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The Entrapment of Paclitaxel in PLGA Nanoparticles Increases its Cytotoxicity against Multiresistant Cell Line

Vanya Bojat[1*], Vadim Balabanyan[1] and Renad Alyautdin[1]

[1]Department of Pharmacology of Pharmaceutical Faculty, I.M. Sechenov First Moscow State Medical University (MSMU), Moscow, Russia.

Research Article

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ABSTRACT

Paclitaxel (Ptx) is a taxane anticancer mitotic inhibitor, widely used in oncology for the last 20 years. Poor solubility of Ptx, as a consequence using of toxic solvents such as Cremofor EL, high affinity to P-glycoprotein are associated with serious side effects due to hypersensitivity reactions, low bioavailability and low therapeutic index. Development of new delivery solvent-free forms of Ptx is one of the key research problems in modern cancer chemotherapy.

Ptx loaded into polylactic-co-glycolic acid (PLGA) nanoparticles (Ptx-PLGA-Nps) (size 200-300 nm) have been prepared using nanoprecipitation method. Impact of technological parameters on Ptx encapsulation efficacy and in vitro drug release was investigated. Drug encapsulation was determined using HPLC. Citotoxic activity and cell accumulation of nanosomal formulation of Ptx was studied on multiresistant cell line Jurkat WT (cells of human T-limphoblastic leucosis). Obtained results suggest that formulation of PLGA Ptx nanoparticles have above 90-98% drug encapsulation efficacy, higher cell accumulation and cytotoxic activity.

Keywords: Paclitaxel; nanoparticles; multiresistance; P-glycoprotein;

**Corresponding author: Email: vzivkovic@mail.ru;*

1. INTRODUCTION

Paclitaxel (Ptx) is a taxane originally derived from the bark of the Pacific yew tree *Taxus brevifolia* and now obtained semisynthetically from a taxane precursor derived from the needles of the European yew. Paclitaxel's antineoplastic action arise from induction of microtubule formation and stabilization of microtubules thereby disrupting normal cell division in the G2 and M phases of the cell cycle (Spratlin and Sawyer, 2007). Common use of the poorly soluble Ptx is limited in oncology by the high toxicity of its dosage form, which becomes even higher by the addition of the solvents such as Cremophor® EL (polyoxyethylated castor oil) and dehydrated alcohol (Xie and Wang, 2005). Ptx is a substrate for P-glycoprotein, a factor presumably responsible for the multiple drug resistance of tumor cells (Chavanpatil et al., 2006). Although the permeability of tumour capillaries is higher than normal, P-glycoprotein effectively decreases Ptx concentrations in neoplastic tissues (Gallo et al., 2003). Therefore, the search for ways to overcome tumour cell resistance factors and reduce Ptx toxicity presents a topical problem.

Using of nanoparticle carriers for drug targeting is known to enhance intracellular transport of these substances, including cases in which P-glycoprotein is expressed in the membranes. Indeed, nanoparticles can undergo endocytosis, which ensures effective intracellular transport of drug substances, including P-glycoprotein substrates, such as Ptx. The increased permeability of newly formed tumor capillaries also results in accumulation of nanoparticles and their elimination from the bloodstream of the tumor growth area (Fonseca et al., 2002; Lacoeuille et al., 2007; Stinchcombe, 2007). Coating nanoparticles with some surfactants such as polysorbate 80 inhibits P-glycoprotein (Alyautdin et al., 1997; Ambruosi et al., 2006; Sparreboom et al., 2002; Zhang et al., 2003). Although commonly used in oncological practice, the nanosomal form of Ptx based on albumin nanoparticles (Abraxane, Abraxis BioScience, USA) has only moderately increased the bioavailability and safety of Ptx (Gradishar et al., 2005; Hawkins et al., 2008; Ibrahim et al., 2002, 2005; Nyman et al., 2005; Socinski, 2006).

Copolymers of lactic and glycolic acids (PLGA) most widely use for the preparation of nanoparticles. They are biodegradable and biocompatible polymers; polylactic-co-glycolic nanoparticles ensure effective sorption and controlled release of drug substances. A number of publications describe the preparation of Ptx-loaded micro- and nanoparticles, including PLGA particles; their biological activity has been demonstrated both in vitro and in vivo (Koziara et al., 2004; Liggins and Burt, 2004). At the same time, in some studies PLGA nanoparticles per se did not increase the efficacy of Ptx. In particular, Chavanpatil et al. (2003) revealed that the cytotoxic effect of Ptx-loaded nanoparticles was obtained only in the presence of P-glycoprotein inhibitors (Galindo-Rodriguez et al., 2005). Therefore, the inclusion of surfactants inhibiting P-glycoprotein, such as polysorbate-80, into the composition of the dosage form appears justified.

Accordingly, the aim of this study was to obtain Ptx loaded polylactic-co-glycolic acid (PLGA) nanoparticles (Ptx-PLGA-Nps), coated with polysorbate 80, to study their cytotoxic activity against human T-cell lymphoblastic leukemic cells (Jurkat/WT), and to investigate the influence of polysorbate-80 on the cytotoxic effect and cell accumulation of Ptx.

2. MATERIALS AND METHODS

The following products were commercially available: Paclitaxel (Calbiochem, USA), PLGA (50:50) (Absorbable Polymers International, USA), polaxamer 188 and polysorbate 80 (BASF, Germany), Coumarin-6 (Sigma-Aldrich, USA), Propidium iodide (Sigma, USA), MTT solution 5mg/ml (Sigma, USA), LDH test (Roche applied science, Switzerland). All other reagents and solvents had a purity level of at least "c. p."

