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ORIGINAL ARTICLE

# Antitumour effect of combination treatment with Sabarubicin (MEN 10755) and cis-platin (DDP) in human lung tumour xenograft

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**Abstract**
*Purpose* Sabarubicin (MEN 10755), a new disaccaride anthracycline, has shown greater efficacy than Doxorubicin in a large panel of preclinical models and now it is in phase II clinical trials. Its promising antitumour activity promoted considerable interest to combine Sabarubicin with other antitumour agents. Thus, the purpose of this study was to evaluate in vitro cytotoxic effects and in vivo antitumour activities produced by the combination of Sabarubicin and cisplatin (DDP).
*Methods* The antitumour effect of Sabarubicin and DDP association was investigated, in vitro and in vivo, in preclinical models of lung cancer i.e.: the non-small cell lung carcinoma (NSCLC) H460 and the small-cell lung carcinoma (SCLC) GLC4 in terms of synergism, additivity or antagonism in order to establish the best schedule for the combined treatment. Further, the correlation between antitumour activity and the pharmacokinetic parameters of the studied combination was also evaluated.
*Results* The drug combination in vitro was in general more cytotoxic than the single drug alone, indicating the presence of a synergistic effect in both tumour cell lines. Also, in the xenograft experiments a superior antitumoral effect was observed when Sabarubicin was combined with DDP. The antitumour efficacy of Sabarubicin (6 mg/kg q4d × 5) combined with DDP (6 mg/kg q4d × 3) greatly depended on the schedule of administration. In H460 tumour line, the sequential combination was more effective than the simultaneous administration of the two agents, although the antitumour efficacy was not dependent on the sequence of combination. On the other hand, a strong sequence-dependent effect was observed when Sabarubicin was combined with DDP in SCLC, GLC4. In particular, the highest value of LCK = 6.7 was obtained when administration of DDP followed by 24 h that of Sabarubicin. Pharmacokinetics of Sabarubicin in combination with DDP was evaluated at 6 mg/kg for both drugs with different sequential schedule. The experimental data showed no evidence for pharmacokinetics drug–drug interaction.
*Conclusion* These preclinical results indicate the potential for a strong antitumour activity in lung tumours of the combination Sabarubicin and DDP. In particular, in SCLC the best response should be given by a sequence with administration of Sabarubicin followed 24 h later by that of DDP. Clinical trials based on these results are ongoing.

**Keywords** Sabarubicin · Cis-platin · Lung cancer xenograft · Combination treatment

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## Introduction

Lung cancer is the most frequent and most lethal cancer worldwide. Only in the USA it accounts for almost one third of all cancer deaths in males (32%) and in females (25%). Despite some progress in recent decades, lung cancer remains the leading cause of death in most developed countries. The outlook for patients following diagnosis is poor—80% die within 1 year with only 5–15% surviving at 5 years [1–4].

Lung cancer is usually classified as NSCLC or SCLC. SCLC accounts for 20–25% of all bronchogenic malignancies

and is characterised clinically by a propensity for early metastases and initial high-response rates to chemotherapy and radiotherapy. NSCLC is less sensitive to chemotherapy and surgical resection is the treatment of choice. Most SCLCs acquire multi-drug resistance, whereas NSCLCs tend to be intrinsically resistant to chemotherapy. Less than 5% of SCLC patients currently survive 5 years past initial diagnosis; whereas, the 5-year survival rate for patients diagnosed with NSCLC is about 15% [5–7].

Combination chemotherapy has significantly improved the response rate for a variety of human tumours and the establishment of an optimal regimen for combination therapies with currently used and newly developed drugs is an important step to achieve higher response and longer survival [8].

A combination of several agents may be more effective than the best single agent for several reasons: (a) separate agents have different limiting toxicity and can therefore be combined at doses close to their maximum single-agent levels; (b) the combination of two or more agents may result in a drug synergy in their efficacy; (c) last but not least, combination of drugs characterised by a different mechanism of action may be a useful tool for overcoming drug resistance [9–11].

Many clinical trials showed that cisplatin (DDP)-based therapy with other cytotoxic agents is the best combination chemotherapy for lung cancer, since it improves survival rates significantly in patients affected by this disease [11]. In the case of patients with SCLC, a distinction should be drawn between those with limited stage disease (LD) and those with extensive disease (ED). Roughly, one-third present LD, while the remainder are ED. Surgery and radiation therapy have been used as a single modality or in combined modality therapy, although they have resulted in few cures. The standard treatment strategy for LD–SCLC and ED–SCLC is chemoradiotherapy and chemotherapy, respectively [8].

