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Potential CYP3A-mediated drug-drug interactions and excretion of the antibody-drug conjugate brentuximab vedotin in patients with hematologic malignancies that are CD30-positive

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Abstract
An antibody-drug conjugate (ADC) called benuximab vedotin preferentially transports monomethyl auristatin E (MMAE) into cells that express CD30. This study assessed the excretion of MMAE and the possibility for CYP3A-mediated drug-drug interactions with brentuximab vedotin. 56 patients with CD30-positive hematologic malignancies received two 21-day cycles of intravenous brentuximab vedotin (1.2 or 1.8 mg/kg). Additionally, each patient was given either a potent inhibitor (ketoconazole), an efficient inducer (rifampin), or a sensitive CYP3A substrate (midazolam). Midazolam exposures were unaffected by benuximab vedotin. Concomitant rifampin or ketoconazole had no effect on ADC exposures, however MMAE exposures were reduced with

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rifampin and more when using ketoconazole. The study's short-term safety profile for brentuximab vedotin was mostly in line with previous clinical findings. Nausea, exhaustion, diarrhea, headache, pyrexia, and neutropenia were the most frequent side effects. After receiving brentuximab vedotin, approximately 23.5% of intact MMAE was recovered over the course of one week; all other

species were below the quantitation limit. Feces are the main means of excretion (median 72% of recovered MMAE). These findings imply that MMAE is a substrate of CYP3A but neither brentuximab vedotin (1.8 mg/kg) nor MMAE are CYP3A inducers or inhibitors.

Keywords
Biotechnology, Oncology, Clinical Pharmacology, Clinical Trials, Pharmacokinetics, and Drug Metabolism

INTRODUCTION

An essential technique for raising the therapeutic index of strong cytotoxic medicines is the use of antibody-drug conjugates (ADCs), which are made up of an antibody, a cytotoxic agent, and a stable linker. The cytotoxic agent's biodistribution is changed and its apparent half-life is extended upon conjugation to the antibody.1,2 After internalizing into antigen-expressing cells, the ADC releases the cytotoxic agent while remaining stable in circulation. This allows for targeted delivery to tumors without exposing normal tissues to high systemic concentrations of the cytotoxic agent. Human CD30, a cell-surface protein frequently present on Hodgkin lymphoma (HL) and non-Hodgkin lymphomas including anaplastic large cell lymphoma (ALCL), but typically missing on normal cells with the exception of activated T and B cells, is the target of benuximab vedotin (ADCETRIS®), an ADC.4,5 A protease-cleavable linker binds the anti-CD30 antibody cAC106,7 to the microtubule-disrupting cytotoxic drug monomethyl auristatin E (MMAE).8

MMAE is released by lysosomal proteases after internalization into CD30-positive cells, causing cell cycle arrest and apoptosis.9,10 The usefulness of targeted delivery was demonstrated when brentuximab vedotin produced intratumor MMAE concentrations in a mouse xenograft model that were 1 to 2 orders of magnitude higher than an equimolar dosage of unconjugated MMAE. When 1.8 mg/kg brentuximab vedotin was given intravenously every three weeks for up to sixteen cycles, pivotal clinical trials showed objective response rates of 75% and 86% for patients with relapsed/refractory HL and relapsed/refractory systemic ALCL, respectively,11,12 along with an acceptable safety profile.

Total antibody (TAb, the sum of ADC and cAC10), ADC, and released MMAE were monitored to determine the pharmacokinetics (PK) of benuximab vedotin.13–15 Within the therapeutic dose range, the PK of all three analytes is roughly dose proportionate. For ADC, peak concentrations usually happened at the end of infusion, while for MMAE, they usually happened two to three days after the injection. TAb had the most exposure and a PK profile that was comparable to the ADC's. After the initial dose, MMAE exposures dropped and, on a mass basis, were around 2000 times lower than those of the ADC.15. There have also been reports of much larger exposures of other ADCs compared to their corresponding small molecule components (16–19). The relative exposure ratio is probably determined by the drug-linker stability and clearance rates of the particular ADCs.

