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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

by aspiration. The erythrocytes were then washed three times in cold isotonic phosphate-buffered saline (PBS) with centrifugation for 5 min at 5000 rpm (MIKRO20 centrifuge, Hettich, Germany). The isolated erythrocytes were collected until used for the PTX loading (Alanazi et al., 2011).

2.2.2. Entrapment of PTX into erythrocytes

A hypotonic preswelling method was used for the loading of human erythrocytes with PTX. For this purpose, 1 volume of washed packed erythrocytes was transferred gently to a test tube, and then 4 volumes of hypotonic 0.6% NaCl were added. This mixture was incubated at 0 °C for 5 min and then centrifuged; then, 1 volume of plasma containing the required PTX concentration was added to 1 volume of preswelled cells and incubated for 10 min at 0 °C. Finally, the erythrocytes were resealed by the addition of 0.06 volumes of KCl (1.5 M) and incubated at 37 °C for 30 min (Humphreys et al., 1981). Sham-encapsulated erythrocytes were prepared as described but the PTX solution replaced by distilled water. Loaded amount, the total amount of PTX encapsulated in the final packed erythrocytes. Efficiency of entrapment, the percentage ratio of the loaded amount of PTX to the amount added during the entire loading process. Cell recovery, the percentage ratio of the hematocrit value of the final loaded cells to that of the initial packed cells, measured on equal volumes of two suspensions (Hamidi et al., 2007b).

2.2.3. PTX analysis

PTX was extracted from the plasma samples by the addition of an equal volume of acetonitrile, and the mixture was centrifuged for 15 min at 13,000 rpm. The supernatant was used for PTX quantitation. The assay was performed using a μ Bondapak-C18 column (150 mm × 4.6 mm i.d.) with a mobile phase consisting of acetonitrile and 20 mM phosphate buffer (pH 5) (50:50 v/v), a flow rate of 1 ml/minute, and UV detection at 229 nm. The chromatography data were analyzed with the Empower™ Program (Waters, USA). Hydrocortisone acetate was used as an internal standard. The detection limit of PTX was in the range of 0.1–40 μg/ml (Mowafy et al., 2012).

2.2.4. In vitro characterization of PTX-loaded erythrocytes

A series of tests were carried out to characterize the nanoparticle-loaded erythrocytes in comparison with the unloaded, sham-encapsulated, and the free drug loaded ones.

2.2.5. Hematological indices

Control erythrocytes, sham-loaded erythrocytes, and PTX-loaded erythrocytes were hematologically characterized. The mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), the mean corpuscular hemoglobin concentration (MCHC) and the hematocrit (Hct) were measured using a hematology analyzer. To estimate the morphological variation between normal and PTX-loaded erythrocytes, both normal and PTX-loaded erythrocyte samples were examined using a scanning electron microscope (Hamidi et al., 2007b).

2.2.6. In vitro release of hemoglobin and PTX from carrier erythrocytes

The in vitro release of both hemoglobin and PTX from carrier erythrocytes was evaluated as follows: packed PTX-loaded erythrocytes were diluted 1:10 mL using autologous plasma and then mixed by several gentle inversions. The mixture was aliquoted into Eppendorf tubes. The samples were incubated at 37 °C and rotated vertically. Samples were removed at 0.5, 1, 2, 4, 8, 12, 24 and 48 h and then centrifuged at 3000 rpm for 5 min. One hundred microliters of the supernatant was separated for PTX assays, and the remaining portion was centrifuged for 5 min. The supernatant was used for hemoglobin analysis by measuring the absorbance at 540 nm. The results are expressed as percentages of the absorbance of a completely hemolyzed sample (Hamidi et al., 2007b).

2.2.7. Osmotic fragility

The osmotic fragility test was used to assess the ability of erythrocyte membranes to resist lysis caused by exposure to solutions of NaCl ranging from 0.0 to 0.9 g%. A 25 μL erythrocyte sample was added to each of a series of 2.5 mL saline solutions containing 0.0–0.9 g% NaCl. After gentle mixing and standing for 15 min at room temperature, the erythrocyte suspensions were centrifuged at 5000 rpm for 5 min. The released hemoglobin was expressed as percentage absorbance of each sample in reference to a completely lysed sample prepared by diluting packed cells of each type with 1.5 mL of distilled water (Kraus et al., 1997).