Regarding SCLC chemotherapy the regimens most often used are: Etoposide and DDP (EP; the standard regimen in the United States); Cyclophosphamide, Doxorubicin and Vincristine (CAV); Cyclophosphamide, Doxorubicin, and Etoposide (CAE); or Cyclophosphamide, Doxorubicin, Vincristine, and Etoposide (CAVE). Despite extensive studies no substantial improvement (as prolongation of overall survival) in the chemotherapy of SCLC has been achieved in the last 25 years [8–10].

Recently, in a phase I–II clinical study, some authors have demonstrated a significant response of ED–SCLC to the treatment of DDP combined with the synthetic anthracycline Amrubicin [12]. Nevertheless, no anthracyclines are approved in combination with DDP for the treatment of SCLC, and the gold registered standard therapy remain the association of Etoposide with DDP [13, 14].

Sabarubicin (MEN 10755), a new synthetic disaccharide anthracycline, has shown greater efficacy than Doxorubicin in a large panel of preclinical models [15]. Moreover in a phase II clinical trial it has shown, as a single agent, a good and promising activity on SCLC [16]. Since Sabarubicin is a topoisomerase II poison [17] as well as Etoposide, the antitumour activity of Sabarubicin and DDP association in preclinical models of lung cancer was investigated. DDP is the reference compound in the combination therapy of lung cancer and our study aimed at identifying the best ratio and schedule of the two drugs to get better efficacy and lower toxicity, thus allowing to improve the rational design of protocols for new clinical trials in SCLC and NSCLC [14].

In our study, the antitumour effect of Sabarubicin and DDP association was investigated, in vitro and in vivo, in preclinical models of lung cancer in terms of synergism, additivity or antagonism in order to establish the best schedule for the combined treatment. Further, the correlation between antitumour activity and the pharmacokinetic parameters of the studied combination was also evaluated.

## Materials and methods

### Drugs

Sabarubicin (MEN 10755) (7-*O*-[2,6-dideoxy-4-*O*-(2,3,6-trideoxy-3-amino-α-L-*lyxo*-hexopyranosyl)-α-L-*lyxo*-hexopyranosyl]-4-demethoxy-14-hydroxydaunomycinone hydrochloride), synthesized by Menarini Ricerche-Pisa (Italy), was dissolved in saline (NaCl, 0.9%) immediately prior to use. DDP was purchased from Sigma, dissolved in dimethyl sulfoxide (DMSO) and diluted in saline immediately before use (DMSO final concentration was equivalent to 1%). Daunomycin hydrochloride was purchased by Sigma.

### Tumour lines

NSCLC cell line H460 was purchased from American Type Culture Collection. Human SCLC cell line GLC4 was kindly provided by Dr. E.G.E. De Vries (University of Groningen, The Netherlands). The cells were propagated in RPMI 1640 supplemented with 10% foetal bovine serum, at 37°C in $CO_2$ air incubator.

### Cell growth inhibition

Drug-induced cytotoxic effects were evaluated by using Alamar Blue Assay [18]. In brief, H460 and GLC4 cells were incubated in 96-well microtitration plates at a cellular density to ensure a logarithmic growth throughout the experiments. After cell attachment, fresh medium containing drugs was added to each well for 24 h. Both

agents in the combination test were added in sequence and at the same time. Tumour cells were then placed in drug-free medium for further 48 h. The tumour cells' growth inhibition, calculated on dose–response curves, was analysed according to Chou and Talalay methods [19].

## Combination index assay

The effects of drug combinations in terms of synergy, additivity or antagonism were analysed by the median effect plot [19, 20]. The cytotoxicity of each compound was analysed using the CalcuSyn program, Windows software for dose effect analysis (BIOSOFT®, Copyright, 1996). The combination index (CI) method is based on that described by Chou and Talalay [19]. The ranges of CI are established from those already described [19]. CI $< 1$; $= 1$; and $>1$ indicate synergism, additive effect and antagonism, respectively. The CI was plotted as a function of the fractional affected according to CalcuSyn program.

