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High-dose cyclophosphamide for autoimmunity and alloimmunity

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Abstract High-dose cyclophosphamide (high-CY) is a potent immunosuppressive regimen that is increasingly used to mitigate both autoimmune and alloimmune conditions. Differential expression of aldehyde dehydrogenase between hematopoietic stem cells and lymphocytes accounts for the differential sensitivity of these cells to high-CY and explains why this regimen is immunosuppressive but not myeloablative. This article describes the clinical translation of high-CY for the treatment of autoimmune and alloimmune conditions.

Keywords Cyclophosphamide · Autoimmunity · Bone marrow transplantation · Aplastic anemia

Introduction

Cyclophosphamide was approved as an anti-cancer agent more than 50 years ago, and it remains one of the most successful and widely employed anti-neoplastic drugs [1]. It is also a potent immunosuppressive agent and the most commonly used drug in blood and marrow transplantation (BMT). Cyclophosphamide's unique metabolism is responsible for its distinct immunosuppressive properties; its immunosuppressive properties are the result of differential cellular expression of aldehyde dehydrogenase, the major mechanism of cyclophosphamide inactivation. Increasingly, high-dose cyclophosphamide (100–200 mg/kg administered intravenously over a few days, hereafter referred to as high-CY) has been used to treat a variety of severe refractory autoimmune diseases, and more recently, high-CY has been employed after allogeneic BMT in order to facilitate engraftment and to prevent graft-versus-host disease (GVHD). This article will discuss the clinical translation of high-CY for the treatment of autoimmunity and alloimmunity.

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Pharmacology

High-CY is a prodrug and is converted to 4-hydroxycyclophosphamide and its tautomer aldophosphamide by the hepatic cytochrome P-450 system. These compounds diffuse into cells and are converted into the active alkylating compound, phosphoramide mustard, through simple intracellular decomposition. The major mechanism of high-CY detoxification appears to be the inactivation of aldophosphamide by cellular aldehyde dehydrogenase (ALDH) to form the inert compound, carboxyphosphamide. ALDH1A1 (also known as ALDH1 or retinaldehyde dehydrogenase 1) appears to be the ALDH isoenzyme most responsible for high-CY detoxification [2]. Hematopoietic stem cells are relatively resistant to high-CY because they express high levels of ALDH1A1 [3–5]. Lymphocytes have low levels of ALDH1A1 and are rapidly killed by high-CY. High-CY is therefore highly immunosuppressive but not myeloablative, allowing endogenous hematopoietic stem cells to rapidly reconstitute hematopoiesis.

High-CY and acquired aplastic anemia

Acquired severe aplastic anemia (SAA) is a life-threatening bone marrow failure disorder that presents with severe pancytopenia and a hypocellular bone marrow [6]. Most cases are caused by an immune-mediated attack directed at hematopoietic stem/progenitor cells. In 1972, the first successful allogeneic bone marrow transplant (BMT) for SAA was reported following conditioning with high-CY (50 mg/kg/d $\times$ 4 consecutive days) [7]. By the mid-1970s, allogeneic BMT after conditioning with high-CY became the treatment of choice for young patients with severe aplastic anemia (SAA). Shortly thereafter, several reports of autologous hematopoietic reconstitution following allogeneic BMT for SAA appeared [8, 9]. This suggested that viable hematopoietic stem cells remained in these patients and that the immunosuppressive activity of high-CY allowed the endogenous stem/progenitors to reconstitute hematopoiesis. In 1976, a case report in the New England Journal of Medicine described a complete remission in an SAA patient treated with high-CY without BMT [10]. Since then, multiple clinical trials have demonstrated that high-CY without BMT can induce durable hematologic remissions in patients with SAA [11–14]; the best results occurring in patients who are treatment naïve, but successful treatment of refractory SAA has also been reported following high-CY [15, 16]. The Johns Hopkins group recently reported an overall actuarial survival of 88% in 44 treatment-naïve SAA patients following high-CY therapy; the response rate was 71% with the majority being complete remissions, and the actuarial event-free survival at 10 years was 58% [17]. Death, relapse, evolution to a myelodysplastic syndrome or paroxysmal nocturnal hemoglobinuria, or need for a BMT were defined as events. Patients with refractory SAA fared less well after high-CY therapy; at 10 years, overall actuarial survival, response, and actuarial event-free survival rates were 62, 48, and 27%, respectively. A drawback to the use of high-CY for treating SAA is the slow hematopoietic recovery. For treatment-naïve patients, the median time to a neutrophil count of 0.5×10^9/l was 60 (range, 28–104) days, the median time to last platelet transfusion was 117 (range, 24–640) days, and the median time to last red cell transfusion was 186 (range, 30–1,784) days. For patients with refractory SAA, the median time to a neutrophil count of 0.5×10^9/l was 54 (range, 35–119) days, the median time to last platelet transfusion was 103 (range, 51–751) days, and the median time to last red cell transfusion was 210 (range, 63–796) days. Currently, BMT remains the treatment of choice for young SAA patients who have a suitable matched sibling donor. However, fewer than

