Therapeutics and Clinical Risk Management

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ORIGINAL RESEARCH

A SNP in *CYP2C8* is not associated with the development of bisphosphonate-related osteonecrosis of the jaw in men with castrate-resistant prostate cancer

This article was published in the following Dove Press journal:
Therapeutics and Clinical Risk Management
18 November 2010
Number of times this article has been viewed

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Abstract: A single nucleotide polymorphism (SNP) in *CYP2C8* (rs1934951), was previously identified in a genome-wide association study as a risk factor for the development of osteonecrosis of the jaw (ONJ) in patients receiving bisphosphonates (BPs) for multiple myeloma. To determine if the same SNP is also associated with the development of ONJ in men receiving BPs for bone metastases from prostate cancer, we genotyped 100 men with castrate-resistant prostate cancer treated with bisphosphonates for bone metastases, 17 of whom developed ONJ. Important clinical characteristics, including type and duration of bisphosphonate therapy, were consistent among those who developed ONJ and those who did not. We found no significant correlation between the variant allele and the development of ONJ (OR = 0.63, 95% CI: 0.165–2.42, $P > 0.47$). This intronic SNP in *CYP2C8* (rs1934951) does not seem to be a risk factor for the development of bisphosphonate-related ONJ in men with prostate cancer. It is important to note that this is only the second study to investigate the genetics associated with BP-related ONJ and the first to do so in men with prostate cancer. More studies are needed to identify genetic risk factors that may predict the development of this important clinical condition.

Keywords: bisphosphonates, ONJ, *CYP2C8*, polymorphism

Introduction

Approximately 90% of men with advanced prostate cancer develop bone metastases.[1] Skeletal metastases can cause serious morbidities, including pathological fracture and severe pain.[2] In prostate cancer, skeletal complications are often compounded by anticancer treatment, particularly androgen deprivation therapy (ADT), which has been shown to cause treatment-induced bone loss.[2,3] Bisphosphonates (BPs) are potent osteoclast inhibitors that have been used to treat various bone diseases, including skeletal complications from bone metastases in men with prostate cancer.[2–7]

Due to their relatively increased rate of bone turnover, the mandible and maxilla often contain high levels of BPs.[8,9] A high incidence of osteonecrosis of the jaw (ONJ) in patients treated with BPs was first reported in 2003.[10] Since that time, multiple studies have supported the role of bisphosphonates in the development of ONJ.[8,11–16] In 2007, 3 criteria for the diagnosis of bisphosphonate-related ONJ were established: exposed bone in the maxillofacial region that remained unhealed for a minimum of 8 weeks, exposure to BPs, and no history of radiation therapy in the craniofacial region.[17]

Because BP-related ONJ was only recently identified, much remains unknown about the condition, including the true incidence. The most commonly reported

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incidence range is 6% to 15% in men with prostate cancer,[9] though other studies have reported slightly higher rates.[18,19] Also, little is known about risk factors for its development. There are some established risk factors, including prior dental procedure, especially dental extraction,[14,16,20,21] type of BP,[11,21] and length of BP treatment, with increased treatment duration positively correlated with risk.[11,14,16,20,21] Some studies suggest that concurrent treatments such as chemotherapy,[14,16] corticosteroids,[14,20] and antiangiogenic therapy[18,22,23] may also increase risk. Other studies have found no such correlation.[21,24] Therefore, markers that better predict the development of ONJ are needed to identify patients who are susceptible to the condition, especially in the setting of prostate cancer, where men often receive many of these therapies concurrently with BPs.

One area that has remained mostly unexplored is the link between genetics and bisphosphonate-related ONJ. In the first exploration of the genetic risk factors for ONJ, Sarasquete et al recently employed a genome-wide association study to identify single nucleotide polymorphisms (SNPs) associated with the development of this condition in patients with multiple myeloma receiving BP therapy. This study resulted in the identification of an intronic SNP in *CYP2C8* as the first possible genetic risk factor for developing bisphosphonate-related ONJ.[25] Based on these results, we hypothesized that the same SNP may be associated with the development of ONJ in men with prostate cancer treated with BPs. To our knowledge, we are the first to investigate the genetics behind the development of BP-related ONJ in men with prostate cancer.

Materials and methods

Patients

We retrospectively genotyped 100 men who received BPs for bone metastases from advanced prostate cancer and who participated in at least 1 clinical trial at the National Cancer Institute (NCI). Patients are derived from participants of 33 clinical trials taking place at the NCI, and many patients underwent multiple clinical trials. All men received zoledronic acid (Zometa®; Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA). Of these, most patients received a standard dose: 4 mg infused over 15 minutes every 3 to 4 weeks. Four men received multiple BPs, though not concurrently. The patient who received a combination of BPs and developed ONJ was treated with zoledronic acid and alendronate (Fosamax®; Merck & Co., Inc., Whitehouse Station, NJ, USA). Two of the men who received a combination of BPs and did not develop ONJ received zoledronic acid and alendronate, and the third patient received zoledronic acid and pamidronate (Aredia®; Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA). In addition to BPs, all men had received androgen deprivation therapy, as well as some combination of chemotherapy, steroids, and antiangiogenic therapy, including sorafenib, thalidomide, AZD2171, and/or bevacizumab. No men received BPs for the treatment of osteoporosis. Data on dental extraction, and oral hygiene were not available; however, it is unlikely that a significant number of patients with advanced castrate-resistant prostate cancer underwent significant dental procedures immediately prior to, or during therapy. This study was approved by the Internal Review Board of the National Cancer Institute.

