. They arrived at 20:00 p.m.and housed into house cages at 15–20 °C and allowed free access to regular rodent diet and water. After 24 h acclimatization period, group C were administered with single dose of metronidazole (0.006 g/kg body weight, p.o.). The group C was exposed to an altitude of 4300 m for a duration of 3 days' continuous intervention with 0.006 g/kg body weight, p.o. of metronidazole everyday.

2.3. Blood gas analysis

1 ml sample of blood was taken from the abdominal aorta using an Arterial Blood Collection Syringe following anesthetization by the injection of 1 ml 10% chloral hydrate into the peritoneal cavity. Blood gas analysis was carried out immediately with automatic blood gas system (RADIOMETER ABL80) [21]. The indicators measured were pH, red blood cell specific volume (Hct), hemoglobin (ctHb) level, buffer base (BB), base excess (BE), actual bicarbonate (AB), standard bicarbonate (SB), anion gap (AG), content of total carbon dioxide ($ctCO_2$), oxygen saturation of arterial blood (sO_2), carbon dioxide tension of arterial blood (pCO_2), oxygen tension of arterial blood (pO_2), sodium ion concentration (cNa^+), potassium ion concentration (cK^+) calcium ion concentration (cCa^{2+}) and chloride ion concentration (cCl^-).

2.4. Preparation of rat hepatic microsomes [4]

The rats were fasted overnight and the abdominal cavity was opened, and then sheared abdominal aorta for the purpose of expelling the blood before removal of the liver. The liver was excised, rinsed with ice-cold saline (0.9% NaCl w/v), weighed and preserved in liquid nitrogen, and brought back to laboratory in lanzhou (1500 m above sea level). The liver microsomes were prepared by differential centrifugation. Liver samples were thawed and weighed, chose the perivenous zone and homogenized in 2 vol of ice-cold 0.15 mol/L KCL-0.2 mol/L sucrose solution. The homogenate was centrifuged at 12,000*g* at 4 °C for 30 min. The supernatant was then centrifuged at 105,000*g* at 4 °C for 60 min. The pellets were reconstituted with 0.05 mol/L Tris-HCl buffer at pH 7.5 containing 0.25 mol/L sucrose and stored at −80 °C until use. The protein concentration of the liver microsomes was determined by a protein quantitative assay using bicinchoninic acid.

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