2.1 Preparation of Ptx- Loaded and Coumarin-6 Loaded NPs

The conventional nanoprecipitation technique was modified in order to prepare the nanoparticles (Fonseca et al., 2002). Briefly, 0.5 to 10 mg of Ptx and 100 mg of PLGA were dissolved in 5 mL of acetone. This solution was mixed with 10 mL of aqueous poloxamer 188 solution; the resulting suspension was stirred while heated (to 50 or 60 °C) using a magnetic mixer over 2 to 3 hours in order to remove the organic solvent; after that, the suspension was filtrated through a "white tape" paper filter (pore dimension, 3 mcm) and lyophilized. Empty nanoparticles (reference formulation) were obtained in the same manner, without including Ptx. Coumarin 6-loaded NPs were prepared similarly for the fluorescent microscopic studies. The polymer solution contained 0.05% (w/v) coumarin-6 as fluorescent marker instead of PTX.

2.2 Characterization of NPs

NPs mean size was determined by PCS using Coulter N4MD (Coulter Electronics, U.K). The photon correlation spectroscopy measurements were carried out at a scattering angle of 90°, temperature 25 °C, viscosity 0,01 P and average index of refraction 1,333. Each 1ml sample was suitably diluted with 2 ml of distillated water, aliquot (50mcl) was placed in a quartz cuvette, containing 3ml of distillated. The size analysis consisted of 30 measurements per sample, and the results are expressed as mean size ± SD.

2.3 Encapsulation Efficacy

The content of Ptx in dry-lyophilized samples was determined by HPLC, following methanol processing of the sample to dissolve Ptx and destroy the nanoparticles. The Ptx yield (expressed as a percentage) was calculated as the ratio of the entrapped Ptx in NPs to the amount used in their preparation.

2.4 In Vitro Release Study

Aliquots of 50 mg of freeze-dried and cryoprotected PTX-loaded NPs were placed in a screwcapped tube and suspended in a) 5 ml of isotonic PBS pH 7.4 and b) 1% of polysorbate 80 in PBS. The tube was placed in a water bath at 37.2°C under magnetic stirring. At fixed time intervals, three tubes for each batch were removed and ultracentrifuged (microfilters Microcon 30kDa) at 15,000 g for 20min. The supernatants were extracted with 3×5 ml-aliquots of dichloromethane. The solvent aliquots were dried 10min at 120°C. The resulting solutions were analyzed by HPLC to determine the drug concentration. Calibration curve was linear in concentration range 0,5-100mcg/ml.

2.5 Cell Line Experiment

Jurkat WT cells were kindly provided by Russian Scientific Centre of Oncology (Russian Academy of Medical Sciences).

Jurkat WT cells in the exponential growth phase, placed in a fresh culturing medium (glutamine-free 90% RPMI 1640, 10% FBS – embryonal calf serum, vitamins, essential amino acids, L-glutamine), were re-cultured in the wells of a 96- or 24-well plate (50,000 to 100,000 cells per well) and incubated at CO2 concentration 5% and at temperature 37°C; aliquots of the tested forms were added after 12-hour incubation until Ptx concentrations of 10^{-4} M to $5*10^{-7}$ M were obtained. The cells were incubated in the presence of different drug concentrations at 37°C and CO_2 concentration 5% for 24 hours. Intact cells incubated under the same conditions without were used as controls.

2.6 In Vitro Particle Cell Uptake: Fluorescent Microscopic Studies

Jurkat cells were seeded in 24-well slides. At 80% confluency, medium was replaced with 1 ml of coumarin-6 loaded particle suspension (250 µg/ml). After 2 h of incubation, cells were fixed with 70% ethanol solution and kept at 37°C for 20 min. Subsequently PBS was used to wash wells for three times and 1 µg/ml PI was added to stain cell nucleus for 30–40 min. PI was washed three times using PBS and the glass slides were observed by fluorescence microscopy (Vashishtha and Dimmock, 1981). Coumarin-6-loaded particles and PI-staining cell nucleus showed green colour and red colour, respectively.

2.7 Study of the Cytotoxic Activity of Ptx and Its Nanosomal Dosage Forms

2.7.1 Dosage forms

Lyophilized nanoparticle samples were re-dispersed in distilled water or in 2 % polysorbate-80 solution until a homogeneous suspension was obtained. Four dosage forms were examined: free paclitaxel (Ptx), nanoparticles + polysorbate 80 (NPs+PS80), paclitaxel + nanoparticles (Ptx+NPs), and paclitaxel + nanoparticles + polysorbate 80 (Ptx+NP+PS80).

2.7.2 MTT test

The MTT test was performed according to Wataha et al. (1992). The MTT test was used to evaluate cell viability; this test is based on the ability of dehydrogenases found in live cells to convert the pale-yellow water-soluble 3-(4,5-dimethyltriazole-2-yl)2,5-diphenyl-2-H-tetrazolium bromide (MTT) into water-insoluble blue crystals of formazan. The absorption of resulting formazan is directly proportional to the number of living cells.

