Paclitaxel + 17AAG co-encapsulated in nanoemulsion: overcoming drug resistance

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INTRODUCTION

The anticancer chemotherapy is aimed at the tumor cell elimination via pharmacological induction of apoptosis or mitotic catastrophes. Another effective approach is administration of cytostatic drugs causing cell cycle arrest in tumors. For example, paclitaxel (taxol) is a potent cytotoxic and cytostatic drug which is often used for treatment of human ovarian or breast cancer.

Unfortunately, some fractions of drug-treated cancer cells may survive by selecting for adaptive mutations or reprogramming cellular regulation to involve alternative pathways that confer drug resistance. This is one of the major problems in chemotherapy because such survived cancer cells acquire tolerance to many drugs and become germs of the refractory and relapsing tumors.

Because functional inhibition of heat shock protein 90 (Hsp90) breaks multiple Hsp90-dependent pathways ensuring tumor cell progression and survival, pharmacological Hsp90 inhibitors, such as 17-N-allilamino-17-demethoxygeldanamycin (17AAG or tanespimycin), may repress tumors and be synergistic with other anticancer drugs (Stravopodis D. et al. 2007; Kabakov A. 2009). In addition, Hsp90 catalyzes functioning of membrane P-glycoprotein (Bertram J. et al. 1996) that performs cell clearance from entered toxins and contributes to multidrug resistance of tumors. The Hsp90 inhibition with 17AAG may suppress this P-glycoprotein-mediated mechanism thus increasing the general efficacy of chemotherapy (Radujkovic A. et al. 2005).

Co-administration of 17AAG with paclitaxel may be used in combinative schemes of anticancer chemotherapy (Ramalingam S. et al. 2008). While both the drugs are poorly soluble and require oil-based formulations, we co-encapsulated them into oil-in-water nanoemulsions to overcome the P-glycoprotein-mediated drug resistance and achieve the synergism in antitumor effects.

MATERIALS AND METHODS

In the present comparative study, we used paclitaxel (or paclitaxel-rhodamine-123) and 17AAG (tanespimycin) as single agents or in combination with each other. Both the drugs were simultaneously encapsulated into oil-in-water nanoemulsion by means of serial sonication of their joint solution in polyoxyl castor oil (Cremophor) mixed with water phase.

Human tumor-derived cell lines, HeLa (cervical cancer) and MCF-7 (breast cancer), were taken for our *in vitro* experiments with the drug (co-)treatments. Additionally, murine Ehrlich ascites carcinoma growing in a peritoneal cavity of mice (Kabakov A. et al., 1995) was herein used for modeling of the *in vivo* drug (co-)administration.

The drug-induced cytotoxicity (post-treatment tumor cell death/survival) was assessed in TUNEL, annexin V-staining, and clonogenic or MTT-assays using commercially available kits and standard protocols from manufacturers. In the case of *in vivo* growing ascites Ehrlich carcinoma, portions of the cell-containing ascites fluid were harvested by a syringe from the living animals; then the cell death percentage was determined in the harvested samples.

17AAG-induced inhibition of the pumping-drug-out function of P-glycoprotein was evaluated on duration of retaining of paclitaxel-rhodamine-123 inside the drug-treated tumor cells which are gradually liberated from the label. The integral analyses of the rhodamine fluorescence intensity per cell were performed on a flow cytometer (FACS Vantage) according to routine techniques.

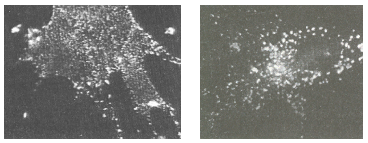
RESULTS AND DISCUSSION

Formation of the stable oil-in-water nanoemulsions containing the drug(s) was confirmed by optical and physico-chemical analyses. No signs of intermolecular interactions between the two co-encapsulated drugs were detected. Thus, paclitaxel and 17AAG (tanespimycin) seem quite compatible with each other as components of the same nanoemulsive formulation.

The antitumor effects of such nanoemulsions were tested in both *in vitro* and *in vivo* oncology-relevant models. Comparison of the effects has revealed obvious advantages of the nanoemulsions containing both the drugs (paclitaxel + 17AAG). In the case of such combining, the nanoemulsive formulations were well entrapped by tumor cells (**Figs. 1, 2**) while significantly longer retaining of pacltaxel inside treated HeLa cells (data not shown), MCF-7 cells (**Table 1**) and Ehrlich ascites carcinoma cells (**Table 2**) took place.

