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Research Paper

Trastuzumab and Liposomal Doxorubicin in the Treatment of MCF-7 Xenograft Tumor-Bearing Mice: Combination Does Not Affect Drug Serum Levels

Dawn N. Waterhouse,[1,3] Tetyana Denyssevych,[1] Norma Hudon,[1] Stephen Chia,[1] Karen A. Gelmon,[1] and Marcel B. Bally[2]

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Purpose. We assessed the combination of doxorubicin or liposomal doxorubicin with trastuzumab for alterations in peak serum drug levels, as these agents are increasingly being paired in the treatment of aggressive breast cancer. We hypothesized that trastuzumab would exhibit a slower rate of elimination from the serum when in combination with liposomal doxorubicin based on the known effects of liposomal doxorubicin on phagocytic cells of the mononuclear phagocyte system (MPS), which are responsible in part for the uptake and degradation of antibodies.
Methods. Doxorubicin and trastuzumab serum levels were assessed following injection of free doxorubicin, liposomal doxorubicin, or trastuzumab into female RAG2-M mice bearing subcutaneous MCF-7$^{HER\text{-}2}$ tumors. The effects of combination drug treatment on tumor growth were compared to single-agent treatment.
Results. Peak serum trastuzumab levels were not altered as a result of addition of doxorubicin therapy, nor were doxorubicin levels altered over 24 h as a result of coadministration of trastuzumab. Liposomal doxorubicin administration did result in serum doxorubicin levels 200- to 1000-fold higher than with injection of free doxorubicin.
Conclusions. For the specific combination of trastuzumab with doxorubicin, either in free or liposomal form, coadministered in mice, there was no impact of one drug on the other in terms of peak serum drug levels or efficacy.

KEY WORDS: cancer drug pharmacology; drug clearance, hepatic; drug targeting, liposomes; liposomes, pharmacokinetics; oncology; PEGylation; pharmacokinetics/pharmacodynamics.

INTRODUCTION

The current paradigm for optimal treatment of many types of cancer is that rational combinations of two or more agents may give higher response rates, longer time to relapse, and potentially enhanced quality of life. The rationale behind this is simple: there are many classes of anti-cancer drugs and combining drugs from different classes will lead to differing and non-overlapping mechanisms of cell toxicity or cytostasis and reduced likelihood of cancer cell resistance (1). Rationales for combination chemotherapy include the potential for sequential or concurrent attack on biochemical or cell-signaling pathways, manipulation of drug transport or drug metabolism systems, use of metabolites to preferentially rescue normal cells from anti-metabolite effects, metabolic modulation, and approaches involving cell synchronization and recruitment (2,3). Finally, these combinations may be utilized without reduction in the maximum tolerated dose levels employed, and in some cases may even allow for dose reduction without a resultant loss in efficacy. This effect may be termed *therapeutic synergism* and is an increasingly attainable target with the advent of biological drugs including monoclonal antibodies, gene therapy and vaccines.

When developing combination regimens, it is important to be aware of how each drug in the regimen may affect the pharmacokinetic or pharmacodynamic profile of the other agent(s). Such awareness may lead to the necessity of altered schedules of administration or even different administration routes. There are many types of potential drug–drug interactions: i) P-gp inhibitors leading to increases in systemic exposure and tissue distribution of drug levels, associated with increased toxicity (4); ii) induction or inhibition of metabolic enzymes by one drug leading to altered metabolism of the second; as well as iii) chemical interaction of two or more drugs such as antisense oligonucleotides and doxorubicin (5). A recent paper outlined altered pharmacokinetics and tissue distribution of carboplatin and gemcitabine following dexamethasone treatment (6). Thus, depending on the drugs being combined, novel interactions may emerge. Of particular interest to our research group are combination effects associ-

[1] BC Cancer Agency.
[2] University of British Columbia.
[3] To whom correspondence should be addressed. (e-mail: dwater@bccrc.ca)

ABBREVIATIONS: AC, doxorubicin and cyclophosphamide; AUC, area under the curve; i.p., intraperitoneal; PBS, phosphate-buffered saline; TMB, tetramethylbenzidine.

ated with use of liposomal anticancer drugs, such as doxorubicin and vincristine, in regimens that also include other therapeutics such as antibodies. More specifically, liposomal formulations of doxorubicin are being combined with Herceptin (7) and liposomal formulations of vincristine are being combined with rituximab (8). This interest is due, in part, to the therapeutic potential of these agents when used in combination as well as the fact that both therapeutic agents have the potential to be eliminated by cells of the MPS.

Over the last several years, several biological agents have entered clinical trials and clinical use for the treatment of breast cancer or its resultant metastases. These include trastuzumab (Herceptin) (reviewed in 9), the bispecific antibody MDX-H210 (anti-FcgammaRI × anti-HER-2/neu) (10), and bevacizumab (11), a monoclonal antibody targeting vascular endothelial growth factor. Promising results in clinical trials have been noted with these and other monoclonal antibodies in the treatment of breast cancer, non-Hodgkin lymphoma and chronic lymphocytic leukemia (12,13) and metastatic colorectal cancer (14). Further, it has been recognized that the true value of monoclonal antibodies as therapeutics will be realized when these agents are paired with other therapeutics, such as anthracyclines, cyclophosphamide (15,16) or endocrine therapy (17). Thus it is anticipated that antibodies will continue to play an increasingly important role in the treatment of cancer, but it is assumed that these antibodies will serve as a complementary component in an existing combination of cytotoxic drugs.

Trastuzumab is a humanized monoclonal antibody targeted to the extracellular domain of the HER-2/neu protein, and has been shown in clinical trials to produce objective tumor responses in 15–21% of the 25–30% of human breast cancer patients whose tumors overexpress HER-2/*neu*, and who had relapsed following chemotherapy for metastatic breast cancer (18). In breast and ovarian carcinoma, overexpression of this receptor tyrosine kinase is associated with younger patient age, earlier disease recurrence, lymph node involvement and increased level of metastases, resistance to endocrine therapy and poor survival (19–25). High levels of HER-2/*neu* have also been detected in a range of further malignancies, including prostate, lung, uterine serous papillary, gastriomas and thyroid carcinomas (26–31). It has been postulated that trastuzumab works by antagonizing the function of the growth signaling properties of the HER-2/neu system. However it has also been suggested that it may signal immune cells to attack and kill tumor cells, and in addition, may enhance the cytotoxicity of other chemotherapeutic agents (32).

