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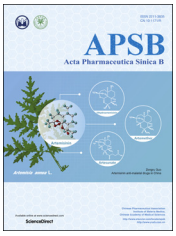

ORIGINAL ARTICLE

# Intracellular pharmacokinetic study of zidovudine and its phosphorylated metabolites

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**Abstract** Zidovudine (AZT), the first drug approved by the US Food and Drug Administration for the treatment of human immunodeficiency virus (HIV) infection, is metabolized in the host cells to 5′-AZT triphosphate (AZT-TP) which inhibits HIV reverse transcriptase. As the pharmacokinetics of AZT and its phosphorylated metabolites in human peripheral blood mononuclear cells (hPBMCs) is limited, the aim of this study was to determine the pharmacokinetic parameters of AZT and its phosphorylated metabolites in hPBMCs from 12 healthy Chinese male subjects after a single oral dose of 600 mg of AZT. Blood samples were collected prior to drug administration, then at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8 and 10 h after drug administration. Mononuclear cells collected by Ficoll-Hypaque density gradient centrifugation were used for determination of AZT and metabolites [AZT monophosphate (AZT-MP), AZT diphosphate (AZT-DP) and AZT-TP] and the plasma was used to evaluate the pharmacokinetics of AZT. Plasma concentration of AZT peaked within 0.583 h and intracellular concentrations of AZT, AZT-MP, AZT-DP and AZT-TP peaked within 1.083, 1.500, 1.417 and 1.583 h, respectively. AZT in plasma was eliminated rapidly with $t_{1/2}$ of 2.022 h, and AZT-MP, AZT-DP and AZT-TP were eliminated with $t_{1/2}$ of 13.428, 8.285 and 4.240 h, respectively. The plasma concentration of the phosphorylated metabolites was not quantifiable.



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## 1. Introduction

Nucleoside reverse transcriptase inhibitors (NRTIs) were the first agents shown to be safe and effective for the treatment of patients infected with HIV, and they remain cornerstones of most treatment regimens today. These drugs are selectively phosphorylated to their active triphosphate moieties (NRTI-TP) in human peripheral blood mononuclear cells (hPBMCs)[1]. Zidovudine (3'-azido-3'-deoxythymidine, AZT) was the first drug for the treatment of human immunodeficiency virus (HIV) infection approved by the US Food and Drug Administration (FDA). The parent compound AZT must be metabolized in the host cells to 5'-AZT triphosphate (AZT-TP), which acts as a competitive inhibitor of HIV reverse transcriptase or is incorporated into the viral genome, terminating DNA chain elongation[2,3]. It remains an important NRTI component of many first-line highly active antiretroviral therapies (HAART) used today[4].

AZT is converted inside the cell into AZT monophosphate (AZT-MP) by thymidine kinase. Thymidylate kinase in turn converts AZT-MP into AZT diphosphate (AZT-DP), which is further phosphorylated to AZT-TP, presumably by pyrimidine nucleoside diphosphate kinase. AZT monophosphate is the predominant compound in human cells exposed to AZT, suggesting that thymidylate kinase is the rate limiting enzyme in the synthesis of active AZT-TP[2,5]. Most measurements of AZT are performed in plasma or serum, but because AZT is phosphorylated to its active form inside the cells, measurements of plasma levels of AZT may not directly correlate with antiviral activity or toxicity. Therefore, it is important to determine the intracellular levels of AZT and its metabolites.

Recently, several studies have shown that the intracellular concentration of the triphosphate moieties of NRTIs correlated better with the virologic response than did the levels of the parent compound in plasma[6,7]. Long-term use of high-dosage AZT caused damage to many tissues, including a mitochondrial skeletal muscle myopathy, a dilated cardiomyopathy and hepatotoxicity associated with mitochondrial DNA depletion[8]. These conditions are related to AZT use and not to the progression of acquired immunodeficiency syndrome (AIDS) since when patients experiencing one or more of these adverse effects discontinued AZT therapy, the adverse effects would resolve[9,10]. In modern therapeutic regimens, AZT is given at much lower doses and in combination with other drugs. Thus, these adverse effects have become fairly rare, but hematological toxicities, such as anemia and lipodystrophy are observed commonly in combination therapy[11-13].

Detection and quantitation of the NRTI-TP concentrations in hPBMCs will lead to a better understanding of the pharmacokinetic and pharmacodynamic characteristics of these agents, which will optimize treatment with AZT or combination therapy. The measurement of AZT-TP remains difficult because of the interference from endogenous nucleoside triphosphates. In this study, the pharmacokinetics of AZT and its phosphorylated metabolites was determined in hPBMCs.

## 2. Materials and methods

### 2.1. Materials and subjects

Zidovudine tablets (300 mg) were purchased from Northeast Pharmaceutical Group Shenyang No. 1 Pharmaceutical Co., Ltd. (Shenyang, China). Reference material of Zidovudine was purchased from Yidu HEC Pharma Co., Ltd. (Wuhan, China). Reference material of Zidovudine phosphate compounds was purchased from Moravek Biochemicals (CA, USA). All other reagents were commercially available and of analytical grade.

**Table 1** Baseline demographic characteristics of volunteers.

| Parameter | Value[a] | Range |
|---|---|---|
| Age (year) | 23.1 ± 1.5 | 20–28 |
| Height (m) | 1.7 ± 0.1 | 1.60–1.84 |
| Body weight (kg) | 64.8 ± 3.0 | 49–78 |

[a]Each value represents the Mean ± SD, $n = 12$.

Twelve healthy Chinese male subjects were recruited and assessed for inclusion in the study. The subjects were underwent a standardized screening procedure to confirm their eligibility. The demographic characteristics of the study group are given in Table 1. All subjects gave their written informed consent to participate in the study after they had been well informed about the study objectives, method and possible risks. After a physical examination, subjects with any abnormality of the cardiovascular, respiratory, abdominal or central nervous system were excluded. Blood pressure (BP) and heart rate (HR) were measured. Additionally, an electrocardiogram was conducted and a general examination of the subject was performed to exclude any illness or abnormality.

### 2.2. Study design

This study was carried out in accordance with the International Conference on Harmonization (ICH) guidance on general considerations for clinical trials[14]. The dose in this study was selected based on the daily dosage regimen of AZT (600 mg) according to the terms of safety and therapeutic efficacy. The pharmacokinetics of AZT was determined following the administration of a single oral dose of 600 mg of AZT. An open-label design was used to evaluate the pharmacokinetics of AZT in the selected subjects after an overnight fast of at least 12 h. They received a single oral dose of 600 mg AZT followed by 240 mL of water. They received standardized meals 4 h and 10 h after drug administration.

### 2.3. Sample collection

For the pharmacokinetic assessments, blood samples (20 mL) were collected from an indwelling intravenous catheter inserted into the antecubital vein of the forearm of each subject prior to drug administration (blank), then at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8 and 10 h after drug administration in heparinized tubes. The blood samples were centrifuged and the plasma was harvested and transferred to polystyrene microcentrifuge tubes and stored at –20 °C until assay.

The remaining blood cells without plasma are used for the separation of mononuclear cells by the Ficoll-Hypaque density-gradient centrifugation method. First, the blood cells were diluted (1:1 blood cells/diluted solution). Second, a volume of 3–4 mL Ficoll-Hypaque solution was added into 15-mL conical centrifuge tubes using a sterile pipet, then the diluted blood cells (6–8 mL) were slowly layered by placing the tip of the pipet containing the diluted blood cells 0.7–1.5 cm above the level of Ficoll-Hypaque