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ORIGINAL ARTICLE

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# A phase I clinical and pharmacokinetic study of the multi-drug resistance protein-1 (MRP-1) inhibitor sulindac, in combination with epirubicin in patients with advanced cancer

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**Abstract** *Purpose:* Multi-drug resistance mediated by ATP-binding cassette trans-membrane protein pumps is an important cause of cancer treatment failure. Sulindac has been shown to be a competitive substrate for the clinically important resistance protein, multi-drug resistance protein-1 (MRP-1), and thus might enhance the anti-cancer activity of substrate chemotherapeutic agents, e.g. anthracyclines. *Methods:* We conducted a dose-escalating, single arm, prospective, open label, non-randomised phase I trial of epirubicin (75 mg/m$^2$) in combination with escalating oral doses of sulindac (0–800 mg) in patients with advanced cancer to identify an appropriate dose of sulindac to use in future resistance studies. Anthracycline and sulindac pharmacokinetics were studied in cycles 1 and 3. *Results:* Seventeen patients (8 breast, 3 lung, 2 bowel, 1 melanoma, 1 renal, 1 ovarian and 1 of unknown primary origin, 16/17 having had prior chemotherapy) were enrolled. Eight patients received a full six cycles of treatment; 14 patients received three or more cycles. Dose-limiting toxicity was observed in two patients at 800 mg sulindac (1 renal impairment, 1 fatal haemoptysis in a patient with advanced lung cancer), and sulindac 600 mg was deemed to be the maximum tolerated dose. Sulindac had no effect on epirubicin pharmacokinetics. Among 15 patients with evaluable tumour, two partial responses were seen (malignant melanoma and breast cancer). Four others had prolonged stable disease. *Conclusion*: Epirubicin 75 mg/m$^2$ and sulindac 600 mg are the recommended doses for phase II studies for these agents in combination.

**Keywords** Clinical trial · MDR · MRP-1 · Phase I · Resistance modulation · Sulindac · Epirubicin

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## Introduction

Some cancer cells display resistance to a variety of structurally and functionally unrelated chemotherapeutic drugs, a phenomenon, referred to as "multi-drug resistance" (MDR). One potentially important mechanism of MDR involves trans-membrane cellular efflux pumps of the ATP-binding cassette (ABC) superfamily [1]. The molecular structure and function of several of these pumps have been elucidated. The first to be recognised was P-glycoprotein (P-gp) [2], an important physiological cellular defence mechanism which is found in healthy intestinal, renal and hepatic tissue, and in the blood–brain barrier. P-gp is also over-expressed in a wide variety of human tumours where it may contribute to tumour cell resistance to several groups of chemotherapeutic agents, prominently–naturally occurring cytotoxic drugs [3]. The ability of these pumps to extrude anti-cancer agents can be inhibited in vitro by the co-administration of competing non-cytotoxic substrates (e.g. cyclosporine A, verapamil), prompting speculation that it might also be possible to

overcome drug resistance in the clinic. However, despite promising results in single arm studies, randomised trials, in which patients received chemotherapy with or without putative modulators of P-gp, have all been negative [4, 5]. More recent studies involving highly specific inhibitors (e.g. elacridar and tariquidar) have also been negative [6].

These generally disappointing results prompted speculation that MDR may be a poor clinical target. While this may be true, it is also possible that the very heterogeneity of resistance phenotypes might limit the benefit which inhibition of one system might achieve. According to this interpretation, clinically meaningfully reversal of resistance might necessitate the selective inhibition of multiple pumps. In addition to P-gp, several other ABC transporters have been shown to mediate multiple drug resistance. One of these, Multi-drug Resistance Protein (MRP-1), has also been extensively characterized [7]. Despite an overlap in substrate specificities with P-gp, MRP-1 is a structurally distinct pump, with a different mechanism of drug efflux. MRP-1 also appears to play a protective role in normal physiology, and in particular in defending the integrity of certain physiological barriers, e.g. blood–brain and blood–testis [8]. However, in knockout animals the absence of the MRP-1 gene has much less toxicological impact for an organism than does loss of P-gp [9].