## Evaluation of antitumor activity

Tumours originated from subcutaneous (sc) in vivo injection of 10–20 $\times$ $10^6$ cells. Tumour cells were suspended in 0.2 ml of 0.9% NaCl sterile solution and injected sc into the right flank of female nude mice. Tumour growth was followed by calibre measurement of length and width at predetermined (twice weekly) time. Tumour volume (TV) was calculated using the formula [21]:

$$\text{Volume in mm}^3 = \left(\text{width}^2 \times \text{length}\right)/2.$$

Both drugs were administered *i.v.* at a dose volume of 10 ml/kg at different dosages on H460 and GLC4 tumour lines. The dosages for Sabarubicin obtained by previous studies were: 4 and 6 mg/kg body weight as single administration with q4d $\times$ 5 schedule corresponding, respectively, to 20 and 30 mg/kg as total dose. On DDP treatment, as well as for Sabarubicin, the dosages were: 4 and 6 mg/kg body weight as single administration with a q4d $\times$ 3 schedule and corresponding, respectively, to 12 and 18 mg/kg as total dose. The timing and the sequence of drug administration are shown in the scheme of Table 1. Drug treatment started when tumours were approximately 50–100 $mm^3$ in volume. The following effects achieved by the drug treatment were evaluated:

- Tumour volume inhibition % (TVI%) in treated versus control mice determined at the nadir of tumour volume in the treated groups.

**Table 1** Cytotoxic activity (μM) of Sabarubicin and DDP on human carcinoma cell lines

| Cell lines | Sabarubicin | DDP |
|---|---|---|
| H460 | 0.076 ± 0.012 | 0.20 ± 0.03 |
| GLC4 | 0.036 ± 0.022 | 0.16 ± 0.03 |

$IC_{50}$ values (mean ± SE) were determined from dose–response curves from at least three independent experiments

- Log Cell Kill (LCK) in treated mice according to the formula [22]: $(T - C)/(Dt \times 3.32)$ where T and C were the days taken by the tumours in treated and control mice, respectively, to reach a predetermined volume specified in each experiment. Dt is the tumour-doubling time calculated for each tumour line, from semilogarithmic best-fit curve of tumour volume in the control group plotted versus time, when the growth of tumour was in the exponential phase.

Toxicity considered as toxic deaths correspond to the number of mice deaths showing no measurable tumour mass or mice dead before the first mice-death of the control group.

## Pharmacokinetics study

Female NIH/Swiss mice (20–25 g) purchased at Charles River had free access to food and water. Both DDP and Sabarubicin were administered i.v. at dosage corresponding to 6 mg/kg at a dose volume of 10 ml/kg. The drugs were administered at 24 h interval, in one group first Sabarubicin and then DDP and in another group vice versa. Blood samples were collected from anaesthetised animals (5 per time point) by cardiac puncture at 0, 5, 10, 20, 60 min, and 2, 3, 5, 24, 48, 72 h after i.v. administration into heparinised polypropylene micro-tube.

## Plasma sample preparation and analysis

Plasma samples were thawed at room temperature and one composite sample per time point of each group was prepared by pooling the five original samples. For the analysis of Sabarubicin, 50 μl of 1 μg/ml Daunomycin (internal standard) and 1 ml of acetonitrile were added to 100 μl of each plasma sample. Proteins were removed by centrifugation at 14,900$\times g$ at 4°C for 10 min. Supernatant was evaporated to dryness and reconstituted in 80 μl of mobile phase. For the determination of total DDP by flow injection inductively coupled plasma mass spectrometry (FI-ICP-MS), plasma samples were diluted 50-fold with a nitric acid (1%) and Triton X-100 (0.05%) mixture containing 5 ng/ml of iridium used as internal standard. Free DDP was determined after

plasma ultrafiltration at 5,000×*g* for 1 h at 4°C with a Centricon ultrafiltration system (NMWL 10000 cut-off, Millipore Corporation, Ireland) followed by dilution as described above.

Sabarubicin concentrations were determined by high-performance liquid chromatography as reported previously [23]. A 20-µl aliquot of each deproteinated plasma sample was injected into the analytical column (Phenomenex Luna® C-18, 3 µm, 4.6 × 150 mm) and eluted at room temperature and at a constant flow rate of 1 ml/min with a mobile phase of 0.01 M sodium dihydrogen phosphate/acetonitrile (70:30, v/v) adjusted to pH 3 with orthophosphoric acid. The HPLC system consisted of a model 125 solvent delivery pump (Beckman Coulter®), equipped with a model 234 automatic sample injector (Gilson®) and a spectrofluorimetric detector (Shimadzu®, model RF-10AXL) set at 490 and 570 nm (excitation and emission wavelengths, respectively). The entire system was controlled by the "32 Karat" software (Version 5.0, Beckman Coulter®). The quantification limit of the method was 4 ng/ml. Total and free DDP concentrations were determined by measuring platinum (as $^{195}$Pt) with ICP-MS [24, 25] using FI as the sample introduction method. Platinum and iridium working solutions were prepared in diluted hydrochloric acid (ultrapure, 1%) from standard solutions at 1,000 µg/ml (BDH, Poole, UK). The instrumentation used was a Sciex Elan DRC II spectrometer (Perkin–Elmer, Norwalk, CT, USA), equipped with a Meinhard nebuliser and a cyclonic spray chamber. Samples (40 µl) were introduced into the spectrometer by means of a FIAS-400MS (Perkin–Elmer) FI system. The limit of quantification of the method was 2 ng/ml.

