Semi-Mechanism-Based Pharmacokinetic/Pharmacodynamic Model for the Combination Use of Dexamethasone and Gemcitabine in Breast Cancer

YIN YUAN,[1] XUAN ZHOU,[1] YUPENG REN,[1] SHUPEI ZHOU,[2] LIJIE WANG,[1] SHUANGMIN JI,[1] MING HUA,[2] LIANG LI,[1,3] WEI LU,[1,3] TIANYAN ZHOU[1,3]

[1]School of Pharmaceutical Science, Peking University, Beijing 100191, China
[2]The Department of Laboratory Animal Science, Health Science Center, Peking University, Beijing 100191, China
[3]State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, China

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ABSTRACT: Our study aimed at the investigation of *in vivo* anticancer effect of the combination use of dexamethasone (DEX) and gemcitabine (GM) as well as the development of pharmacokinetic/pharmacodynamic (PK/PD) models in MCF-7 xenograft model. Further, simulations were conducted to optimize doses and administration schedules. The inhibitory effect of different doses and administration schedules were investigated in MCF-7 xenograft model. Semi-mechanism-based PK/PD models were established based on the preclinical data to characterize the relationship between plasma concentration and the time course of the drug response quantitatively. The PK/PD models were further applied to predict and optimize doses and administration schedules, which would lead to tumor stasis by the end of the treatment. Synergistic effect was observed in the PD study *in vivo* and further confirmed by the estimated combination index ψ obtained from PK/PD models. The optimum dose regimen was selected as DEX 2 mg/kg, qd and GM 10 mg/kg, q2d based on the simulation results. In summary, the PD interaction between DEX and GM was demonstrated as synergism by both experimental results and modeling approach. Dosage regimens were optimized as predicted by modeling and simulations, which would provide reference for preclinical study and translational research as well. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:4399–4408, 2015

Keywords: pharmacokinetic/pharmacodynamic models; simulation; drug interaction; cancer chemotherapy; *in silico* modeling

INTRODUCTION

Breast cancer has been considered as the most frequently diagnosed cancer and the leading cause of mortality among women around the world.[1] Chemotherapy is one of the most common therapeutic strategies for breast cancer either before or after operation.[2] Gemcitabine (GM) is an analog of deoxycytidine, which acts as an inhibitor of ribonucleotide reductase and incorporates into cellular DNA, thereby leading to the depletion of dATP and the inhibition of DNA synthesis.[2,3] It has extensive use in the treatment of breast cancer, locally advanced or metastatic pancreatic cancer, ovarian cancer and non-small-cell lung cancer as well.[4–6] However, because of the cytotoxic mechanism of GM, side effects such as neutropenia, anemia, and thrombocytopenia are inevitable,[7] which should be reduced or avoided in the optimization of anticancer therapy.

The combination use of different anticancer drugs with multiple mechanisms has become increasingly investigated and achieved incredible progress in oncology.[8,9] Therefore, the combination therapy of GM with other molecules is expected to achieve better efficacy with low toxicity. As one of the most widely used synthetic glucocorticoids (GCs), dexamethasone (DEX) has been demonstrated with anticancer potency in breast cancer xenograft model as well as in other tumor models (i.e., glioma, metastatic brain tumor, and melanoma).[10–14] According to the previous research progress, there is a great diversity of mechanisms related to the antitumor efficacy of DEX. DEX can reduce the uptake of cytotoxic drugs and hematotoxicity in host tissues while enhancing the drug response, which enables DEX to act as chemoprotectant and chemosensitizer during chemotherapeutics.[15] It has also been reported that DEX can decrease estrogen response by inducing the activity of estrogen sulfotransferase through activating GC receptor in the upstream, thus suppressing the breast tumor growth *in vivo*.[10] Moreover, DEX can be used as a potential VEGF inhibitor by restraining the expression of HIF-1α, thus exerting anti-angiogenic effects and inhibiting the tumor growth.[16,17] Based on the above-mentioned possible mechanisms of both drugs, we assumed that the combination use of DEX and GM could produce a synergistic effect in the treatment of breast cancer.

The combination use of drugs will surely lead to the combination of doses and schedules, which closely related to efficacy and toxicity. However, using experimental approach to testify and optimize the dosage regimens remains many limitations. PK/PD modeling and simulation provides a feasible approach to realize quantitative optimization of treatment schedules with high efficiency. Based on experimental PK and PD data, the PK/PD model is developed and testified, and the estimated

Abbreviations used: GM, gemcitabine; GCs, glucocorticoids; DEX, dexamethasone; PK/PD, pharmacokinetic/pharmacodynamic; PK, pharmacokinetics; PD, pharmacodynamics; OFV, objective function value; VPC, visual predictive check.