The cytotoxic agent component of an ADC may be susceptible to metabolism-based drug-drug interactions (DDIs), even though brentuximab vedotin is an antibody-based medication and DDIs

involving antibodies are generally rare. Nonclinical data suggested that MMAE is a substrate of P-glycoprotein (P-gp) and CYP3A, and that MMAE may inhibit CYP3A but not other CYP isoforms.22 Given that CYP3A plays a role in the metabolism of the majority of small molecule pharmaceuticals, it is crucial to comprehend the possibility of CYP3A-based DDIs

in order to guide the administration of brentuximab vedotin to patients taking concurrent medications. This article presents the findings of a clinical investigation that used a prototypical substrate and CYP3A modulators to assess the CYP3A-mediated DDI potential of brentuximab vedotin. Additionally, the main MMAE excretion pathway in individuals with CD30-positive hematologic malignancies was identified.

METHODS

Patients

Patients had to have quantifiable, CD30-positive hematologic malignancies that were confirmed by histology. Patients who have had at least one previous systemic chemotherapy regimen and have relapsed, resistant, or progressing disease, as well as those who are new to treatment and cannot handle severe or potentially curative regimens, may be eligible. Patients who had an allogeneic stem cell transplant within 100 days or an autologous stem cell transplant within 4 weeks before the first dose of the study drug, concurrent corticosteroid therapy (≥20 mg/day prednisone equivalent), or an active systemic viral, bacterial, or fungal infection requiring antimicrobial therapy within 2 weeks before the first dose of the study drug were not allowed to participate in the study. Patients with active acute or chronic graft-versus-host disease, New York Heart Association Class III or IV congestive heart failure, primary cutaneous ALCL, hemodialysis, or chronic ambulatory peritoneal dialysis were excluded from the study. Additionally, patients with absolute neutrophil counts <1000/μL, platelets <50,000/μL, serum bilirubin or creatinine >1.5 times the upper limit of normal, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >2.5 times the upper limit of normal, or Eastern Cooperative Oncology Group (ECOG) performance status >1 were excluded. Strong CYP3A inhibitors and effective inducers were forbidden starting four weeks before to the study drug's initial dose and continuing for the duration of the investigation. Prior to any study-specific operations, all patients provided written informed permission in compliance with the Declaration of Helsinki.

Study Design

Seven clinical locations around the US participated in this phase 1 study (ClinicalTrials.gov NCT01026415). The Western Institutional Review Board (Olympia, WA) and the local Institutional Review Boards at Wayne State University (Detroit, MI), Healthcare Corporation of America (HCA)-HealthONE (Glendale, CO), New York University Medical Center (New York, NY), St. Francis Hospital and Health Center (Beech Grove, IN), and City of Hope National Medical Center (Duarte, CA) all examined and approved the protocol.

An open label, parallel arm, one-sequence crossover design was employed in the investigation (Figure 1). The main goals were to evaluate the impact of brentuximab vedotin on the PK of a sensitive CYP3A substrate (midazolam); evaluate the impact of a CYP3A inducer (rifampin) on the PK of brentuximab vedotin (ADC and MMAE); evaluate the impact of a CYP3A inhibitor (ketoconazole) on the PK of brentuximab vedotin (ADC and MMAE); and identify the main route

of MMAE. Secondary goals included evaluating safety and tolerability, to determine the excreted metabolite or metabolites of MMAE, and to evaluate the prevalence of antitherapeutic antibodies (ATA). This two-cycle trial did not assess efficacy.

About 12 evaluable patients were to be enrolled in each treatment arm, and the treatment assignment was to be carried out in a sequential manner, starting with the midazolam arm, followed by the rifampin arm, and finally the ketoconazole arm. Treatment arm assignment went according to plan in the majority of cases. However, because of the quick enrollment, none of the three treatment arms were included in distinct phases. Throughout the study, patient evaluability was continuously determined. If a prior treatment arm was determined to require an additional patient due to non-evaluability, the next available patient was allocated to that treatment arm as a replacement patient.Six excretion-evaluable individuals were the target of the excretion analysis, which was carried out in a subset of patients from the rifampin arm. The concentrations of MMAE and associated metabolites in all urine and fecal samples were measured in an in-patient environment for one week (24-hour pools of each) following the initial therapy with brentuximab vedotin (1.8 mg/kg) and before rifampin was administered.

Study Treatment

On the first day of two 21-day cycles, an IV infusion of benuximab vedotin (1.8 mg/kg in the midazolam and rifampin arms; 1.2 mg/kg in the ketoconazole arm) was given over about 30 minutes. In this study, patients were given a maximum of two cycles of brentuximab vedotin; however, in a different experiment, patients who showed clinical benefit and were free from intolerable toxicity might receive longer treatment.

