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ORIGINAL ARTICLE

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Cytotoxic activity of a new paclitaxel formulation, Pacliex, in vitro and in vivo

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Abstract *Background*: The paclitaxel formulation, Taxol (Bristol-Myers Squibb), is one of the most effective anticancer agents used today. However; it is associated with serious side effects believed to be caused by the Cremophor EL used for its formulation. *Aim*: To evaluate the cytotoxic activity of a new paclitaxel formulation, Pacliex (developed by Oasmia Pharmaceutical, Uppsala, Sweden), a mixed micelles preparation in which an amphiphilic synthetic derivative of retinoic acid replaced Cremophor EL/ethanol vehicle. *Method*: In this study, three model systems were used to evaluate the cytotoxic activity of Pacliex and other paclitaxel preparations. The cytotoxic activities of Pacliex, Taxol and paclitaxel in ethanol were investigated against a panel of ten human tumor cell lines using the fluorometric microculture cytotoxicity assay (FMCA). Low- and high- proliferating in vitro hollow fiber model of two cell lines, the leukemia CCRF-CEM and the myeloma RPMI 8226/S cell lines, were used to assess the cytotoxic activity of the three formulations. The in vivo hollow fiber model of the two cell lines was used for assessment of Pacliex and Taxol activity. The [3-4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay was utilized to analyze the in vitro and in vivo hollow fiber data. *Result*: Pacliex was somewhat more effective than Taxol in the more sensitive cell lines. The activity of Taxol was more pronounced in the resistant cell lines due to an additive effect of the vehicle used. The three formulations showed similar activity in both the low- and high-proliferating in vitro hollow fiber cultures. The in vivo hollow fiber cytotoxic activity of Pacliex was similar to that of Taxol. Putting all the results together, it was found that all the three formulations had similar in vitro and in vivo activity. *Conclusion*: The three in vitro and in vivo models confirmed the similarity of the cytotoxic activities of Pacliex and Taxol. Considering the above, Pacliex could be an interesting alternative Cremophor EL-free formulation of paclitaxel.

Keywords Paclitaxel · Pacliex · Taxol · In vitro · In vivo · Hollow fiber

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Introduction

The clinically available formulation of the taxane, paclitaxel, Taxol (Bristol-Myers Squibb) has antitumor activity against ovarian and breast carcinoma as well as a variety of other tumors [5, 23]. Paclitaxel is highly lipophilic and insoluble in water. In the Taxol formulation, paclitaxel is dissolved in a 50:50 v/v mixture of Cremophor EL and dehydrated ethanol. Problems with this formulation are the well-known toxicity of Cremophor EL, the hypersensitivity reactions to Cremophor EL [29], and the fact that Cremophor EL may also contribute to the toxic effects of paclitaxel such as neurotoxicity [30].

Efforts are currently being made to develop alternative paclitaxel formulations to bypass the limitations of the current formulation. Some of these alternatives involve the use of a prodrug incorporated in lipid emulsion [19] or in liposome [4], conjugated with a polymer [20], in a nanoparticle colloidal suspension [14] or in mixed micelles [1] have shown promising preclinical and clinical results.

Pacliex is a new Cremophor EL-free paclitaxel formulation developed by Oasmia Pharmaceutical, Uppsala, Sweden. Pacliex comprises a complex between paclitaxel and the surfactant Oasmia (XR17) as a freeze-dried powder that can form mixed micelles after reconstruction in saline-based Oasmia vehicle (OV). Oasmia surfactant consists of an equimolar mixture of two isoforms of an amphiphilic synthetic derivative of retinoic acid that can solubilize water-insoluble substances such as paclitaxel. A clear micellar solution of paclitaxel is formed as a result of mixing the above-mentioned components. A clear micellar solution of Pacliex, formed as a result, is suitable for parenteral administration.