### Pharmacokinetic parameters

The pharmacokinetic parameters of Sabarubicin and total and free DDP were calculated from the mean plasma concentration versus time curves according to a compartment-model independent analysis [26]. The following parameters were calculated: the area under the concentration–time curve from time zero to the time of the last quantifiable concentration (AUC 0–*t*), the plasma clearance (CL), the terminal plasma half-life ($t_{1/2}$) and the apparent volume of distribution ($V_z$).

## Results

### Combination of Sabarubicin and DDP in vitro is more active than each single drug

The cytotoxic effects of Sabarubicin plus DDP were evaluated in GLC4 and H460 tumour cell lines. GLC4 tumour line appeared slightly more sensitive to cytotoxic effect of both compounds (Table 1). Three different sequences of drug combinations were used: DDP that preceded Sabarubicin; Sabarubicin that preceded DDP and, finally, both compounds incubated simultaneously. Results were evaluated by computational analysis on the basis of the Chou and Talalay method [19]. In H460 tumour cell line, the data indicated the presence of a synergistic effect in all tested conditions (Fig. 1). However, the best synergistic effect was detected when Sabarubicin preceded DDP (Fig. 1, middle panel). The effect observed in terms of CI curve in the remaining two conditions was similar, but with a lower order of magnitude (Fig. 1, upper and lower panel).

Figure 2 shows the results obtained in the SCLC, GLC4. Also in this tumour line a synergistic effect was found mainly when DDP preceded Sabarubicin or when both compounds were used simultaneously (Fig. 2, upper and lower panel, respectively). A neutral or slight antagonism was instead observed when Sabarubicin was given before DDP (Fig. 2, middle panel).

### Additive effect of Sabarubicin and DDP combination in vivo on H460 tumour xenograft

For the studies of drug combinations in tumour xenografts the schedules indicated in the Table 2 were used.

The growth of H460 tumour was quite resistant to chemotherapy. However, it was more sensitive to the antitumour effect of Sabarubicin than to that of DDP: in fact, values of TVI% and LCK were 80% and 0.8 and 59% and 0.3, respectively (Table 3). The result of combination studies of Sabarubicin plus DDP at their dose of 4 mg/kg on H460 tumour line indicated that the combination treatment was more effective than the treatment with the single agents, hence suggesting an additive effect. However, the tumour, after a temporary reduction, started to grow again shortly after the last treatment, about 15 days after tumour implantation (Fig. 3). In this tumour line the antitumour activity was independent of the sequence used for the combination, resulting in a comparable additive effect in the two sequential combinations (Fig. 3; Table 3).

### Synergistic effect of Sabarubicin and DDP combination in vivo on GLC4 tumour xenograft

As single agent the antitumour activity of Sabarubicin was lower with respect to DDP in this tumour line. In fact, TVI% and LCK were 75% and 1.2 LCK and 96% and 2.6, respectively (Table 3; Fig. 4). A substantial increase in antitumour activity was evident with the combination. This synergism became outstanding (LCK = 6.7) when Sabarubicin preceded DDP (Table 3; Fig. 4). Differently from

