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Circulating tumor cell count during zoledronic acid treatment in men with metastatic prostate cancer: a pilot study

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Purpose: Recent clinical trials have demonstrated that zoledronic acid (ZOL) significantly prolongs survival in prostate cancer patients undergoing androgen deprivation therapy. This pilot study investigated the influence of ZOL on circulating tumor cell (CTC) counts in prostate cancer patients in association with prostate-specific antigen (PSA) used as a serum biomarker.
Methods: Patients with metastatic castration-resistant prostate cancer (CRPC) who were CTC-positive (n=4) were enrolled in treatment with ZOL between April 2012 and December 2013. CTCs were detected using the Cell Search System. The study evaluated CTC fluctuations at 1, 2, and 3 months versus baseline, as well as patient outcomes and adverse events.
Results: Two patients showed evidence of temporally decreased CTCs after ZOL treatment. Instead of decreasing the number of CTCs, the PSA level did not go down during the ZOL treatment. One patient could not undergo ZOL treatment due to rapid disease progression.
Conclusions: Although CTC count arguably provides useful information about patients undergoing ZOL treatment, the positive influence of ZOL may be limited to temporary effects for CRPC.

Keywords: Zoledronic acid, Circulating neoplatic cells, Prostate neoplasms

INTRODUCTION

Increasingly malignant cancer cells create an intravascular influx as they permeate blood vessels [1]. Several studies have confirmed that circulating tumor cells (CTCs) in the blood of breast, colorectal, and now prostate, cancer patients are a predictive factor of a poor prognosis [2-5]. Recent review articles suggest that tumor progression-specific CTCs not only identify cancer, but also its activity level [2-5]. Blood CTC count is strongly related to the prognosis for patients who have undergone therapy for castration-resistant prostate cancer (CRPC). CRPC patients, among whom pretreatment levels were 5 CTCs/7.5-mL blood, showed a significantly higher survival rate than baseline patients whose values were 5 or more CTCs/7.5-mL blood [6]. These findings suggest that CTC count may be a potential marker predicting treatment outcome and survivability in CRPC patients. They also clarify that patients whose high CTC counts undergo a marked decrease may expect an improved survivability, and that patients whose low CTC counts markedly increase may expect a worsening survivability [6]. The implication, therefore, is that CTC may act as a useful biomarker for patient condition and

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therapy, suggesting continuation of favorable treatment or an early alteration of ineffective treatment.

Zoledronic acid (ZOL) is a bisphosphonate widely recognized as a treatment for bone metastasis and hypercalcemia of malignancy. It is also administered to breast and prostate cancer patients, along with those suffering from multiple myeloma, to prevent skeletal-related events (SRE) following bone metastasis [7-12]. Several trials have shown that early-stage administration of bisphosphonate preserves bone mass, demonstrating its efficacy against bone loss. A placebo-controlled randomized clinical trial showed that SRE incidence decreased following ZOL administration in men with metastatic prostate cancer who had received hormone refractory therapy [9]. ZOL not only elevates bone mineral density (BMD) in prostate cancer patients, it also prevents bone loss and has demonstrated efficacy against SREs.

Moreover, bisphosphonate administration has been shown to aid survivability of various cancers including bladder and prostate cancer patients in multiple prospective randomized trials [13-17]. A Myeloma IX study demonstrated that this survivability was not only due to reduced bone fractures, but also reflected a direct ZOL antitumor effect [13]. ZOL facilitates inhibition of the chief enzyme in the mevalonate pathway (known to stimulate angiogenesis and promote cell survival), and is postulated to have a direct effect on tumors. *In vitro* studies have demonstrated that joint use of ZOL and a chemotherapy agent such as docetaxel or doxorubicin produces synergistic antitumor activity. ZOL administration has shown to improve overall survivability in breast cancer patients with bone metastasis, with data from several recent breast cancer adjuvant treatment studies showing ZOL antitumor efficacy in early-stage patients [18-20].

We administered ZOL to cancer patients with bone metastasis three times in 4-week intervals, studying the effects on CTCs over a 12-week period. We reasoned that if ZOL could be shown to reduce CTCs in CRPC patients with bone metastasis, its direct anti-tumor effect in prostate cancer patients might also be demonstrable.

MATERIALS AND METHODS

1. Patients

We conducted a prospective single-arm, open-label study in CRPC patients with bone metastasis and no history of bisphosphonate treatment. All patients gave written informed consent, and approval was obtained from the hospital Research Ethics Board. CRPC patients all had confirmed bone metastasis to at least one site. Patients followed a treatment protocol of intravenous ZOL administration in 4-week intervals (on days 0, 28, and 56) at our hospitals (Teikyo University School of Medicine, Sapporo Medical University School of Medicine, and Juntendo University, Graduate School of Medicine). Blood was collected prior to each ZOL administration for CTC and PSA measurement, with an additional collection during the 4th week following the third administration to facilitate weekly safety assessments. During the trial, no change was made in tumor treatment.

