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A Limited Sampling Method for the Estimation of Serum Calcitriol Area under the Curve in Cancer Patients

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Calcitriol (1α, 25-dihydroxyvitamin D₃) has potent antiproliferative, prodifferentiative, and proapoptotic effects that have the potential to be exploited to treat human cancers. The evaluation of calcitriol as an anticancer agent either alone or in combination with dexamethasone or cytotoxic drugs is ongoing. Therapeutic efficacy of intermittent calcitriol therapy in combination with docetaxel in androgen-independent prostate cancer has been reported. Preclinical data indicate that antitumor effects are related to the dose of calcitriol; serum calcitriol AUC and/or peak calcitriol concentrations (C_max) may be important pharmacokinetic determinants of antitumor activity.

We have previously reported substantial interpatient variation in serum calcitriol C_max and AUC, as well as a nonlinear relationship between dose and AUC at high oral (PO) calcitriol doses. The pharmacological basis of these pharmacokinetic (PK) characteristics is unknown but may include variability in calcitriol absorption and metabolism. Because of the large variability in serum calcitriol C_max and AUC, the measurements of serum calcitriol levels in cancer patients will be required to optimize antitumor effects.

The lack of an inexpensive, simple, specific, and sensitive assay to measure serum calcitriol levels limits PK studies of this drug. High-performance liquid chro-
We have developed and validated an LSM for estimating serum calcitriol concentrations in human serum samples. HPLC assay with fluorometric detection is impractical for analyzing numerous serum calcitriol PK samples because of the multiple pre- and postfluorescence labeling solid-phase extractions and two HPLC separations required. Calculitriol PK studies are very expensive because of the high cost of the [1\(^{25}\)]-1\(\alpha\), 25-dihydroxyvitamin D\(_3\), RIA kits and the large number of samples required. Our ongoing calcitriol PK studies require 12 to 16 blood samples, approximately 100 mL of blood, and a 15- to 24-hour stay in the hospital. The reasons for developing a limited sampling method (LSM) for the estimation of serum calcitriol AUC are twofold: first, it should be more acceptable to patients if they need to give fewer blood samples over a shorter time. Second, the cost of PK studies would clearly be much lower if fewer samples could be used. We have developed and validated an LSM for estimating serum calcitriol AUC based on pretreatment and two additional blood samples.

**PATIENTS AND METHODS**

**Study Patients**

Patients studied were enrolled in phase I trials of calcitriol at the University of Pittsburgh Cancer Institute. All patients had advanced solid tumors for which no standard therapy was available. All patients had prior cancer chemotherapy but were off any treatment for at least 3 weeks at the time of study. Additional eligibility criteria included adequate bone marrow, kidney, and liver function, as evidenced by WBC \(\geq 4000/\mu\text{L}\), platelets \(\geq 100,000\), serum creatinine \(\leq 1.5\ \text{mg/dL}\), bilirubin \(\leq 2\ \text{mg/dL}\), and SGOT \(\leq 45\ \text{IU/L}\). Patients with albumin-corrected serum calcium \(\leq 10.5\ \text{mg/dL}\) or past medical history of kidney stones were excluded. All study protocols were reviewed and approved by the Biomedical Institutional Review Board of the University of Pittsburgh Medical Center. Patients signed a written informed consent before participating.

**Calcitriol Treatment and Blood Sample Collection for PK Studies**

Our LSM for estimating calcitriol AUC was developed from day 1 PK data derived from the phase I trial of subcutaneous (SC) calcitriol in advanced solid-tumor patients. Calcitriol dose ranged from 2 to 10 \(\mu\text{g}\) and was administered on an every-other-day schedule (QOD); 7 mL of blood for PK studies was collected before and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 hours after the first dose of calcitriol. Blood samples were centrifuged for 10 minutes at 1400 rpm at 4°C and serum stored at –20°C until assayed for calcitriol.

The clinical utility of the LSM was further evaluated in advanced solid-tumor patients enrolled in the three additional PO calcitriol trials (Table I). In the calcitriol + paclitaxel (D + T) and calcitriol + dexamethasone (D + Dex) trial, PK blood samples were collected prior to and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 18, 20, 22, and 24 hours after calcitriol administration. For the calcitriol + carboplatin (D + C) trial (carboplatin administered 24 h before the first dose of calcitriol), samples were collected before and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 hours. The preparation of calcitriol used in these trials was the commercially available caplet (0.5 \(\mu\text{g/caplet}, \text{Rocaltrol, Hoffman-LaRoche}\). The dose of caplet calcitriol administered was 4 to 38 \(\mu\text{g}\). Caplet-formulated calcitriol was changed to liquid calcitriol (formulated as 1-\(\mu\text{g/mL}\) calcitriol in palm oil) because ingestion of a large number of caplets required to deliver high calcitriol doses was not well tolerated. The dose of liquid calcitriol was 8 to 36 \(\mu\text{g}\).