Genotyping

DNA was extracted from plasma using a QIAamp Ultra-Sens Virus Kit (Qiagen, Valencia, CA, USA) and stored at 4°C. Genotyping was conducted via direct nucleotide sequencing at the *CYP2C8* locus (G > A transition; rs1934951) using nested PCR. The primary PCR primers used were F1 5′-ACTACTTCTCCTCACTTCTGGAC-3′ and R1 5′-TCAAGGCAGGTAAGGAAAGATCAG-3′. The secondary PCR primers were F2 5′-TCCAAATATCTTT GACCCT GGC-3′ and R2 5′-ATGTATCTAGTGGCA GAGTTCAG-3′. A 20 µL reaction was prepared for primary PCR amplification and a 50 µL reaction was prepared for secondary PCR amplification. Each reaction consisted of 1 × PCR buffer, 2 mmol/L of each of the 4 deoxynucleotide triphosphates, 1.5 mmol/L magnesium chloride, 20 mmol/L of the forward and reverse primers, 1 unit of Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), and 5 µL of template DNA. Primary PCR conditions were 94°C for 5 minutes, followed by 20 cycles of 94°C for 30 seconds, 68°C for 30 seconds, and 72°C for 30 seconds, with a final extension step at 72°C for 7 minutes. Secondary PCR conditions were 94°C for 5 minutes, followed by 40 cycles of 94°C for 30 seconds, 64°C for 30 seconds, and 72°C for 30 seconds, with a final extension step at 72°C for 7 minutes. Direct nucleotide sequencing PCR was performed using the Big Dye Terminator Cycle Sequencing Ready Reaction kit V3.1 on an ABI Prism 3130xl Genetic Analyzer (Applied BioSystems, Foster City, CA, USA). The secondary PCR primers were also used for sequencing.

Analysis

Statistical analysis was performed using analysis of variance and the Chi-squared and Fisher's exact tests using NCSS software (NCSS, Kaysville, Utah, USA) and Microsoft Excel

(Microsoft, Redmond, WA, USA). The a priori *P* value for an association was considered to be ($P < 0.05$).

Results

One hundred men with advanced prostate cancer treated with BPs for bone metastases were genotyped for an intronic *CYP2C8* G > A SNP (rs1934951) previously shown to be associated with the development of BP-related ONJ in patients with multiple myeloma.[25] Patient characteristics, including type and duration of BP treatment, are described in Table 1, and genotypic and allelic frequencies are presented in Table 2 along with ONJ status. All patients received chemotherapy, steroids, and antiangiogenic therapy. Most of the patients (94%) received only zoledronic acid, and this was consistent among those who developed ONJ and those who did not. Although we were unable to determine the exact duration of BP treatment for many men, we were able to determine in 94% of cases whether the men had received BPs for greater than or less than a year. The racial breakdown was consistent between the 2 groups (those who developed ONJ and those who did not), the majority (approximately 80%) being Caucasians. The median age of all men at their initial prostate cancer diagnosis was 59 years of age (range: 43–75), and this was similar between the 2 groups (Table 1).

Genotypic and allelic frequencies are presented in Table 2. All genotypes were found to be in Hardy–Weinberg equilibrium. There was no statistically significant relationship between the genotype and development of ONJ ($P > 0.65$), and the variant allele did not correlate with the development of ONJ (odds ratio = 0.63, 95% confidence interval: 0.165–2.42, $P > 0.47$). In fact, of the 3 homozygous variant men, none developed ONJ. Finally, there were no associations between type or duration of BP, race, or age of prostate cancer diagnosis and ONJ development ($P > 0.40$ for all characteristics).

Table 1 Patient characteristics

BP	ONJ (+) n = 17	ONJ (–) n = 83	All n = 100
Zoledronic acid	16 (94.1%)	78 (94.0%)	94 (94.0%)
Ibandronate	0 (0%)	1 (1.2%)	1 (1.0%)
Pamidronate	0 (0%)	1 (1.2%)	1 (1.0%)
Combination	1 (5.9%)	3 (3.6%)	4 (4.0%)
BP duration			
≥1 year	15 (88.2%)	63 (75.9%)	78 (78.0%)
<1 year	2 (11.8%)	14 (16.9%)	16 (16.0%)
Unknown	0 (0%)	6 (8.4%)	6 (6.0%)
Race			
Caucasian	14 (82.4%)	66 (79.5%)	80 (80.0%)
African American	2 (11.8%)	10 (12.0%)	12 (12.0%)
Hispanic	1 (5.9%)	5 (6.0%)	6 (6.0%)
Asian	0 (0%)	2 (2.4%)	2 (2.0%)
Median age at diagnosis (range)	60 (48–75)	59 (43–75)	59 (43–75)

Abbreviations: BP, bisphosphonate; ONJ, osteonecrosis of the jaw.