Patients also received ketoconazole, rifampin, or midazolam, depending on the therapy arm they were assigned. On Day 3 and Day 3 of Cycle 1, concurrent intravenous midazolam (1 mg) was given for at least two minutes. During Cycle 1, Day 14 through Cycle 2, Day 21, concurrent rifampin (600 mg) was administered orally once daily. From Cycle 1, Day 19 through Cycle 2, Day 21, concurrent ketoconazole (400 mg) was administered orally once daily.

Since both brentuximab vedotin and midazolam were given intravenously at the trial site, patient compliance with their administration was closely monitored. At every planned clinic visit, patient diaries were reviewed and any unused tablets or empty bottles were reconciled to determine compliance with rifampin and ketoconazole. Home healthcare nurse services were also offered to help guarantee adherence to the PK blood collection schedule, evaluate oral medication administration compliance, and emphasize the significance of protocol compliance.

Safety Assessments

Regular hematological and serum chemistry testing (performed at local laboratories), evaluation of adverse events, and evaluation of ATA (performed at a central laboratory) were all part of the safety assessments. The Medical Dictionary for Regulatory Activities (MedDRA), version 13.0, was used to summarize adverse events. A standardized MedDRA query (SMQ) was used to define peripheral neuropathy events. The National Cancer Institute's Common Terminology Criteria for Adverse Events, version 3.0, were used to grade laboratory results and adverse events.

Sample Collection and Handling

Blood samples for midazolam analysis were taken in the midazolam arm before each midazolam infusion, within 1 minute of the end of the infusion, at 15, 30, and 45 minutes, and at 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 hours postdose. Before every midazolam dose, as well as before and after the first dose of brentuximab vedotin infusion, blood samples were taken for brentuximab vedotin analysis.Blood samples were taken for brentuximab vedotin analysis during both treatment cycles in the rifampin and ketoconazole groups. MMAE samples were taken prior to the dose, at the conclusion of the infusion, at 2, 4, 8, 12, 24, and 36 hours, and at 2, 3, 4, 7, 10, 14, 17, and 21 days after infusion. Blood samples were taken prior to the infusion, at the conclusion of the infusion, at 2, 4, and 36 hours, and at 3, 7, 14, 17, and 21 days after the infusion in order to perform ADC and TAb tests. Before each dose of brentuximab vedotin, 14 days (rifampin) or 19 days (ketoconazole) after infusion in cycle 1, and 7, 14, and 21 days after infusion in cycle 2, blood samples were taken for trough rifampin and ketoconazole analysis.

Blood samples for ATA analysis were taken at the end-of-treatment visit and on Day 1 of each treatment cycle before brentuximab vedotin was administered in all three treatment arms. Samples processed within an hour after collection were used to evaluate ADC, TAb, and ATA in serum and MMAE in plasma.

Patients who took part in the study's excretion phase also had their urine and feces collected in addition to their blood. Every urine and fecal sample was gathered into a different container and chilled within 30 minutes. Urine samples were combined and well mixed at the conclusion of each 24-hour period, the total volume was noted, and aliquots were frozen. Each 24-hour period's fecal samples were likewise frozen; the total 24-hour fecal samples were weighed and homogenized in water before analysis. Samples of feces and urine were sent frozen to a central laboratory for analysis.

Bioanalytical Methods

Covance, Inc. (Chantilly, VA and Madison, WI) used validated assays to assess all analytes. An enzyme-linked immunosorbent assay (ELISA) in a sandwich format was used to assess ADC quantities in serum. A monoclonal anti-MMAE antibody was used for capture, and a monoclonal anti-idiotypic-cAC10 (anti-ID30, specific for the antigen-combining site of brentuximab vedotin) was used for detection. Using anti-ID30 for both capture and detection, TAb concentrations in serum were assessed using an ELISA immunoassay in a bridging format. Both tests have quantitation limits ranging from 12.5 to 400 ng/mL. Liquid chromatography and tandem mass spectrometry (LC-MS/MS) were used to determine the concentrations of unconjugated MMAE in plasma, urine, and fecal homogenates. Solid-phase extraction (plasma) or liquid-liquid extraction (urine and fecal homogenates) were used to remove free MMAE and the internal standard D8-MMAE from the biological matrix. Gradients containing 0.1% formic acid in water and acetonitrile were used to separate the extracts on a 50 × 3 mm Betasil silica-100 or Aquasil C18 (Thermo Scientific, Waltham, MA) column. Multiple reaction monitoring transitions of 718 to 686 (MMAE) and 726 to 694 (D8-MMAE) were used for detection on an API5000 (Sciex, Framingham, MA). The quantitation limits were 5 to 2500 ng/g for feces, 0.1 to 50 ng/mL for urine, and 0.025 to 1 ng/mL for plasma. MMAE metabolites were detected using LC-MS/MS of neat and 10-fold liquid-liquid extracts of concentrated urine and feces, with structures verified by high resolution mass analysis and parent ion fragmentation. An electrochemiluminescence test with a sensitivity of 4 ng/mL anti-brentuximab vedotin monoclonal antibody and a drug tolerance

