# Cytotoxic Effect of Paclitaxel Incorporated in Nanoparticles Based on Lactic and Glycolic Acid Copolymer

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Paclitaxel dosage form on nanoparticles of 200-300 nm based on lactic and glycolic acid copolymer was obtained by the co-precipitation method. The possibility of controlled release of paclitaxel at pH 7.4 for 24 h was studied *in vitro*. Studies on Jurkat/WT human T-lymphoblastic leukemia cells showed that incorporation of paclitaxel in the nanoparticles led to a 4-fold increase of its cytotoxicity ($6.8\times10^{-6}$ M) in comparison with paclitaxel solution. The efficiency of compositions containing polysorbate-80 was comparable to that of non-modified nanoparticles containing paclitaxel.

**Key Words:** *paclitaxel, nanoparticles; Jurkat; P-glycoprotein*

Paclitaxel is a taxan group chemical drug which inhibiting depolymerization of tubulin microtubules and arresting the cell in the metaphase, which determines its antiangiogenic and antiapoptotic effects. Wide use of poorly soluble paclitaxel in oncology is impeded by high toxicity of its dosage form, including that caused by addition of the solvent. Paclitaxel is a substrate for P-glycoprotein, a factor largely determining multiple drug resistance of tumor cells [4]. Despite high permeability of capillaries in the tumor, P-glycoprotein effectively reduces the concentration of paclitaxel in tumor tissues [8]. Therefore, the search for approaches to overcoming tumor cell resistance factor control and reduction of paclitaxel toxicity is an important problem.

Drug incorporation in nanosized carriers leads to improvement of drug delivery into the cells even if their membranes express P-glycoprotein. Nanoparticles are liable to endocytosis, this providing effective intracellular transport of drugs, including the P-glycoprotein substrates (such as paclitaxel). High permeability of new capillaries of the tumor also leads to accumulation and release of nanoparticles from the vessels in the tumor growth zone. Coating of nanoparticles by surface-active substances inhibits P-glycoprotein. The nanosomal form of paclitaxel based on albumin nanoparticles, despite wide use in practical oncology, just slightly increased drug bioavailability and safety.

Lactic and glycolic acid copolymers (LGAC) are widely used for obtaining nanoparticles. These are biodegraded and biocompatible polymers. Nanoparticles on this basis provide effective adsorption and regulated release of drugs. Preparation of micro- and nanoparticles with paclitaxel, including LGAC nanoparticles, has been described and their biological activity has been demonstrated *in vitro* and *in vivo* [10,11]. On the other hand, LGAC-based nanoparticles *per se* did not increase the efficiency of paclitaxel. For example, the cytotoxic effect of paclitaxel-containing nanoparticles has been demonstrated only in the presence of P-glycoprotein inhibitors [4]. It is therefore justified to add P-glycoprotein inhibitors (such as polysorbate-80) to the surfactant dosage form.

We studied the effects of various technological parameters on physicochemical characteristics of LGAC nanoparticles containing paclitaxel, for example, on the drug release. At paclitaxel:LGAC ratio

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of 1:100 and lower, nanoparticles were synthesized in fact without loss of the drug and polymer. The resultant dosage form was resistant to freezing and subsequent lyophilization. The suspension did not loose the aggregative stability after at least 3 freezing (-20°C)/defrosting (20°C) cycles, and addition of water or polysorbate-80 solution to lyophilized preparation led to formation of homogeneous suspension. Hence, experimental dosage form is stable and fit for oral and intravenous use [1].

We studied cytotoxic activity of nanoparticles towards human Jurkat/WT T-lymphoblastic leukemia cells and the effects of polysorbate-80 on the cytotoxic effect of paclitaxel.

## MATERIALS AND METHODS

Paclitaxel (Calbiochem), LGAC with 50/50 proportion of components and characteristic viscosity of 0.37 in hexafluoroisopropanol (Absorbable Polymers International) were used in the study. The degree of the rest reagents and solvent purity was at least "chemically pure".

Nanoparticles were obtained by a modified co-precipitation method [6]. Paclitaxel (0.5-30 mg) and LGAC (100 mg) were dissolved in 5 ml acetone. This solution was mixed with 10 ml 1% water solution of poloxamer-188. The resultant suspension was mixed at 50-60°C on a magnetic mixer for 2-3 h in order to remove the organic solvent, after which the suspension was filtered through a "white band" paper filter (3-μ pores) and lyophilized. Empty nanoparticles (control form) were obtained similarly without paclitaxel.

Paclitaxel content in lyophilized specimens was measured by chromatography after methanol treatment dissolving paclitaxel and destroing the nanoparticles. Paclitaxel release (in percent) was evaluated as the proportion of paclitaxel found in the sample to that used for synthesis.