Drug(s) in nano-emulsion	Fluorescence per cell (%)		Apoptotic cells (%)	
	16 h	***24 h***	***16 h***	***24 h***
Paclitaxel alone	46±7	20±3	18±4	31±5
Paclitaxel + 17AAG	61±8*	39±5*	26±5*	58±7*

Table 1: Levels of paclitaxel-rhodamine-123 and apoptosis in MCF-7 breast cancer cells treated with nanoemulsions containing paclitaxel-rhodamine-123 without or with 17AAG. The data are mean ± S.E. of 4 independent measurements; * - significant difference from unmarked values, $p < 0.05$.



A **B**

Figure 1: Binding onto cell surface (A) and subsequent intracellular uptake (B) of the fluorescent labeled nanoemulsion containing co-encapsulated paclitaxel-rhodamine-123 and 17AAG.
(Object: cultured MCF-7 breast cancer cells.)

Duration of the intracellular drug retaining nicely correlated with the percentage of apoptosis in the nanoemulsion-treated HeLa cells (not shown), MCF-7 breast cancer cells (**Table 1**) and Ehrlich ascites carcinoma cells growing *in vivo* (**Table 2**). The same tendency was found in other cell death/viability determinations, such as clonogenicity and MTT-assay (data not shown). The improved antitumor effects of the two-drug-containing nanoemulsions appear to be associated with

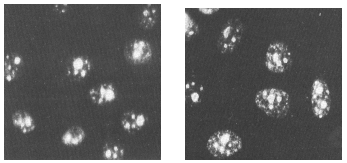
the 17AAG-induced inhibition of the Hsp90-dependent pumping-drug-out function of membrane P-glycoprotein in target cancer cells.

As generally accepted biomarkers of the 17AAG-induced Hsp90 inhibition, we revealed the specific depletion of Akt and Raf-1 in the tumor cells treated with the two-drug-containing nanoemulsions while no depletion of these marker proteins was found in the tumor cells treated with the nanoemulsive formulation containing paclitaxel alone (data not shown). In addition to the 17AAG-provoked dysfunction of P-glycoprotein, depletion of Akt and/or Raf-1 can also contribute to the intensification of apoptosis achieved under the co-encapsulation of paclitaxel and 17AAG into the same nanoemulsion.

Importantly, both the active drug uptake (**Fig. 2B**) and the longer drug retaining and the synergism in drug-induced cytotoxicity (see **Table 2**) were also manifested in the *in vivo* model following intraperitoneal injection of the two-drug-containing nanoemulsions in mice with ascites Ehrlich carcinoma.

Drug(s) in nano-emulsion	Fluorescence per cell (%)		Apoptotic cells (%)	
	16 h	***24 h***	***16 h***	***24 h***
Paclitaxel alone	54±6	28±3	20±3	45±6
Paclitaxel + 17AAG	70±7*	48±5*	29±4*	62±7*

Table 2: Levels of paclitaxel-rhodamine-123 and apoptosis in Ehrlich carcinoma cells growing in mice. Nanoemulsions containing paclitaxel-rhodamine-123 without or with 17AAG were injected into peritoneal cavity of mice. The data are mean ± S.E. of 4 independent measurements; * - significant difference from unmarked values, $p < 0.05$.



A **B**

Figure 2: Uptake of the fluorescent labeled nanoemulsion with co-encapsulated paclitaxel-rhodamine-123 and 17AAG by Ehrlich carcinoma cells treated *in vitro* (A) or *in vivo* (B) as a result of intraperitoneal injection.

Besides the enhancement of apoptosis (**Table 2**), the *in vivo* co-administration of paclitaxel and 17AAG against murine Ehrlich carcinoma has demonstrated the synergistic cytotoxicity in MTT-assays on the tumor cell samples harvested from mice (data not shown).

CONCLUSIONS

Our study provides the so called "proof-of-principle" for an approach when paclitaxel and 17AAG are administered in combination, as components of the same nano-emulsive formulation, to achieve the improved therapeutic effect against cancer. Herein, the co-encapsulation of paclitaxel and 17AAG into the oil-in-water nanoemulsions enables to resolve the problems of solubility and bioavailability, so that both the poorly soluble drugs are effectively delivered into target tumor cells and affect these cells.

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The synergism in cytotoxicity under combining of paclitaxel and 17AAG appears to be caused by (i) overcoming the P-glycoprotein-mediated multidrug resistance and (ii) promoting the apoptotic response in cancer cells, while both these causes are a result of the functional inhibition of Hsp90 with 17AAG.

Overall, such an approach with co-encapsulation of a hydrophobic toxin and 17AAG into the same nanoemulsive formulation seems quite promising to be used in terms of modern anticancer chemotherapy.

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