of 3125 ng/mL brentuximab vedotin was used to identify ATA in serum. For samples that tested positive in the screening assay, ATA titer and specificity were assessed. ATA was then verified by pre-exposure to saturating doses of brentuximab vedotin prior to assay. LC-MS/MS was used to test midazolam in plasma; the lowest limit of quantification was 0.1 ng/mL.

Pharmacokinetic and Statistical Analyses

Noncompartmental analysis was used to determine the PK parameters (AUC, Cmax, Tmax, and t1/2). All pharmacokinetic calculations were performed using WinNonlin 5.2 Enterprise Edition (Pharsight, Mountain View, CA). Geometric mean ratios (GMRs) of the AUC and Cmax with 90% confidence intervals (CIs) were summarized in order to assess drug interactions. Log-transformed PK parameters were used to calculate the GMR and 90% CIs, which were then back-transformed for display.

The period influence on MMAE PK was estimated using an exploratory study. Data from this study and the first brentuximab vedotin clinical trial were merged to create an analysis of covariance (ANCOVA) model.The model took into account 13 covariates, including baseline albumin, weight, and sex. The model's main presumptions were that the errors are normally distributed, the error variance-covariance is constant across studies and dose levels, and the GMR of Cycle 2 to Cycle 1 is independent of brentuximab vedotin dose level, with or without rifampin/ketoconazole.

Continuous variables were summarized using descriptive statistics. For a few PK parameters, the geometric mean and the geometric mean coefficient of variation (CV) were given. Categorical variables were summarized using percentages and frequencies. SAS® version 9.2 (SAS Institute Inc., Cary, NC) was used to compute summary statistics and ANCOVA results.

Evaluability Criteria

All PK-evaluable patients were included in the PK analyses, and all excretion-evaluable patients were included in the excretion studies. Doses of brentuximab vedotin and midazolam within 10% of the intended dose level were deemed acceptable for evaluability; prohibited medications were manually identified; and missing PK samples and doses of rifampin/ketoconazole were manually reviewed to evaluate the possible impact on PK results. If a patient could not be evaluated, they were replaced.

Evaluable patients in the midazolam arm had sufficient samples available for PK parameter estimation, received the first dose of brentuximab vedotin and both doses of midazolam at the intended dose levels, and did not take any medications that were prohibited by protocol during the evaluation period (study entry through Cycle 1, Day 4). A patient was included in the analysis if they satisfied all evaluability criteria and had estimable midazolam AUC0-∞ and/or Cmax ratios. Similarly, during the evaluation period (study entry through Cycle 2, Day 22), evaluable patients in the rifampin and ketoconazole arms received both doses of brentuximab vedotin at the intended dose level, received sufficient doses of rifampin/ketoconazole for PK evaluation, had no medications prohibited by protocol, and had sufficient PK samples available for PK parameter estimation. Patients in the rifampin and ketoconazole arms were included in the analyses if they satisfied all evaluability requirements and if the AUC0-∞ ratio and/or Cmax ratio for MMAE and/or ADC were estimable.

During the evaluation period (study entry through Cycle 1, Day 8), evaluable patients for the excretion portion of the study received the first dose of brentuximab vedotin at the intended dose level, provided adequate matched urine and fecal samples for evaluation, and did not receive any medications prohibited by protocol. For each collection day, matched urine and fecal samples were needed for a patient to be included in the excretion analysis.

RESULTS

Patients

The period of data collection was December 2009–June 2010. 56 participants were enrolled, given at least one dose of the study medication, and had their safety assessed. 59% of the patients in the study population (N = 56) were men, 86% were white, and the median age of the patients was 33.5 years (range: 16 to 71 years). As evidenced by an ECOG performance level of 0 (57%) or 1 (43%), patients who entered the trial were often mobile and capable of carrying out daily tasks without help. One patient (2%) had relapsed/refractory peripheral T-cell lymphoma, three patients (5%) had relapsed/refractory systemic ALCL, and fifty-two patients (93%) had relapsed/refractory HL. The median interval between the first diagnosis and the first brentuximab vedotin treatment was 38 months (range: 9 to 162). 43 patients (77%) had previously received an autologous or allogeneic hematopoietic stem cell transplant, and the patients had a median of three previous systemic chemotherapy regimens (range, 1 to 13).