MRP-1 may be responsible for drug-resistance in a broad range of tumour types, and is generally associated with poorer prognosis [10–13]. Although some P-gp inhibitors have also been shown to modulate MRP-1, there have been no clinical trials of specific MRP-1 inhibitors in oncology. In 1998 our group [13], and later others [14, 15], showed that organic anions including the NSAIDs, sulindac and indomethacin could inhibit MRP-1. Sulindac and its two primary metabolites, sulindac sulfide and sulindac sulfone, can all inhibit MRP-1-mediated drug resistance and efflux effectively at levels reported to be achievable in the blood [16]. Other possibly beneficial properties of sulindac include high oral bioavailability and a relatively long circulatory half-life, especially the metabolites which also inhibit MRP-1 [13, 17]. We have further demonstrated the ability of sulindac to enhance the anti-tumour activity of MRP-1 substrates in a xenograft tumour model over-expressing this pump [17]. There are several literature reports of clinical trials involving non-MRP-1 substrate chemotherapeutic agents with sulindac and/or metabolites including exisulind (sulindac sulfone). These trials are distinct from our own in that they sought to improve cancer drug action by inhibiting cyclooxygenase and/or increase the level of tumour apoptosis and all use protocols where there is prolonged administration of the sulindac agent [18, 19].

To identify an appropriate dose of sulindac to use in cancer resistance circumvention, we report the results of a phase I evaluation of single dose sulindac in combination with epirubicin, a widely active chemotherapy drug, which is also an MRP-1 substrate.

## Patients and methods

### Study design

The trial was a prospective, open-label, single arm, phase I evaluation of escalating doses of sulindac, administered in combination with a fixed standard dose of epirubicin (75 mg/m$^2$).

Eligibility criteria included: advanced incurable cancer; histologic or cytologic documentation of malignancy; measurable or evaluable disease; Karnofsky performance status of > 70%; no active cardiac disease or hypertension currently requiring treatment; resting systolic blood pressure > 100 mm Hg and heart rate > 50 per min; normal haematological evaluations including absolute neutrophil count: $> 1.0 \times 10^9\ l^{-1}$, platelet count $> 100 \times 10^9\ l^{-1}$, haemoglobin > 9 g/dl, normal prothrombin time and normal activated partial thromboplastin time; normal serum bilirubin and calcium; negative pregnancy test for females; adequate nutritional status and age greater than 18 years. All patients were required to sign an informed consent document indicating full understanding of the risks and implications of the study for them. Specific exclusion criteria included prior anthracycline or anthracenedione exposure, prior cardiac radiotherapy, active brain metastases, pregnant or lactating females and the presence of any other serious medical or psychiatric disease. Patients could not have been part of another clinical study in the previous 16 weeks and were not allowed to be treated with any other anti-cancer therapies during the study.

This trial was conducted to ICH/GCP standard following approval by the St. Vincent's University Hospital (Dublin, Ireland) Ethics Committee and the Irish Medicines Board, and conformed to the Helsinki declaration and associated World Medical Association amendments.

Pre-treatment evaluation included: clinical evaluation; blood biochemical profile; differential blood count; urine analysis and radionuclide gated blood pool cardiac ejection fraction (MUGA) or echocardiogram; relevant tumour assessments. Troponin I is an experimental marker of cardiac-specific damage [20, 21]. Associated with the sampling for drug analysis, two 10 ml heparinised blood samples were taken: one at the start of the treatment schedule and one 26 h later (24 h post-epirubicin administration) for analysis of cardiac Troponin I levels on cycle 1 and cycle 3 of the treatment schedule.

Successive cohorts of patients received escalating doses of sulindac (0–800 mg) orally with a fixed 75 mg/m$^2$ IV dose of epirubicin. Patients were not required to be in a fasting state. There was no intra-patient dose escalation. A minimum of three patients were treated at each dose level. In the event that any patient experienced grade 3 toxicity at any given level, an additional two patients were recruited at that level. Three patients must have completed at least two cycles of therapy prior to the study proceeding to the next level. Patients were

expected to receive six cycles of therapy approximately 21 days apart; however, not all patients received the full number of cycles due to disease progression or drug toxicity. The level 1 group received epirubicin alone, level 2 received 200 mg sulindac plus epirubicin, level 3 received 400 mg sulindac plus epirubicin, level 4 received 600 mg sulindac plus epirubicin and level 5 received 800 mg sulindac plus epirubicin. A level 6 of 1,000 mg of sulindac was planned but the study was terminated due to two dose-limiting toxicities emerging in level 5.

A standard blood biochemical assessment of each patient was performed in advance of each cycle of therapy. Cardiac ejection fraction was assessed on completion of the study with an additional follow-up within 3 months where the patient was available.

