Influence of trastuzumab on epirubicin pharmacokinetics in metastatic breast cancer patients

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Background: Anthracycline cardiotoxicity is increased by the contemporaneous administration of trastuzumab. The mechanism by which it occurs is as yet unknown. The aim of this study was to evaluate whether trastuzumab modifies the pharmacokinetics of epirubicin and its metabolites.

Patients and methods: Women with HER2-positive metastatic breast cancer were treated with epirubicin 75 mg/m² i.v. bolus followed by docetaxel 75 mg/m² in a 1-h infusion, every 3 weeks for six cycles, and trastuzumab (once at 4 mg/m², then 2 mg/m² weekly thereafter) in a 30-min infusion. Epirubicin pharmacokinetic data of seven patients were evaluated at the first cycle of therapy (baseline, with trastuzumab administered 24 h after epirubicin), and at the sixth cycle (i.e. 15 weeks after baseline, with trastuzumab administered immediately before epirubicin).

Results: No pharmacokinetic change in the parent compound epirubicin was detected. The area under the plasma concentration–time curve (AUC0–24 h) was 1230 ± 318 [mean ± standard deviation (SD)] at the first cycle and 1287 ± 385 h·µg/l at the sixth. The mean (±SD) maximum plasma concentration (Cmax) and the terminal elimination half-life at the first cycle (1303 ± 490 µg/l and 12.5 ± 3.1 h, respectively) were similar to those obtained at the sixth cycle (1229 ± 580 µg/l and 11.5 ± 2.9 h, respectively). Pharmacokinetic data of epirubicin metabolites evaluated at the first and sixth cycle of chemotherapy were superimposable without any statistical difference.

Conclusion: Enhanced anthracycline cardiotoxicity related to trastuzumab administration was not linked to pharmacokinetic interferences with epirubicin and its metabolites.

Key words: advanced breast cancer, epirubicin, pharmacokinetic, trastuzumab

Introduction

HER2 is a type I receptor tyrosine kinase. HER2 abnormalities resulting in gene amplification and receptor overexpression have been shown to be associated with an aggressive form of breast cancer with reduced overall and disease-free survival [1]. HER2 overexpression is observed in approximately one-third of breast cancer patients [2].

HER2 is a marker of aggressive disease forms, and is intrinsically involved in the development and maintenance of breast cancer. These features, together with the fact that HER2 is a membrane-associated receptor, made it an ideal candidate for targeted therapy. A humanized monoclonal antibody, trastuzumab, was developed and clinical trials started in early 1990. Trastuzumab inhibited tumor growth when used alone, but had synergistic or additive effects when used in combination with several antineoplastic agents [3]. Efficacy was clearly documented in metastatic breast cancer patients. Slamon et al. [4] showed that the addition of trastuzumab to chemotherapy was associated with significantly better responses and longer survival than chemotherapy alone.

The most serious complication of trastuzumab was cardiotoxicity, especially when it was administered in combination with anthracyclines. Reported incidences of 28% (40/143) and 19% (27/143) of these cases were categorized as New York Heart Association (NYHA) class III or IV, respectively. The development of cardiotoxicity was unexpectedly high and had not been reported in early studies of trastuzumab as a single agent or in studies of combination treatment in animals.

The mechanism by which trastuzumab treatment leads to an increased incidence of cardiac dysfunction in patients treated with anthracyclines has not yet been defined, but could include any of the following: immune-mediated destruction of cardiomyocytes, drug–drug interaction with anthracyclines, defects in ErbB2 signaling required for maintenance of cardiac contractility, interference with cardiomyocyte survival signals, or indirect consequences of trastuzumab-mediated effects outside the heart [5].

Cardiotoxicity induced by anthracyclines may be exacerbated by pharmacokinetic interactions between some drugs. For instance, clinical pharmacological findings have shown that paclitaxel influences the pharmacokinetic behavior of doxorubicin when delivered in both 24- and 3-h infusions [6, 7]. Generally, paclitaxel induces a higher plasma concentration of both doxorubicin and its main cardiotoxic metabolite, doxorubicinol. Therefore, exploring possible pharmacokinetic interactions between trastu-
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Values out of range are underlined.