Eight patients in the rifampin arm were evaluable for the excretion analysis, and 45 patients were evaluable for PK: 15 in the midazolam arm, 14 in the rifampin arm, and 16 in the ketoconazole arm (Figure 1). Eleven patients could not be evaluated for PK because of insufficient PK samples (n = 4), insufficient dosages of ketoconazole or rifampin (n = 4), use of restricted concurrent drugs (n = 2), and early cessation due to an adverse event (n = 1). Due to insufficient urine and fecal samples, four out of the twelve patients who took part in the excretion section of the trial could not be evaluated. The evaluable patients' baseline features and demographics (Table 1) were generally comparable to those of all recruited patients in the appropriate treatment arm.

54 (96%) of the 56 patients treated with brentuximab vedotin got both doses and finished the study in accordance with protocol. Due to Grade 5 (fatal) adverse effects and illness progression, two individuals stopped their therapy early. In a different trial, fifty individuals (89%) received prolonged treatment. All 16 individuals in the midazolam arm got both of the scheduled doses of midazolam. Two of the 19 patients in the ketoconazole arm and three of the 21 patients in the rifampin arm could not be evaluated.

for PK as they weren't given enough ketoconazole or rifampin.

Effect of brentuximab vedotin (MMAE) on midazolam PK

By comparing the midazolam PK parameters between midazolam given alone and two days after brentuximab vedotin infusion (the anticipated Tmax for MMAE), the impact of brentuximab vedotin on the PK of midazolam was assessed (Table 2 and Figure 2). The Cmax GMR and accompanying 90% CI were 1.15 and 0.76–1.74, while the midazolam AUC0-∞ GMR (midazolam + brentuximab vedotin/midazolam alone) and corresponding 90% CI were 0.94 and 0.81–1.10. Both the t1/2 and the midazolam Tmax were comparable with and without brentuximab vedotin.

Effect of rifampin on brentuximab vedotin and MMAE PK

By comparing ADC and MMAE exposures between brentuximab vedotin alone and coadministered with a daily dosage of rifampin, the impact of rifampin on the PK of brentuximab vedotin was assessed (Table 2 and Figure 2).

For ADC, the Cmax GMR and associated 90% CI were 0.93 and 0.81–1.06, while the AUC0-∞ GMR (brentuximab vedotin with rifampin/brentuximab vedotin alone) and accompanying 90% CI were 1.04 and 0.87–1.24. Both the ADC Tmax and the t1/2 were comparable with and without rifampin.

The AUC0-∞ GMR and related 90% CI for MMAE were 0.54 and 0.43–0.68, while the Cmax GMR and accompanying 90% CI were 0.56 and 0.42–0.76. Concomitant rifampin decreased the median MMAE Tmax (3.00 [range, 0.99–5.01] alone vs. 1.49 [range, 1.33–4.02] in combination); nonetheless, there was significant overlap across the ranges. Both with and without rifampin, the MMAE t1/2 was comparable.An exploratory analysis that was not included in the statistical analysis plan was carried out to account for the period effect on the PK of MMAE because MMAE exposures decreased with successive doses,15 to around 50% to 80% relative to the first dose (data on file). With concurrent rifampin, the adjusted GMR (90% CI) for MMAE AUC0-∞ using a model-based approach was 0.69 (0.49, 0.98).

Effect of ketoconazole on brentuximab vedotin and MMAE PK

By comparing the brentuximab vedotin PK parameters between brentuximab vedotin alone and coadministered with a daily dose of ketoconazole, the impact of ketoconazole on the PK of brentuximab vedotin was assessed (Table 2 and Figure 2).

For ADC, the Cmax GMR and associated 90% CI were 0.99 and 0.75–1.31, whereas the AUC0-∞ GMR (brentuximab vedotin with ketoconazole/brentuximab vedotin alone) and accompanying 90% CI were 1.07 and 0.95–1.19. Both with and without concurrent ketoconazole, the ADC Tmax and t1/2 were comparable.