Early studies with trastuzumab paired the antibody with doxorubicin, however in a 1998 trial involving 469 women (33), cardiac dysfunction was observed in 27% of women receiving doxorubicin, cyclophosphamide (AC) and trastuzumab, *versus* only 8% in women receiving AC only. This unforeseen increase in cardiotoxicity led to further trials with trastuzumab being paired with cytotoxic agents other than anthracylines, such as paclitaxel (34), gemcitabine (35), and docetaxel (36). Trastuzumab has been granted approval in the United States and several European countries, and it is being paired with chemotherapeutics in standard treatment protocols. A potential concern with this therapy is that it may either cause cardiac dysfunction on its own, or amplify the cardiac toxicity observed with the use of anthracyclines (37) as mentioned above, necessitating further exploration of the potential for enhanced cardiac toxicity. Recognizing however, the benefits of increased time to progression with the pairing of trastuzumab and doxorubicin, recently initiated trials are exploring the combination of trastuzumab with pegylated liposomal formulations of doxorubicin (38) (ECOG-3198 and NCI-G00-1878). Ideally, these new combinations will be without the additional toxicity currently noted with the trastuzumab/doxorubicin combination.

Liposomal encapsulation of doxorubicin affords several benefits over the free form of the drug. Of foremost importance, liposomal formulations buffer the acute cardiotoxicity of doxorubicin (39,40), while also extending plasma circulation time and enhancing drug accumulation in malignant tissues (41). There are two approved liposomal formulations of doxorubicin; Myocet, composed of egg phosphatidylcholine and cholesterol, and Doxil® (Caelyx), which incorporates PEG-modified phosphatidylethanolamine as well as hydrogenated soya phosphatidylcholine and cholesterol (reviewed in 42). This paper will focus on pegylated liposomal doxorubicin, as this formulation has been most widely studied in the treatment of breast cancer, both as a single agent, and in combination regimens, and shows great promise in the treatment of platinum and taxane resistant tumors (43).

When considering the use of combinations of liposomal doxorubicin with an antibody, a potential unique interaction may arise due to elimination mechanisms involving cells of the MPS. It is known for example, that doxorubicin encapsulation in liposomes can result in increased doxorubicin delivery to phagocytic cells which in turn, are killed by the encapsulated drug (44). This has been found to be true for both conventional liposome formulations and those which incorporate either the ganglioside G_{M1} or PEG in order to achieve extended circulation lifetimes (45,46), which have decreased, yet still significant recognition by the MPS (47). The metabolism of trastuzumab is not fully understood at present, however it is hypothesized that IgG clearance through cells of the MPS may be involved (48). Given this, it is possible that liposomal doxorubicin administration may decrease elimination of an antibody or alter its route of elimination. If this proved to be true, it would be important to determine how such an interaction altered the therapeutic and toxicological effects of the drugs when used in combination.

The data reported in this paper presents a carefully constructed series of murine studies assessing the therapeutic effects of doxorubicin both in free and liposomal form, trastuzumab, and combinations of the two drugs. Doxorubicin and trastuzumab have previously been found to exhibit additive effects when used in combination both *in vitro* (49) and *in vivo* (50). The data presented provides an approach to identifying optimal concentrations of two active agents to be used in a combination regimen. Importantly, this paper also addresses the question of whether the addition of liposomal doxorubicin to trastuzumab treatment affected the peak serum levels of trastuzumab administered intraperitoneally, and similarly, whether trastuzumab affected the serum levels of intravenous liposomal doxorubicin over 24 hours. The results suggest that the drugs can be combined without an impact on maximum serum concentrations of either drug or the absorption phase of trastuzumab.

MATERIALS AND METHODS

Cell Lines and Culture

MCF-7^{HER-2} and MCF-7^{NEO} cells were a kind gift from Dr. M. Alaoui-Jamali (McGill University, Montreal, Quebec) (51). All cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Stem Cell Technologies, Vancouver, BC, Canada), supplemented with 2 mM L-glutamine (Stem Cell Technologies), 10% FBS (Hyclone, Logan, UT) and 100 μg/ml Geneticin. Cells were maintained at 37°C and 5% CO_2 in a humidified atmosphere.

Reagents and Chemicals

Trastuzumab (Hoffman-La Roche, Mississauga, ON, Canada), doxorubicin hydrochloride [Faulding (Canada) Inc., Vaudreuil, QC] and liposomal doxorubicin (Caelyx, Sequus Pharmaceuticals, CA, USA) were provided by the BC Cancer Agency Pharmacy and diluted in 5% dextrose USP injection (Baxter Corp., Toronto, ON) prior to use.

In Vivo Models

Female RAG2-M mice were purchased from Taconic (Germantown, NY, USA) and used for experiments at between 6 and 8 weeks of age. Average weight was 22 g. All animal protocols were approved by the University of British Columbia Animal Care Committee, and studies performed in accordance with guidelines established by the Canadian Council on Animal Care and with the "Principles of Laboratory Animal Care" (NIH, 1985).

Drug Serum Levels

A group of 76 female RAG2-M (three mice for each time point), were given bolus intravenous tail vein injections of doxorubicin at 5 mg/kg or liposomal doxorubicin at 2.5 mg/kg and intraperitoneal (i.p.) injections of trastuzumab at 0.3 mg/kg. Lower doses of liposomal doxorubicin were given due to enhanced efficacy of doxorubicin in this formulation. Trastuzumab was administered i.p. to copy the route of administration in efficacy studies, and studies have demonstrated rapid accumulation in the circulating blood pool following this route of administration (52). Blood samples collected by cardiac puncture following CO_2 asphyxiation of mice, were allowed to clot at room temperature (RT) and centrifuged to separate the serum. Serum was immediately frozen for further evaluation by ELISA (trastuzumab) and HPLC (doxorubicin).