*Including adjuvant chemotherapy.

ALP, alkaline phosphatase; Bil, total bilirubin; BUN, blood urea nitrogen; Crea, creatinine; CT, chemotherapy; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; LVEF, left ventricular ejection fraction.
zumab and anthracyclines, as a potential explanation of the clinical findings, is clearly warranted [8].

Due to its decreased cardiotoxicity, epirubicin has replaced doxorubicin in recent clinical trials, and the association of epi-
rubicin and docetaxel has been demonstrated not to be more cardiotoxic than epirubicin alone [9]. This was essentially explained by the absence of pharmacokinetic interference between docetaxel and epirubicin [10, 11].

The objective of the present study was to investigate the inter-
actions of trastuzumab administration on the pharmacokinetics of epirubicin and its metabolites.

Patients and methods

This study was conducted at the Department of Medical Oncology of the Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy. All clinical and pharmacokinetic protocols were approved by the Protocol Review Committee and by the Ethics Committee of the institute. Written informed consent was obtained from all patients before entry into the study.

Patient selection

Patients affected by metastatic breast cancer that overexpressed HER2 were eligible for the study. The level of expression of HER2 was determined by immunohistochemical analysis (Hercept Test). Only patients with a score of 2+ or 3+ were eligible. Other eligibility criteria were performance status <1, no other serious medical or psychiatric illness that would preclude intensive treat-
ment, and/or informed consent. All patients had to have normal bone marrow, liver and kidney functions. A normal left ventricular ejection fraction (LVEF) evaluated by angioscintigraphy (MUGA scan) was also required.

Treatment plan and drug administration

Epirubicin plus docetaxel administration. Based on our previous experience [9, 12], chemotherapy consisted of epirubicin 75 mg/m² given as an i.v. bolus immediately followed by docetaxel 75 mg/m² in a 1-h infusion. Cycles were repeated every 3 weeks. A maximum of eight cycles were planned. Trastuzumab administration. Trastuzumab 4 mg/m² was administered in a 90-min infusion on the first administration, and 2 mg/m² in a 30-min infusion on the following administrations. Trastuzumab was administered weekly and continued until disease progression.

On day 1 of the first cycle of chemotherapy, patients received epirubicin plus docetaxel administration, and trastuzumab was administered 1 day later. On day 1 of the following cycles, trastuzumab was administered immediately before the epirubicin and docetaxel regimen administration.

Epirubicin, purchased as a sterile lyophilized powder in 50-mg vials, was dissolved in 25 ml of normal saline, and administered intravenously over 10 min.

Docetaxel, purchased as a sterile solution in vials containing 80 mg, was dissolved in 2 ml polysorbate 80 (Tween 80). Docetaxel was diluted in a 13% ethanol solution to a concentration of 10 mg/ml. The drug was diluted in 250 ml of 5% dextrose and infused over 1 h immediately after the epirubicin injection. The premedication schedule consisted of prednisone 50 mg starting 12 h before and continuing for 36 h after docetaxel administration, and ranitidine 300 mg orally the day before, the same day and then for 2 days after docetaxel administration. All patients received granisetron 3 mg i.v. the day of chemotherapy as antiemetic treatment.

Roche (Milan, Italy) supplied trastuzumab as a freeze-dried preparation at a nominal quantity of 150 mg in a single-dose vial for parenteral administration (powder for solution for infusion). For intravenous administration, each vial was reconstituted with 7.0 ml of sterile water for injection, yielding a solution of 22 mg/ml trastuzumab. Reconstituted trastuzumab was then added to 250 ml of 0.9% sodium chloride for injection and infused.

In order to prevent clinically significant cardiotoxicity, patients were fol-
lowed using a MUGA scan at baseline: every two cycles during chemotherapy, and every 3 months during treatment with trastuzumab alone. Patients were removed from the treatment regimen if they developed cardiac toxicity as defined previously [13].