A model system of ten human tumor cell lines representing defined types of drug resistance has been used in our laboratory for the initial in vitro evaluation and preliminary mechanistic classification of anticancer agents [7]. The hollow fiber assay [13] was developed at the National Cancer Institute, US, as a preliminary rapid in vivo screening method for evaluating novel compounds emerging from in vitro cell line screening. The hollow fiber model in immunocompetent rats [15] provides the possibility of studying the activity of cytotoxic drugs against different tumor types within the same individual animal in parallel with monitoring hematological toxicity and pharmacokinetics. Furthermore, it has been shown that tumor cells can be cultured inside hollow fibers up to several weeks to form a heterogeneous slowly proliferating in vitro solid tumor model [3, 12].

In this study, we used these three model systems to evaluate the cytotoxic activity of the new paclitaxel formulation Pacliex and to compare its cytotoxic activity to that of Taxol and paclitaxel (Indena) dissolved in ethanol.

Materials and methods

Reagents and drugs

A carbonate buffered culture medium RPMI-1640 (HyClone, Cramlington, UK) supplemented with 10% inactivated FCS, 2 m*M* glutamine, 50 μg/ml of streptomycin and 60 μg/ml of penicillin was used throughout. Fluorescein diacetate (FDA, Sigma, St Louis, Mo.) was dissolved in DMSO and kept frozen (−20°C) as a stock solution protected from light. 2.5% of protamine sulfate (1%; Sigma). MTT ([3-4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Sigma) was used for the hollow fiber staining procedure. The 5 mg/ml stock solution of MTT in phosphate-buffered saline (PBS; pH 7.4 Hyclone) was kept frozen (−20°C) and protected from light. Pacliex, the Oasmia surfactant XR17 and the OV were freshly prepared at Oasmia Pharmaceutical. The OV contained 0.15 *M* sodium chloride (saline) plus 5 m*M* sodium acetate. Paclitaxel (Indena) was reconstituted to 5 mg/ml in methanol which was further diluted in ethanol, 1:1.5 v/v. Taxol 6 mg/ml was obtained from the hospital pharmacy. Taxol and paclitaxel were diluted further with PBS to the final concentrations used. The three formulations are referred to as Pacliex, Taxol and paclitaxel in the following text.

Human tumor cell-line panel and drug exposure

A human cell line panel of four sensitive parental cell lines, five drug-resistant sublines representing different mechanisms of resistance, and one cell line with primary resistance were used to evaluate the activity of the formulations (Table 1) [7].

Taxol, Pacliex, paclitaxel and XR17 were tested at six concentrations in fivefold serial dilutions from a maximum of 200 μg/ml for the more resistant cell lines, NCI-H69, H69AR, RPMI 8226/Dox40 and ACHN. For the rest of the cell lines, the maximum concentration tested was 1.6 μg/ml. Drug solutions at ten times the desired concentrations were placed at 20 μl/well in 96-well microtiter plates (Nunc, Roskilde, Denmark). Each drug and concentration was tested in triplicate. Six wells with cells but without drugs served as control and six wells with only culture medium as blank. Cell seeding density varied between 10×10^4 and 20×10^4 cells/well.

The fluorometric microculture cytotoxicity assay (FMCA) and quantification of results

FMCA is based on the measurement of fluorescence generated from hydrolysis of FDA to fluorescent fluorescein by cells with intact plasma membranes as described in detail previously [16]. The plates were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 72 h. At the end of the incubation period the plates were centrifuged (1000 rpm, 5 min) and the medium was removed by aspiration. After one wash in PBS,

Table 1 The panel of ten human tumor cell lines used in the study, their origin, the agents used for subline selection and the proposed mechanisms of resistance

Parental line	Resistant line	Origin	Selecting agent	Resistance associated with
CCRF-CEM	CEM/VM-1	T cell leukemia	Teniposide	Topoisomerase II
U-937-GTB	U-937-vcr	Histocytic lymphoma	Vincristine	Tubulin
RPMI 8226/S	RPMI 8226/LR5	Myeloma	Melphalan	Glutathione
RPMI 8226/S	RPMI 8226/Dox40	Myeloma	Doxorubicin	Pgp
NCI-H69	H69AR	Small cell lung cancer	Doxorubicin	MRP
ACHN	–	Renal adenocarcinoma		

100 μl/well of FDA dissolved in a physiological buffer (10 μg/ml) was added. The plates were incubated for 45 min and the fluorescence generated from each well was measured in Fluoroscan II (Labsystems Oy, Helsinki, Finland). The fluorescence generated is proportional to the number of intact cells in the well.