The AUC0-∞ GMR and related 90% CI for MMAE were 1.34 and 0.98–1.84, whereas the Cmax GMR and accompanying 90% CI were 1.25 and 0.90–1.72. Both with and without concurrent ketoconazole, the MMAE Tmax and t1/2 were comparable. The adjusted GMR (90% CI) for MMAE AUC0-∞ with concurrent ketoconazole was 1.73 (1.22, 2.46) using a model-based approach to account for the MMAE period effect.

Effect of ATA on brentuximab vedotin PK

At least one postbaseline ATA result was positive for five of the fourteen PK-evaluable patients in the rifampin arm and eight of the sixteen PK-evaluable patients in the ketoconazole arm. To find out if removing these patients would have an impact on the GMR analyses for brentuximab vedotin, a study was conducted. The GMRs with and without individuals who had positive postbaseline ATA results were largely consistent for both therapy arms (data not shown). Because there were fewer individuals, it was to be expected that variability increased when these patients were eliminated.

Safety

53 out of 56 participants (95%) had at least one treatment-emergent adverse event over the course of the study's two treatment cycles. Nausea (30%), fatigue (29%), diarrhea, headache, and pyrexia

(23% each), and neutropenia (20%) were the most frequently reported treatment-emergent side effects. Twenty-two individuals (39%) in all had at least one adverse event that was at least Grade 3. After receiving one dose of brentuximab vedotin, one post-allogeneic transplant patient died after cerebral hemorrhage, CMV reactivation, pancytopenia worsened by Streptococcus pneumoniae sepsis and pneumonia, and concomitant multisystem organ failure. Uncertainty surrounded the clinical relevance of CMV reactivation. During the study, 30% of patients experienced a most severe adverse event of Grade 3, and 7% experienced a most severe adverse event of Grade 4 (neutropenia and/or thrombocytopenia). Neutropenia (18%) and anemia (7%) were the most frequent adverse events ≥ Grade 3. No one else.

27% of patients had pre-existing Grade 1 peripheral neuropathy, while 18% of patients experienced treatment-emergent peripheral neuropathy during the study's two treatment cycles. All treatment-emergent peripheral neuropathy was sensory in character, with the exception of Grade 1 gait impairment. During the trial, one patient's pre-existing peripheral neuropathy deteriorated to Grade 2, whereas all other patients had Grade 1 (asymptomatic) peripheral neuropathy.

Reduced absolute neutrophil count (16 patients, 29%), low white blood cell count (7 patients, 13%), elevated ALT (6 patients, 11%), and low hemoglobin, low lymphocyte, and low platelet counts (5 patients, 9% each) were the most frequently reported laboratory abnormalities with baseline to postbaseline changes from Grade 1 or 2 to ≥ Grade 3.

Some numerical differences were observed, despite the fact that the study was not intended to objectively assess differences between treatment arms. For instance, compared to the midazolam arm (4 patients, 25%), the incidence of adverse events ≥ Grade 3 during the study was higher in the rifampin and ketoconazole arms (9 patients, 43% and 9 patients, 47%, respectively). Additionally, compared to the midazolam and rifampin arms (7 patients, 44% and 10 patients, 48%, respectively), the ketoconazole arm had a greater frequency of gastrointestinal disturbances (such as nausea, diarrhea, and vomiting) (13 patients, 68%). To ascertain if these data might be connected to the co-administration of ketoconazole or rifampin, post hoc analyses were carried out (Table 3).

The overall incidence of adverse events for the rifampin arm did not differ between Cycles 1 and 2 (18 patients, 86% in Cycle 1 vs. 17 patients, 85% in Cycle 2), and the incidence of adverse events ≥ Grade 3 was comparable between Cycles 1 and 2 (6 patients, 29% vs. 4 patients, 20%, respectively).
Pre- and post-ketoconazole, the overall incidence of adverse events for the ketoconazole arm was 84% (16 patients); the incidence of adverse events ≥ Grade 3 increased after the start of ketoconazole treatment (4 patients, 21% before vs. 7 patients, 37% after). The only events > Grade 3 recorded for more than one patient in the ketoconazole arm were anemia (n = 2; 1 patient pre- and 1 patient post-ketoconazole) and neutropenia (n = 3; all post-ketoconazole). Research on gastrointestinal conditions revealed that the prevalence of Following the start of ketoconazole treatment, there was an increase in diarrhea (1
patient, 5% pre- vs. 6 patients, 32% post-) and vomiting (3 patients, 16% pre- vs. 6 patients, 32% post-), but not in nausea (5 patients, 26% both pre- and post-ketoconazole) (Table 3). Peripheral neuropathy events (2 patients, 11% before vs. 1 patient, 5% after), elevated ALT (1 patient, 5%

both before and after), elevated AST (1 patient, 5% before vs. 2 patients, 11% after), neutropenia (0 patients, 0% before vs. 3 patients, 16% after), fatigue (4 patients, 21% before vs. 5 patients, 26% after), pyreximab vedotin. There were no obvious causes of the numerical discrepancies found.