Doxorubicin Analysis by HPLC

Doxorubicin was assayed as in Embree *et al*. (53). Briefly, prior to HPLC analysis 0.1 ml serum samples were extracted using 0.3 ml acetonitrile before evaporation to dryness using a nitrogen stream at 37°C. Samples were reconstituted into mobile phase. The HPLC system consisted of a Waters Alliance 2695 separation module and fluorescence detector, Waters 474 (Waters Corp., Milford, MA, USA). Doxorubicin was quantified on a Waters Symmetry C18 column (3.5 μm, 4.6 × 75 mm) with a C18 guard column. Mobile phase was 78% of 16 mM ammonium formate at pH 3.5, 15% acetone and 7% isopropanol at a flow rate of 1.0 ml/min; column temperature 40°C, sample temperature 5°C. The Doxorubicin excitation wavelength was 480 nm; emission wavelength was 580 nm.

ELISA

ELISA plate wells (Nunc Maxisorb, Roskilde, Denmark) were coated with 50 ng/well of rabbit monoclonal antibodies to the Fc fragment of human IgG (ICN Biomedicals Inc., Aurora, Ohio). After 24 h of incubation at 4°C, plates were washed thoroughly with 0.05% Tween 20 (Sigma, Saint Louis, MO, USA) in phosphate buffered saline (PBS) (washing buffer) and free-binding sites were saturated with 1% bovine serum albumin (Sigma) in PBS. Analytes were diluted 1:300 in PBS which contained 1% bovine serum albumin (Sigma) and 0.05% Tween 20 (assay buffer). Subsequently the diluted samples were added into the wells which were incubated at room temperature for 2 h. Each plate contained a series of standards (trastuzumab diluted from 2.5 to 30 ng/ml), normal human IgG (ICN Biomedicals, Aurora, OH) as positive control, serum samples spiked with trastuzumab at 3, 7, 12, and 22 ng/ml as quality controls, and negative controls. The plates were washed six times prior to rabbit anti-human IgG (whole molecule)-HRP conjugates (Sigma) prepared at 1:40,000 dilution being added 100 μl/well for 1 h at room temperature. Plates were washed, then developed with tetramethylbenzidine (TMB) substrate (Pierce-MJS Biolynx, Brockville, ON) for 15–30 min at RT. The reaction was stopped by addition of 2 M H_2S0_4 and absorbance measured at 450 nm in an MRX microplate reader (Dynex Technologies, Chantilly, VA, USA). The concentrations of trastuzumab were calculated by liner regression analysis of optical density values, the standard curve serving as a reference. The lower limit of detection of these assays was 2.5 ng/ml ($r^2 > 0.998$).

Efficacy Studies

MCF-7^{HER-2} cells were harvested in exponential growth phase, washed and resuspended at a concentration of 2×10^7 cells per 100 μl DMEM media. As the *in vivo* growth of this cell line is estrogen dependent, on the day prior to cell inoculation, mice had 60 day release 1.5 mg β-estradiol pellets (Innovative Research of America, FL, USA) implanted below the skin through a small incision on the upper back. Incisions were closed with surgical staples. On study day zero, 1×10^7 cells were injected subcutaneously on the back of female RAG2-M mice. Tumor growth and body weight were monitored three times per week with calipers. Tumor size in cubic millimeters was calculated using the formula 1/2 [length (mm)] × [width (mm)]^2. Of note, the MCF-7 cell line does not form solid tumors in mice without estrogen supplementation (54).

Treatment was initiated when average tumor size was 50–100 mm^3. Trastuzumab in sterile water was administered intraperitoneally every Tuesday and Friday for a total of five weeks at doses ranging from 0.03 to 10 mg/kg. Free or liposomal doxorubicin was administered intravenously (via

the lateral tail vein) once weekly over five weeks at doses ranging from 1.5 to 4.5 and 2.5 to 12.5 mg/kg, respectively. Control animals were injected on an identical schedule with sterile water. The injection volume was 200 μl per 20 g mouse.

Statistical Analysis

Means were compared using one-way ANOVA followed by the post-hoc comparisons of means test described by Scheffé using STATISTICA software (Tulsa, OK, USA). Differences were considered significant at the probability level equal to or less than 0.05 ($p \leq 0.05$).

RESULTS

Inhibition of MCF-7^{HER-2} Xenograft Tumor Growth with Trastuzumab

While traditionally, treatment with chemotherapeutics has been at or near the maximal tolerated dose of the drug, it is not always necessary to use such high doses to achieve maximal therapeutic effects. In fact, a primary rationale behind the development of synergistic or additive drug combinations is the achievement of optimal therapeutic effects with substantially lower drug dose. To determine the optimal dose for trastuzumab, a dose titration was performed in female RAG2-M mice bearing established subcutaneous MCF-7^{HER-2} tumors. Treatment was initiated on day 17. Maximum weight loss was 8% of initial body weight, noted in mice receiving 10 mg/kg trastuzumab dose (data not shown). Mice in other groups experienced either no weight loss or to a lesser degree. Other signs of drug-related toxicity were not noted. As shown in Fig. 1, mice receiving the 0.03 or 0.1 mg/kg dose did not exhibit any delay or reduction in tumor growth. Mice treated at 0.3, 1.0 or 10 mg/kg exhibited delays in tumor growth where doses of 10 mg/kg resulted in regression of established tumors.