Quality criteria for a successful analysis included a fluorescence signal in the control wells of more than ten times the mean blank value, a mean coefficient of variation (CV) in the control wells of less than 30% and the CVs for triplicate experimental wells of less than 8%.

Cell survival is presented as a survival index (SI), defined as the fluorescence in the experimental wells expressed as a percentage of that in the control wells, with values in the blank wells subtracted. All data from two or three experiments were included in the analysis.

In vitro hollow fiber model

The hollow fiber procedure was modified from that described by Hollingshead et al. [13]. Briefly, polyvinylidene fluoride (PVDF) hollow fibers (500 kDa molecular weight cut-off, 1 mm inner diameter; Spectrum, Laguna Hills, Calif.) were flushed with and incubated in 70% ethanol for 72 h and autoclaved in deionized water. On the filling day, the fibers were washed with supplemented medium, flushed with air and filled with cell suspension using a 0.9-mm needle and a 1-ml syringe. CCRF-CEM and RPMI 8226/S cell lines (1×10^6 cells/ml) were cultured inside the hollow fibers for 3 or 14 days prior to a 72-h drug exposure [12]. Pacliex, Taxol and paclitaxel were tested at six concentrations obtained by fivefold serial dilutions from a maximum of 0.1 μg/ml. Duplicate fibers were incubated in six-well plates containing 3 ml drug solution in medium. The effect of Cremophor EL and XR17 on 3-day and 14-day hollow fiber cultures was tested at 8.8 μg/ml and 0.15 μg/ml, respectively, corresponding to their contents in the highest concentrations of Taxol and Pacliex used in this study. Cell growth was followed in duplicate fibers on day 3, 6, 10, 14 and 17. Two independent experiments were performed and data from four fibers for each concentration were pooled. The densities of living cells were assessed as described below.

In vivo hollow fiber model

CCRF-CEM and RPMI 8226/S cell lines (2×10^6 cells/ml) were cultured for 48 h inside the hollow fibers in vitro prior to subcutaneous implantation in the back of six anesthetized male Sprague-Dawley rats (weighing on average ± SD 284 ± 10 g; Charles River, Uppsala, Sweden) [15]. Each rat carried four fibers of each cell type. Pacliex and Taxol were prepared within 24 h prior to drug administration and were diluted to a final paclitaxel concentration of 0.5 mg/ml. For placebo treatment normal saline was used. One day after implantation the rats received slow bolus injections of 4 mg/kg of Taxol ($n=2$), Pacliex ($n=2$) or saline ($n=2$) into the jugularis vein using catheters (PE-50 polyethylene tubing, 0.58 mm inner diameter). Five days after drug administration the fibers were retrieved from the back of the anesthetized rats, collected in six-well plates filled with culture medium and stained within 4 h. The rats were acclimatized for 1 week before the start of the procedure and had free access to standard pellet food and water throughout the experiment. The in vitro cell growth of the cell lines was followed in parallel to the in vivo part, and assessed in duplicate fibers on the filling, implantation and retrieval days. Two independent experiments were performed. The Animal Ethics Committee in Uppsala approved the study (C67/99 and C53/02).

Assessment of density of living cells in hollow fibers

The density of living cells was evaluated by staining with MTT which is converted by metabolically active cells to insoluble blue formazan crystals. The procedure as described in detail previously [15], was modified by omitting the trypsin washing step for the in vivo fibers and using 500 μl instead of 250 μl of DMSO for extraction. The fibers were incubated in six-well plates with 3 ml of supplemented medium and 200 μl MTT at 37°C for 4 h. The staining medium was then replaced by PBS with 2.5% of protamine sulfate and the plates were incubated at 4°C overnight. The fibers were cut into two pieces and put into 24-well plates to dry until the end of the experiment. The formazan was extracted with 500 μl DMSO for 4 h at room temperature. Of the 500 μl extract, 150 μl was transferred to a flat-bottomed 96-well plate for reading the absorbance at 570 nm in a microplate spectrophotometer (SPECTRAmax PLUS, Molecular Device Corporation, Sunnyvale, Calif.). Blank values for DMSO were subtracted from each reading. Extraction and reading of all fibers from one experiment were performed at the end of that experiment.

