Bendamustine is a DNA alkylating agent approved for the treatment of chronic lymphocytic leukemia (CLL) and B-cell non-Hodgkin lymphoma. There are scant reports on bendamustine-induced immune hemolytic anemia occurring mainly in CLL patients. We report a case of immune hemolytic anemia that developed after exposure to bendamustine in a 70-year-old female with CLL who was previously exposed to fludarabine. Previous exposure to fludarabine is a common finding in the majority of reported cases of bendamustine drug-induced immune hemolytic anemia (DIIHA), including our case. Bendamustine should be suspected as the cause of any hemolytic anemia that develops while on this drug, especially in CLL patients treated previously with fludarabine.

KEYWORDS: CLL; Bendamustine; Hemolytic anemia

CASE DESCRIPTION

The patient is a 70-year-old female diagnosed with chronic lymphocytic leukemia (CLL) in 2005 (Rai stage I) and followed up conservatively without treatment. In 2008, due to development of constitutional symptoms related to her disease, she received two cycles of fludarabine and cyclophosphamide with satisfactory response. Five to six months later, she developed direct antiglobulin test (DAT)-negative hemolytic anemia that responded to a course of steroids. In 2010, the patient developed marked and symptomatic splenomegaly, and was started on chemoimmunotherapy consisting of bendamustine and rituximab. She tolerated the first cycle fairly well. However, due to infusion reaction during the second cycle of rituximab, it was subsequently discontinued and she remained on single agent bendamustine. She received a total of four cycles of bendamustine, with the last cycle in June 2010 resulting in a favorable response and a remarkable decrease in the size of the spleen. Notably, following the third cycle of bendamustine, she had mild hyperbilirubinemia in the 2–2.5 mg/dL range, accompanied by decreased haptoglobin at 24 mg/dL, and elevated absolute reticulocyte count of 200,000/L, albeit with stable hemoglobin and LDH levels, and negative DAT. This hemolytic process was felt to be related to the significant reduction of spleen size as a response to chemotherapy. Another possible explanation would be immune hemolytic anemia related to bendamustine that was inhibited by the recent exposure to rituximab. Over the following six to eight months, the bilirubin levels slowly trended down and stabilized at around 1.4–1.5 mg/dL, and haptoglobin levels normalized. The patient remained relatively asymptomatic from her disease and was managed by observation at regular office visits, CBC testing and periodic abdominal ultrasound to re-assess liver and spleen size. In January 2011, her white blood cell...
BENDAMUSTINE-INDUCED ANEMIA

Case Report

Bendamustine-induced anemia secondary to a drug in 1953, neutropenia.

Evidence of drug-induced immune thrombocytopenia or per million of the population, which exceeds the incidence of drug-induced immune hemolytic anemia (DIIHA). Therefore, confirmed drug-induced immune hemolytic anemia (DIIHA) is reported by serologic evidence of immune mechanism for the hemolytic process. Therefore, confirmed drug-induced immune hemolytic anemia (DIIHA) is identical to idiopathic warm autoimmune hemolytic anemia (WAIHA). After removing the offending drug, a hematological response will be noticed in one to two weeks, but the DAT will remain positive for several months. The prototype drug is methyl-dopa, but today the most common drug to cause WAIHA is fludarabine. Proving that a certain drug causes WAIHA in vitro is difficult as in vitro testing is of no value; the only clue is resolution of hemolysis after the drug is stopped, but this can be confounded by a concomitant use of steroids.

Bendamustine hydrochloride is an alkylating agent first designed in 1963 by Ozegowski and Krebs in East Germany, and rediscovered in the 1990s. In 2008, it received FDA approval for first line treatment of patients with CLL as well as for patients with indolent B-cell non-Hodgkin lymphoma (NHL) that progressed during or within six months of treatment with rituximab or a rituximab-containing regimen. Chemically, bendamustine is composed of three structural components: the mechlorethamine (nitrogen mustard) group, which is structurally similar to cyclophosphamide and chlorambucil, and gives the drug its alkylating properties; a benzimidazole ring, which is similarly configured to some purine analogs such as 2-chlorodeoxyadenosine, and represents a unique facet of the molecule; and finally, a butyric acid side chain. The purpose of adding this purine-like to the nitrogen mustard was to include the antimetabolite properties shown for benzimidazole, but no evidence exists to confirm antimetabolite functionality.