Four to six hours before the end of the calculated incubation period, 10 mcL of the MTT solution (5 mg/mL stock solution, Sigma, USA) were added to each of the cell cultures that had developed for a certain time in the wells of 96-well flat-bottomed plates either containing or not containing cytotoxins (reference and tested samples). After the incubation period, the cells were precipitated by centrifugation the plates at 1,000 rpm over 5 to 7 minutes. The supernatant was carefully removed; 60 mcL of DMS (Sigma, USA) were added into each of the wells; the sediments were re-suspended and incubated for 30 minutes at 37°C; after that, the optic density of the formazan solution was determined immediately using a vertically

scanning spectrophotometer (Titerteck Multiscan MCC/340, Flow Lab., USA) at wave length 540 nm.

Survival was expressed as the percentage of viable cells in treated samples relative to not treated control cells. All the experiments were repeated three times in triplicate.

2.7.3 LDH test

LDH test is colorimetric assay for the quantification of cell death and cell lysis, based on the measurement of lactate dehydrogenase (LDH) activity released from the cytosol of damaged cells into the supernatant. The amount of water soluble red formazan formed in the assay is proportional to the number of lysed cells. 0,1% solution of Triton X-100 was added to experimental and controlled cells 4-6 hours before end of incubation. After incubation cells were centrifugated at 1000 rpm for 5-7 min. Supernatant was taken and in each well 10 mcl of tetrazolium salt was added. Precipitates were resuspended and incubated 30min. at 37°C. The absorbance was measured at wavelength 590nm (Koh and Choi, 1987).

2.7.4 ATP test

ATP test is used to determine the number of viable cells in culture based on quantitation of the ATP present, which signals the presence of metabolically active cells. Luciferase catalyzes reaction of emission of ATP and light which is measured on luminometer in relative light units (RLU). 100 mcl of reaction mixture containing luciferin and luciferase (CellTiter-Glo®) was added to control and experimental cells 30 min. before end of incubation (Batiuk et al., 2001). Luminescence was measured using luminometer Microlumat LB 96P (Berthold, Germany).

3. RESULTS AND DISCUSSION

3.1 Biopharmaceutical Characteristics of Ptx Loaded PLGA NP

Nanoparticles were obtained by means of the so-called nanoprecipitation technique (Fonseca et al., 2002; Galindo-Rodriguez et al., 2005). The water-insoluble Ptx and PLGA carrier were dissolved in a water-mixable organic solvent (acetone); the resulting organic phase was added to an aqueous phase containing a surfactant (poloxamer 188). As the organic solvent is removed, the solubility of Ptx and the polymer decreases, which results in formation of particles measuring less than a micrometre (250 to 300 nm), provided that appropriate synthesis conditions are ensured.

We have studied effects of various technological parameters on the physicochemical properties of the dosage form, and in particular on drug substance yield. We have demonstrated that Ptx: PLGA ratio of 1 : 100 or below ensured a nanoparticle synthesis that was associated with virtually no loss of the drug substance and the polymer. It could be explained by a possibility of PLGA to sorb defined quantity of some substance. After saturation of sorbent rise of quantity of sorb substance has no influence on encapsulation efficiency. Our experiment showed encapsulation efficiency equal to 99.5% (Table 1) with particle size about 250-300nm.

Table 1. Influence of various technological parameters on the paclitaxel encapsulation efficiency; NP – nanoprecipitation, DCM – dichloromethane; PTX – paclitaxel, PLGA – polylactic-co-glycolic acid

Obtaining method	Organic phase			Aqueous phase	Encapsulation of PTX, %	Loss of polymer
	PTX	PLGA	Solvent	1% Poloxamer 188		
NP	0,5 mg	100 mg	Acetone	5 ml	99,5	No
NP	1 mg	100 mg	Acetone	5 ml	91,4	No
NP	3 mg	100 mg	Acetone	5 ml	9,2	No
NP	10 mg	100 mg	Acetone	5 ml	4,7	No

The obtained dosage form proved resistant to freezing and subsequent lyophilization: the suspension did not lose its aggregate stability after at least three "-20 °C – room temperature" cycles, while the addition of water or polysorbate-80 solution to the lyophilized powder resulted in formation of a homogeneous suspension. The nanosomal form was able to maintain steady release of Ptx over 24 hours in phosphate buffer (pH 7.4), i.e., in a medium that imitates blood plasma and intracellular fluid. The addition of polysorbate-80 into the incubation medium as a solubilizer resulted in a somewhat higher Ptx release rate as compared with the unmodified phosphate buffer (Figure 1).