The cell densities in the treated fibers from the in vitro experiments are expressed as a survival index (SI%), defined as the absorbance of the treated fibers as a percentage of that in control fibers.

The cell densities of the retrieved fibers from the animals are expressed as net growth (%), defined as:

$$\frac{(\text{absorbance on the retrieval day} - \text{mean absorbance on the implantation day})}{\text{mean absorbance on the implantation day}}.$$

Net growth values were then subjected to an outlier analysis performed according to Grubb's test for detecting outliers (http://www.graphpad.com/quickcalcs/GrubbsExplain.cfm). A significant outlier is a value found to be too far from the other values and

considered unreliable. Outliers were excluded from the further analysis and are not included in the results. Data from repeated in vivo experiments ($n = 2$, fiber numbers 12–16) were pooled and hence a net growth of −100% represents total cell kill, while a value greater than 0% represents growth of the cells in the fiber compared with the implantation day.

Curve fitting

Concentration-effect data from the cell line panel and from the in vitro hollow fiber model were fitted to a sigmoid dose–response equation with variable slope using nonlinear regression in the GraphPad Prism software (GraphPad Software, San Diego, Calif.). IC_{50} (the concentration giving 50% of the maximum effect), bottom plateau (%) and slope parameters were estimated by the curve fitting. For some of the data sets, estimations of baseline and maximum effect were not possible from the data; therefore 0% and 100% cell survival were set as the maximum effect and baseline, respectively.

Statistical calculation and correlation analysis

The estimated log IC_{50}, the bottom plateau and the net growth were compared between the three groups using one-way ANOVA. Student's *t*-test was used for the in vivo part and when necessary for the other parts of the study. Sets of ten log IC_{50} values from the all cell line panels for each drug were correlated using Pearson's correlation coefficient.

Results

Cell line panel

Pacliex, Taxol, paclitaxel and XR17 concentration–response curves in CCRF-CEM and RPMI 8226/S cell lines are shown in Fig. 1. Pacliex showed a slightly higher effect than Taxol in both cell types, whereas XR17 was nontoxic at the tested concentrations.

The activity of the three formulations, expressed as IC_{50}, for all cell lines and the Pacliex/Taxol activity ratios are presented in Table 2. The IC_{50} values for Pacliex were significantly lower ($P < 0.05$) than those for Taxol against CCRF-CEM, RPMI 8226/S cells and the subline RPMI 8226/LR5. The two formulations were equally active with no significant difference ($P > 0.05$) between the IC_{50} values of the cell lines U-937-GTB; CEM/VM-1 and H69AR. The IC_{50} values for Pacliex were found to be significantly higher ($P < 0.05$) than those for Taxol in the more resistant cell lines U-937-vcr,

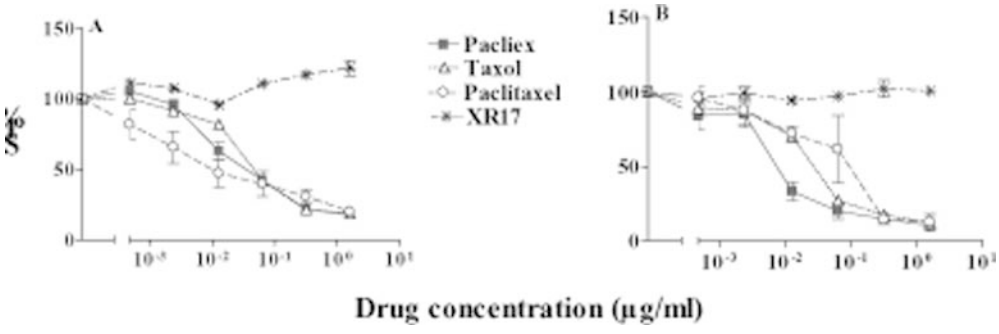

Fig. 1a, b Concentration–response curves generated from a 72-h exposure of (**a**) CCRF-CEM and (**b**) RPMI 8226/S cell suspension cultures in 96-well plates to Pacliex, Taxol, paclitaxel and XR17. The results are presented as a means ± SEM of two or three independent experiments, with six to nine SI% data points