The following patients were excluded from this study: those with a corrected serum calcium level of less than 8.0 mg/dL or more than 12.0 mg/dL, those with a history of malignancy, and those with (upper or lower) tooth or jaw infection or combined dental disease. This study was conducted following permission from the Ethics Committee, the informed consent of the participants, and screening for ten patients. The four patients in whom CTCs were detected agreed to participate, and were enrolled in the study.

2. Blood sample collection and CTC analysis

Venous blood was collected at the clinical site into Cell-Save Preservative Tubes (Veridex, Raritan, NJ, USA) containing cellular preservatives and ethylenediaminetetraacetic acid as an anticoagulant. Blood was collected from patients before and after zoledronic acid treatment. In addition, routine laboratory analyses were conducted including PSA, lactate dehydrogenase, alkaline phosphatase, hemoglobin, and calcium. CTCs were isolated and processed from 7.5-mL peripheral venous blood using CellSearch System (Veridex, Raritan, NJ, USA) according to the manufacturer's protocol. Two independent well operators performed a blind evaluation of the selected cells. CTCs were identified by nuclear and cytokeratin staining and the absence of the leukocyte typical CD45 epitope.

RESULTS

Ten metastatic CRPC patients who were candidates for this study were examined for CTCs; 4 individuals were found to have CTCs and were administered ZOL. Patient backgrounds are shown in Table 1.

Adverse events were evaluated as follows. Case 1: The patient was found to have grade 1 facial edema and requested termination of ZOL. Case 2: The patient experienced rapid disease progression, resulting in a change of therapy two months following initial ZOL administration. Two of the four patients were able to complete the study. Case 3: One patient had a CTC count of 3 prior to initial ZOL administration, decreasing to 1 two months later. In the third month, however, his CTC count

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Table 1. Characteristics of patients

No.	Age (yr)	Therapy	Histology	Stage	Adverse event
1	83	MAB	Unknown	cT4N1M1	Edema
2	77	MAB	Gleason 4+3	cT2N0M1	None
3	80	MAB	Gleason 4+3	cT3N1M1	None
4	77	MAB	Gleason 3+4	cT2N0M1	None

MAB, maximum androgen blockade.

rose to 5. Case 4: The patient's CTC count was 20 prior to ZOL administration, decreasing to 3 during the first month of the study.

Changes in CTC count before and after ZOL treatment are shown in Table 2. None of these patients showed a decreased PSA as a result of ZOL administration.

DISCUSSION

There is a growing body of evidence that bisphosphonates have anticancer activity. Their effect in the bone is mainly due to inhibition of bone resorption [21]. Several preclinical and animal model studies have demonstrated ZOL to exert anticancer activity, inhibiting tumor cell adhesion [22], invasion [23], proliferation and angiogenesis [24] and inducing apoptosis [25,26]. ZOL may affect the invasive behavior of metastatic cells in secondary sites through both direct and indirect effects and have the ability to interact with tumor cells at each step of the metastatic process.

We thus designed a preliminary study examining the effects of ZOL treatment on tumor cell count in the blood of CRPC patients with metastatic prostate cancer. Two of the four patients in whom CTCs were detected showed a decreased CTC count following ZOL treatment, suggesting that ZOL may be able to reduce CTC count in blood. However, absolutely no ZOL-derived antitumor effect (in terms of reduced CTC count) was noted in patients whose disease progressed rapidly, or in those with multiple bone metastases and an extremely high CTC count prior to initial ZOL administration. A recent report affirmed the efficacy of ZOL in breast cancer patients with tumor cells in bone marrow [27]. Authors reported that ZOL improved the prognosis of such patients, as tumor cells were detected in 16% of the patients two years after receiving surgery with a single adjuvant therapy, while no tumor cells were detected in the experimental group receiving ZOL as an additional adjuvant therapy [27].

The challenge lies in the short ZOL administration period; as new cancer cells are continually supplied to blood in bone metastatic CRPC patients, evaluating the ongoing antitumor effect of ZOL is difficult. CTC count has been reported as a predictive factor in CRPC prognosis [28]. CTCs were not detected in patients with relatively low PSA values whose disease was progressing slowly. The current findings should serve as important information in the design of any future large-scale clinical trial.

Table 2. CTC counts and PSA in patients with metastatic castration- resistant prostate cancer during zoledronic acid treatment

	Prior to study	1 Month	2 Months	3 Months
Case 1				
CTC count	10	15	[a]	
PSA	56.3	96.1	[a]	
Case 2				
CTC count	47	146	900	[b]
PSA	21.25	283.8	686.5	[b]
Case 3				
CTC count	3	2	1	5
PSA	51.3	157.1	165.8	199.9
Case 4				
CTC count	20	3	7	36
PSA	11.2	16.7	24.2	33.8

CTC, circulating tumor cell; PSA, prostate-specific antigen.
[a]Discontinuance with adverse event. [b]Discontinuance with disease progression.

CONFLICT OF INTEREST

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