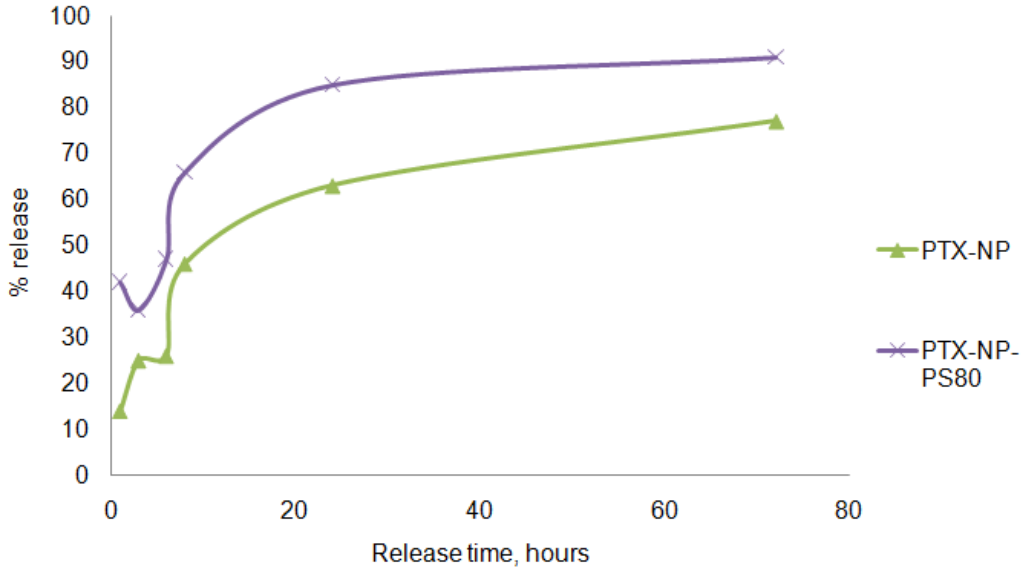

Fig. 1. In vitro release of paclitaxel-loaded from NPs and PS80 coated NPs

The use of polysorbate-80 was due not only to its ability to solubilize the poorly soluble Ptx. Polysorbate-80 is known to be capable of inhibiting P-glycoprotein, the membrane protein responsible for transporting xenobiotics back into the extracellular space (Zhang et al., 2003; Gelperina et al., 2002). P-glycoprotein is expressed by human T-cell lymphoblastic leukemic cells (Jurkat WT), and therefore we were interested in comparing the cytotoxic activity of Ptx-containing nanoparticles coated with polysorbate 80 versus free Ptx.

3.2 Citotoxic Activity of PTX Loaded PLGA NPs in Jurkat Cells

It is a clear fact that the therapeutic effects of the drug loaded into PLGA NPs would depend on internalization and sustained retention of the NPs by the tumor cells. Although in vivo and in vitro biological processes could be very much different, in vitro investigation can provide some preliminary knowledge to understand the advantages of the NP drug formulation versus the free drug. Therefore, first of all we studied ability of PLGA NPs to accumulate inside of multiresistant Jurkat cells. Cells were incubated for 2 h with PLGA NPs loaded with coumarin-6 and then investigated by fluorescent microscopy. Nuclei were stained with PI. No fluorescence was detected from cells not exposed to coumarin-6 proving the absence of cell or NS auto-fluorescence (data not shown). The fluorescence of coumarin-6 loaded PLGA NPs (green) was closely located around the nuclei (red) indicating a significant accumulation of PLGA NP by Jurkat cells (Figure 2).

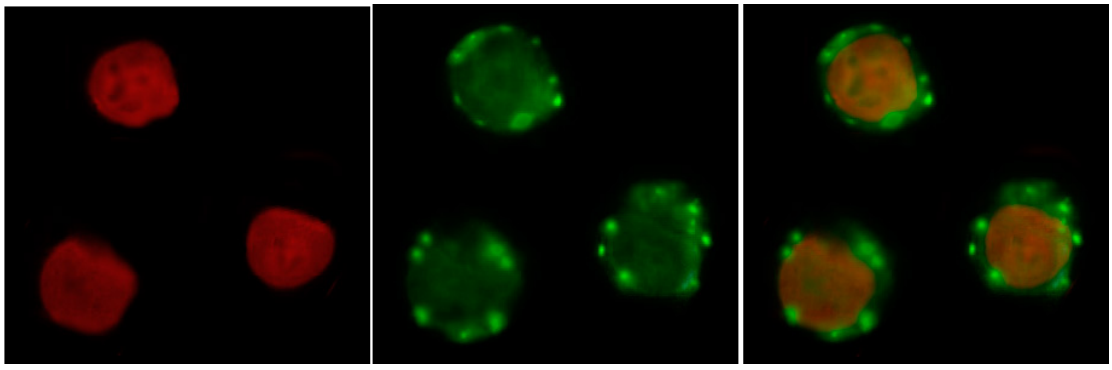

Fig. 2. Intracellular accumulation of NPs in Jurkat cells.
Nuclei were stained with PI and are visible in red (2A). The uptake of coumarin-6-loaded NS is visible in green (2B). Figure 2C displays an overlaying image obtained combining the FITC and the PI filters. A representation of two experiments is shown. Magnification: 63 ×

In order to determine the effect of the nanoparticles formulations on the cells as well as the sensitivity of Jurkat cells to Ptx, the cytotoxic effect of Ptx was evaluated by its inhibitory effect on the cell viability. The cytotoxic activity and subsequent therapeutic potential of Ptx-NPs was thereafter evaluated by incubating Jurkat cells within increasing range of concentrations from $5*10^{-7}$ to $1*10^{-4}$ M for 24 h. Different formulations of Ptx were used: free Ptx, NP-loaded Ptx and NP-loaded Ptx, coated with polysorbate 80. In the present in vitro study the cytotoxicity of different formulations was evaluated using three assays measuring different parameters. The assays were the MTT, the LDH, and the ATP assays. The cytotoxic effect of the various Ptx forms in MTT test is presented on Figure 3. In accordance with literary data the medicine that inhibits 50% (IC_{50}) of living cells possesses cytotoxic activity. At the same time cytotoxicity of the medicine should revealed at low concentrations 10^{-4} - 10^{-5}M. Rise of IC_{50} up to 10^{-4} M brings to increasing of toxic effect of medicine to healthy organs and tissues. Empty PLGA NPs were not cytotoxic to cells at all concentrations (data not shown). MTT test findings have shown that free Ptx within range of testing concentration haven't reach IC_{50}, at the same time nanoformulations of Ptx reached IC_{50} at more lower concentrations $6.8*10^{-6}$M. Ptx-NP and Ptx-NP-PS80 reduced cell growth in a significant manner ($p < 0.01$, Student's t test) by approximately 70% after 24 h of exposure. Clear dose-response relationship was observed for both NPs-Ptx and Nps-Ptx-PS80. Decreasing of free Ptx concentrations from $1*10^{-4}$ M to $6.8*10^{-6}$ M resulted in a