Pharmacokinetic studies

Each patient underwent pharmacokinetic analysis on day 1 of the first cycle of chemotherapy [i.e. epirubicin plus docetaxel administration alone (ED) group] and on day 1 of the sixth cycle of chemotherapy [i.e. trastuzumab administered immediately before the epirubicin plus docetaxel administration (TED) group]. Each patient, therefore, acted as their own control.

For plasma analysis of epirubicin and its metabolites, heparinized venous blood samples were collected before treatment and at various times thereafter. Plasma samples were obtained at 5, 15, 30 and 60 min, and at 2, 3, 4, 6 and 24 h following epirubicin i.v. bolus. Blood samples were centrifuged immediately at room temperature, and plasma was separated and stored in aliquots at −20°C until analysis.

Sample analysis and pharmacokinetic calculations

Concentrations of epirubicin and its metabolites in plasma were determined as described previously [10] according to Maessen et al. [14]. The pharmacokinetics of the epirubicin metabolites epirubicinol (EOL), 7-deoxydoxorubicinone (7d-Aone), epirubicinol-glucuronide (EOL-glu) and epirubicin-glucuronide (EPI-glu) were evaluated.

All pharmacokinetic data were analyzed using an integrated computer system (Siphar program, Simed) on an IBM/IC computer. Values obtained by non-compartmental analysis (statistical moment theory) were considered, and the maximum peak plasma concentration (Cmax) was put on par with the mean concentration in the plasma samples following drug administration. Pharmacokinetic parameters were calculated using standard formulas [15]. Because it was demonstrated that the pharmacokinetics of epirubicin is at least linear for doses up to 150 mg/m² [16], in the case of dose reduction at the sixth cycle, pharmacokinetic data were normalized for higher doses of epirubicin, i.e. 75 mg/m².

Statistical analysis

Pharmacokinetic data were compared using the non-parametric Wilcoxon matched-pairs signed-ranks test for paired data. A probability of \( P < 0.05 \) was considered significant. Version 5.0.1 of SPSS for Windows software was used for the statistical analysis.

Results

Eight consecutive patients complying with the above criteria were entered in to the study. One patient underwent pharmacokinetic evaluation at the first cycle, but discontinued the treatment before the sixth cycle of chemotherapy due to medical decision. This patient at the second cycle suffered from pulmonary infection and thereafter she received three further cycles of chemotherapy with 25% dose reduction. At the fifth cycle of chemotherapy, a tumor evaluation was performed. The patient had stable disease and then stopped chemotherapy because no further clinical benefit was expected as a result of continuation of treatment. Therefore, pharmacokinetic data from seven patients were evaluable. Two of them had a 25% dose reduction at the sixth cycle and pharmacokinetic data were normalized according to the methods described above. Pharmacokinetic determination was performed at the sixth cycle of chemotherapy. Due to some delay in the administration of
chemotherapy, this evaluation was actually done on average at week 16 (range 15–17 weeks), instead of at week 15 as planned.

Main patient characteristics are listed in Table 1.

### Pharmacokinetics

Mean plasma concentration–time curves and estimated pharmacokinetic data of epirubicin at the first and sixth cycle of chemotherapy are shown in Figure 1. The mean ± standard deviation (SD) systemic clearance (CL) of epirubicin when administered at the first cycle was 64.4 ± 16.2 l/h/m². The area under the concentration–time curve (AUC0–24 h) of the plasma epirubicin concentration–time curve ranged from 804 to 1776 h·µg/ml (mean 1230 ± 318 h·µg/l), and the Cmax ranged from 449 to 1818 µg/l (mean 1303 ± 490 µg/l). All these values, and the mean terminal elimination half-life (t½elim) of epirubicin (12.5 ± 3.1 h) were in good agreement with our previous data [10] obtained in patients with advanced breast cancer. The administration of trastuzumab did not modify epirubicin pharmacokinetic parameters of epirubicin significantly (Figure 1; Table 2). Mean plasma concentration–time curves of EOL (Figure 1), 7d-Aone and glucuronides were also superimposable, and no difference was evident between the first and the sixth cycle of chemotherapy.