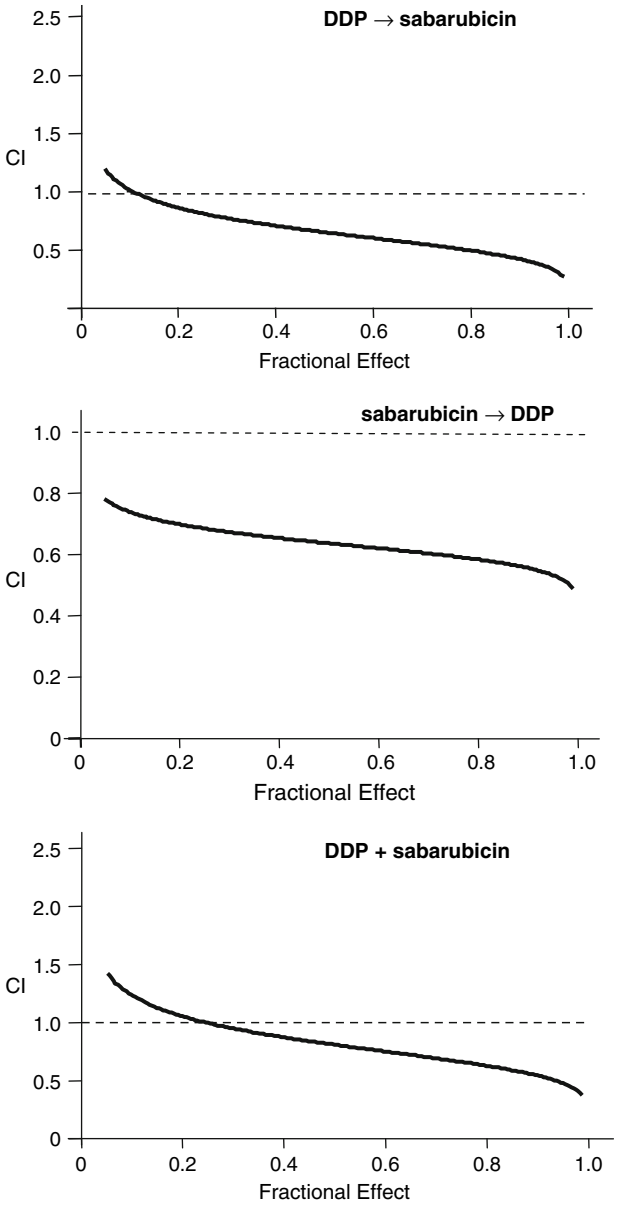


**Fig. 1** Data resulting from combination of Sabarubicin and DDP in H460 tumour cell line, analysed by the median effect analysis program. *Upper panel* sequential combination: first DDP and after Sabarubicin; *middle panel* sequential combination: first Sabarubicin and after DDP; *lower panel* simultaneous exposure of DDP and Sabarubicin

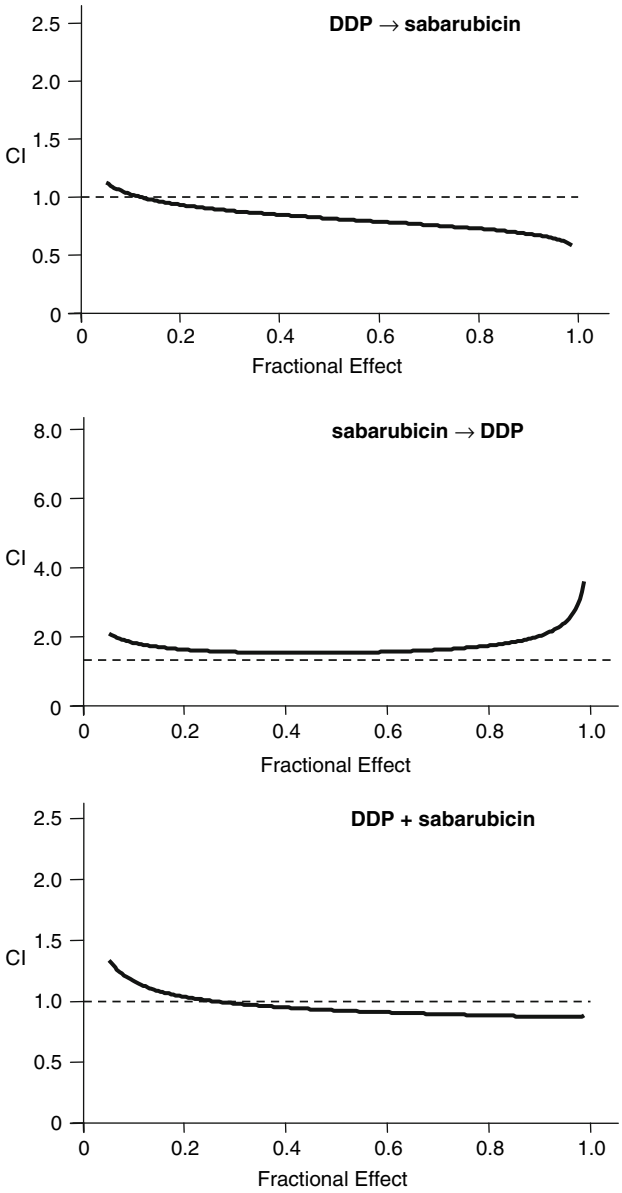


**Fig. 2** Data resulting from combination of Sabarubicin and DDP in GLC4 tumour cells, analysed by the median effect analysis program. *Upper panel* sequential combination: first DDP and after Sabarubicin; *middle panel* sequential combination: first Sabarubicin and after DDP; *lower panel* simultaneous exposure of DDP plus Sabarubicin