NCI common toxicity criteria were used to assess the severity of patient toxicity. Dose-limiting toxicity was defined in advance in the trial protocol as follows:

Nadir platelet count below $25 \times 10^3\ l^{-1}$ for more than 5 days, nadir granulocyte count less than $0.5 \times 10^3\ ml^{-1}$ with fever lasting more than 7 days or requiring administration of IV antibiotics. Development of grade 3 renal or hepatic toxicity or grade 2 renal or hepatic toxicity lasting more than 7 days.

Failure of serum creatinine to return to a value of $< 150$ μmol/l prior to the next schedule treatment. Failure of hepatic function to recover to the point that bilirubin is $< 1.5 \times$ the upper limit of normal. Development of grade II cardiac toxicity. Development of any other irreversible non-hematologic toxicity (NHT) greater than grade 1 (except alopecia), or any NHT greater than grade 2. A treatment delay of more than 14 days due to toxicity.

NCI-Response Evaluation Criteria in Solid Tumors (RECIST) guidelines were used to assess tumour response.

## Drug administration schedule

On the day of treatment patients received an IV dose of 3 mg granisetron as an anti-emetic. Level 1 patients (epirubicin alone) initially received no steroid medication; however, despite granisetron pre-medication, the emergence of grade 3 and 4 nausea and vomiting necessitated the inclusion of a standard dexamethasone administration scheme for all patients. This consisted of 16 mg IV dexamethasone immediately after granisetron administration followed by a reducing oral dexamethasone dose, 8 mg *b.i.d* the day after chemotherapy treatment, 4 mg *b.i.d.* on day 3 and a single 4 mg dose on day 4.

Immediately after the anti-emetic therapy, patients received the dose of sulindac appropriate to their drug level in standard 200 mg tablets of Clinoril™, i.e. level 1 received no sulindac while level 5 received four tablets. Sulindac was only administered once per cycle. Two hours later, patients received their dose of epirubicin as an IV infusion in normal saline/D5 W. Patients who developed neutropenic infection/fever on any cycle received ciprofloxacin 500 mg twice a day administered from days 8 to 15 (inclusive) on all subsequent cycles. With any neutropenic infection recurring on a subsequent cycle, despite use of prophylactic antibiotics, granulocyte colony-stimulating factor was administered in standard dosage on days 3 through 11 of subsequent cycles.

Eight 10 ml blood samples were taken at specific intervals for PK analysis as follows: 1 h after granisetron administration (55 min after patients received their sulindac dose and 60 min prior to receiving epirubicin). Taking the epirubicin administration as the start time, further 10 ml samples were taken 20, 30, 60, 180, 240, 360 and 1,440 min later. Although most of the 20-min sample were taken at the appropriate time, the duration of epirubicin administration for three of the patients was longer than 20 min so no 20-min sample was provided. The minor variation in sampling was incorporated into the model used as indicated in Pharmacokinetic analysis section. Clotted blood was centrifuged and the serum removed and stored frozen until analysis.

## Drug quantification

Epirubicin, two epirubicin metabolites (dihydroxyepirubicin and dihydroxyadriamycinone), sulindac and the two primary metabolites of sulindac (sulindac sulfide and sulindac sulfone) levels were quantitated in patient serum using reverse phase HPLC analysis. Modifications of the methods developed and validated by our laboratory were used to quantitate the anthracycline [13] and sulindac species were quantitated by combining the methods of Grgurinovich et al. [22] and Swanson et al. [23]. The details of these methods and their validation will be reported elsewhere (manuscript in preparation).

## Pharmacokinetic analysis

Epirubicin serum concentration–time profiles were analysed using the computer program WinNonlin Professional (v.2.1, Pharsight Corporation, Mountain View, Ca). To account for variations in the duration of infusions, the time profile for each patient was normalised to zero (min) and the dose was assumed to be administered as an intravenous bolus injection. Serum drug concentrations ($C_p$) were described using a biexponential equation:

$$C_p = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t}$$

where estimated intercept coefficients ($C_1$ and $C_2$) and exponents ($\lambda_1$ and $\lambda_2$) were used to calculate primary pharmacokinetic parameters, including: area under the concentration–time curve (AUC), total systemic clearance (CL), central volume ($V$), and steady-state volume of distribution ($V_{ss}$) [24].

Individual patient profiles were analysed, and goodness of fit and model selection were assessed by standard model fitting criteria [25]. Pharmacokinetic parameters for each group were reported as mean ± standard deviation and significance of differences were determined using a one-way ANOVA followed by a post hoc Dunnetts *t* test, where the control group did not receive sulindac ($P$ value $\leq 0.05$ was considered significant).