At baseline, eighteen patients had no confirmed ATA; nevertheless, at one or both postbaseline timepoints, they tested positive. All verified positive ATA results in this investigation had titers ≤ 125, with one exception. Before the second dosage of brentuximab vedotin, one patient's ATA titers were 3125, and at the conclusion of treatment, they were 125. Due to Grade 1 hypertension and Grade 2 episodes of dyspnea, back discomfort, and chills, the patient's second dose was stopped. Four more patients experienced infusion-related responses after their second brentuximab vedotin infusion: three were negative at baseline but had positive ATA titers at both postbaseline timepoints, and one was negative for ATA at all three timepoints.

Excretion

Over the course of a week, urine and feces contained around 23.5% of the MMAE administered during a brentuximab vedotin infusion. The median percentage of recoverable MMAE excreted in feces was 72% (range: 59% to 77%), with the remaining MMAE excreted in urine. The mean and cumulative excretion of MMAE throughout time are shown in Figure 3.Tandem and high resolution mass spectrometry were used to identify MMAE metabolites in urine and feces. In liquid-liquid extracts of unconcentrated pee and feces, MMAE was the only species found. However, eight MMAE metabolites were found after the liquid-liquid extracts were concentrated ten times. Supplemental Figure S1 displays the metabolites and metabolic pathways.

DISCUSSION

The results of a clinical experiment that assessed brentuximab vedotin's potential for CYP3A-mediated medication interactions are presented in this paper. Since CYP3A is one of the main enzymes involved in drug metabolism, understanding this interaction is crucial to giving patients taking concurrent drugs advice when administering brentuximab vedotin. Furthermore, the main human pathway of MMAE excretion was identified.

The creation of an ADC presents challenges that are both comparable to and different from those related to standard antibody or small molecule drug development. Like other antibody-based treatments, the ADC is not anticipated to be subject to P450 hepatic metabolism in terms of DDIs; nevertheless, the ADC's small molecule component may be metabolized.24. The cytotoxic agent's apparent half-life is a few days after an ADC is administered due to formation-limited kinetics. In contrast to a few days for the development of small molecule medications, this lengthy apparent half-life hinders the clinical assessment of DDI potential and requires continuous dosing of a DDI perpetrator on the order of weeks, as performed in this investigation.

The potential for CYP3A inhibition was found at concentrations many orders of magnitude greater than those attained clinically, and MMAE was a time-dependent inhibitor, despite in vitro studies suggesting that MMAE does not increase CYP3A. Therefore, midazolam was used as a probe substrate to assess the potential of brentuximab vedotin (MMAE) to modify CYP3A. In order to reduce the possibility of significant sedation and amnesia in the presence of a CYP3A inhibitor, a low dose of midazolam was used in this study.25,26 When midazolam was given at the

approximate Tmax for MMAE, benuximab vedotin had no effect on the AUC0-∞ of midazolam: the 90% CI for the AUC0-∞ GMR was within bioequivalence bounds (0.80, 1.25). Although the point estimate for the Cmax GMR was near to 1, the Cmax variability does not rule out the potential of an interaction because the 90% CI for the Cmax GMR stretched past both the upper and lower bioequivalence bounds. These results support the conclusion that brentuximab vedotin, administered at a dose of 1.8 mg/kg, and resultant MMAE levels neither inhibit nor induce CYP3A.

The main mechanism for MMAE metabolism in the liver is CYP3A. Thus, the effects of a potent inhibitor (ketoconazole) and an efficient inducer (rifampin) were assessed. The 90% CI for both the AUC0-∞ and Cmax GMRs were within bioequivalency constraints for ADC when rifampin and brentuximab vedotin were given together. Rifampin coadministration reduced MMAE exposures by around 46%, and the upper 90% CI bounds for the AUC0-∞ and Cmax GMRs were less than 1. The 90% CI for the AUC0-∞ GMR of ADC was within bioequivalency boundaries when ketoconazole and brentuximab vedotin were coadministered, whereas the 90% CI for the Cmax GMR stretched over both bounds, indicating more variability; both GMRs for ADC were near 1. Coadministration of ketoconazole increased MMAE exposures by approximately 34%, and the upper 90% CI bounds for the AUC0-∞ and Cmax GMRs were larger than 1. An investigation on the period influence on MMAE PK based on the lower MMAE concentration in subsequent cycles as opposed to