There are ten reports of bendamustine-related hemolytic anemia in the literature. Nine of these cases occurred in CLL patients and one in a follicular lymphoma (FL) patient. Only two cases took place in a treatment naïve patient. Bendamustine dose ranged between 70 and 100 mg/m². DAT was negative in three cases, not available in two, and positive in the other five cases. Five patients were previously treated with fludarabine, and four of these had prior hemolytic anemia related to fludarabine. There are no reports of bendamustine-induced hemolytic anemia in malignancies other than CLL (except one case of FL as noted above). This is in agreement with the known association of CLL with autoimmune and drug-induced hemolytic anemia (DIIHA), including two of the most

Discussion

After the first report of an immune mediated hemolytic anemia secondary to a drug in 1953, the list of drugs implicated in this phenomena continued to expand, reaching 125 in 2007 in an update by Garratty et al. Many of these reports were not supported by serologic evidence of immune mechanism for the hemolytic process. Therefore, confirmed drug-induced immune hemolytic anemia (DIIHA) is quite rare and its incidence is estimated to be one per million of the population, which exceeds the incidence of drug-induced immune thrombocytopenia or neutropenia. There are two antibody types, and therefore two primary mechanisms, by which a drug can induce an immune hemolytic anemia. The first type is drug-dependent, reacting with RBCs in vitro in the presence of a drug. Hemolysis will cease after the drug is stopped. The second type of antibody is drug-independent, which will react with RBCs in vitro without the presence of a drug. Such antibodies appear to be autoantibodies rather than antibodies to the drug itself, producing a hematologic picture similar to idiopathic warm autoimmune hemolytic anemia (WAIHA). After removing the offending drug, a hematological response will be noticed in one to two weeks, but the DAT will remain positive for several months. The prototype drug is methyl-dopa, but today the most common drug to cause WAIHA is fludarabine. Proving that a certain drug causes WAIHA in vitro is difficult as in vitro testing is of no value; the only clue is resolution of hemolysis after the drug is stopped, but this can be confounded by a concomitant use of steroids.

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frequently used agents in this disease (chlorambucil and fludarabine). The exact mechanism leading to the development of immune hemolysis in CLL is unclear. Existing evidence suggests acquired T-cell dysregulation mediating autoimmunity, and for CLL cells producing inhibitory cytokines such as IL10 and IL6, which alter immunologic tolerance.13–15

In our case report, there are a number of findings that support the diagnosis of bendamustine DIIHA. First, the chronological association with exposure to bendamustine. Second, the exclusion of other potential etiologies such as other drugs or viral infections. Third, the concomitant response of CLL to treatment, both clinical and hematological, which renders the patient’s leukemia a less probable cause. Fourth, a history of exposure to fludarabine. As suggested by other reports7 and from observations of previous case reports where five out of ten cases were fludarabine-exposed patients – including four who developed fludarabine-related hemolytic anemia – it seems that prior exposure to and/or hemolytic anemia induced by fludarabine is emerging as a risk factor for hemolytic anemia upon future administration of bendamustine. The structural similarity between the two agents, more specifically the purine ring, might serve as a sensitizing factor leading to a more pronounced immunologic reaction upon subsequent exposure to the other drug. Fifth, a history of hemolytic episode in association with previous bendamustine use. The possibility of bendamustine-induced immune hemolytic anemia during the above mentioned episode should still be entertained despite a negative DAT which could have been masked by the recent exposure to rituximab, a phenomena that was previously described in the literature.16 Notably, given that the eluate from the patient’s RBC was positive for drug-independent autoantibodies, the possibility of coinciding AIHA secondary to CLL should also be considered in this case. One can postulate that a leukemic clone emerged after suppression of the other clones via effective chemotherapy and triggered an autoimmune process that resulted in hemolytic anemia. However, the conversion of DAT from a negative to a positive after bendamustine exposure is further support of the involvement of bendamustine in the hemolytic event. In the absence of a laboratory test or method that can reliably distinguish drug-independent/drug-mediated immune hemolytic anemia from AIHA related to CLL, the diagnosis of bendamustine-induced immune hemolytic anemia in this case remains equivocal, albeit very suspicious. A possible mechanism for immune hemolysis related to bendamustine is depletion of CD4 cells – in a similar fashion to the lymphopenic effects of the other purine analogues – which can lead to failure of control of autoreactive T-cells.

This case adds to the accumulating data which indicate that bendamustine administered to CLL patients can potentially induce an autoimmune hemolytic event, especially in those who previously received and developed a hemolytic reaction to fludarabine. Therefore, should such an event occur while a patient is on bendamustine, withholding the drug and avoiding future exposure should be strongly considered.

**CONFLICT OF INTEREST**

None declared.

**REFERENCES**