### Cardiotoxicity

Cardiological events occurred in four out of the eight patients treated. At the seventh cycle of chemotherapy, one patient experienced an asymptomatic decline in LVEF (43%). She stopped chemotherapy and continued weekly trastuzumab, without requiring any cardiological therapy. The other three patients had cardiotoxicity after the end of chemotherapy, during trastuzumab administration. The first patient showed a congestive heart failure (CHF) (NYHA class III) with a decline in LVEF of 44% after 9 months of weekly trastuzumab alone. The second patient showed a CHF (NYHA class III) with a decline in LVEF of 42% after 2 months of weekly trastuzumab alone. The third patient experienced an asymptomatic decline in LVEF at 39% after 4 months of weekly trastuzumab alone.

Pharmacokinetic data of epirubicin (mean ± SD) evaluated in these four patients at the first and sixth cycles of chemotherapy were, respectively, Cmax 1203 ± 657 and 1053 ± 587 µg/l, AUC0–24h 1201 ± 410 and 1320 ± 375 h·µg/l, CL 64.4 ± 16.2 and 61.1 ± 14.0 l/h/m², and t½elim 13.5 ± 3.8 and 11.7 ± 4.0 h.

### Discussion

The mechanism by which trastuzumab treatment leads to an increased incidence of cardiac dysfunction in patients treated with anthracyclines has not yet been defined. A strong expression of HER2 receptors was not observed in the tissue obtained from heart biopsies from 60 patients with cardiac dysfunction, nor from 25 breast cancer patients with or without previous anthracycline
treatment [17]. Nonetheless, cardiotoxicity seems to be related to myocardial uptake of trastuzumab [18]. In fact, it was observed that, in a group of 20 patients, seven had myocardial uptake of radiolabeled trastuzumab. Six out of the seven patients developed cardiotoxicity, and the seventh had episodes of cardiac arrhythmia during the administration of trastuzumab. In the 13 patients without myocardial uptake, no adverse cardiac effect occurred. Recent animal studies have demonstrated that in ErbB2-deficient conditional mutant mice there is a progressive onset of multiple independent parameters of dilated cardiomyopathy, the same type of cardiac dysfunction evident in patients treated with trastuzumab [5, 19], and that ErbB2-deficient cardiomyocytes are more susceptible to anthracycline-induced cell death [5].

We evaluated the effects of trastuzumab on epirubicin pharmacokinetics in patients receiving an epirubicin plus docetaxel regimen. This regimen was chosen on the basis of both our previous clinical experience and on the lack of pharmacokinetic interactions between anthracycline and taxane. In fact, we had previously shown that the docetaxel administration with epirubicin did not influence epirubicin and epirubicinol pharmacokinetics when compared with treatment using epirubicin alone [10]. Therefore, we could study the interactions of trastuzumab on epirubicin pharmacokinetics even though the anthracycline was combined with the taxane.

Moreover, we chose to evaluate epirubicin pharmacokinetics after six cycles on the basis of the long terminal half-life of trastuzumab and the time needed to achieve steady-state plasma concentration [20]. Trastuzumab plasma concentrations after 16 weeks are close to the concentrations seen at steady-state, which should be achieved after 20 weeks of weekly administration [20].

We enrolled only seven patients in the study, a relatively small sample size. All the pharmacokinetic parameters of epirubicin and its metabolites evaluated were quite similar at baseline compared to those at the sixth cycle, and P values were far from significant. Moreover, we were not able to find evidence for any trends in other directions. On the other hand, when a drug–drug interaction such as occurred here is evident, e.g. between paclitaxel and doxorubicin, eight patients is a sufficient number to highlight this interaction [6].

Our data are supported further by the fact that no pharmacokinetic interaction was observed in patients who experienced cardiotoxicity. In fact, we were not only able to observe any significant difference in epirubicin pharmacokinetics using the intra-patient evaluation, but we were also able to observe no effect when comparing patients with cardiotoxicity with those without (data not shown).

Our results did not show any interaction determined by trastuzumab on epirubicin pharmacokinetics; therefore it is unlikely that the increased cardiotoxicity observed with the association of trastuzumab and anthracyclines is due to changes in anthracycline pharmacokinetics.

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References