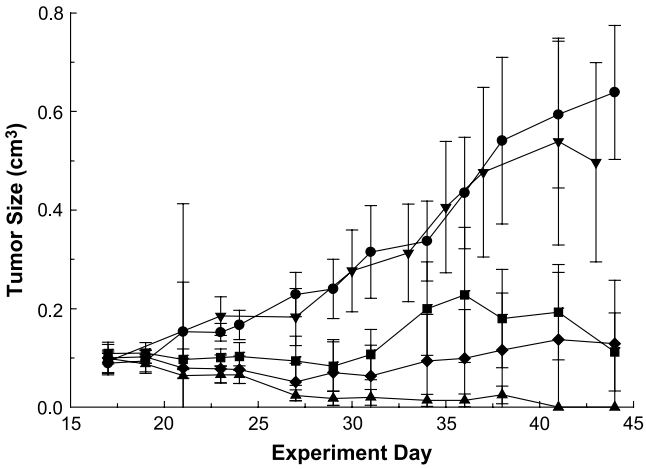

Fig. 1. MCF-7^{HER-2} xenograft tumor growth in female RAG2-M mice following intraperitoneal treatment with saline (●) or trastuzumab at 0.03 (▼), 0.3 (■), 1.0 (♦) or 10.0 (▲) mg/kg. Treatment was twice weekly over a period of 5 weeks. Data points represent the mean ± standard error of the mean from groups of 4–5 mice.

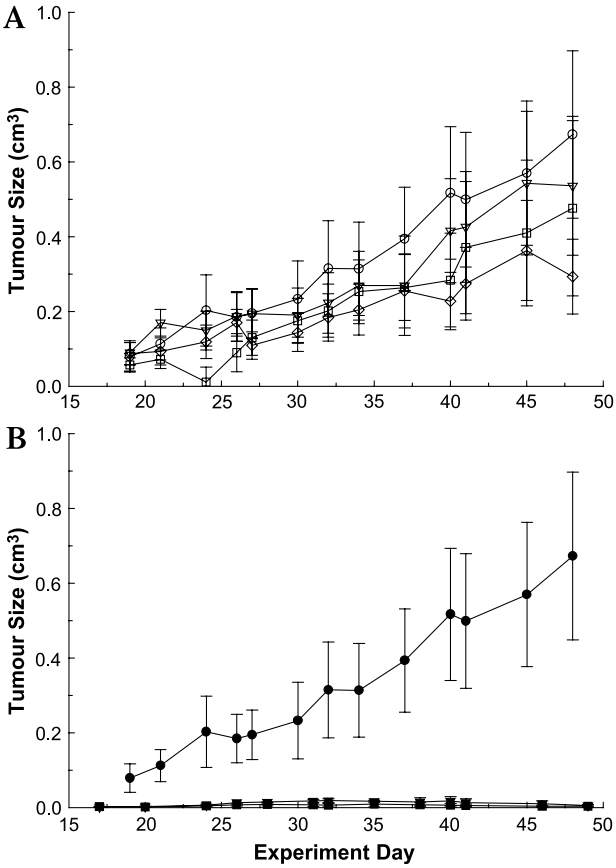

Fig. 2. MCF-7^{HER-2} xenograft tumor growth in female RAG2-M mice following intravenous treatment with saline (○; ●), (A) free doxorubicin at 1.5 (▼), 3.0 (□), or 5.0 (◇) mg/kg, or (B) liposomal doxorubicin at 2.5 (▼) or 5.0 mg/kg (■) once weekly over a period of 5 weeks. Data points represent the mean ± standard error of the mean from groups of 4–5 mice.

Inhibition of MCF-7^{HER-2} Xenograft Tumor Growth with Free and Liposomal Doxorubicin

Similar to the studies described in Fig. 1, it was important to establish dose response curves for doxorubicin and Caelyx (the liposomal formulation of doxorubicin) used in these studies. Female RAG2-M mice with established MCF-7^{HER-2} tumors were treated once weekly for 5 weeks. The dose response curve for free doxorubicin is provided in Fig. 2A. At the maximum tolerated dose of free doxorubicin, 5.0 mg/kg which caused a maximum of 15% body weight loss, tumor progression was delayed. Control animals exhibited 400 mg tumors on day 37 after tumor cell inoculation. Animals treated with doxorubicin at 5.0 mg/kg exhibited 400 mg/kg tumors on day 45. The therapeutic effects of Caelyx were significantly better than free doxorubicin (Fig. 2B). Caelyx was tolerated at doses three times that which could be given for free drug, indicating reduced toxicity of liposomal doxorubicin. At this dose and at doses as low as 2.5 mg/kg, almost complete inhibition of tumor growth was observed (Fig. 2B; day 49 tumor size ~6 mm^3 on day 49 postinoculation).

Given the results shown in Figs. 1 and 2, the combination studies summarized in Fig. 3 are not unexpected. Since the therapeutic activity of Herceptin alone is exceptional,

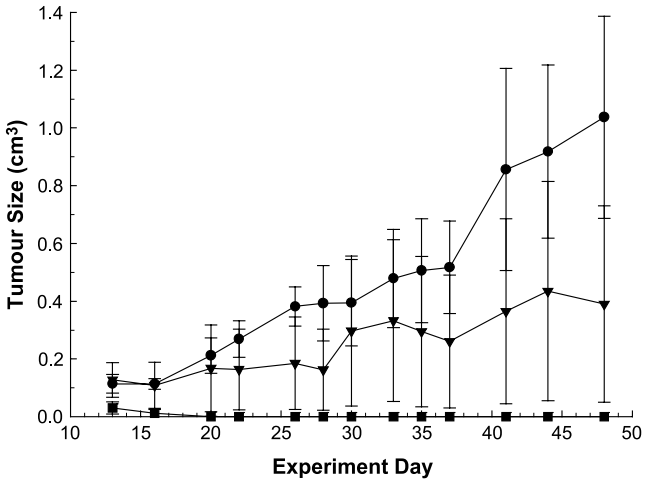

Fig. 3. MCF-7^{HER-2} xenograft tumor growth in female RAG2-M mice following intraperitoneal treatment with saline (●), intravenous combination of trastuzumab and free doxorubicin [0.3 and 5.0 mg/kg (▼)], or trastuzumab and liposomal doxorubicin [0.3 and 2.5 mg/kg (■)] once weekly over a period of 5 weeks. Data points represent the mean ± standard error of the mean from groups of 4–5 mice.

even when administered at 0.3 mg/kg, when it was combined with free doxorubicin administered at its maximum therapeutic dose (5 mg/kg), no tumor growth was observed over the 50 day time course. Similarly, when 0.3 mg/kg Herceptin was combined with Caelyx given at 2.5 mg/kg, the established 100 mg tumors regressed to an immeasurable size. These studies were not designed to assess the therapeutic interactions between Herceptin and free or liposomal doxorubicin; studies that would require additional dose titration studies with the combinations. Studies assessing interactive effects for example will require a broad dose titration of the combined drugs using fixed dose combinations shown in Fig. 3 and stepwise reductions to dose levels which provide a range of therapeutic responses between controls and no growth. However, the results obtained at the combined doses provide a rationale for selected doses used to assess whether Herceptin combined with doxorubicin or Caelyx influences the serum drug levels of the other drug when used in the combination.