H460, the antitumour activity observed on GLC4 tumour line was strongly dependent on the sequence used for the drug combination. A temporary reduction in the growth of tumour volume was observed in the schedule in which DDP preceded Sabarubicin with values of TVI = 99% and LCK = 3.8. However, a more pronounced and long-lasting inhibition of tumour growth was observed when Sabarubicin preceded DDP as showed by the best result in terms of LCK = 6.7 (Table 3). This schedule and dose timing resulted in a tumour volume under the threshold of measurability ($<10$ mm$^3$) up to 55 days after tumour implantation (Fig. 4). It is to be noted that these schedules and dosage were well tolerated in all mice treated and no toxic effects were observed.

## Pharmacokinetic results

In order to evaluate whether the promising combination of DDP and Sabarubicin was based on pharmacokinetic interaction the plasma concentrations of DDP and Sabarubicin

**Table 2** Schematic representation of the treatment schedule and dose for DDP and Sabarubicin in the xenograft studies

| Drugs | Schedule | Days of treatment starting from tumour cells inoculation | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 4 | 5 | 8 | 9 | 12 | 13 | 16 | 20 |
| DDP | q4d × 3 | ▲ | | ▲ | | ▲ | | | |
| Sabarubicin | q4d × 5 | ● | | ● | | ● | | ● | ● |
| Sabarubicin + DDP | | ● | ▲ | ● | ▲ | ● | ▲ | ● | ● |
| DDP + Sabarubicin | | ▲ | ● | ▲ | ● | ▲ | ● | ● | ● |

**Table 3** Antitumour activity of Sabarubicin in combination with DDP on GLC4 and H460 tumour xenograft

| Tumour xenograft | Compound | Dose (mg/kg) | Schedule | TVI (%) | LCK (1 g) | no. of deaths/total no. mice |
|---|---|---|---|---|---|---|
| H460 | DDP | 4 | Q4d × 3 (a) | 59 | 0.3 | 0/5 |
| | Sabarubicin | 4 | q4d × 5 (b) | 80 | 0.8 | 0/5 |
| | Sabarubicin + DDP | 4 + 4 | (a) + (b) | 95 | 1.3 | 1/5 |
| | DDP + Sabarubicin | 4 + 4 | (b) + (a) | 94 | 1.3 | 0/5 |
| GLC4 | DDP | 6 | q4d × 3 (a) | 96 | 2.6 | 0/5 |
| | Sabarubicin | 6 | q4d × 5 (b) | 75 | 1.2 | 0/5 |
| | Sabarubicin + DDP | 6 + 6 | (b) + (a) | 99 | 6.7 | 0/5 |
| | DDP + Sabarubicin | 6 + 6 | (a) + (b) | 99 | 3.8 | 0/5 |

were measured using a dose level of 6 mg/kg according to the two sequential combination schedule i.e.: (1) first DDP and Sabarubicin after 24 h; (2) first Sabarubicin and DDP after 24 h.

Pharmacokinetic data are summarized in Table 4. Plasma concentrations of free and total DDP were measurable up to 72 h after administration, whereas those of Sabarubicin were measurable up to 48 h. The values of clearance of the Sabarubicin and DDP were quite similar to that reported in the literature [27, 28]. The kinetic of Sabarubicin and DDP remain unchanged regarding the schedule used and no kinetic interactions were observed.