Sulindac and metabolite serum concentration-time profiles were generated using Sigma plot.(version 9, Systat, Point Richmond, CA). Individual curves were generated and compared. Gross comparisons were also generated by averaging the results for each drug/metabolites and the times at which the respective samples were taken.

### Immunohistochemical analysis of P-gp and MRP-1 protein expression

Formalin-fixed paraffin-embedded material was available for 12 of the patients included in this trial. Representative 5 μm sections of tissue blocks were cut using a microtome, mounted onto poly-L-lysine-coated slides and dried overnight at 37°C. Slides were stored at room temperature until required for staining. Immunohistochemical studies were performed broadly in accordance with the method of Hsu et al. [26], using an avidin–biotin horseradish peroxidase (HRP) conjugated kit (ABC) plus an appropriate secondary antibody. Procedures were optimised in our laboratory, as recently reported [10]. Specimens which had, in previous studies, been shown to express MRP-1 or P-gp were included as positive controls. Negative control slides in which sections were immunostained, as outlined, but primary antibody was omitted, were included in all experiments.

MRP-1 and P-gp immunohistochemical staining was evaluated semi-quantitatively according to the percentage of cells showing specific immunoreactivity, and the intensity of this immunoreactivity. A semi-quantitative measurement was used in which overall positivity of the tumour was assessed and a score of 0 was given where no staining was evident, + where up to 1–24% of cells showed positive staining; a score of ++ was given where 25–49% cells showed positive staining, a score of +++ where 50–74% of cells showed positive staining and ++++ for staining of 75% or more of cells. The intensity of immunoreactivity was scored as weak, medium or intense.

## Results

### Patient characteristics

A total of 17 patients were enrolled in the trial. Patient demographics were as follows: 11 females, 6 males; age range 41–78 years with a mean age of 61 years. Eight patients had breast carcinoma, three had cancers of lung and two of bowel origin, one of renal, one of ovarian origin, one melanoma and one whose primary tumour site was unknown. Sixteen of the patients had received prior chemotherapy, hormone or biological therapy. The median number of treatment cycles delivered was six (range one–six). Twelve patients (three at each of levels 1–4) received at least three cycles of protocol therapy. The breakdown of patient cycles and reasons for treatment discontinuation are outlined in Table 1.

### Toxicity

The treatment schedule was well tolerated with no treatment-specific toxicological differences between the epirubicin alone level and levels 2–4. Summary toxicities and their incidence are reported in Table 2. No patients needed treatment reduction or prophylactic of G-CSF support. Two of three patients who were treated with 800 mg of sulindac developed dose-limiting toxicities as defined by the trial protocol. One, a patient with advanced non-small cell lung cancer, developed a fatal haemoptysis 5 days after their third cycle of therapy, and a second patient developed significant renal impairment prompting withdrawal after 1 cycle of therapy. The second patient demonstrated elevated creatinine levels; however, this patient's creatine levels were elevated before therapy and he also received genamicin to treat febrile neutropenia. The level below (level 4, 600 mg) was deemed to be the maximum tolerated dose of sulindac in combination with 75 mg/m$^2$ epirubicin.

### Cardiac troponin levels

In general, there was a small fall in ejection fraction (as assessed by MUGA or Echocardiogram) over the course of therapy in the majority of patients on the trial as is common with anthracycline treatment. However, no consistent treatment-specific decline was evident in patients. At no time before or during therapy did the levels of Troponin I levels in any patients rise above the lower threshold of quantification (0.03 μg/l).

### Pharmacokinetics

#### *Epirubicin pharmacokinetics*

The time-course of epirubicin serum concentrations showed polyexponential disposition kinetics following intravenous infusion, and a standard biexponential pharmacokinetic model was found to capture the observed data well (Fig. 1). Pharmacokinetic parameters were estimated with relatively good precision and summary statistics are reported in Table 3. Mean total systemic clearance was 19.5 l/h in the control group and values ranged from 10.1 to 25.9 l/h in the presence of sulindac. No significant differences in epirubicin PK

**Table 1** Patient therapy cycles

| All patients, epirubicin 75 mg/m$^2$ + | Cycle 1 | Cycle 2 | Cycle 3 | Cycle 4 | Cycle 5 | Cycle 6 |
|---|---|---|---|---|---|---|
| Level 1,0 mg sulindac | 3 | 3 | 3 | 2 *patient withdrew | 2 | 2 |
| Level 2, 200 mg sulindac | 3 | 3 | 3 | 2 *patient progressed | 2 | 2 |
| Level 3, 400 mg sulindac | 4 | 4 | 3 *patient progressed | 3 | 3 | 3 |
| Level 4, 600 mg sulindac | 4 | 3 *patient progressed | 3 | 3 | 3 | 2 *patient progressed |
| Level 5, 800 mg sulindac | 3 | 2 *patient removed due to DLT | 2 *2nd DLT | | | |