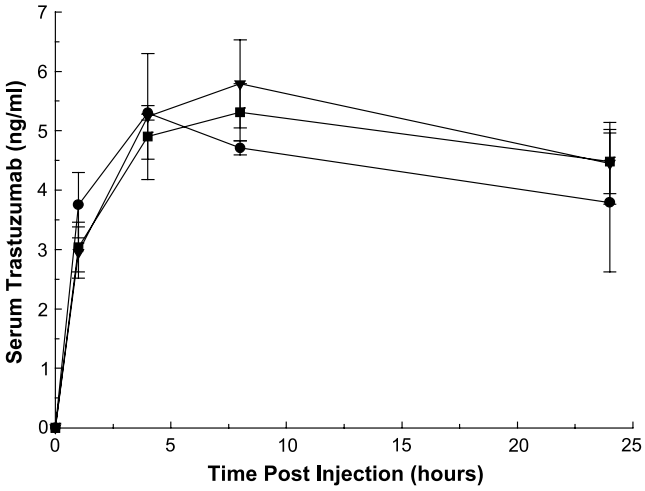

Fig. 4. Serum trastuzumab levels in female RAG2-M mice following treatment with trastuzumab only at 0.3 mg/kg (●), or coadministered with free doxorubicin at 5.0 mg/kg (▼) or liposomal doxorubicin (■). Data points represent the mean ± standard error of the mean from groups of 4–5 mice.

Serum Drug Levels

These studies were conducted to assess any impact of free or liposomal doxorubicin on the serum levels of trastuzumab or conversely, any impact of trastuzumab administration on the levels of free or liposomal doxorubicin. Figure 4 shows the serum trastuzumab levels following a single bolus intraperitoneal injection of trastuzumab (0.3 mg/kg) administered within one hour of intravenous injection with either free or liposomal doxorubicin (5.0 and 2.5 mg/kg, respectively). The results demonstrate an increase in serum trastuzumab levels over eight hours post administration, with slow elimination between 10 and 24 h, the longest timepoint assessed. At 24 h, the serum trastuzumab concentration was 4.45 ± 0.69 ng/ml when in combination with free doxorubicin, and 4.48 ± 0.54 ng/ml when in combination with liposomal doxorubicin. The lower limit of quantitation of trastuzumab

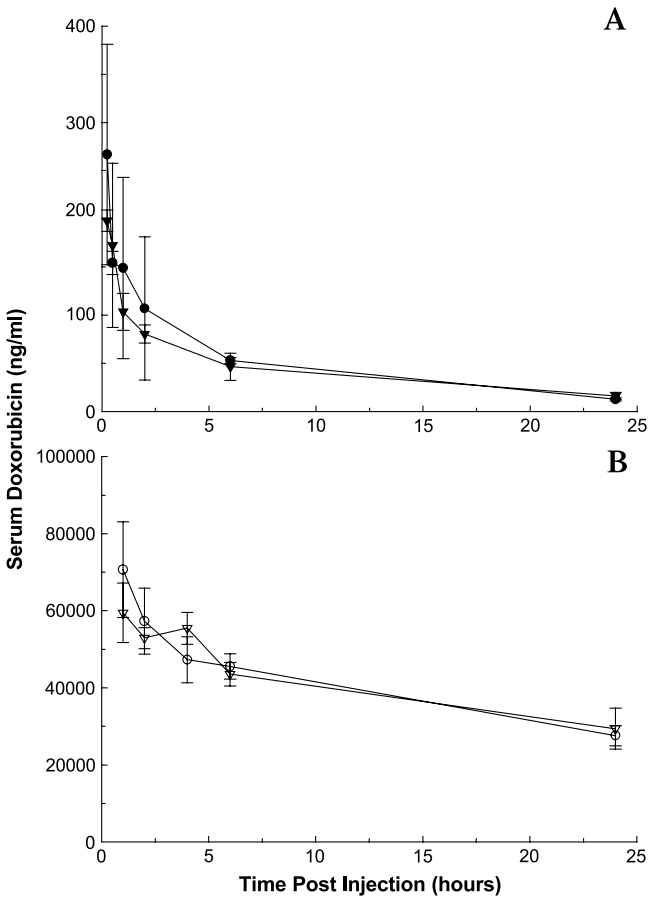

Fig. 5. Serum doxorubicin levels in female RAG2-M mice following treatment with (A) free doxorubicin only at 5.0 mg/kg (●), free doxorubicin coadministered with trastuzumab at 0.3 mg/kg (▼), (B) liposomal doxorubicin only (●), or liposomal doxorubicin coadministered with trastuzumab (▼). Data points represent the mean ± standard error of the mean from groups of 4–5 mice.

was 2.5 ng/ml, and the level of this therapeutic antibody was below the limit of the trastuzumab assay at 48 h. There was no significant difference in the absorption phase, peak serum levels or elimination over 24 h between mice treated with trastuzumab only, or those treated in combination with either free or liposomal doxorubicin. The mean AUC (0–24 h), as determined by the trapezoidal rule, for saline, free doxorubicin or Caelyx treated animals was 103.47, 117.74, and 112.17 ng trastuzumab·h/ml, respectively.