2.2.8. Turbulence fragility

Aliquots of 0.5 mL of packed erythrocytes of each of the three types were suspended in 10 ml of PBS in polypropylene test tubes and shaken vigorously using a multiple test tube orbital shaker at 2000 rpm for 4 h. To evaluate the time course of hemoglobin release, 0.5 ml portions of each suspension were withdrawn at 0, 1, 2, 3, 4 and 5 h. The samples were centrifuged at 1000*g* for 10 min, and the absorbance of each supernatant was determined spectrophotometrically at 540 nm. The percent hemoglobin release was determined relative to that of a completely lysed suspension with the same cell fraction (i.e., 0.5 ml packed cells added to 10 ml of distilled water). To compare the turbulence fragilities of the different types of erythrocytes, the turbulence fragility index (TFI) was used. This value is calculated as the shaking time required to produce 20% hemoglobin release from erythrocytes (Hamidi et al., 2007b).

2.2.9. The effects of PTX on erythrocyte oxidative markers

2.2.9.1. Effect of PTX on glutathione. The effect of PTX on glutathione (GSH) content was estimated according to the method of Ellman, 1959. Briefly, 0.1 mL of 25% TCA was added to 0.5 mL of lysate to precipitate the protein, and the samples were centrifuged to obtain the supernatant. Then, 0.1 mL of supernatant was incubated with 2.0 mL of freshly prepared 0.6 mmol/L 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB). The absorbance of the resulting yellow complex was measured at 412 nm against a blank to which the hemolysate had not been added. A standard curve for GSH was obtained and used to calculate the GSH content in the samples.

2.2.9.2. Effect of PTX on erythrocyte lipid peroxidation. The effect of PTX on erythrocyte lipid peroxidation was demonstrated by the spectrophotometric measurement of malondialdehyde (MDA). A mixture of 200 μL of 8% sodium dodecyl sulfate, 200 μL of 0.9% thiobarbituric acid, and 1.5 mL of 20% acetic acid was added to 200 μL erythrocyte

lysate samples; then 1.9 mL of distilled water brought the volume to 4 mL. After boiling for 1 h, the mixture was cooled and 5 mL of an *n*-butanol and pyridine (15:1) solution was added. The mixture was centrifuged at 5000 rpm for 15 min and the absorbance was measured at 532 nm. Quantification of MDA levels was performed using tetraethoxypropane as a standard (Ohkawa et al., 1979).

2.2.9.3. Effect of PTX on erythrocyte protein oxidation. The effect of PTX on erythrocyte protein oxidation was determined by measuring protein carbonyl (PCO) formation as described by Levine et al. (1994). Erythrocytes were hemolysed, and proteins were precipitated by the addition of 10% TCA. The proteins were resuspended in 1.0 mL of 2 M HCl for the blank, and 2 M HCl containing 2% 2,4-dinitrophenyl hydrazine for test samples. After incubation for 1 h at 37 °C, protein samples were washed with alcohol and ethyl acetate, and reprecipitated by the addition of 10% TCA. The precipitated protein was dissolved in 6 M guanidine hydrochloride solution and absorbance was measured at 370 nm. Calculations were made using the molar extinction coefficient of $22 \times 10^3\ M^{-1}cm^{-1}$ and the results are expressed as nmol carbonyls formed per mg protein. The total protein in the erythrocyte pellets was measured according to the method of Lowry et al. (1951).

2.2.10. Statistical analysis

The results are expressed as the mean ± S.D. of each group. Data analysis was carried out by one-way ANOVA followed by the Tukey–Kramer's test for multiple comparisons. A 0.05 level of probability was used as the criterion for significance.

3. Results and discussion

Table 1 shows the loaded amount, the entrapment efficiency and the percent cell recovery. The HPLC method was used to estimate the PTX content of the supernatants after the incubation of erythrocytes with PTX. The obtained data indicate that 148.8 μg of PTX was loaded, with an entrapment efficiency of 46.36%. This amount is notable in comparison to those values reported in the literature for a variety of drugs (Shavi et al., 2010; Hamidi et al., 2011). The observed cell recovery of approximately 75.94% is comparable to the recovery results for various drugs reported in other studies (Magnani et al., 2002; Rossi et al., 2005).

Table 1 Loading parameter of PTX into human erythrocytes.

Parameters	Mean	S.D.
Loaded amount (μg)	148.8	10.1
Entrapment efficiency (%)	46.36	5.25
Cell recovery (%)	75.94	8.44

Data expressed as mean ± S.D. six sample /group.