**Table 2** Summary of the number and episodes per patient of grade 1–2 and grade 3–4 non-haematological chemotherapy-related toxicities documented on study

| Toxicity | Episodes (patients) | | | | |
|---|---|---|---|---|---|
| | Level 1 (*15 cycles*) | Level 2 (*15 cycles*) | Level 3 (*20 cycles*) | Level 4 (*18 cycles*) | Level 5 (*7 cycles*) |
| Infection | | | | | |
| Grade 1–2 | 1 (1) | 2 (1) | | | |
| Grade 3–4 | 2 (2) | | 3 (3) | 3 (3) | |
| Dyspnoea | | | | | |
| Grade 1–2 | | | | 2 (1) | 1 (1) |
| Grade 3–4 | | | 1 (1) | 1 (1) | |
| Nausea | | | | | |
| Grade 1–2 | 10 (3) | 7 (2) | 14 (4) | 8 (2) | 1 (1) |
| Grade 3–4 | 2 (2) | | | | |
| Vomiting | | | | | |
| Grade 1–2 | 9 (3) | 5 (1) | 6 (2) | | |
| Grade 3–4 | 1 (1) | | | 4 (2) | |
| Fatigue | | | | | |
| Grade 1–2 | 7 (2) | 10 (3) | 9 (3) | 7 (2) | 5 (3) |
| Grade 3–4 | | | | | |
| Stomatitis | | | | | |
| Grade 1–2 | 2 (1) | 2 (1) | 2 (1) | | 2 (1) |
| Grade 3–4 | | | | | |
| Pain | | | | | |
| Grade 1–2 | 3 (3) | | 3 (2) | 9 (3) | 3 (1) |
| Grade 3–4 | 1 (1) | | | | |
| Diarrhoea | | | | | |
| Grade 1–2 | | | | 4 (1) | |
| Grade 3–4 | | | | | |
| Constipation | | | | | |
| Grade 1–2 | 1 (1) | 9 (3) | 8 (4) | 6 (2) | 4 (2) |
| Grade 3–4 | | | | | |
| Bowel obstruction | | | | | |
| Grade 1–2 | | | | | |
| Grade 3–4 | | | | 1 (1) | |
| Pulmonary embolus | | | | | |
| Grade 1–2 | | | | | |
| Grade 3–4 | | 1 (1) | | | |
| Alopecia | | | | | |
| Grade 2 only | (3) | (3) | (4) | (4) | (2) |
| Haemoptysis | | | | | |
| Grade 1–2 | | | | | |
| Grade 3–4 | | | | | 1[a]Fatal |
| Serum creatinine | | | | | |
| Grade 1–2 | | | | | 1 (1)[b] |
| Grade 3–4 | | | | | |
| Neurosensory | | | | | |
| Grade 1–2 | 1 (1) | | | | |
| Grade 3–4 | | | | | |
| Pleural effusion | | | | | |
| Grade 1–2 | 1 (1) | | | | |
| Grade 3–4 | | | | | |

The total number of chemotherapy cycles administered per level is indicated in italics

[a]DLT (dose-limiting toxicity): fatal haemoptysis

[b]DLT: failure of serum creatinine to return to a value of $< 150$ μmol/l prior to next schedule treatment