gradual decrease in the drug's cytotoxicity, and percent of viable cells at concentration $6.8*10^{-6}$ M was found to be 97% of that seen in intact controls. It is apparent that Ptx is ineffective at higher dilutions.

Results of assessment of cytotoxicity obtained in LDH test correlate with those obtained in MTT test (Figure 3 and 4). Free Ptx couldn't reach level of IC_{50}. We observed significant differences in cell viability between free Ptx vs. Ptx-NPs and Ptx-NPs-PS80 ($p \leq 0,01$).

In ATP test nanoform of Ptx coated with polysorbat 80 reached IC_{50} at minimal concentration $1*10^{-6}$M (Figure 5). At the same time free Ptx didn't reach IC_{50} after 24h of incubation in testing concentration and percent of viable cells didn't fall lower than 70%. Nanoform of Ptx without coating reached IC_{50} at higher concentrations $6.8*10^{-6}$M.

Paclitaxel is the first drug from the group of taxanes that is used in oncology for more than 10 years. As of today Ptx is fistly approved for treatment of breast, ovarian and lung (non-small-cell) cancers and AIDS-related Kaposi's sarcoma. However the clinical use of Ptx is limited by its poor water solubility and therefore systemic administration of this drug relies upon concomitant use of solubilazer Cremophor EL®. Unfortunately, Cremophor EL® use is also associated with patient toxicity because of poor tolerance and hypersensitivity reactions. Another obstacle in clinical use of Ptx is the fact that Ptx is substrate for P-glycoprotein responsible for drug efflux from the tumor cell. To overcome these difficulties, clinicians have attempted to prolong infusion schedules, to use pre-medication regimen and to increase the dosage of Ptx respectively. However, to improve the efficacy of Ptx in anti-cancer therapy, a potential solution must involve reformulation of the drug into better-tolerated and selectively delivered vehicles. Accordingly, a number of strategies to develop alternative formulations of PTX are in progress, including the use of albumin NP, pro-drugs (Xyotax and Taxoprexin), co-solvents (Genexol PM), emulsions, liposomes and microspheres (Hennenfent and Govindan, 2006).

Development of new nanoformulation of paclitaxel with improved solubility, pharmacokinetics and bioavailability remains to be a promising task for a better treatment of wide range of malignancies. In fact NP is easily prepared with biodegradable polymers and is highly stable in biological fluids and during storage (Fonseca et al., 2002; Musumeci et al., 2006).

In our experiment we solve the problem of paclitaxel poor solubility by loading paclitaxel into polymeric biocompatible and biodegradable PLGA nanoparticles. Nanoprecipitation technique that we used for preparing nanoparticles enabled to get stable colloidal system with paclitaxel encapsulation efficiency 99,5%, size of nanoparticles 250-300nm. Due to size-related effect of increased permeability and retention nanoparticles concentrate in highly neovascularized tumor tissues, thus allowing passively targeted drug release (Feng et al., 2004). Effect of increased permeability and retention is not always to determine drug efficacy, because optimal release is also necessary. In particular optimum drug release from NPs can provide therapeutically active concentration within tumor cells during the necessary time period.