The suspension was diluted to paclitaxel concentration of 2 μm/ml in 70 ml medium for release. The composition of medium for release was as follows: phosphate buffer (pH 7.4), 1% polysorbate-80 in phosphate buffer (pH 7.4). Pure paclitaxel served as the control. Medium for release was incubated in a thermostat shaker at 37.2°C. Aliquots of the suspension were collected after certain periods, ultrafiltered through Microcon microfilters (30 kDa, 20 min, 15,000*g*), an aliquot of ultrafiltered suspension was transferred into a penicillin flask, dried (10 min, 120°C), and paclitaxel was measured by chromatography.

Lyophilized specimens of nanoparticles were re-dispersed in water for injections or in 2% polysorbate-80 solution (Twin) to obtain a homogeneous suspension. Hence, four forms were studied: paclitaxel, nanoparticles+Twin, paclitaxel+nanoparticles, and paclitaxel+nanoparticles+Twin.

Cells Jurkat/WT in the exponential growth phase in fresh nutrient medium (90% RPMI 1640 without glutamine, 10% FCS, vitamins, essential amino acids, L-glutamine) were reinoculated into wells of 96- or 24-well plates (50-100 thousand cells per well) and incubated at 5% $CO_2$ and 37°C. After 12-h incubation, aliquots of the studied forms were added into the wells to paclitaxel concentrations of $10^{-4}$-$5\times10^{-7}$ M. The cells were incubated with different concentrations of the test drugs at 37°C and 5% $CO_2$ for 24 h. Intact cells incubated under the same conditions without preparations served as the control.

The cytotoxic effects of the drugs were evaluated by the MTT test. This test is based on the capacity of living cell dehydrogenases to transform pale yellow water-soluble 3-(4,5-dimethyltriasole-2-yl)2,5-diphenyl-2-H-tetrasoleum bromide (MTT) into water-insoluble blue formasane crystals. The volume of resultant formasane, evaluated by colorimetry after its dissolution in organic solvents, characterizes the intensity of redox processes in cell cultures and is an indirect characteristic of active biomass.

Stock MTT solution (5 mg/ml; Sigma) was added (10 μl) into cell cultures growing in the wells of 96-well flat-bottom plates with or without cytotoxins (control and experimental samples) 4-6 h before the end of incubation. After incubation, the cells were precipitated by centrifugation of plates at 1000 rpm (5-7 min). The supernatant was collected and DMSO (60 μl/well; Sigma) was added. The precipitate was resuspended and the plates were incubated (30 min, 37°C), after which optical density of formasane solution was directly measured on a vertical scanning Titerteck Multiscan MCC/340 spectrophotometer (Flow Lab.) at λ=540 nm. The results were processed using Elisafit software.

## RESULTS

Nanoparticles were obtained by the so-called co-precipitation method [6,7]. Paclitaxel and LGAC carrier insoluble in water were dissolved in an organic solvent mixed with water (acetone). The resultant organic phase was added to the water phase containing surface-active substance (poloxamer-188). Solubility of paclitaxel and polymer decreased with removal of the organic solvent, which led, with correctly selected parameters of synthesis, to the formation of particles of submicron size (250-300 nm).

The nanosomal form provided smooth release of paclitaxel for 24 h in phosphate buffer (pH 7.4), that is, in medium imitating blood plasma and intracellular liquid (Fig. 1). Addition of polysorbate-80 into

the incubation medium as the solubilizer provided a higher rate of paclitaxel release in comparison with nonmodified phosphate buffer (Fig. 1).

The use of polysorbate-80 was explained not only by its ability to dissolve poorly soluble paclitaxel. Polysorbate-80 inhibited P-glycoprotein, a membrane protein responsible for reverse transport of xenobiotics to extracellular environment. In addition, polysorbate-80 adsorbed apolipoprotein E from the liquid phase. This was assumed to be one of the mechanisms providing the transport of nanoparticles of different nature, including LGAC nanoparticles, through the blood-brain barrier [2,8-10,15]. Since P-glycoprotein was expressed by Jurkat-WT human T-lymphoblastic leukemia cells, it was interesting to compare cytotoxic activities of paclitaxel-containing nanoparticles not only with free paclitaxel, but also with compositions containing polysorbate-80.

The cytotoxic effects of various paclitaxel forms are presented in Fig. 2. Reduction of paclitaxel concentration from $10^{-4}$ M to $6.8\times10^{-6}$ M led to gradual reduction of its cytotoxicity. In the presence of $6.8\times10^{-6}$ M paclitaxel, the level of viable cells was 90% of intact control (growth medium with phosphate buffer instead of the drugs). Obviously, paclitaxel in higher dilutions is ineffective. Hence, the efficiency of nanosomal drugs was studied starting from the concentration of $6.8\times10^{-6}$ M.

The nanosomal forms of paclitaxel at this concentration of the drug exhibited a significantly higher cytotoxic effect: no more than 30-40% cells survived. A difference, though less significant, was demonstrated for lower dilutions: $10^{-6}$ and $5\times10^{-7}$ M (Fig. 2).