The major hematological indices of the control, sham-loaded and PTX-loaded erythrocytes are shown in Table 2. These parameters, which are measured as part of routine clinical hematology tests, may provide some useful estimates of the biological state of the erythrocytes. The results of the present study showed that significant changes in erythrocyte volume were caused by the entrapment process in sham and PTX loading, as indicated by the MCV values. However, both the MCH and the MCHC decreased following the exposure of the erythrocytes to the loading procedure, in sham and PTX loaded erythrocytes. The overall loss of hemoglobin from the erythrocytes upon loading procedure was expected because the procedure is destructive in nature. In similar studies (Kravtzoff et al., 1990; Garin et al., 1996; Hamidi et al., 2001), all of these parameters were found to be lower in carrier erythrocytes than in normal unloaded cells. This finding is consistent with the results of Hamidi et al. (2001).

The osmotic fragility of the studied erythrocytes is shown in Fig. 1. This test is a marker of possible changes in the integrity of the cell membrane caused by the loading procedure. Moreover, the osmotic fragility test measures the resistance of these cells to changes in the osmotic pressure of the surrounding media. The entrapment of PTX in cells significantly increases the osmotic fragility of the cells, as shown in Fig. 1. Likewise, Hamidi et al., 2001 reported that the osmotic fragility curves changed from an S-shape in the case of control erythrocytes to nearly linear in the case of drug-loaded erythrocytes. This indicates that the PTX-loaded cell population is more heterogeneous in terms of cell membrane resistance to changes in the extracellular osmotic pressure than are normal unloaded erythrocytes. Several studies have demonstrated that the osmotic fragility of drug-loaded erythrocytes is greater than that of unloaded cells and that this change is accompanied by a change in the fragility curves from sigmoidal to somewhat linear (Hamidi et al., 2001; Garin et al., 1996; Jain and Jain, 1997). In contrast, a decrease in osmotic fragility has been reported in some cases, a result that has been explained by a reduction in the average cell volume upon loading. This reduction in cell volume leads to a decrease in the intracellular osmotic pressure (Kravtzoff et al., 1990).

The turbulence fragility test is used to exploit the mechanical strength of the erythrocyte membranes. In the present study, this test was mainly carried out by shaking the cell suspensions vigorously (Hamidi et al., 2001). The hemoglobin released was measured at different times. The results indicated that the turbulence fragility of the PTX-loaded erythrocytes

Table 2 Hematological parameters of control erythrocytes, sham erythrocytes and PTX loaded erythrocytes.

Parameters	Control erythrocytes	Sham- erythrocytes	PTX- loaded erythrocytes
MCV (fl)	84.5 ± 0.60	96.6 ± 1.30[a]	109 ± 1.50[a]
MCH (pg)	30.5 ± 0.48	25.6 ± 0.15[b]	22.3 ± 0.18[b]
MCHC (g/dl)	33.4 ± 0.14	23.7 ± 0.55[b]	21.4 ± 0.49[b]

Data expressed as mean ± S.D. six sample /group.
[a] Significant increase at $P < 0.01$.
[b] Significant increase at $P < 0.01$.

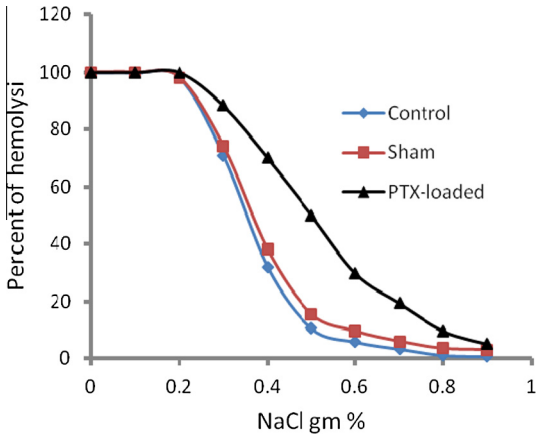

Figure 1 Hemolysis profile of unloaded erythrocytes, sham erythrocytes and erythrocytes loaded with PTX. Data represent the mean ± S.D. (n = 3).