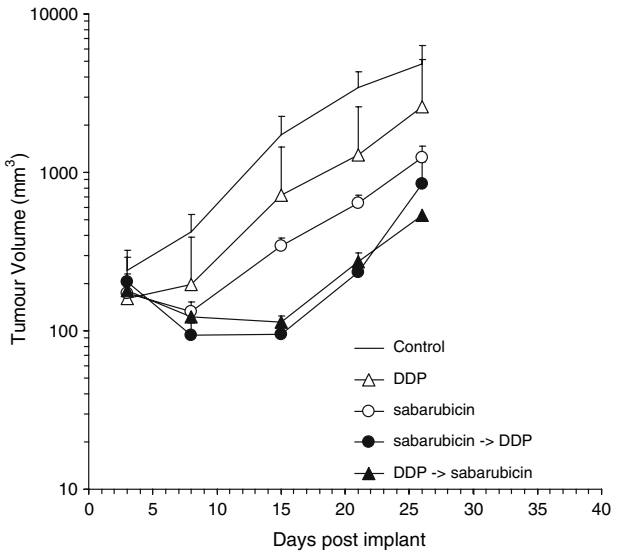


**Fig. 3** Tumour growth inhibition in H460 tumour xenograft obtained by Sabarubicin and DDP alone or in combination treatment. (*open triangle*) DDP, 6 mg/kg alone; (*open circle*) Sabarubicin, 6 mg/Kg alone; (*filled circle*) Sabarubicin that preceded DDP; (*filled triangle*) DDP that preceded Sabarubicin. Each point on the graph indicated the average value of tumour volume obtained from five tumours + S.E

## Discussion

Anthracyclines have been employed in the treatment of tumours for many years and research continues to seek agents with the same efficacy and reduced toxicity [29]. Doxorubicin and Epirubicin are classified as active agents for SCLC. Doxorubicin has been used as a constituent of combination therapy for SCLC in the CAV (cyclophosphamide, Doxorubicin and Vincristine) and CAP (cyclophosphamide, Doxorubicin and Cisplatin) regimens. Epirubicin has shown 50% and 48% response rates in two clinical studies in previously untreated patients with ED–SCLC [14]. However, current combination modalities containing Doxorubicin or Epirubicin are not registered and are not in use in the therapy of SCLC, while the preference is for the combination between DDP and Etoposide.

Sabarubicin, a novel disaccharide anthracycline, displays an interesting preclinical profile characterised by potent topoisomerase II-mediated DNA strand breaks [17] and a significant antitumour activity against tumour naturally resistant to Doxorubicin [15]. Results from a recent clinical trial in ED–SCLC have demonstrated an excellent antitumour acivity of Sabarubicin, as single agent, in terms of response rate and overall survival comparable to the combination DDP/Etoposide [16].

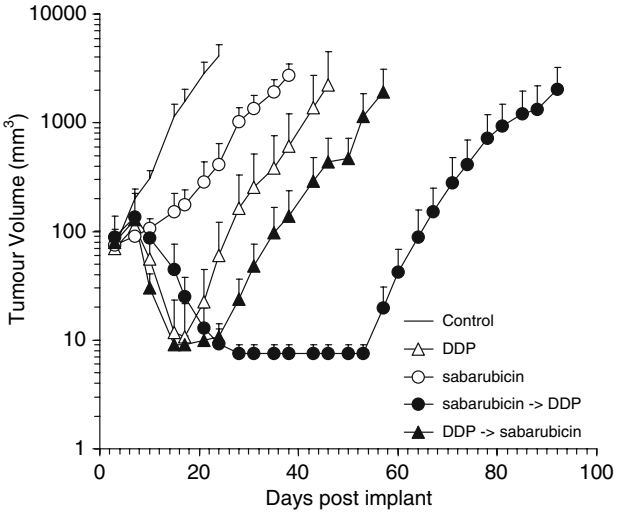


**Fig. 4** Tumour growth inhibition in GLC4 tumour xenograft obtained by Sabarubicin and DDP alone or in combination treatment. (*open triangle* DDP, 6 mg/kg alone; (*open circle*) Sabarubicin, 6 mg/Kg alone; (*filled circle*) Sabarubicin that preceded DDP; (*filled triangle*) DDP that preceded Sabarubicin. Each point on the graph indicated the average value of tumour volume obtained from five tumours + S.E

So far, extensive clinical studies have explored the potential of DDP-based combination therapy and the common approach is to combine DDP with one, two or more known non-platinum anticancer drugs that have a favourable therapeutic index [8, 30]. The association of DDP with anthracyclines is unusual in lung cancer therapy and only a few preclinical studies are available, whereas other DDP-based combinations are preferred, i.e. with Gemcitabine and Taxol [3].

Several in vitro and in vivo tumour-cell models have been examined to ascertain whether they are reliable in predicting clinical utility. It is a common opinion that human xenografts have a superior clinical predictive value for cancer drug development with respect to mouse allograft model, in particular for NSCL and ovarian cancers when panels of xenografts were used [31, 32].

Moreover, the importance of these models is enhanced when they are exploited as a useful ''filter'' for schedule selection and protocol design of a subsequent clinical trial [33].