Table 2 IC_{50} values (SEM) estimated from the concentration–effect curves obtained from a 72-h exposure of ten cell lines to Pacliex, Taxol and paclitaxel as well as Pacliex/Taxol activity ratios (the data presented are pooled data from two or three independent experiment yielding six to nine SI% data points) (the IC_{50} of XR17 was not achieved in any of the ten cell lines used)

Cell line	IC_{50} (SEM) (µg/ml)			Pacliex/Taxol activity ratio[a]
	Pacliex	Taxol	Paclitaxel	
CCRF-CEM	0.018 (0.07)*	0.034 (0.04)	0.005 (0.17)	0.5
CEM/VM-1	0.055 (0.05)	0.045 (0.06)	0.079 (0.21)	1.2
U-937-GTB	0.041 (0.10)	0.028 (0.02)	0.077 (0.09)	1.5
U-937-vcr	0.965 (0.05)*	0.290 (0.09)	1.49 (0.10)	3.3
RPMI 8226/S	0.007 (0.11)*	0.023 (0.07)	0.054 (0.18)	0.3
RPMI 8226/LR5	0.006 (0.08)*	0.018 (0.04)	0.013 (0.20)	0.3
RPMI 8226/Dox40	21 (0.04)*	14 (0.05)	> 200	1.5
NCI-H69	128 (0.06)*	29 (0.14)	> 200	3.2
H69AR	0.147 (0.12)	0.213 (0.14)	0.425 (0.36)	0.7
ACHN	> 200	38 (0.08)	> 200	> 5

*$P < 0.05$, vs Taxol

[a]Obtained by dividing the IC_{50} for Pacliex with that of Taxol

NCI-H69 and RPMI 8226/Dox40. ACHN showed sensitivity to Taxol only. The IC_{50} values were not achieved for paclitaxel in NCI-H69 and RPMI 8226/Dox40 cell lines. The overall difference in the IC_{50} values between Pacliex and Taxol was around three times or less in all cell lines except ACHN.

The relationship between the activities of the three formulations was evaluated by correlating their log IC_{50} values in all ten cell lines. The activity of Pacliex, Taxol and paclitaxel produced high correlation coefficients (Pearson $r = 0.98$).

In vitro hollow fiber model

Figure 2 displays the concentration–effect curves of Pacliex, Taxol and paclitaxel in 3-day and 14-day in vitro hollow fiber cultures of CCRF-CEM (Fig. 2a, b) and RPMI 8226/S (Fig. 2c, d) cell lines. The insets show the cell proliferation inside the hollow fibers during the experimental period. The proliferation slowed down after 10 days incubation. Table 3 presents the estimated IC_{50} values, maximum effect (bottom plateau %) and slope for all three formulations. For RPMI 8226/S, there was a tendency for the 14-day culture curves to be shallower than the 3-day culture curves. The bottom plateau level was significantly higher (38–47% and 28–30% viable cells) in the 14-day than in the 3-day culture curves (14–18% and 11–13% viable cells, $P < 0.05$) for CCRF-CEM and RPMI 8226/S, respectively. In terms of IC_{50} values, there was no significant difference ($P > 0.05$) between the activities of each formulation in the 3-day and 14-day cultures of the two cell lines.