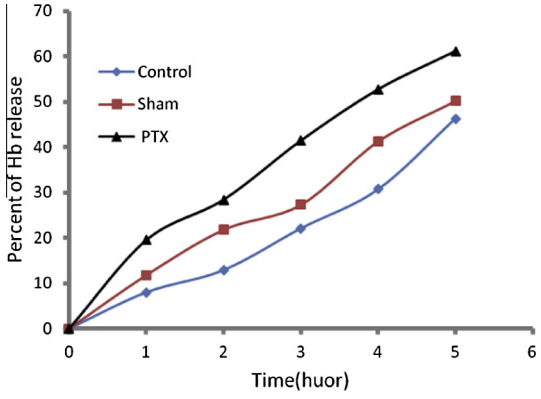

Figure 2 Turbulence shock test of control sham and PTX-loaded erythrocytes. Data represent the mean ± S.D. (n = 3).

was greater than that of the control and sham-loaded erythrocytes. As shown in Fig. 2, the turbulence fragility index values for unloaded, sham-loaded and PTX-loaded erythrocytes were 3, 2, and 1 h, respectively. Similarly, other studies have shown that the turbulence fragility of the erythrocytes as drug vehicles increases significantly relative to that of normal control cells (Talwar and Jain, 1992; Jain and Jain, 1997). These results indicate that the resistance of the erythrocytes to vigorous turbulent flow shows a decreasing trend from control cells to PTX-loaded erythrocytes. These results indicate that erythrocytes become more fragile during the loading process and that this fragility is enhanced by PTX encapsulation.

An increase in erythrocyte hemolysis destabilizes the heme structure in hemoglobin molecules, leading to a release of free iron ions that generate more free radicals. Moreover, the presence of PTX in the media surrounding the erythrocytes promotes the production of reactive oxygen species (ROS). Furthermore, several studies have reported that PTX stimulates ROS production. Varbiro et al., 2001 demonstrated that PTX mediates ROS production and Ramanathan et al., 2005 demonstrated that PTX increases the levels of superoxide, hydrogen peroxide and nitric oxide. Furthermore, antioxidants attenuate the anticancer activity of PTX *in vivo* and in vitro (Fukui et al., 2010). Accordingly, it is relevant to investigate the susceptibility of erythrocytes to PTX-induced oxidative damage. GSH is the main antioxidant in erythrocytes that protects proteins and lipids from oxidative damage. The oxidation of such molecules can result in the loss of cell membrane integrity (Jain, 1984). The present results showed that, relative to native erythrocytes, PTX-loaded cells contain a significantly less GSH (Table 3).

The high polyunsaturated fatty acid content of erythrocyte membranes renders them more sensitive to ROS attack (Chiu, 1989). In the present study, the observed increase in MDA level was attributed to the peroxidation of PUFAs. These findings are similar to those of Hadzic et al. (2010), who demonstrated that PTX enhances lipid peroxidation. The oxidative modification of proteins may be one of the factors responsible for the altered membrane asymmetry of oxidized erythrocytes (Dumaswala et al., 1997). Oxidative stress renders membrane-bound proteinases unable to remove oxidatively damaged proteins from the cell membrane (Beppu et al., 1994). In the present work, a significant increase in protein carbonyl (PCO) content was found in erythrocytes loaded with PTX. It has been demonstrated that PTX causes the accumulation of H_2O_2 and that this accumulation is a crucial step in PTX-induced cell death (Alexandre et al., 2007). Likewise, a previous report showed that the exposure of erythrocytes to ROS increases protein oxidation (Pandey et al., 2009). Dumaswala et al. (1997) reported that antioxidant membrane-bound proteinases are lost due to oxidative stress. Similar effects were reported under oxidation conditions by Bukowska et al. (2008).

It has also been reported that the increase in protein oxidation is due to the oxidation of thiol groups and their subsequent formation of disulfide bonds (Robaszkiewicz et al., 2008). Thus, the induction of oxidative stress by PTX in carrier erythrocytes by increasing ROS formation decreases the GSH content and consequently increases lipid and protein oxidation. The oxidative stress alters the asymmetry within the erythrocyte membrane, and the rapid elimination of oxidized

Table 3 GSH, PCO and MDA of control erythrocytes, sham erythrocytes and PTX loaded erythrocytes.

Parameters	Studied groups		
	Control	Sham-Erythrocytes	PTX-Loaded Erythrocytes
GSH	9.848 ± 2.66	8.690 ± 2.32	5.368 ± 1.43[a]
PCO	2.602 ± 1.02	3.947 ± 1.39	8.572 ± 3.22[b]
MDA	15.53 ± 3.84	20.44 ± 3.77	38.85 ± 10.24[b]

Data expressed as mean ± S.D. six sample /group.
[a] Significant decrease at $P < 0.01$.
[b] Significant increase at $P < 0.01$.

