Erythrocytes as Carriers of Indinavir: Preparation, Characterization, In vitro and In vivo Pharmacokinetic Evaluation in Rats

D. V. R. N. Bhikshapathi1*, A. Shiva Krishna1, M. Ramesh2, V. V. Rajesham1, G. Suresh1 and S. Jyothi Sri3

1CMR College Pharmacy, Kandlakoya, Medchal road, Hyderabad-501401, Telangana state, India, 2Jubilant Biosys Limited, Yeshwanthpur, Bengaluru-560022, Karnataka state, India, and 3MLR College of Pharmacy, Dundigal, Hyderabad-500043, Telangana state, India.

Received November 09, 2016; accepted December 12, 2016

ABSTRACT

Indinavir is a protease inhibitor of the human immuno-deficiency virus. Indinavir is commercially available as capsule of 200 mg and 400 mg. Adult dose is 800 mg every 8 h, i.e., 2400 mg per day is equivalent to 6 capsules per day. No other dosage form is available in the market. Sustained release dosage form of indinavir can produce maximum therapeutic effect with minimum side effects and achieve better patient compliance. Various carriers have been used for the drug targeting among which cellular carriers such as erythrocytes offer greater potential advantages than other system. The drug is never free in circulation thus reducing toxicity and the drug half-life in circulation increases thus kinetic patterns. Antiretroviral-loaded erythrocytes offer a promising therapy against HIV owing to their potential to deliver this kind of drugs to macrophages and reticulo-endothelial (RES) tissues. The aim of the present investigation was to develop and optimize antiretroviral indinavir encapsulated in rat erythrocytes. In this study, the encapsulation of indinavir by rat erythrocytes prepared and compared with indinavir dissolved in normal saline. The prepared formulations were administered to rats by intravenous route and plasma samples was analysed by LC-MS/MS technique. The pharmacokinetic parameters were calculated using WinNonlin software. The prepared indinavir loaded erythrocytes showed enhanced bioavailability in equal dose due to higher extent of absorption owing to its retention in erythrocytes and releasing the drug slowly. Indinavir demonstrated a sustained release from loaded erythrocytes over a period of 36 h, which suggests a potential use of the erythrocyte as a slow systemic release system for antiretroviral drugs.

KEYWORDS: Indinavir; erythrocytes; LC-MS/MS; Reticulo-endothelium system; HIV.

Introduction

Erythrocytes are known as red blood cells and have been extensively studied for their potential capabilities as drug carriers for drugs and drug loaded microspheres. Such drug loaded erythrocytes were prepared simply by collecting the blood from the organism of interest, separating erythrocytes from the plasma, entrapping the drug in the erythrocytes and rescaling the resultant cellular carriers (Green and Widder 1987; Ropars et al., 1987; Lewis and Alpar 1984). Hence, these carriers are called resealed erythrocytes. The overall process is based on the response of these cells under osmotic conditions. Upon re-injection, the drug loaded erythrocytes serve as slow circulating depots and target the drug to the drugs to reticulo endothelial system (RES) (Ropars et al., 1987; Lewis and Alpar 1984; Zimmermann 1983; Jain and Jain 1997).

Erythrocytes are the most abundant cells in the human body (approximately 5.4 million cells/mm³ blood in a healthy male and approximately 4.8 million cells/mm³ blood in a healthy female). These cells were described in human blood samples by Dutch scientist Lee Van Hock in 1674. Hope Seyer identified haemoglobin and its crucial role in oxygen delivery to various parts of the body (Telen and Lee 1993). Erythrocytes are biconcave discs with an average diameter of 7.8 µm, a thickness of 2.5 µm in periphery, 1 µm in the centre, and a volume of 85-91 µm³ (Guyton and Hall 1996). They lack a nucleus and other organelles. Their plasma membrane encloses haemoglobin a heme-containing protein that is responsible for CO₂ and O₂ binding inside the erythrocytes. The main role of erythrocytes is the transport of O₂ from the lungs to tissues and the CO₂ produced in the tissues back to lungs. Because a nucleus is absent, all the intracellular space is available for O₂ transport. Also because mitochondria are absent and because energy is generated anaerobically in erythrocytes, these cells do not consume oxygen they are carrying. Erythrocytes live only about 120 days because of wear and tear on their plasma membranes as they squeeze through the narrow blood capillaries. Worn-out erythrocytes are removed from the circulation and destroyed in the spleen and liver (RES), and the breakdown products are recycled (Torotra and Grabowski...