There was no significant difference ($P > 0.05$) between the IC_{50} values of the three formulations in the 3-day cultures in either of the two cell lines. Taxol was more

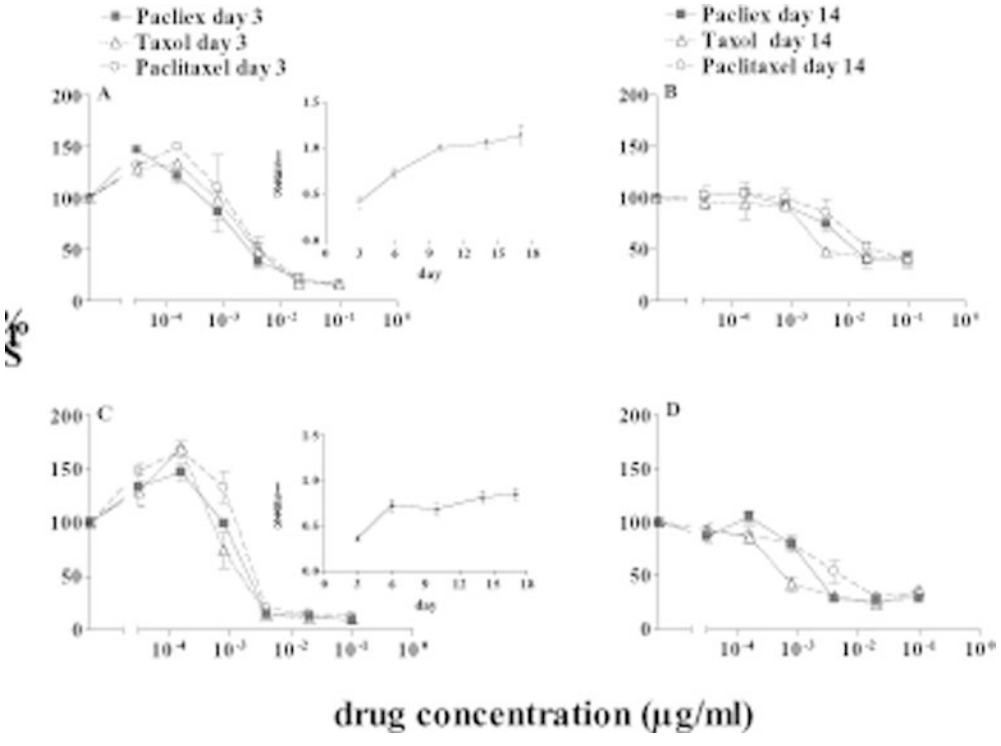

Fig. 2a–d The concentration–effect curves of Pacliex, Taxol and paclitaxel in 3-day and 14-day in vitro hollow fiber cultures of (**a**,**b**) CCRF-CEM and (**c**,**d**) RPMI 8226/S cell lines after a 72-h exposure. Insets are the growth curves of the two cell lines (two experiments, four fibers, means ± SEM)

Table 3 The IC_{50} values, the bottom plateau and the slope estimated from the concentration–effect curves obtained following a 72-h exposure of 3-day and 14-day hollow fiber cultures of CCRF-CEM and RPMI 8226/S to Pacliex, Taxol and paclitaxel (two experiments, data from four fibers were pooled)

Cell line	Culture time (days)	Pacliex			Taxol			Paclitaxel		
		IC_{50} (μg/ml) (SEM)	Bottom plateau (%)	Slope	IC_{50} (μg/ml) (SEM)	Bottom plateau (%)	Slope	IC_{50} (μg/ml) (SEM)	Bottom plateau (%)	Slope
CCRF-CEM	3	0.002 (0.14)	16	−1.4	0.003 (0.09)	14	−1.6	0.003 (0.16)	18	−2
	14	0.005 (0.09)	39*	−1.9	0.002 (0.11)	38*	−2.4	0.006 (0.17)	47*	−2.1
RPMI 8226/S	3	0.001 (0.2)	11	−3.7	0.001 (0.08)	11	−3	0.002 (0.2)	13	−3.7
	14	0.001 (0.16)	28*	−1	0.0004 (0.09)	29*	−1.9	0.002 (0.16)	30*	−1.1

*$P < 0.05$, vs corresponding 3-day culture

effective ($P < 0.05$) than Pacliex and paclitaxel in the 14-day cultures. However, the difference between Taxol and the other two formulations was evident at only one concentration in each cell line (0.0004 μg/ml for CCRF-CEM, and 0.0008 μg/ml for RPMI 8226/S). Cremophor EL and XR17 had no effect in 3-day and 14-day in vitro hollow fiber cultures of CCRF-CEM and RPMI 8226/S cell lines at the concentrations used in the formulations in this experiment (data not shown). The bottom plateau values of the three formulations in CCRF-CEM and RPMI 8226/S 14-day hollow fiber cultures were significantly higher than in the cell line cultures ($P < 0.01$). There was no difference in the bottom plateau values of the three formulations between 3-day hollow fiber and cell line cultures (data not shown). The IC_{50} values for the three formulations in the cell line cultures were significantly higher ($P < 0.05$) than those from the hollow fiber cultures except for paclitaxel in CCRF-CEM cultures (Tables 2 and 3).