Similar experiments were performed in animals given free doxorubicin or Caelyx, with serum being analyzed for doxorubicin levels over time (Fig. 5). These results highlight two points. First, comparing the serum levels of doxorubicin following injection of free drug (Fig. 5A) or Caelyx (Fig. 5B) clearly shows that drug levels are 200- to 1000-fold higher after administration of the liposomal drug. Second, coadministration of trastuzumab with doxorubicin did not alter the serum concentrations of either free or liposomal doxorubicin. The AUC (0–24 h) of free drug and drug encapsulated in liposomes was 0.85 and 919.54 μg·h/ml respectively. These values were 0.77 and 919.88 μg·h/ml respectively when the drugs were given with trastuzumab.

DISCUSSION

To effectively design combination regimens, several parameters must be considered. First, the optimal dose of the two (or more) drugs to be used must be defined. Second, the potential for drug–drug interactions altering the pharmacokinetics/biodistribution of the drugs needs to be established. And third, the therapeutic potential of the drug combination needs to be established. While a majority of chemotherapeutic regimens have used drugs at or near their maximal tolerated dose, thereby hoping to achieve maximal therapeutic benefit, there may be rationale for using lower doses and potentially expanding the therapeutic window by selecting drug combinations that are synergistic. To further optimize combination regimens, doses of drugs may have to be titrated against each other at varying ratios, to achieve optimal results. Our laboratory is focusing on demonstration of the potential of fixed dose combination products, but this approach to combination therapy must first rely on selection of drugs which have the potential to act synergistically and which do not exhibit interactions that may adversely affect their activity *in vivo*.

The majority of regimens in use in the treatment of advanced, metastatic breast cancer include two or more therapeutic agents. Traditionally, these have been cytotoxic agents, however more recently these are likely to include at least one biologically targeted agent. Pharmacokinetic drug–drug interactions are a critical point in design of optimal treatment regimens and play an important role in development of clinical trials and treatment protocols. Interactions can result in altered absorption due to chelation, complex formation or pH effects, altered distribution if the two agents use similar binding sites, altered transport, enhanced or decreased metabolism, or altered excretion (55). In this study, we specifically focused on the serum drug levels of doxorubicin and trastuzumab following coadministration of these two drugs. Any alteration in levels noted may have indicated a need to adjust the schedule of administration of the drugs to achieve maximum benefit. No alterations in trastuzumab peak serum levels or elimination over 24 h were noted as a result of coadministration with either free or liposomal doxorubicin, nor were alterations in doxorubicin serum levels noted following co-administration of trastuzumab. A more sensitive assay for trastuzumab may have allowed us to further characterize the elimination phase for this antibody drug. Assessment of the disposition of active metabolite of doxorubicin, doxorubicinol, may also have provided a more subtle indication of differences in the metabolism of free vs. liposomal doxorubicin with or without trastuzumab. Further, it will be important to assess how the combination influences tissue distribution of the individual drugs. Regardless, these preliminary data suggest that the combination of trastuzumab and liposomal doxorubicin will be therapeutically effective and that plasma elimination data suggest that these agents, which are both eliminated in part by MPS cells, are not adversely affected when co-administered.

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REFERENCES

1. J. H. Goldie. New information on drug resistance: implications for the adjuvant treatment of breast cancer. *Recent Results Cancer Res.* **115**:8–16 (1989).
2. E. Frei III. Acute leukemia in children. Model for the development of scientific methodology for clinical therapeutic research in cancer. *Cancer* **53**:2013–2025 (1984).
3. Beers, M. H. (ed.), *Merck Manual of Medical Information*, Merck Research Laboratories, Whitehouse Station, NJ, 2003.
4. J. H. Lin. Drug–drug interaction mediated by inhibition and induction of P-glycoprotein. *Adv. Drug Deliv. Rev.* **55**:53–81 (2003).
5. D. Lopes and L. D. Mayer. Pharmacokinetics of Bcl-2 antisense oligonucleotide (G3139) combined with doxorubicin in SCID mice bearing human breast cancer solid tumor xenografts. *Cancer Chemother. Pharmacol.* **49**:57–68 (2002).
6. H. Wang, M. Li, J. J. Rinehart, and R. Zhang. Dexamethasone as a chemoprotectant in cancer chemotherapy: hematoprotective effects and altered pharmacokinetics and tissue distribution of carboplatin and gemcitabine. *Cancer Chemother. Pharmacol.* **53**:459–467 (2004).
7. A. C. Wolff, M. Bonetti, and J. A. Sparano. Cardiac safety of trastuzumab (H) in combination with pegylated liposomal doxorubicin (D) and docetaxel (T) in HER2-positive metastatic breast cancer (MBC): preliminary results of the Eastern Cooperative Oncology Group trial E3198. *Proc. Am. Soc. Clin. Oncol.* **22**:A-70, (2003).
8. M. A. Rodriguez, A. Sarris, K. East, N. H. Dang, L. Fayad, A. Goy, F. Hagemeister, B. Pro, J. Romaguera, F. Samaniego, A. Younes, and F. Cabanillas. A phase II study of liposomal vincristine in CHOP with rituximab for elderly patients with untreated aggressive B-cell non-Hodgkin's lymphoma (NHL). *ASCO Gen. Meet.* Abstract #1132 (2002).
9. F. Montemurro, G. Valabrega, and M. Aglietta. Trastuzumab-based combination therapy for breast cancer. *Expert Opin. Pharmacother.* **5**:81–96 (2004).
10. R. Repp, H. H. van Ojik, T. Valerius, G. Groenewegen, G. Wieland, C. Oetzel, B. Stockmeyer, W. Becker, M. Eisenhut, H. Steininger, Y. M. Deo, G. H. Blijham, J. R. Kalden, J. G. van de Winkel, and M. Gramatzki. Phase I clinical trial of the bispecific antibody MDX-H210 (anti-FcgammaRI (anti-HER-2/

