Development of a novel nanoparticulate form of paclitaxel for cancer treatment

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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.

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. Coating nanoparticles with some surfactants such as polysorbate 80 inhibits P-glycoprotein. Although commonly used in oncological practice, the nanosomal form of Ptx based on albumin nanoparticles (Abraxane). 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[1].

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.
Nanoparticles were obtained by means of the so-called nanoprecipitation technique[2]. 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% with particle size about 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[3]. 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.

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[4] .
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 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[5].

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

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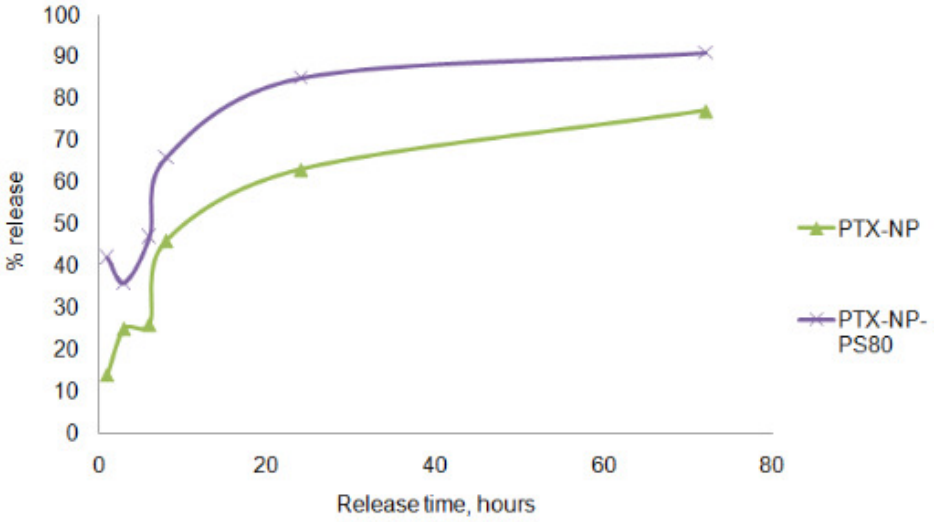
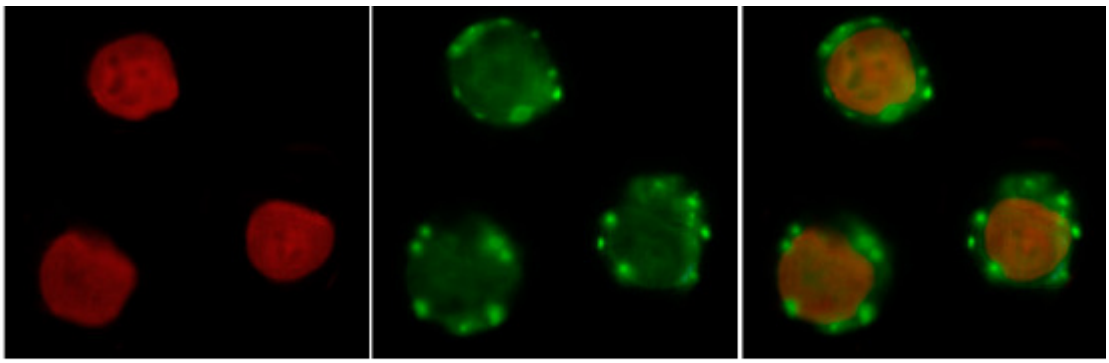
Figures

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



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



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 ×