In vivo hollow fiber model

In the two experiments, 13–16 out of 16 fibers were retrieved in each group. Two fibers in the Taxol group, one from each cell line, were judged as being outliers and were hence excluded from the analysis.

The cytotoxic effects of Pacliex, Taxol and placebo on CCRF-CEM and RPMI 8226/S cell lines are illustrated in Fig. 3 a, b. Pacliex and Taxol induced similar growth inhibition of CCRF-CEM cells and killing of RPMI 8226/S cells in in vivo hollow fiber cultures ($P > 0.05$). The net growth in the control rats indicates a more rapid in vivo proliferation of CCRF-CEM leukemia cells line of RPMI 8226/S myeloma cells. Figure 3 c,d shows the in vitro proliferation of CCRF-CEM and RPMI 8226/S cells inside the hollow fibers during the experimental period.

Discussion

Paclitaxel is a highly promising drug against ovarian and breast cancer [23], but several drawbacks are associated its clinical formulation Taxol caused by the formulation vehicle Cremophor EL/ethanol. This has stimulated research to develop alternative formulations based on vehicles other than Cremophor EL/ethanol. Cremophor EL is a polyethoxylated triglyceride of unsaturated ricinoleic acid. Unsaturated fatty acids have been found to be toxic to tumor cells, probably through the effect of free radicals generated from fatty acids peroxidation of membrane lipids [2, 8], and they may directly increase the lipid fluidity of the surface membrane of the cells [21]. Cremophor EL has been shown to enhance paclitaxel activity [6, 10] and might itself have a cytotoxic effect in vitro [9]. The XR17 surfactant is a derivative of retinoic acid. Retinoids have been shown to regulate the proliferation and death of a variety of normal and malignant cells [18]. Synergistic cytotoxicity exhibited by combination treatment of some retinoids with paclitaxel has been reported recently [27, 28]. However, formulations of paclitaxel with natural or synthetic retinoids are not yet described [24]. Therefore it seemed to be interesting to develop a combination of paclitaxel and XR17 in