**RIA for Serum 1,25-Dihydroxyvitamin D\(_3\) Concentrations**

Serum calcitriol concentrations were measured using the [1\(^{25}\)]-1,25-dihydroxyvitamin D\(_3\), RIA kit from DiaSorin Labs (Stillwater, MN) as previously described.

**Calculation of Pharmacokinetic Parameters**

Pretreatment serum calcitriol level for each individual patient was subtracted from serum calcitriol concentration measured at each sampled time point before calculating corrected PK parameters. Serum calcitriol \(C_{\text{max}}\) and time to \(C_{\text{max}}\) (\(t_{\text{max}}\)) were determined by visual inspection of serum calcitriol concentration versus time curves. Corrected serum calcitriol AUC\(_0\) \(\rightarrow 12\ \text{h}\) and AUC\(_0\) \(\rightarrow 24\ \text{h}\) values were calculated by the trapezoidal rule using the PHARM/PCS computer program. Linear regression analyses were performed using the NCSS statistical software package.

**Development of Limited Sampling Model Method**

The 34 patients enrolled in the phase I trial of SC calcitriol were split into training and evaluation data.
sets based on calcitriol dose and chronological order of enrollment, and pharmacokinetic data were subjected to linear regression analysis. The result was that 17 patients, representing all range of doses, were assigned to the training data set. The remaining 17 patients were assigned to the evaluation data set. The training data set was used to develop the prediction rule by estimating regression parameters. The evaluation data set was used to assess the prediction.

The development of a prediction rule by this approach requires at least $2p + 25$ subjects, where $p$ is the number of regression coefficients thought necessary for good prediction. Using this guideline, we have sufficient patients to develop a predictive rule based on at most three coefficients, which would translate into an intercept and two coefficients for the limited sampling times. The first step was to fit straight-line regression models to predict the logarithm of corrected AUC$_{0 \rightarrow 12\, h}$ using individual log calcitriol concentrations at the following time points: baseline, hour 1, hour 2, hour 3, hour 4, hour 6, and hour 8. The AUC$_{0 \rightarrow 12\, h}$ and serum concentrations were logged to meet two standard regression assumptions: first, the relationship between the log serum concentrations and log AUC$_{0 \rightarrow 12\, h}$ was linear. Second, the residual distributions from these model fits were a good fit by normal distributions. After the linear relationship was estimated using the training data set, the fit was tested using the evaluation data set. For each patient in the evaluation data set, his or her log serum concentration was used to predict the log AUC$_{0 \rightarrow 12\, h}$. The measure of prediction error used was the mean squared error (MSE) over the 17 evaluation set patients:

$$\text{MSE} = \frac{\sum_{i=1}^{17} \left( \log \text{AUC}_i - \hat{\log \text{AUC}}_i \right)^2}{17},$$

where $\log \hat{\text{AUC}}_i$ is the predicted log AUC for the $i$th patient.

RESULTS AND DISCUSSION

Prediction Based on One Time Point Serum Calcitriol Measurement

Since the lower MSE is equivalent to smaller prediction error, the best single predictor of log AUC$_{0 \rightarrow 12\, h}$ was the log serum calcitriol at hour 6 (Table II). This predictor was used as the first term in further models that used two time points to predict log AUC$_{0 \rightarrow 12\, h}$. In addition, the hour 4 measurement was used in the same fashion to see whether a good prediction could be obtained while releasing the study subject 2 hours earlier. The predictive equations for the model based on the 4-hour and 6-hour measurements were as follows:

$$\log \text{AUC} = 1.582 + 0.734 \log (\text{calcitriol}) \text{ at hour 4},$$

$$\log \text{AUC} = 1.316 + 0.879 \log (\text{calcitriol}) \text{ at hour 6}.$$
Log AUC = 1.309 + 0.3969 \cdot \log (\text{calcitriol}) 
\text{at hour 2} + 0.4643 \cdot \log (\text{calcitriol}) \text{ at hour 4},

Log AUC = 1.125 + 0.3756 \cdot \log (\text{calcitriol}) 
\text{at hour 2} + 0.5859 \cdot \log (\text{calcitriol}) \text{ at hour 6}.