In Cycle 1, it was projected that coadministration of ketoconazole could raise MMAE AUC0-∞ by approximately 73%. In the absence of concurrent ketoconazole, the increase in MMAE levels for subsequent cycles is anticipated to be around 38% or less in relation to Cycle 1 MMAE levels since MMAE levels drop to 80% or less after Cycle 1.In Cycle 1, ketoconazole treatment may raise MMAE AUC0-∞ by about 73%. In the absence of concurrent ketoconazole, the increase in MMAE levels for subsequent cycles is anticipated to be around 38% or less in relation to Cycle 1 MMAE levels since MMAE levels drop to 80% or less after Cycle 1.

Rifampin is a recognized inducer of P-gp, and ketoconazole has been linked to P-gp inhibition. Both drugs can alter P-gp expression in addition to CYP3A.27 Since MMAE was found to be a P-gp substrate in in vitro investigations, the study's findings cannot rule out P-gp's possible involvement in modifying MMAE clearance. It is uncertain how much P-gp modulation affects MMAE disposition in comparison to CYP3A modulation.

When combined, the rifampin and ketoconazole arms' data show that neither a potent CYP3A inhibitor nor an effective CYP3A inducer had an impact on the ADC, and that ADC exposures were constant across Cycles 1 and 2. Furthermore, the data imply that MMAE is a CYP3A substrate. Therefore, individuals receiving brentuximab vedotin along with powerful CYP3A inhibitors should be cautiously watched for side effects.

The study's safety evaluation results are mostly in line with those of other clinical trials including brentuximab vedotin.11–14 Nausea, tiredness, diarrhea, headache, pyrexia, and neutropenia were the most frequently reported treatment-emergent side effects.During the two treatment cycles of this trial, 18% of patients experienced treatment-emergent peripheral neuropathy, which was primarily sensory and Grade 1 (asymptomatic). Positive postbaseline ATA results were seen in four out of five individuals whose second brentuximab vedotin infusion was stopped because to

infusion-related events. Due to an adverse event, one patient stopped receiving treatment. In a different research, 89% of the 54 patients (96%) who finished the study according to protocol went on to get prolonged treatment with brentuximab vedotin.

Post hoc analysis revealed that numerical variations in safety findings among treatment arms were unlikely to be caused by interactions of brentuximab vedotin with midazolam, rifampin, or ketoconazole, despite the fact that the study was not intended to assess differences across treatment arms. Individual factors such pre-existing gastrointestinal issues and concurrent use of ketoconazole and other potentially contributing drugs may have contributed, at least in part, to the greater incidence of vomiting and diarrhea seen in the ketoconazole group. For adverse events frequently seen in brentuximab vedotin clinical trials, no consistent patterns were found, and no obvious contributory factors were found. Although the safety of long-term CYP3A modulator administration during prolonged therapy with brentuximab vedotin was not addressed in this two-cycle study, the short-term safety profile was broadly in line with previous clinical data.
This study's excretion section did not achieve mass balance. Based on the data collected, the primary route of excretion of MMAE in humans is via feces (median 72% of total excreta), which is consistent with preclinical results in a rat excretion study. The most common species found in excreta was MMAE, and only MMAE metabolites were found.
when the samples were concentrated ten times. The concentration of MMAE in excreta is therefore probably at least ten times greater than that of its metabolites. The CYP3A DDI profile of MMAE and brentuximab vedotin was described in this study. When brentuximab vedotin is given to people at a dose of 1.8 mg/kg, the data collectively indicate that MMAE is neither an inhibitor nor an inducer of CYP3A; instead, MMAE is a substrate of CYP3A and possibly a substrate of P-gp. The findings of this study might also be applicable to other MMAE-based ADCs that attain circulating concentrations of unconjugated MMAE and ADC comparable to those seen for brentuximab vedotin. While concurrent treatment of rifampin or ketoconazole did not seem to significantly alter the safety profile of brentuximab vedotin, individuals taking powerful CYP3A inhibitors concurrently with brentuximab vedotin should be cautiously watched for adverse events. Feces are the main way that people excrete MMAE.

Supplementary Material

For additional information, see the PubMed Central Web version.

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