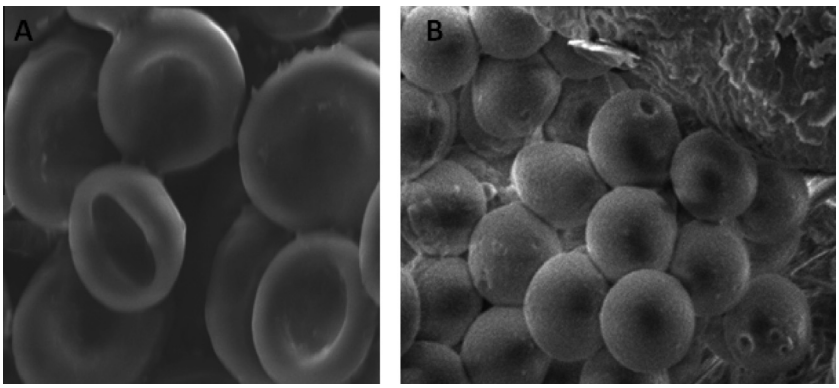

Figure 3 Scanning electron microscope graphing of (a) unloaded erythrocytes (b) erythrocytes loaded with PTX, Magnification X 5000. Note spherocytes formation with PTX loading.

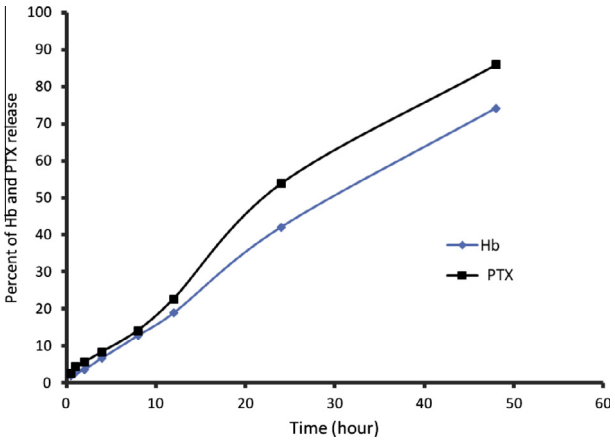

Figure 4 Percent of released hemoglobin(Hb) and PTX in plasma from loaded erythrocytes at 37 °C. Data represent the mean ± S.D. (n = 3).

erythrocytes from the circulation is accelerated by the alteration of this asymmetry (Jain, 1984). Another report stated that oxidatively modified erythrocytes are flagged for phagocytosis (Tyurina et al., 2000). Moreover; oxidative modification accelerates erythrocyte clearance from the circulation by macrophage (Mandal et al., 2002). This may accelerate the targeting of PTX loaded erythrocytes to RES.

The primary morphological change in PTX-loaded cells revealed by SEM was the transformation of loaded cells from biconcave to spherocyte shape, as illustrated in Fig. 3. The spherical shape of loaded cells makes them more fragile, and fragile cells are destroyed and rapidly cleared from the circulation by macrophages (Talwar and Jain, 1992).

Approximately 81% of the loaded PTX was released from the erythrocytes into the plasma within 48 h as observed in Fig. 4. The factors that determine drug release from carrier erythrocytes are size and the ionization of the drug molecule (Eichler et al., 1985). PTX release from the loaded erythrocytes is an important factor that affects the plasma concentration profile of this drug upon the re-injection of these carrier cells. The efflux of PTX from carrier cells followed zero-order kinetics during the entire experimental period. In addition, the release profile of PTX is remarkably consistent with that of hemoglobin. These findings are consistent with the results published by Hamidi and Tajerzadeh (2003) and Shavi et al. (2010).

Thus, carrier erythrocytes may be a good candidate for targeted delivery to the RES. In fact, it is possible to retain the PTX inside the carrier erythrocytes until the carriers are trapped in the RES. Therefore, controlling the life-span of carrier erythrocytes can be an effective method to achieve the desired profile of delivery, i.e., to obtain a suitable RES-targeting delivery system. PTX diffused through the lipid bilayer into the plasma, as observed for lipophilic drugs (Lewis and Alpar, 1984). The drug apparently diffused readily because cell membrane lysis was not essential for the release of PTX from loaded erythrocytes.

4. Conclusions

The results of the present study revealed that PTX was loaded successfully in human erythrocytes with acceptable loading parameters. Approximately 81% of the loaded PTX was released from the erythrocytes into the plasma within 48 h. PTX loading decreased the GSH level and increased lipid and protein oxidation. Further studies are required to demonstrate erythrophagocytosis of PTX-loaded erythrocytes. The relative impacts of the various in vitro findings on the overall *in vivo* drug delivery efficacy of these cellular carriers remain to be evaluated in future studies.

Conflict of interest

The authors have declared that no conflict of interest exists.

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