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ORIGINAL ARTICLE

Engineering erythrocytes as a novel carrier for the targeted delivery of the anticancer drug paclitaxel

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Abstract Paclitaxel (PTX) is formulated in a mixture of Cremophor EL and dehydrated alcohol. The intravenous administration of this formula is associated with a risk of infection and hypersensitivity reactions. The presence of Cremophor EL as a pharmaceutical vehicle contributes to these effects. Therefore, in this study, we used human erythrocytes, instead of Cremophor, as a pharmaceutical vehicle. PTX was loaded into erythrocytes using the preswelling method. Analysis of the obtained data indicates that 148.8 μg of PTX was loaded/mL erythrocytes, with an entrapment efficiency of 46.36% and a cell recovery of 75.94%. Furthermore, we observed a significant increase in the mean cell volume values of the erythrocytes, whereas both the mean cell hemoglobin and the mean cell hemoglobin concentration decreased following the loading of PTX. The turbulence fragility index values for unloaded, sham-loaded and PTX-loaded erythrocytes were 3, 2, and 1 h, respectively. Additionally, the erythrocyte glutathione level decreased after PTX loading, whereas lipid peroxidation and protein oxidation increased. The release of PTX from loaded erythrocytes followed first-order kinetics, and about 81% of the loaded drug was released into the plasma after 48 h. The results of the present study revealed that PTX was loaded successfully into human erythrocytes with acceptable loading parameters and with some oxidative modification to the erythrocytes.

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1. Introduction

Paclitaxel (PTX) is an anticancer drug that is used against human solid tumors (e.g., for advanced breast, ovarian and non-small-cell lung cancers) either alone or in combination with other treatments. PTX stabilizes microtubule assembly

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through non-covalent interactions with the cytoskeleton, thereby blocking cell division (Rowinsky et al., 1993).

PTX has a high molecular weight (MW; 854 Da) and a very low aqueous solubility (Nicolaou et al., 1994). Moreover, PTX does not contain any functional groups that can be ionized by pH changes or that allow salt formation to increase its solubility. Therefore, the development of PTX formulations has been challenging (Singla et al., 2002).

PTX has a low level of oral bioavailability owing to its poor solubility, to the effects of intestinal and liver cytochrome P450-metabolizing enzymes and to the effects of phosphorylated glycoprotein (PgP). Several studies have shown that the oral bioavailability of PTX can be greatly improved when the drug is administered in combination with PgP inhibitors (Woo et al., 2003). PgP inhibitors improve the oral bioavailability of PTX by enhancing PTX absorption and decreasing its elimination (Van Asperen et al., 1998). However, the use of PgP inhibitors is limited in humans because of the risk of adverse cardiac and immunosuppressive effects (Woo et al., 2003).

Therefore, lipid-based formulations, such as self-microemulsifying drug delivery systems (SMEDDS), were developed to increase PTX solubilization and absorption (Gao et al., 2003). PTX SMEDDS formulations have greater bioavailability than orally administered PTX formulations (Yang et al., 2004). Furthermore, lipid nanocapsules were developed to allow the solubilization of PTX, increase its absorption (Heurtault et al., 2002), inhibit PgP and reverse multidrug resistance mechanisms (Coon et al., 1991).

PTX for intravenous infusion is formulated in a 1:1 v/v mixture of Cremophor EL and dehydrated alcohol. The intravenous (IV) administration of this formula is associated with a risk of catheter-related infection and hypersensitivity reactions. It is well established that the use of Cremophor EL as a pharmaceutical vehicle contributes to these effects (Van Zuylen et al., 2001). Thus, much research is being carried out to identify alternative intravenous formulations that do not use Cremophor EL (Wissing et al., 2004).

The available alternative delivery systems utilize multi-component structures such as cells (Hamidi et al., 2007a). Erythrocytes represent one of the most promising biological drug delivery systems (Millan, 2004). Erythrocytes are biodegradable and biocompatible, and they are able to circulate throughout the body. In addition, their degradation products are reusable (Pierigè et al., 2008). According to the preferred therapeutic approach, erythrocytes are used either as carriers for the sustained release of the drugs or to target the drugs to specific organs (Hamidi et al., 2007b). The maintenance of the normal oxidant/antioxidant balance in erythrocytes during drug encapsulation may help to produce loaded cells with characteristics similar to those of normal erythrocytes (Alanazi, 2010). In this case, such drug-loaded cells can be used as slow-release carriers for the entrapped drugs (Hamidi et al., 2007b). In contrast, the modification of loaded erythrocytes results in their accelerated removal and targeting to the reticuloendothelial system (RES) (Alanazi et al., 2011).

Osmotic stress can alter erythrocyte morphology and thereby accelerate their removal from the circulation by the RES (Minetti et al., 2007). The major difficulty associated with the use of erythrocytes as extended drug carriers thus involves their uptake *in vivo* by the RES Hamidi et al., 2007a). This accelerated uptake may be attributed to the oxidation of lipids and proteins in the erythrocyte membrane (Zwaal and Schroit, 1997). An increase in protein oxidation is a feature of erythrocyte aging (Robaszkiewicz et al., 2008). The reported side effects of PTX include anemia, which may result from the decreased formation of new erythrocytes or from the accelerated clearance of circulating erythrocytes (Lang et al., 2006). Accelerated clearance, in turn, may be the result of stress-induced eryptosis, which is characterized by cellular shrinkage, phosphatidylserine externalization and cellular protease activation (Lang et al., 2006).

Exposure of erythrocytes to a hypotonic solution creates pores in the erythrocyte membrane, allowing drugs to pass through the pores and become permanently entrapped after the cells have been resealed with a specific isotonic buffer solution. Hypotonic dilution has been widely studied as a technique for the drug loading of erythrocytes. This method has previously been used for the entrapment of anticancer drugs (Mishra and Jain, 2002). The loading of anticancer drugs into erythrocytes may increase the uptake of the drug by cancer cells (Gaudreault et al., 1989).

Many approaches have been proposed to improve the therapeutic effects of paclitaxel and to reduce its side effects, including the use of micellar carriers, soluble polymers, PTX-soluble prodrugs, and polymeric nanocapsules (Zhao et al., 2010).

The objective of this study was to utilize human erythrocytes as a pharmaceutical vehicle for PTX delivery. PTX was loaded into erythrocytes by the preswelling method. Additionally, the effects of PTX on oxidative status, osmotic fragility and hematological indices were determined.

2. Materials and methods

2.1. Materials

Paclitaxel was obtained from David Bull Laboratories, Victoria, Australia. Hydrocortisone acetate was obtained from Fluka AG, Buchs, Switzerland. Methanol and acetonitrile (AnalaR® with 99.8% purity) were purchased from BDH, Pool, England. The water used in this study was obtained from a Milli-Q water purification system (Millipore, Bedford, MA).

Reduced glutathione (GSH), oxidized glutathione (GSSG), and thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO). Guanidine hydrochloride was obtained from Winlab (UK). All other chemicals used were of high analytical grade.

A stock solution of PTX was prepared by dissolving the drug in methanol containing 0.1% acetic acid. This solution was protected from light and stored at −20 °C prior to use. Autologous plasma was used for drug dilution as required.

VWR vortex mixer was obtained from Scientific Industries Inc. (Bohemia, NY). The Spectro UV–Vis Split Beam PC, model UVS-2800 was obtained from Labomed, Inc. (Culver City, CA).

2.2. Methods

2.2.1. Erythrocyte isolation and PTX loading

Blood samples from apparently healthy volunteers were collected in heparinized tubes. Informed consent was obtained from all volunteers. The plasma and buffy layer were detached