neu) in combination with Filgrastim (G-CSF) for treatment of advanced breast cancer. *Br. J. Cancer* **89**:2234–2243 (2003).
11. B. Ramaswamy and C. L. Shapiro. Phase II trial of bevacizumab in combination with docetaxel in women with advanced breast cancer. *Clin. Breast Cancer* **4**:292–294 (2003).
12. L. M. Weiner. Monoclonal antibody therapy of cancer. *Semin. Oncol.* **26**:43–51 (1999).
13. M. von Mehren, G. P. Adams, and L. M. Weiner. Monoclonal antibody therapy for cancer. *Ann. Rev. Cancer* **54**:343–369 (2003).
14. B. Gatto. Monoclonal antibodies in cancer therapy. *Curr. Med. Ther.–Anti-Cancer Agents* **4**:411–414 (2004).
15. D. J. Slamon, B. Leyland-Jones, S. Shak, H. Fuchs, V. Paton, A. Bajamonde, T. Fleming, W. Eiermann, J. Wolter, M. Pegram, J. Baselga, and L. Norton. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* **344**:783–792 (2001).
16. L. A. Emens and N. E. Davidson. Trastuzumab in breast cancer. *Oncology* **18**:1117–1128 (2004).
17. R. Bell, S. Verma, M. Untch, D. Cameron, and I. Smith. Maximizing clinical benefit with trastuzumab. *Semin. Oncol.* **31**:35–44 (2004).
18. M. A. Cobeigh, C. L. Vogel, D. Tripathy, N. J. Robert, S. Scholl, L. Fehrenbacher, V. Paton, S. Shak, G. Lieberman, and D. Slamon. Efficacy and safety of Herceptin (humanized anti-HER2 antibody) as a single agent in 222 women with HER2 overexpression who relapsed following chemotherapy for metastatic breast cancer. *Proc. Am. Soc. Clin. Oncol.* Abstract #376 (1998).
19. D. C. Allred, A. K. Tandon, G. M. Clark, and W. L. McGuire. Her2/neu oncogene amplification and expression in human mammary carcinoma. In T. G. Pretlow II and T. P. Pretlow (eds.), *Biochemical and Molecular Aspects of Selected Cancers*, Vol. 1, Academic Press, Inc., San Diego, CA, USA; London, England, UK, 1991, pp. 75–97.
20. A. Berchuck, A. Kamel, R. Whitaker, B. Kerns, G. Olt, R. Kinney, J. T. Soper, R. Dodge, D. L. Clarke-Pearson, and P. Marks. Overexpression of HER-2/neu is associated with poor survival in advanced epithelial ovarian cancer. *Cancer Res.* **50**:4087–4091 (1990).
21. W. B. Carter, J. B. Hoying, C. Boswell, and S. K. Williams. HER2/neu over-expression induces endothelial cell retraction. *Int. J. Cancer* **91**:295–299 (2001).
22. A. Harlozinska, J. K. Bar, R. Wenderski, and M. Bebenek. Relationship between c-erbB-2 oncoprotein, epidermal growth factor receptor, and estrogen receptor expression in patients with ductal breast carcinoma. Association with tumor phenotypes. *In vivo* **10**:217–222 (1996).
23. A. H. McCann, P. A. Dervan, M. O'Regan, M. B. Codd, W. J. Gullick, B. M. J. Tobin, and D. N. Carney. Prognostic significance of c-erbB-2 and estrogen receptor status in human breast cancer. *Cancer Res.* **51**:3296–3303 (1991).
24. M. Piccart, C. Lohrisch, A. Di Leo, and D. Larsimont. The predictive value of Her-2 in breast cancer. *Oncology* **61**:73–82 (2001).
25. D. J. Slamon, W. Godolphin, L. A. Jones, J. A. Holt, S. G. Wong, D. E. Keith, W. J. Levin, S. G. Stuart, J. Udove, A. Ullrich, and M. A. Press. Studies of HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* **244**:707–712 (1989).
26. J. A. Kern, D. A. Schwartz, J. E. Nordberg, D. B. Weiner, M. I. Greene, L. Torney, and R. A. Robinson. P185neu expression in human lung adenocarcinomas predicts shortened survival. *Cancer Res.* **50**:5184–5187 (1990).
27. M.-C. Hung and Y.-K. Lau. Basic Science of HER-2/neu: a review. *Semin. Oncol.* **26**:51–59 (1999).
28. Y. Wen, M.C.-T. Hu, K. Makino, B. Spohn, G. Bartholomeusz, D.-H. Yan, and M.-C. Hung. Her-2/neu promotes androgen-independent survival and growth of prostate cancer cells through the Akt pathway. *Cancer Res.* **60**:6841–6845 (2000).
29. C. Ensinger, R. Kremser, P. Obrist, D. Kendler, and H. Erler. Her2/neu overexpression in differentiated thyroid carcinomas. *Proc. AACR* **43**:717 Abstract #3553 (2002).
30. S. U. Goebel, M. Iwamoto, M. Raffeld, F. Gibril, W. Hou, J. Serrano, and R. T. Jensen. Her-2/neu expression and gene amplification in gastriomas: correlations with tumor biology, growth, and aggressiveness. *Cancer Res.* **62**:3702–3710 (2002).
31. A. D. Santin, S. Bellone, M. Palmieri, M. Gokden, J. J. Roman, S. Pecorelli, M. J. Cannon, L. F. Hutchins, J. G. Agha, D. L. Dunn, T. J. O'Brien, and P. P. Groesbeck. HER2/neu overexpression in uterine serous papillary cancer. *Proc. AACR* **43**:716–717, Abstract #3552 (2002).
32. M. Fornier, P. Munster, and A. D. Seidman. Update on the management of advanced breast cancer. *Oncology* **13**:647–664 (1999).
33. D. J. Slamon, B. Leyland-Jones, S. Shak, H. Fuchs, V. Paton, A. Bajamonde, T. Fleming, W. Eiermann, J. Wolter, M. Pegram, J. Baselga, and L. Norton. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* **344**:783–792 (2001).
34. S. Gori, M. Colozza, A. M. Mosconi, E. Franceschi, C. Basurto, R. Cherubini, A. Sidoni, A. Rulli, C. Bisacci, V. De Angelis, L. Crino, and M. Tonato. Phase II study of weekly paclitaxel and trastuzumab in anthracycline- and taxane-pretreated patients with HER2-overexpressing metastatic breast cancer. *Br. J. Cancer* **90**:36–40 (2004).
35. S. Stein, A. DeMichele, S. Domchek, and K. Fox. Gemcitabine and trastuzumab combinations for patients with metastatic breast cancer overexpressing HER2/neu. *Clin. Breast Cancer* **4**:S117–S120 (2004).
36. F. Montemurro, G. Choa, R. Faggiuolo, M. Donadio, M. Minischetti, A. Durando, A. Capaldi, G. Vietti-Ramus, O. Alabiso, and M. Aglietta. A phase II study of three-weekly docetaxel and weekly trastuzumab in HER2-overexpressing advanced breast cancer. *Oncology* **66**:38–45 (2004).
37. M. S. Ewer, H. R. Gibbs, J. Swafford, and R. S. Benjamin. Cardiotoxicity in patients receiving transtuzumab (Herceptin): primary toxicity, synergistic or sequential stress, or surveillance artifact? *Semin. Oncol.* **26**:96–101 (1999).
38. S. K. Rigatos, D. Tsavdraris, A. Athanasiadis, J. G. Stathopoulos, and G. P. Stathopoulos. Paclitaxel and liposomal doxorubicin (Caelyx) combination in advanced breast cancer patients: a phase II study. *Oncol. Rep.* **10**:1817–1819 (2003).
39. T. Safra. Cardiac safety of liposomal anthracyclines. *The Oncologist* **8**:17–24 (2003).
40. M. E. O'Brien, N. Wigler, M. Inbar, R. Rosso, E. Grischke, A. Santoro, R. Catane, D. G. Klieback, P. Tomczak, S. P. Ackland, F. Orlandi, L. Mellars, L. Alland, and C. Tendler. CAELYX Breasts Cancer Study Group. *Ann. Oncol.* **15**:440–449 (2004).
41. A. Gabizon, R. Catane, B. Uziely, B. Kaufman, T. Safra, R. Cohen, and F. Martin. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. *Cancer Res.* **54**:987–992 (1994).
42. F. Muggia and A. Hamilton. Phase III data on Caelyx in ovarian cancer. *Eur. J. Cancer* **37**:S15–S18 (2001).
43. D. N. Waterhouse, P. Tardi, L. G. Mayer, and M. Bally. A comparison of liposomal formulations of doxorubicin with drug administered in free form. *Drug Safety* **24**:903–920 (2001).
44. T. Daemen, G. Hofstede, M. T. Ten Kate, I. A. Bakker-Woudenberg, and G. L. Scherphof. Liposomal doxorubicin-induced toxicity: depletion and impairment of phagocytic activity of liver macrophages. *Int. J. Cancer* **61**:716–721 (1995).
45. M. J. Parr, S. M. Ansell, L. S. Choi, and P. R. Cullis. The presence of GM1 in liposomes with entrapped doxorubicin does not prevent RES blockade. *Biochim. Biophys. Acta* **1168**:249–252 (1993).
46. M. J. Parr, D. Masin, P. R. Cullis, and M. B. Bally. Accumulation of liposomal lipid and encapsulated doxorubicin in murine lewis lung carcinoma: the lack of beneficial effects by coating liposomes with poly(ethylene glycol). *J. Pharmacol. Exp. Ther.* **280**:1319–1327 (1997).
47. I. A. Bakker-Woudenberg. Long-circulating sterically stabilized liposomes as carriers of agents for treatment of infection or for imaging infectious foci. *Int. J. Antimicrob. Agents* **19**:299–311 (2002).
48. E. D. Lobo, R. J. Hansen, and J. P. Balthasar. Antibody pharmacokinetics and pharmacodynamics. *J. Pharm. Sci.* **93**: 2645–2668 (2004).
49. M. Pegram, S. Hsu, G. Lewis, R. Pietras, M. Beryt, M. Sliwkowski, D. Coombs, D. Baly, F. Kabbinavar, and D. Slamon. Inhibitory effects of combinations of HER-2/neu