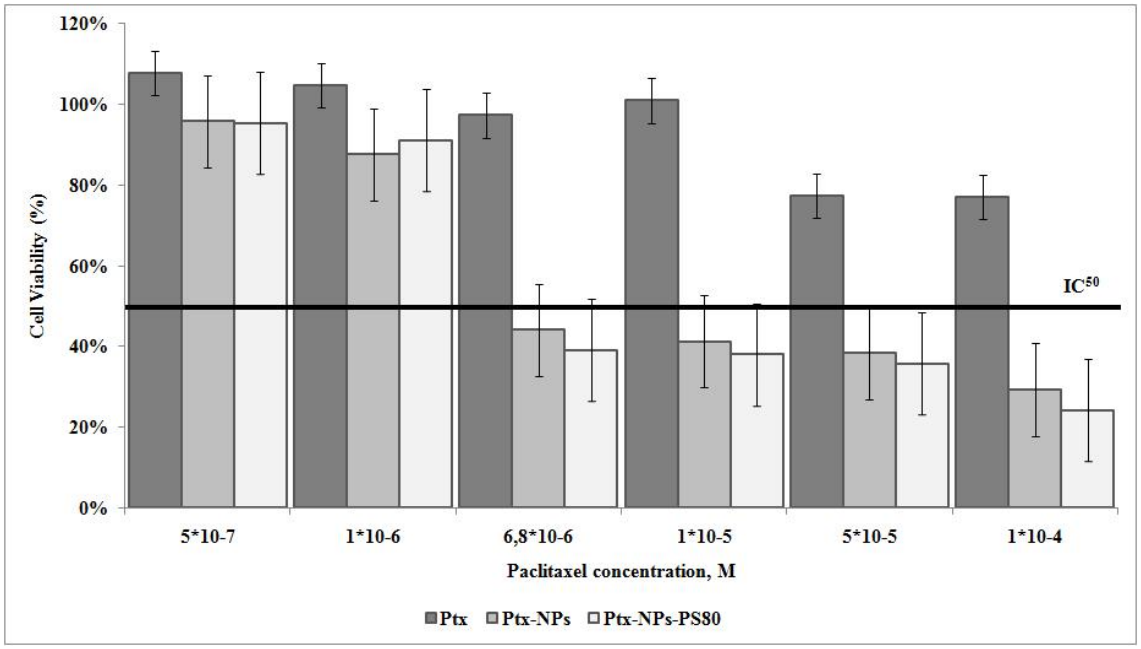

Fig. 3. In vitro cell viability in MTT test

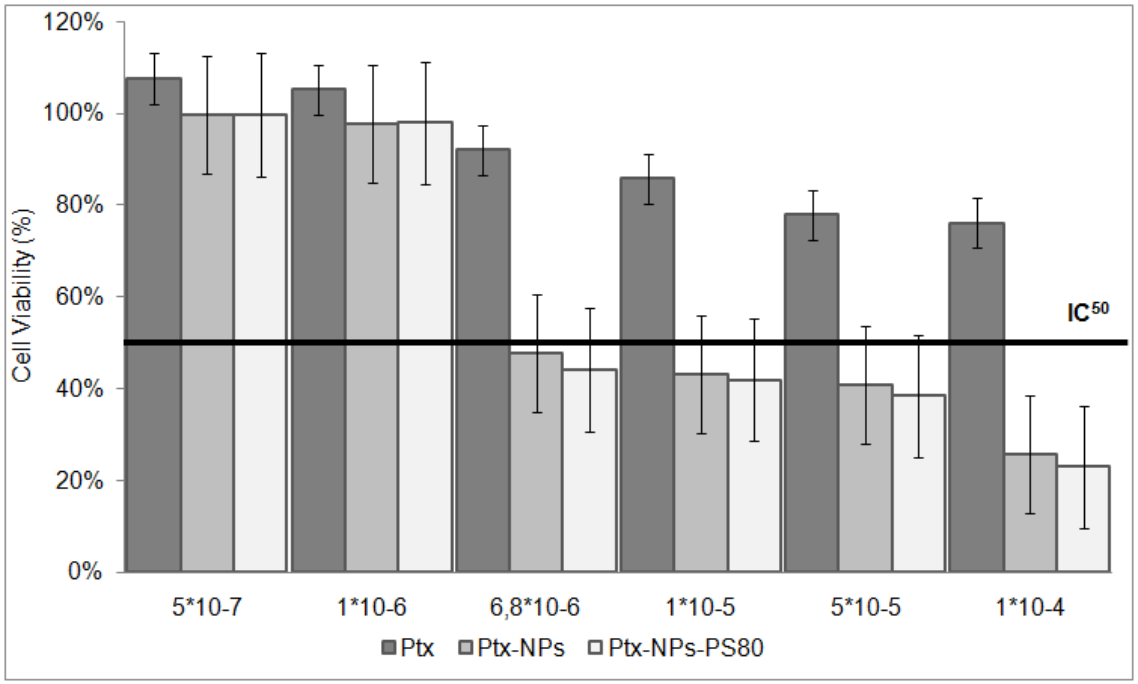

Fig. 4. In vitro cell viability in LDH test

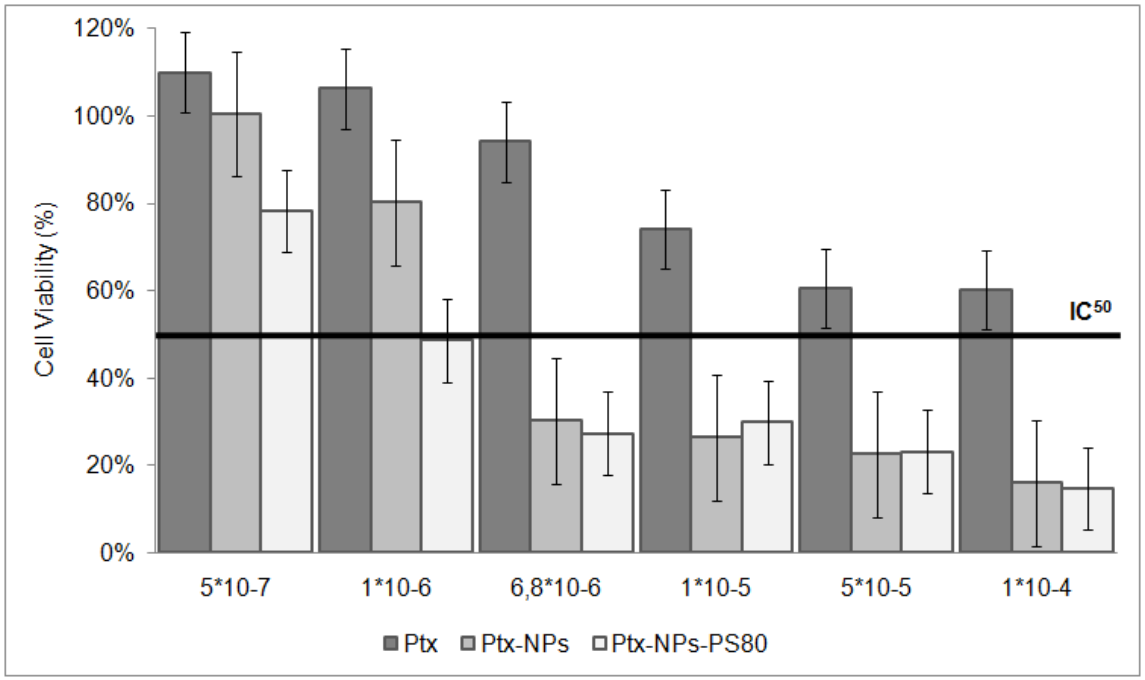

Fig. 5. In vitro cell viability in ATP test

In our in vitro release study different formulations of Ptx had different release. The amount of Ptx released from Ptx-NP-PS80 and Ptx-NP after 24h was approximately 85% and 65% correspondingly. Such release could be explained by diffusion or erosion of the matrix and depends on different parameters (for example particle size and/or molecular weight of the polymer). The release behavior of the nanoformulations used in this study showed a biphasic pattern, with an initial relevant burst effect followed by a slower and more constant release.

The main problem for NPs clinical use is their thermodynamic instability and precipitation of the suspension. As a result of this water removal is necessary by usage of freeze-drying method. We have demonstrated preparing of high stable aggregate nanosuspension with addition of cryoprotector mannitol.

Biopharmaceutical characteristics of prepared nanoform of Ptx favored further cytotoxicity and accumulation study on the multiresistant line of T-leukemic cells (Jurkat WT). Jurkat WT is a multiresistant cell line that produces P-glycoprotein, one of the main component of the multi drug resistance (MDR) responsible for drug efflux from the cell (Martel et al., 1997; Myrick et al., 1999). As a substrate for p-glycoprotein paclitaxel is effluxed out of the multiresistant cell and its efficacy is reduced accordingly. As of today Ptx is not approved for treatment of leukemia and along with multiresistance that was a challenge for our study to use T-leukemic cells as a biological model.

Cytotoxicity and indirect overcoming of P-glycoprotein by nanoform of Ptx was studied using three biochemical assays: MTT, LDH and ATP. In all the three assays tested in this study, the cytotoxicity of Ptx-NPs was higher than that of free Ptx. Jurkat cells were sensitive to common Ptx-NPs as well as Ptx-NPs-PS80. Nanosomal forms of paclitaxel reached IC_{50} at lower concentration $6.8*10^{-6}$M comparing with free Ptx. A different mechanism of transport of PTX-NS in respect to the free drug can be claimed to explain such differences. In fact, drug encapsulation in PLGA NS caused a rapid internalization of the system inside the cells by endocytosis, resulting in a higher cellular uptake. Once inside the cells, NS escaped the endo-lysosomal pathway and entered the cytoplasm where they are retained for a longer time (Chavanpatil et al., 2003).