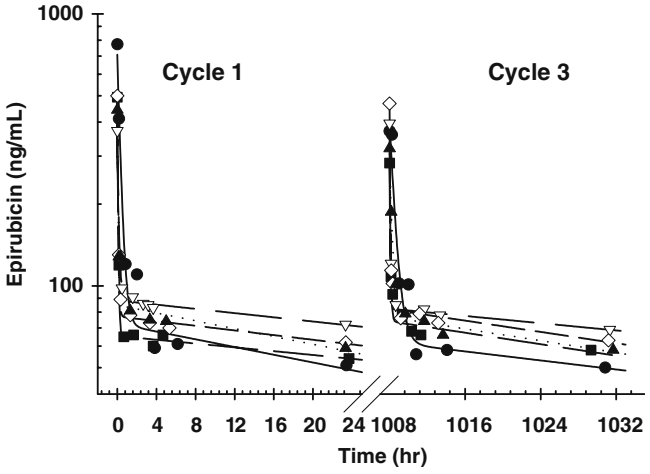


**Fig. 1** Epirubicin pharmacokinetics. A representative serum concentration–time profile of epirubicin after administration of 75 mg/m$^2$ with 0 (*filled circles*), 200 (*open inverted triangles*), 400 (*filled squares*), 600 (*open diamonds*), and 800 (*filled triangles*) mg of sulindac, administered orally 2 h prior to epirubicin administration is shown. *Symbols* represent the observed data for one patient from each of the five treatment groups. Epirubicin serum concentrations were described normalizing the time for cycle 1 to 0 h and cycle 2 to 1,008 h and using a two compartment pharmacokinetic model with bolus input and first-order elimination. The *solid* and *dashed lines* show the model fit for each treatment group

parameters or epirubicin metabolite serum profiles were observed between the patients administered sulindac (200–800 mg, po) and those who received epirubicin alone. Due to data limitations, we did not perform a full PK analysis of epirubicin metabolite PK parameters.

### *Sulindac pharmacokinetics*

Serum levels of sulindac and metabolites exhibited significant inter-patient and intra-patient variation. In the level 2 group (200 mg sulindac) serum sulindac concentration peaked above 3 μg/ml (8.4 μM) in four of six cycles measured and lower but significant amounts of sulindac sulfide and sulindac sulfone were also measured in all patients. Correspondingly higher concentrations of sulindac and metabolites were evident in patients who received larger doses of sulindac. Figure 2 illustrates sulindac and metabolite levels measured in patients who received the 600 mg maximum tolerable sulindac dose (level 4).

### Tumour expression of drug resistance proteins

Tumour blocks of 12 patients were available and analysed by immunohistochemical for the expression of MRP-1 and P-gp. The results are indicated in Table 4.

### Response to therapy

Of the 15 patients with evaluable disease, two had a partial response [one with melanoma treated at the 600 mg sulindac dose whose tumour stained weakly positive for MRP-1 protein and one breast cancer treated at the 400 mg dose of sulindac (tumour block unavailable). The duration of these responses was 11 and 8 months, respectively]. Four others had stable disease at the completion of therapy (breast—epirubicin alone group, unknown primary—200 mg sulindac group, non-small cell lung—600 mg sulindac group and ovarian—600 mg group). Nine patients showed disease progression on study. No specific correlations were noted with the limited amount of immunohistochemistry data available.

## Discussion

These results suggest that sulindac can be safely administered at an oral dose of 600 mg with epirubicin 75 mg/m$^2$. Granisetron pre-medication alone was not sufficient to adequately control epirubicin-associated nausea and vomiting in the epirubicin alone group but inclusion of a standard dexamethasone treatment regimen led to more complete control of this reaction in all groups. The extent and incidence of treatment-related toxicity evident in our study was as might be expected with a 75 mg/m$^2$ epirubicin dose and, with the exception of the DLTs evident in the 800 mg dose, did not

**Table 3** Model predicted epirubicin pharmacokinetic parameter estimates

| Treatment group | Sulindac (mg) | Estimated PK parameter (SD) | | | |
|---|---|---|---|---|---|
| | | AUC (μg h/l) | Cl (l/h) | *V* (l) | $V_{ss}$ (l) |
| Level 1 | 0 | 7,612 (1,856) | 19.5 (5.6) | 235 (39) | 1,604 (264) |
| Level 2 | 200 | 13,687 (3,316) | 10.1 (1.8) | 338 (46) | 1,593 (188) |
| Level 3 | 400 | 6,141 (2,056) | 23.1 (7.1) | 313 (48) | 1,471 (292) |
| Level 4 | 600 | 9,467 (4,918) | 16.4 (5.0) | 294 (57) | 1,697 (113) |
| Level 5 | 800 | 5,394 (971) | 25.9 (6.8) | 300 (61) | 1,563 (128) |

No significant differences were determined using a one-way ANOVA followed by a post hoc Dunnetts *t* test, where the control group did not receive sulindac; a $P$ value $\leq 0.05$ was considered significant

*SD* standard deviation (bracketed values), *AUC* area under the concentration–time curve, *Cl* systemic clearance, *V* central volume, $V_{ss}$ steady-state volume of distribution