Potentiation of drug activity is the basis for using combined-protocol treatments. In the present study, the best regimen for combination therapy with Sabarubicin and DDP was investigated, in particular regarding the sequence of combination, in different lung-tumour models both in vitro and in vivo.

The effect of drug combinations observed in vivo in this study is distinct from that observed in in vitro studies. As already reported in the literature, it is a common observation that in vivo effect of combination in tumour xenografts may differ from that observed in vitro [31–33].

The results obtained in this investigation clearly indicate a different response rate depending on the tumour model histotype. Moreover, the antitumour combination efficacy appeared strongly dependent on the sequence of dosing schedule used.

In vitro data for the SCLC GLC4 show that this tumour line was slightly more sensitive than H460, in terms of $IC_{50}$ value, to the antiproliferative effects of both agents, Sabarubicin and DDP. The effects of drug combinations in H460 were always synergic regardless of the schedule used. In GLC4, a slight antagonist effect was observed when Sabarubicin preceded DDP, indicating the importance of sequence of administration by the combination of DDP/Sabarubicin in GLC4 tumour line in vitro. This finding is in agreement with a host of preclinical studies suggesting that a careful design of the sequence and timing of administration of the two agents might be critical to avoid potential antagonism while optimizing the potential therapeutic effects [34–37].

In in vivo studies for H460 tumour line only an additive effect between Sabarubicin and DDP was observed (Table 3). On the other hand, a strong effect of combinations was observed in GLC4 xenograft, especially when Sabarubicin preceded DDP. Since this effect was distinct from that reported in in vitro study, where a neutral or slight antagonistic effect of this drug sequence combination was observed, an attempt was made to elucidate this pattern.

First, the mechanism(s) underlying the schedule-dependent activity of the combination was investigated from a pharmacokinetic point of view. However, the kinetics of both drugs remained unchanged and independent of the treatment schedule. Thus, pharmacokinetic properties and drug–drug interaction cannot explain the striking and

**Table 4** Main plasma pharmacokinetic parameters for Sabarubicin and DDP (total and free) administered 6 mg/kg *i.v.* in two sequential manners in nude mice

| PK parameters | Sabarubicin → DDP | | | DDP → Sabarubicin | | |
|---|---|---|---|---|---|---|
| | total DDP | free DDP | Sabarubicin | total DDP | free DDP | Sabarubicin |
| AUC(0–*t*) (ng*h/l) | 66.0 | 2.6 | 4.6 | 86.9 | 2.9 | 6.1 |
| CL (l/h/kg) | 0.06 | 1.51 | 1.31 | 0.05 | 1.33 | 0.99 |
| $t_{1/2}$ (h) | 79.2 | 45.6 | 9.4 | 65.0 | 62.8 | 9.8 |
| $V_z$ (l/kg) | 6.8 | 99.5 | 17.8 | 4.2 | 120.8 | 14.0 |

long-lasting antitumour effects of Sabarubicin followed by DDP in GLC4 tumours.

Currently, it is well known that antitumour agents kill tumour cells by different mechanisms, the most important being the topoisomerase II poisoning by Sabarubicin [29] and the DNA alkylation and the inhibition of DNA synthesis by DDP [11]. It may be speculated that DDP could increase the persistance of topoisomerase II-mediated breaks induced by Sabarubicin, thus resulting in a more efficient triggering of apoptotic pathways [11, 38].

Since synergistic effects are not observed in vitro in GLC4 cells, it is possible that the expression of critical proteins for the apoptotic mechanism is induced during the in vivo growth of GLC4 tumour [39].

Combination therapy has been the basis for most success stories in cancer treatment, which is understandable when components of a combination have a favourable pharmacological interaction (same target, but different large-organ toxicities). A barrier to major advancements in combination therapy, however, has been the lack of understanding about the intersection of critical signalling pathways. Synergy might be induced through the effect of drugs on the same as well as parallel pathways. Because the number of drug combinations is limitless, a strategy for determining the most promising combinations and prioritizing their evaluation is crucial.

Although the precise molecular mechanism of interaction between Sabarubicin and DDP has not yet been identified, a clear therapeutic gain has been demonstrated in preclinical models. These results point to the potential therapeutic value of Sabarubicin in cancer combination treatment therapy. Further studies will be necessary to clarify the mechanisms operating in vivo in the tumour mass.

Clinical studies based on the combination Sabarubicin/DDP—the schedule of which prescribes the administration of Sabarubicin first and of DDP 24 h later—are currently in progress in ED–SCLC patients.

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