4. CONCLUSION

Incorporation of Ptx in PLGA NPs enhanced its cytotoxic activity in vitro in multiresistant cell line Jurkat WT. Even if these data need to be confirmed in other systems (such as human primary culture or tumor xenografts), this formulation appears as an interesting delivery system for PTX in the treatment of multiresistant cancer.

REFERENCES

Alyautdin, R.N., Petrov, V.E., Langer, K., Berthold, A., Kharkevich, D.A., Kreuter, J. (1997). Delivery of loperamide across the blood-brain barrier with polysorbate 80-coated polybutylcyanoacrylate nanoparticles. Pharm. Res., 14, 325-8.

Ambruosi, A., Khalansky, A.S., Yamamoto, H., Gelperina, S.E., Begley, D.G., Kreuter, J. (2006). Biodistribution of polysorbate 80-coated doxorubicin-loaded [14C]-poly(butyl cyanoacrylate) nanoparticles after intravenous administration to glioblastoma-bearing rats. J. Drug Target, 14(2), 97-105.

Batiuk, T.D., Schnizlein-Bick, C., Plotkin, Z., Dagher, P.C. (2001). Guanine nucleosides and Jurkat cell death: roles of ATP depletion and accumulation of deoxyribonucleotides. Am. J. Physiol. Cell Physiol., 281, 1776–1784

Chavanpatil, M.D., Patil, Y., Panyam, J. (2006). Susceptibility of nanoparticle-encapsulated paclitaxel to P-glycoprotein-mediated drug efflux. Int. J. Pharm., 320(1-2), 150-6.

Feng, S.S., Mu, L., Win, K.Y., Huang, G. (2004). Nanoparticles of biodegradable polymers for clinical administration of paclitaxel. Curr. Med. Chem., 11, 413–24.

Fonseca, C., Simoes, S., Gaspar, R. (2002). Paclitaxel-loaded PLGA nanoparticles: preparation, physicochemical characterization and in vitro anti-tumoral activity. J Control Release, 83, 273-86.

Galindo-Rodriguez, S.A., Puel, F., Briancon, S., Allemann, E., Doelker, E., Fessi, H. (2005). Comparative scale-up of three methods for producing ibuprofen-loaded nanoparticles. Eur. J. Pharm. Sci., 25(4-5), 357-67.

Gallo, J.M., Li, S., Guo, P., Reed, K., Ma, J. (2003). The effect of P-glycoprotein on paclitaxel brain and brain tumor distribution in mice. Cancer Res., 63(16), 5114-7.