It was found that 2% polysorbate-80 did not improve drug efficiency and up to 95% cells remained viable at concentrations of $6.8\times10^{-6}$-$5\times10^{-7}$ M. The efficiency of nanoparticles coated with 2% polysorbate-80 containing LGAC was comparable to that of nonmodified nanoparticles containing paclitaxel. About 40% cells remained viable at drug concentration of $6.8\times10^{-6}$ M, about 80% at concentrations of $10^{-6}$ and $5\times10^{-7}$ M.

We showed that LGAC-based nanoparticles stimulated paclitaxel cytotoxicity towards P-glycoprotein-positive Jurkat cells without polysorbate-80. Our results are in good agreement with published data. Previous experiments showed that polymeric nanoparticles underwent effective endocytosis by Jurkat cells and the endocytosis rate depended, among other things, on the material from which the nanoparticles were made and on the surface properties; nanoparticles based on polylactic acid and its copolymers were captured sufficiently rapidly [12]. Human albumin-based nanoparticles slightly increased the phototoxic effect of pheophorbide against Jurkat cells [5]. The use of

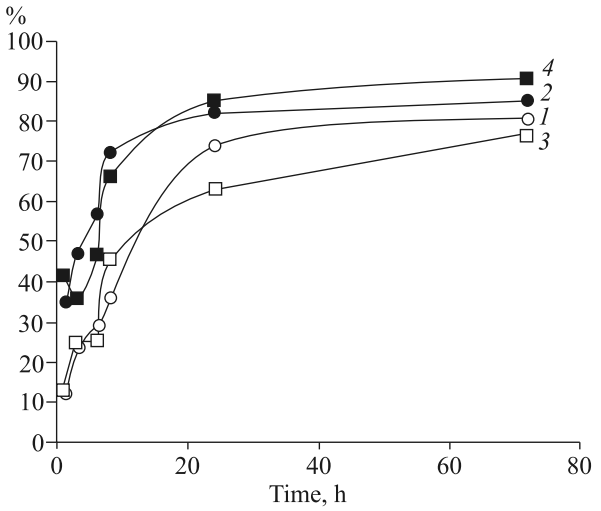


**Fig. 1.** Paclitaxel release from nanoparticles. Ordinate: percentage of released paclitaxel. *1*) paclitaxel; *2*) paclitaxel+1% polysorbate-80; *3*) paclitaxel+nanoparticles; *4*) paclitaxel+nanoparticles+1% polysorbate-80.

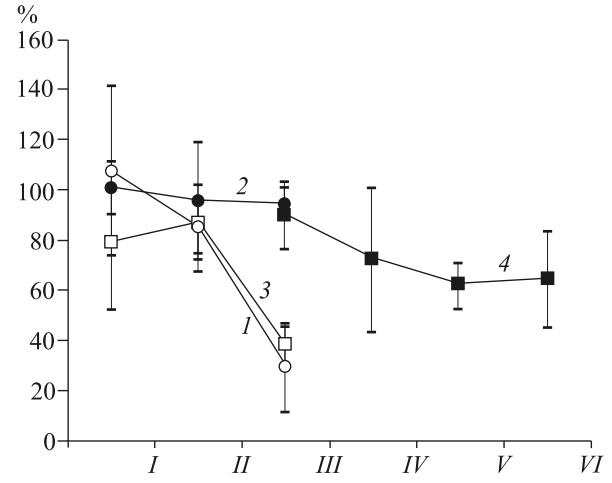


**Fig. 2.** Cytotoxic activities of paclitaxel dosage forms towards Jurkat-WT cells. Abscissa: paclitaxel concentrations. *I*) $5\times10^{-7}$; *II*) $1\times10^{-6}$; *III*) $6.8\times10^{-6}$; *IV*) $1\times10^{-5}$; *V*) $5\times10^{-5}$; *VI*) $1\times10^{-4}$. Ordinate: viable cells. *1*) nanoparticles+paclitaxel; *2*) nanoparticles+2% polysorbate-80; *3*) nanoparticles+paclitaxel+2% polysorbate-80; *4*) paclitaxel.

LGAC nanoparticles stimulated the antiapoptotic and antiproliferative effects of curcumine towards various tumor cells, including leukemic cells [3]. The absence of difference between the cytotoxic effects of LGAC nanoparticles and polysorbate-8-coated LGAC nanoparticles can be attributed to the absence of apolipoproteins E and B in incubation medium or insufficient adsorption of polysorbate-80 by LGAC.

Hence, a new dosage form of paclitaxel was developed, based on lactic and glycolic acids copolymers and fit for oral and intravenous administration with the submicron particle size. The nanoprecipitation method used in our study makes it possible to obtain the dos-

age form virtually without loss of paclitaxel and accessory substances. Controlled release of paclitaxel at pH 7.4 over 24 h was demonstrated, including release in medium containing solubilizer polysorbate-80. The use of nanoparticles led to a 4-fold increase of paclitaxel cytotoxicity towards Jurkat/WI human T-lymphoblastic leukemia cells. Our results obtained *in vitro* suggest further *in vivo* studies of the experimental nanosomal dosage form.

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