antibody and chemotherapeutic agents used for treatment of human breast cancers. *Oncogene* **18**:2241–2251 (1999).
50. R. J. Pietras, M. D. Pegram, R. S. Finn, D. A. Maneval, and D. J. Slamon. Remission of human breast cancer xenografts on therapy with humanized monoclonal antibody to HER-2 receptor and DNA-reactive drugs. *Oncogene* **17**:2235–2249 (1998).
51. C. C. Benz, K. G. Scott, J. C. Sarup, R. M. Johnson, D. Tripathy, E. Coronado, E. M. Shepard, and C. K. Osborne. Estrogen-dependent, tamoxifen-resistant tumorigenic growth of MCF-7 cells transfected with HER2/neu. *Breast Cancer Res. Treat.* **24**:85–95 (1993).
52. S. Palm, R. M. Enmon Jr., C. Matei, K. S. Kolbert, S. Xu, P. B. Zanzonico, R. L. Finn, J. A. Koutcher, S. M. Larson, and G. Sgouros. Pharmacokinetics and biodistribution of (86)Y-trastuzumab for (90)Y dosimetry in an ovarian carcinoma model: correlative microPET and MRI. *J. Nucl. Med.* **44**:1148–1155 (2003).
53. L. Embree, K. A. Gelmon, A. Lohr, L. D. Mayer, A. J. Coldman, P. R. Cullis, W. Palaitis, F. Pilkiewicz, N. J. Hudon, and J. H. Goldie. Chromatographic analysis and pharmacokinetics of liposome-encapsulated doxorubicin in non-small-cell lung cancer patients. *J. Pharm. Sci.* **82**:627–634 (1993).
54. S. M. Shafie. Estrogen and the growth of breast cancer: new evidence suggests indirect action. *Science* **209**:701–702 (1980).
55. Drug–Drug Interactions: Studies *in Vitro* and *in Vivo*. Therapeutic Products Directorate, Health Canada. http://www.hc-sc.gc.ca/hpfb-dgpsa/tpd-dpt/drug_int_e.html (accessed 10/13/04).