Table 2 Genotype frequencies

Genotype at *CYP2C8* rs1934951*	ONJ (+) n = 17	ONJ (–) n = 83	All n = 100
GG	14 (82.4%)	62 (74.7%)	76 (76.0%)
GA	3 (17.6%)	18 (21.7%)	21 (21.0%)
AA	0 (0%)	3 (3.6%)	3 (3.0%)
GA and AA combined**	3 (17.6%)	21 (25.3%)	24 (24%)

Notes: *$P > 0.05$ for all genotypes; **OR = 0.63 (95% CI: 0.165–2.42), $P > 0.47$ for GG vs GA and AA combined.

Abbreviations: CI, confidence interval; ONJ, osteonecrosis of the jaw; OR, odds ratio.

Discussion

This study suggests that there is no relationship between the development of BP-related ONJ in men with prostate cancer and the rs1934951 locus in *CYP2C8*, a potential risk factor previously identified in study of individuals with multiple myeloma.[25] We were also unable to detect any relationship between ONJ development and basic clinical characteristics of men with prostate cancer. Our incidence of ONJ (17%), while high, was consistent with other studies in similar populations.[18,19]

Because nitrogen-containing BPs (eg, zolendronic acid), a group that includes those commonly prescribed for bone involvement in patients with cancer, are not metabolized,[26] it is unlikely that this is a strictly pharmacogenetic relationship per se. However, as Sarasquete et al describe, *CYP2C8* is involved in many endogenous pathways, and alterations in these pathways could increase a person's risk of developing ONJ.[25] For example, *CYP2C8* metabolizes arachidonic acid to epoxyeicosatrienoic acids,[27] and epoxyeicosatrienoic acids are involved in vascular tone and vascular endothelial growth factor (VEGF)-activated angiogenesis.[28] In addition, BPs have antiangiogenic effects in vivo[29,30] and have been shown to suppress VEGF expression in cultured cells.[31] Thus, it is possible that accumulation of microtrauma from osteoclast inhibition by BPs could be enhanced by insufficient vasculature due to both variations in *CYP2C8* and impaired angiogenesis from BP therapy, thus increasing a person's risk of developing ONJ.

It is important to note that the effects of the SNP discussed here are not known. As an intronic SNP, its effects at the protein level are not straightforward; it does not result in an amino acid change, and it does not appear to be in a splice

site. Even if it does not result in a functional change, it could be a marker for another polymorphism. However, HapMap (www.hapmap.org, last accessed 1 Dec, 2009) analysis does not show any significant linkage, though it may be a marker for currently unknown SNPs. Sarasquete et al also suggest that intronic SNPs may affect intronic miRNAs but do not show that this is the case for the investigated SNP.[25]

There are several possible reasons why our study did not find an association between the *CYP2C8* SNP and development of ONJ. First, we genotyped men with prostate cancer while Sarasquete et al[25] genotyped both men and women with multiple myeloma, and an individual's underlying disease may influence the development of ONJ.[32] It is also possible that the SNP identified by Sarasquete et al was a false positive despite their stringent statistical testing and identification of other SNPs within *CYP2C8* that trended toward an association with ONJ though was not statistically significant.[25] We also recognize that there are some limitations to our data. We did not initially select our patient cohort to have matched controls for our cases of ONJ, though important characteristics such as race, age of prostate cancer diagnosis, and type and duration of BP treatment were consistent between those who developed ONJ and those who did not (Table 2). Further, though duration of BP treatment is a demonstrated risk factor for ONJ development,[11,14,16,20,21] we were unable to ascertain exact BP-treatment time-course data for many patients. Instead we could only determine if a patient had received BPs over a time period greater than or less than a year. Finally, we were unable to determine if ONJ was more or less frequent in individual clinical trails given the relatively few patients who developed ONJ, and the large numbers of trials from which we obtained patients. Thus, further studies carefully examining time-course data are warranted. Finally, while all patients received ADT, chemotherapy, steroids, and antiangiogenic therapy at some point in their disease course, the type and duration of treatment as well as their temporal relationship with BP use was heterogeneous. This variation may have positively or negatively affected the likelihood of the development of ONJ.

To our knowledge, this is only the second study to investigate the genetics behind BP-related ONJ and the first to do so in men with prostate cancer. Though our retrospective investigation did not find the same association between the *CYP2C8* rs1934951 SNP and the development ONJ as Sarasquete et al,[25] more studies in independent patient cohorts are necessary to determine if genetic polymorphisms in *CYP2C8* are a risk factor for the development of ONJ. As ONJ causes significant morbidity in men with prostate cancer, identification of genetic variants that may put patients at greater risk should continue to be an area of active investigation.

Acknowledgment

This study was supported in part by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Bethesda, MD, USA.

Disclaimer

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Disclosure

The authors disclose no conflicts of interest.

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