Gelperina, S.E., Khalansky, A.S., Skidan, I.N., Smirnova, Z.S., Bobruskin, A.I., Severin, S.E., et al. (2002). Toxicological studies of doxorubicin bound to polysorbate 80-coated poly(butyl cyanoacrylate) nanoparticles in healthy rats and rats with intracranial glioblastoma. Toxicol Lett., 126, 131–141.

Gradishar, W.J., Tjulandin, S., Davidson, N., Shaw, H., Desai, N., Bhar, P., et al. (2005). Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. J Clin. Oncol., 23, 7794-7803.

Hawkins, M.J., Soon-Shiong, P., Desai, N. (2008). Protein nanoparticles as drug carriers in clinical medicine. Adv Drug Deliv Rev., 60, 876-85.

Hennenfent, K.L., Govindan, R. (2006). Novel formulations of taxanes: a review. Old wine in a new bottle? Ann. Oncol., 17, 735-49.

Ibrahim, N.K., Desai, N., Legha, S., Soon-Shiong, P., Theriault, R.L., Rivera, E., et al. (2002). Phase I and pharmacokinetic study of ABI-007, a Cremophor-free, protein-stabilized, nanoparticle formulation of paclitaxel. Clin. Cancer Res., 8, 1038-44.

Ibrahim, N.K., Samuels, B., Page, R., Doval, D., Patel, K.M., Rao, S.C., et al. (2005). Multicenter phase II trial of ABI-007, an albumin-bound paclitaxel, in women with metastatic breast cancer. J. Clin. Oncol., 23, 6019-26.

Koh, J.Y., Choi, D.W. (1987). Quantitative determination of glutamate mediated cortical neuronal injury in cell culture by lactate dehydrogenase efflux assay. J. Neurosci. Methods, 20, 83–90.

Koziara, J.M., Lockman, P.R., Allen, D.D., Mumper, R.J. (2004). Paclitaxel nanoparticles for the potential treatment of brain tumors. J. Control Release, 99(2), 259-269.

Lacoeuille, F., Hindre, F., Moal, F., Moal, F., Roux, J., Passirani, C., et al. (2007). In vivo evaluation of lipid nanocapsules as a promising colloidal carrier for paclitaxel. Int. J. Pharm., 344, 143-9.

Liggins, R.T., Burt, H.M. (2004). Paclitaxel loaded poly(L-lactic acid) (PLLA) microspheres. II. The effect of processing parameters on microsphere morphology and drug release kinetics. Int. J. Pharm., 281(1-2), 103-6.

Martel, J., Payet, M.D., Dupuis, G. (1997). The MRD1 (P-glycoprotein) and MRP (P-190) transporters do not play a major role in the intrinsic multiple drug resistance of Jurkat T lymphocytes. Leuk Res., 21(8), 743-52.

Musumeci, T., Vicari, L., Ventura, C.A., Gulisano, M., Pignatello, R., Puglisi, G. (2006). Lyoprotected nanosphere formulations for paclitaxel controlled delivery. J. Nanosci. Nanotechnol., 6, 3118-25.

Myrick, D., Blackinton, D., Klostergaard, J., Kouttab, N., Maizel, A., Wanebo, H., et al. (1999). Paclitaxel-induced apoptosis in Jurkat, a leukemic T cell line, is enhanced by ceramide. Leuk. Res., 23, 569–78.

Nyman, D.W., Campbell, K.J., Hersh, E., Long, K., Richardson, K., Trieu, V., et al. (2005). Phase I and pharmacokinetics trial of ABI-007, a novel nanoparticle formulation of paclitaxel in patients with advanced nonhematologic malignancies. J. Clin. Oncol., 23, 7785-93.

Socinski, M. (2006). Update on nanoparticle albumin-bound paclitaxel. Clin. Adv. Hematol. Oncol., 4, 745-6.

Sparreboom, A., Zhao, M., Brahmer, J.R., Varweij, J., Baker, S.D. (2002). Determination of the docetaxel vehicle, polysorbate 80, in patient samples by liquid chromatography–tandem mass spectrometry. J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci., 773(2), 183-190.

Spratlin, J., Sawyer, M.B. (2007). Pharmacogenetics of paclitaxel metabolism. Crit. Rev. Oncol. Hematol., 61, 222-9.

Stinchcombe, T.E. (2007). Nanoparticle albumin-bound paclitaxel: a novel Cremphor- EL-free formulation of paclitaxel. Nanomed., 2, 415-23.

Vashishtha, A.J., Dimmock, J.R. (1981). Application of fluorescence microscopy to measure apoptosis in Jurkat T cells after treatment with a new investigational anticancer agent (N.C.1213). Cell. Mol. Neurobiol., 3, 2011-2014.

Wataha, J.C., Hanks, C.T., Craig, R.G. (1992). *In vitro* synergistic, antagonistic and duration of exposure effects of metal cations on eukaryotic cells. J. Biomed. Mater. Res., 26, 1297–309.

Xie, J., Wang, C.H. (2005). Self-assembled biodegradable nanoparticles developed by direct dialysis for the delivery of paclitaxel. Pharm. Res., 22, 2079-90.

Zhang, H., Yao, M., Morrison, R.A., Chong, S. (2003). Commonly Used Surfactant, Tween 80, Improves Absorption of P-Glycoprotein Substrate, Digoxin, in Rats. Arch. Pharm. Res., 26(9), 768-72.