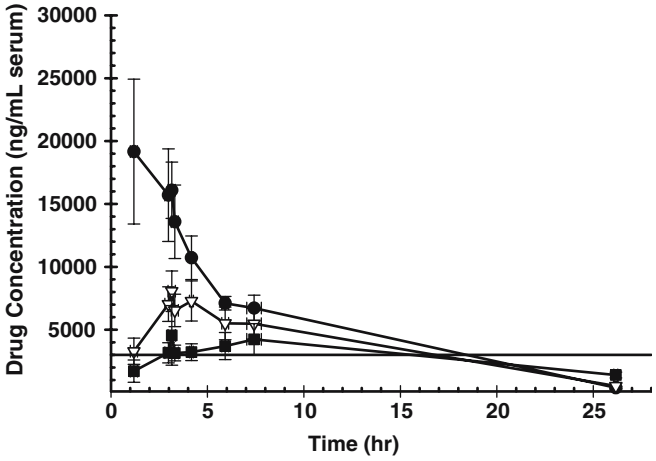


**Fig. 2** Sulindac and metabolite pharmacokinetics. The serum concentration–time profiles of sulindac (*black circles*), sulindac sulfide (*open triangles*), and sulindac sulfide (*black squares*) after oral administration of 600 mg sulindac. The results shown are the average of the values measured with four patients on the first cycle of therapy. A *horizontal line* showing the sulindac concentration producing strong in vitro synergy (3,000 ng/ml) is shown

vary significantly between the epirubicin alone and sulindac plus epirubicin groups. Dose-limiting toxicity was observed in two patients who were treated with 800 mg sulindac. It is possible that one of these toxic events, (haemoptysis, in a patient with lung cancer) was in fact a manifestation of progressive cancer. The second dose-limiting toxicity, renal impairment, occurred in a patient who had elevated creatinine levels prior to protocol chemotherapy and who also received the nephrotoxic antibiotic gentamicin. The trial protocol required these events to be regarded as drug-related as we could not discount the possibility that they were caused by the drug treatment regimen. Pharmacokinetic analysis of sulindac levels indicated that an oral dose of 600 mg of sulindac generated levels of sulindac and MRP-1 inhibitory metabolites well in excess of those required in vitro to inhibit the drug efflux pump (3 μg/ml) [13]. Sulindac did not alter the pharmacokinetics of epirubicin or epirubicin metabolites in our patient group.

Sulindac appears to inhibit MRP-1 specifically and has no interactions with P-gp [13, 17]. In addition, unlike many first and second generation P-gp inhibitors [27, 28], sulindac does not compete with the same cytochrome p450 enzymes that mediate chemotherapy metabolism [16], and this may explain why sulindac does not interfere with chemotherapy elimination. MRP-1 expression has been described in the blood–brain and blood–testes barriers [8]; however, the trial data also showed no signs of increased incidence of side effects including any CNS effects.

Because of the small sample size of each group, the standard deviations of the pharmacokinetic variables calculated are large. Although the mean values change between groups, no sulindac-dose responsive correlation was found. The summary of pharmacokinetic data for epirubicin although consistent between the sulindac treatment groups are not in full agreement with literature values for a 75 mg/m$^2$ dose. In the current study, we observed a generally greater area under the concentration–time curve for epirubicin in all treatment groups, together with a lower rate of clearance than previously reported in the literature [29, 30]. The explanation and implications for this disparity are unclear, but there were no significant differences in epirubicin PK among the treatment groups (including epirubicin alone), there was no evidence of increased toxicity among the patients and the results may therefore reflect minor variations in local drug administration practices and/or the limited sample size within each treatment group, population variation, and possibility of differences due to advanced disease state and previous chemotherapy exposure of the patients.

Our sulindac data were generally in agreement with the limited amount of literature available on circulating levels of this drug [16]. In the fasting state and with conventional doses, sulindac levels generally peak after approximately 2 h with increasing dose or the presence of food tending to lengthen the time to reach peak serum concentration. Hence we dosed with sulindac 2 h before epirubicin administration to ensure maximal or near maximal sulindac and metabolite levels when the chemotherapy drug entered the blood stream. We noted that our findings indicated that in the higher sulindac