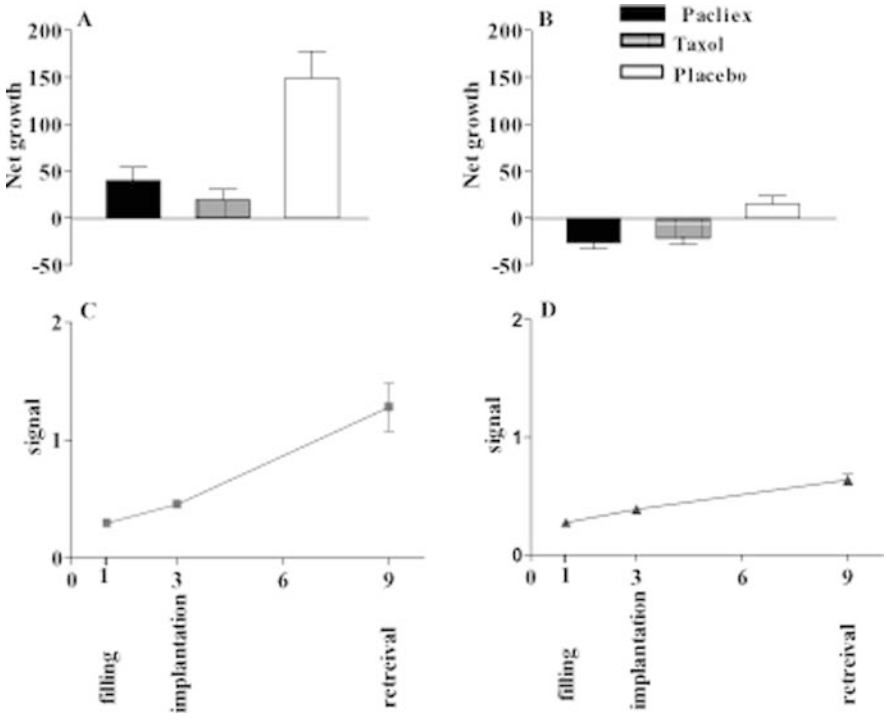

Fig. 3a–d Effects of Pacliex and Taxol on (**a**) CCRF-CEM and (**b**) RPMI 8226/S cells in the in vivo hollow fibers. The in vitro growth of (**c**) CCRF-CEM and (**d**) RPMI 8226/S cells in parallel to the in vivo study (two experiments, number of fibers 12–16, means ± SEM)

order to obtain a water-soluble formulation that is Cremophor EL-free and with a similar activity to Taxol.

In all cell lines tested a close relationship between the activity of Pacliex, Taxol and paclitaxel was found, as shown by the very high correlation coefficients. This was interpreted to mean that paclitaxel is the active constituent that exerts most of the cytotoxic effect seen in vitro. It was remarkable, however, that Taxol and Pacliex were more active than paclitaxel alone in the resistant cell lines. In these resistant cell lines higher concentrations from the three formulations were used, which meant the introduction of higher surfactant concentrations. Cremophor EL alone has previously been shown to have a cytotoxic effect at high concentrations in vitro (IC_{50} 1–7 μg/ml as the corresponding concentrations of Taxol) [6]. This may explain the lower IC_{50} seen with Taxol compared to Pacliex in the resistant cell lines. Conversely, it should be noted that Pacliex is somewhat more effective than Taxol at lower Cremophor EL concentrations. Cremophor EL has a very limited volume of distribution [26] indicating that the cytotoxic activity will not be relevant to the in vivo situation [25, 26]. However, even though XR17 had no cytotoxic effect in any of the cell lines tested, its potentiating effect on paclitaxel activity cannot be excluded. Whether sufficiently high concentrations of XR17 can be reached in vivo to exert a potentiating effect is not known.

The sensitivity difference between the 3-day and 14-day in vitro hollow fiber cell cultures and the shallowness of the 14-day culture concentration–effect relationship may have been due to the limited diffusion through the tumor layers or due to drug resistance related to microenvironmental or proliferative heterogeneity. It has been reported previously that tumor cells show a tendency to form a heterogeneous cell population with necrotic cores when cultured for several weeks inside the hollow fibers [3, 22] and that shallow curves could be an indication of heterogeneous cell populations [12, 17]. It is possible that the three formulations kill the tumor cells in the outer layers leaving the inner ones unaffected as there was no difference in the formulation potency in the 3-day and 14-day cultures. As impaired drug penetration into the tumor is a major obstacle for an effective anticancer drug therapy, it could be argued that formulations that are more active against slowly proliferating hollow fiber cultures possess the highly desirable property of efficient penetration into the tumor cell layers. As evidenced by their similar activity in both low- and high-proliferating cultures, the surfactant/solvent system apparently had no influence on the capability of any of the formulations to penetrate the multiple layers of the tumor mass.

The differences in cell viability observed between low proliferating hollow fiber and cell line cultures is supported by similar observation reported by Frankel et al. [11] when studying the cytotoxic effect of Taxol on monolayer and spheroid cultures of ovarian cancer cells. We believe that the in vitro hollow fiber model could be used to provide information to answer questions regarding drug penetration through tumor cell layers and to elucidate the possible effect of tumor heterogeneity in the response of tumors to cytotoxic drugs that could not be answered from in vitro cell line culture models.

Using three different model systems, we have been able to show that the cytotoxic activity of Pacliex, a new paclitaxel formulation, is similar to that of Taxol. This makes Pacliex, which avoids the Cremophor EL-related toxic effects, is an interesting alternative paclitaxel formulation, and it is currently being prepared for phase I/II clinical trials.

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