Correspondence to: Tianyan Zhou (Telephone: +86-10-82805937; Fax: +86-10-82801717; E-mail: tianyanzhou@bjmu.edu.cn)

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parameters are further utilized to perform simulations, so as to predict drug response under different dose regimens and achieve the optimum schedule. To the best of our knowledge, there have been some examples which take advantage of the established PK/PD models and the estimated parameters to perform simulations based on designed administration schedules. By comparing the predicted drug response of different administration schedules, the modeling approach can realize the optimization of the combination use of anticancer drugs and show preferable predicting prospect in both preclinical and clinical field.[18,19]

The purpose of this study was to investigate the *in vivo* anticancer effect of the combination use of DEX and GM, and to describe the quantitative relationship between plasma concentration and drug response by modeling approach as well as predicting therapeutic effect of different dose regimens and selecting the optimum therapeutic regimen. Semi-mechanism-based pharmacokinetic/pharmacodynamic (PK/PD) models of DEX, GM as well as their combination use in MCF-7 nude mice model were established for the first time in our study. The PK/PD models and the estimated parameters were further utilized to identify the PD interaction between DEX and GM, and to optimize doses and administration schedules, aiming at achieving sufficient efficacy while reducing toxicity.

MATERIALS AND METHODS

Animals

Female BALB/c nude mice (CAnN.Cg-Foxn1^{nu}/CrlVr, Inbred Mice) weighing between 19 and 21 g were purchased from the Animal Service of Health Science Center, Peking University (Haidian district, Beijing, China). The animals were housed under constant standard temperature (25°C–28°C) and humidity between 50% and 60% on a controlled 12 h light/dark cycle. The nude mice had free access to rodent chow and water, except for those used for PK study, which food supply was stopped 12 h before administration. All the animal studies were granted with animal ethics approval by the Institutional Animal Care and Use Committee of Peking University. This research adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised in 1985).

Reagents and Materials

Gemcitabine was purchased from Melone Pharmaceutical Company, Ltd. (Dalian, China), and DEX was purchased from Sigma–Aldrich Company (Nanjing, China). RPMI1640 was bought from Macgene Biotech Company, Ltd. (Beijing, China), and fetal bovine serum was obtained from Gibco (Grand Island, New York, USA).

Cell Culture

MCF-7 human breast cancer cell line was kindly provided by Prof. Wan-liang Lu (School of Pharmaceutical science, Peking University), and cultured in RPMI1640 containing 10% fetal bovine serum. The tumor cells were maintained at 37°C under a mixed atmosphere with 5% CO_2 and 95% air.

PK Study

The PK of DEX was characterized by a two-compartment model with first-order absorption,[20] whereas the PK of GM was characterized by a two-compartment model without absorption compartment since GM was administered via intravenous (i.v.) injection.[21] The PK parameters of DEX were obtained from our former study,[20] and the PK parameters of GM were obtained from literature.[21]

PD Study *in Vivo*

2×10^6 MCF-7 cells were suspended in 200 μL RPMI1640 free of fetal bovine serum and inoculated subcutaneously in the second mammary fat pad on the right flank of nude mice. Tumor diameters were measured by electronic Vernier caliper and tumor volumes were calculated according to the equation: TV (mm^3) = length × width^2 × 0.5.[22] When the tumor volumes reached a mean volume of 50–75 mm^3, the animals were assigned to eight groups randomly ($n = 5$) and began to receive administration. DEX was dissolved in corn oil and administered by intraperitoneal (i.p.) injection, whereas GM was dissolved in PBS (pH 7.4) and given via i.v. injection. The vehicle group received vehicle solution paralleled with the treatment groups. In the 4 monotherapy groups, mice were treated with DEX (2 mg/kg, i.p.) daily (qd) or GM (5, 10, and 15 mg/kg, i.v.) every 3 days (q3d × 3). The three combination groups were all treated with DEX (2 mg/kg, i.p.) daily (qd), whereas different doses of GM (5, 10, and 15 mg/kg, i.v.) were simultaneously administered to different groups every 3 days (q3d × 3), respectively. Tumor volumes and body weights of nude mice were measured and recorded every day during the study.

PK/PD Models

The natural growth of tumor was characterized by the nonlinear model suggested by Koch et al.[23] The model function (Eq. (1)) and differential equation (Eq. (2)) are as follows.

$$f(X_1) = \frac{2\lambda_0\lambda_1 X_1}{\lambda_1 + 2\lambda_0 X_1} \tag{1}$$

$$\frac{dX_1}{dt} = \frac{2\lambda_0\lambda_1 X_1(t)^2}{\left(\lambda_1 + 2\lambda_0 X_1(t)\right) w(t)},\ X_1(0) = w_0 \tag{2}$$

where λ_0, λ_1, and w_0 represent the exponential growth rate, the linear growth rate, and the initial tumor volume, respectively. The model assumes that all tumor cells keep proliferating in vehicle group.

Tumor growth is perturbed upon the effect of anticancer drugs. PK/PD models for single drug treatment were established and presented in Figure 1 (i.e., Fig. 1a for DEX and Fig. 1b for GM), the basic model structure of both models were taken from literature, as previously proposed by Simeoni et al. and Lubo and Balthasar.[24,25]

As DEX was previously reported with the ability to change the inflammatory microenvironment and induce the expression and activity of estrogen sulfotransferase (EST) by activating GC (GR), thus promoting the metabolism and inactivation of estrogen and reducing the stimulation of estrogen on the proliferation of estrogen-dependent MCF-7 breast cancer,[10,26] the PK/PD model of DEX was developed based on its main mechanism as shown in Figure 1a, where DEX exerted antiproliferation effect on MCF-7 breast cancer cells. Therefore, in