**Table 4** Results of immunohistochemical analysis for MRP-1 and P-gp for patients with available tumour biopsies

| Sulindac group (mg) | 1° Tumour type | MRP-1 level | Intensity | P-gp level | Intensity | Response |
|---|---|---|---|---|---|---|
| 0 | Breast | 0 | | ++ | Intense | |
| 0 | Breast | 0 | | ++ | Weak | |
| 200 | Breast | + | Intense | ++++ | Medium | |
| 200 | Breast | 0 | | +++ | Medium | |
| 400 | Breast | 0 | | 0 | | Partial |
| 400 | Breast | + | Intense | 0 | | |
| 400 | Breast | 0 | | ++++ | Intense | |
| 600 | Bowel | 0 | | ++++ | Intense | |
| 600 | Ovarian | + | Intense | + | Weak | Stabilisation |
| 600 | Melanoma | + | Weak | + | Medium | Partial |
| 800 | Lung | 0 | | ++ | Medium | |
| 800 | Bowel | + | Medium | +++ | Medium | |

0 = no staining evident;
+ = 1–24%; ++ = 25–49%;
+++ = 50–74%;
++++ = ≥75%

dose groups, this time to peak was in some instances delayed but the delay was not consistent within patients or within a given dosage group. Likewise in the groups receiving lower sulindac doses, some cycles of sulindac administration seemed to generate peak sulindac and sulfide metabolites approximately 1 h following administration. Such inter- and intra-patient variation in sulindac levels are unlikely to have clinical significance in this instance as epirubicin was administered 2 h following sulindac, a time at which all patients (levels 2–5) showed significant concentrations of the inhibitor which were maintained for a minimum of several hours with greater durations of elevated sulindac levels in patients receiving the greater oral doses.

The optimal schedule for putative drug resistance modulators is unclear. Most early P-gp inhibitor trials used treatment schemes which, if effective, would aim to give rise to prolonged pump inhibition [6]. The newer third generation modulators of P-gp are all irreversible, non-competitive, inhibitors [6, 28]. If most chemotherapy drugs have their major tumour cell cytotoxic action within the first few hours of administration [6], such prolonged inhibition is unlikely to be necessary and could possibly contribute to prolonged drug elimination profiles and thus to increased toxicity of P-gp inhibition. However, since sulindac is a competitive inhibitor of MRP-1, it may also be possible to optimise the ''window'' of MRP-1 inhibition to ensure functional blockade of efflux while the chemotherapy is having its cytotoxic actions but allowing pump activity to recover and assist in the removal of any lingering toxic actions of the drug without the necessity for new pump protein synthesis. Detailed PK analysis of the compartmental distribution of drugs (especially the tumour compartment) under varying conditions of pump inhibition might indicate relevant chronological variables.

Sulindac and metabolites have been demonstrated to have several other useful properties in cancer treatment including chemopreventative (for example, prevention of the emergence of polyps and tumours in familial adenomatous polyposis (FAP), anti-angiogenic (inhibition of new vessel formation in tumour models) and pro-apoptotic activities (sulindac and analogues stimulating cell death in in vitro model), all independent of cyclo-oxygenase inhibition [31–33]. Indeed, a xenograft study conducted as a prelude to this trial showed some anti-tumour activity of prolonged high doses of sulindac as a single agent [17]. Therefore, it is possible that sulindac could have additional anti-cancer activities clinically through mechanisms unrelated to MRP-1 inhibition.

The small patient numbers and variety of tumour types preclude any meaningful analysis of the clinical relevance of the immunohistochemical findings and the level of MRP-1 staining would perhaps appear to be less than has been shown in much larger studies. It is unfortunate that all of the blocks were not available for staining but interesting that one of the responders, the melanoma patient, demonstrated weak MRP-1 staining. The staining results generally support previous findings that expression of one or both pumps is a common phenomenon in cancer [10]. A larger study is required to adequately correlate any positive effects of sulindac therapy with tumour MRP-1 expression and our next planned study will include blocks being made available for immunohistochemical analysis from all patients enrolled.

In summary, our study has shown that a 600 mg oral ''pre''-dose of sulindac can be combined with a fixed dose of 75 mg/m$^2$ epirubicin without affecting the conventional toxicology and pharmacokinetics of this anthracycline chemotherapy drug. This dose generates concentrations of sulindac and metabolites significantly in excess of those required in vitro to inhibit the MRP-1 cancer drug resistance pump. We will shortly initiate a phase II multi-centre trial utilising this treatment combination in an effort to evaluate the relative efficacy of single dose sulindac as an inhibitor of MRP-1 as part of a long-term translational research effort to examine the potential of selective MDR inhibition in patients with resistant cancers receiving chemotherapy treatment.

## Conflict of interest statement

The authors have no conflicts of interest to disclose. Preliminary results of this study were presented at the 2004 Annual Meeting of the American Society of Clinical Oncology.

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