It is clear from MSE results and from plots of predicted versus observed AUC\text{0→12 h} (Figure 1) that the prediction is better using the 2 + 6–hour model than using the 2 + 4–hour model. However, the decision of which model to choose is not only statistical. Two clinical/scientific questions are relevant to the decision and may overrule statistical considerations: first, will patients find it more acceptable to enroll in a 4-hour than a 6-hour study? Second, is the improvement in prediction of the 6-hour data over the 4-hour data clinically significant? If the improvement in enrollment would not be substantial or the difference is clinically significant, then we would recommend the 2 + 6–hour model. Otherwise, the 2 + 4–hour model might be more attractive.

Application of the LSM to PK Data Obtained from Oral Calcitriol Trials

The utility of the 2 + 6–hour LSM to predict AUC\text{0→12 h} of PO-administered calcitriol was further examined using PK data obtained from 32 advanced cancer patients.
treated with 4 to 38 µg of caplet calcitriol and 19 patients receiving 13 to 36 µg of liquid calcitriol. Plots of predicted versus observed serum calcitriol AUC\(_{0\rightarrow12\,h}\) are shown in Figure 2A,B. These results indicate that the LSM developed using SC-administered calcitriol PK data is equally effective in predicting the serum calcitriol AUC\(_{0\rightarrow12\,h}\) of PO-administered calcitriol (caplet or liquid).

Figure 2C,D shows plots of predicted versus observed serum calcitriol AUC\(_{0\rightarrow12\,h}\) when calcitriol was administered 24 hours after carboplatin and when given concurrently with dexamethasone. The results indicate that LSM accurately predicts serum calcitriol AUC\(_{0\rightarrow12\,h}\) in the presence of these drugs. Accurate estimation of calcitriol AUC under these clinical conditions is important for two reasons: first, calcitriol antitumor activity is enhanced when used in combination with dexamethasone, and calcitriol enhances the antitumor activity of carboplatin.\(^{14,15}\) Second, administration of dexamethasone blocks calcitriol-induced hypercalcemia; hypercalcemia was the dose-limiting toxicity in cancer patients receiving SC calcitriol on a QOD treatment schedule.\(^7\)

The results of the predicted versus observed serum calcitriol AUC\(_{0\rightarrow12\,h}\) using the 2 + 4-hour LSM were also significantly correlated (\(r^2 = 0.88-0.96, p > 0.0001\)) for PO (caplet and liquid) calcitriol and when administered with dexamethasone or carboplatin (data not shown).

**Additional PK Information Derived from LSM**

We have previously reported substantial interpatient variability in the shape of the calcitriol concentration versus time curve in the first 12 hours after PO calcitriol and that the shape of this curve between the 12- and 24-hour time points was monotonously flat.\(^8\) This observation has been confirmed in a large number of cancer patients in the present study (data not shown). On the ba-
sis of these observations and the excellent prediction already achieved, we suggest that further improvement in estimating serum calcitriol AUC using the 2 + 4-hour or 2 + 6-hour LSM is unlikely to be achieved by including 12- to 24-hour time point samples.

Extending calcitriol PK sampling to 24 hours in our subsequent studies provided us the opportunity to demonstrate that serum calcitriol AUC$_{0\rightarrow12\text{h}}$ and AUC$_{0\rightarrow24\text{h}}$ were highly correlated ($r^2 = 0.9$, $p = 0.001$) (Figure 3A). Furthermore, serum calcitriol AUC$_{0\rightarrow12\text{h}}$ accounted for more than two-thirds of AUC$_{0\rightarrow24\text{h}}$ in 70% of the cancer patients studied (Figure 3B). These observations suggest that serum calcitriol AUC$_{0\rightarrow24\text{h}}$ may also be estimated using this LSM. The 2 + 6-hour LSM was not designed to provide information on serum calcitriol C$_{\text{max}}$.

Conclusions

We have developed an LSM that requires pretreatment and 2-hour and 6-hour samples for estimating serum calcitriol AUC. This limited sampling strategy is applicable for calcitriol administered SC and PO (caplet or solution). We have demonstrated that 24-hour prior carboplatin or concurrent dexamethasone administration did not influence the predictive value of this LSM. This LSM requires fewer blood samples over a shorter period and is, therefore, more convenient to patients and will reduce the cost of calcitriol PK studies. We suggest that this LSM may serve as a prototype for developing similar strategies for the PK studies of vitamin D$_3$ analogs in cancer patients.

REFERENCES