30% of young SAA patients have a matched sibling donor. For these patients, immunosuppressive therapy with high-CY or antithymocyte globulin and cyclosporine is recommended.

High-CY for treating refractory severe autoimmune disease

Autologous peripheral blood stem cell transplantation (HSCT) following high-CY is an effective therapy for severe refractory autoimmune diseases [18, 19]. The major steps of HSCT include: [1] stem cell mobilization/collection; [2] conditioning with high doses of chemotherapy; and [3] hematopoietic stem cell infusion. The European Group for Blood and Marrow Transplantation (EBMT) and European League Against Rheumatism (EULAR) have established a registry to compile the results of numerous phase I/II studies of HSCT for the treatment of autoimmune disease. In the United States, the results of HSCT for the treatment of autoimmune disease are collected by the Center for International Blood and Marrow Transplant Research (CIBMTR). Together, these groups have compiled data on more than 1,000 patients, and high-CY (200 mg/kg divided over 4 consecutive days), often with other non-myeloablative agents such as anti-thymocyte globulin (ATG), is becoming the preferred conditioning for HSCT in autoimmune diseases. However, the efficacy of autologous HSCT is derived from the immunosuppressive properties of the conditioning regimen; the administration of stem cells is primarily a rescue procedure to hasten hematopoietic recovery. The success of high-CY in treating SAA (in most cases an autoimmune disease) served as the impetus to try high-CY in more classical autoimmune diseases. In contrast to SAA, where hematopoietic recovery after high-CY takes many weeks, hematopoietic recovery after high-CY in classical autoimmune diseases that do not target hematopoietic stem cells (e.g., lupus, multiple sclerosis etc.) is rapid; most patients are neutropenic for less than 1 week. Thus, HSCT is not necessary following high-CY [20]. Importantly, eliminating the steps of stem cell mobilization and reinfusion could be advantageous. Not only can mobilization add to the duration, cost, and toxicity of HSCT [21], but the mobilized product also contains numerous effector cells (auto-reactive lymphocytes) that may contribute to relapse. Additional study of high-CY for treating severe refractory autoimmune disease is necessary; however, durable remissions (several beyond 3 years) have been reported in a variety of diseases including refractory cases of autoimmune hemolytic anemia [22], multiple sclerosis [23, 24], chronic inflammatory polyneuropathy [25, 26], systemic sclerosis [27], myasthenia gravis [28, 29], systemic lupus erythematosis [30, 31], and others [32]. A recent study treated nine relapsing-remitting multiple sclerosis patients with high-CY and followed them for a mean period of 23 months [24]. Eight patients were unresponsive to conventional therapy, and one was treatment naive. All patients developed transient total or near-total pancytopenia as expected followed by hematopoietic recovery in 10–17 days. There were no deaths or unexpected serious adverse events. The investigators reported a statistically significant 39% reduction in disability scores (EDSS) and an 81% decrease in the number of gadolinium-enhancing lesions from the pretreatment MRI scans. Two patients required rescue treatment with other immunomodulatory therapies during the study owing to disease exacerbations.

High-CY for treating alloimmunity

BMT is potentially curative therapy for a variety of life-threatening malignant and non-malignant hematologic diseases. The application of BMT to treat these disorders is often

limited by the lack of suitable donors and sometimes end-organ damage in the prospective patient. Related haploidentical BMT following a reduced-intensity conditioning regimen is a potential method for expanding the potential pool of bone marrow donors; any patients share one HLA haplotype with each biologic parent or child and siblings or half-siblings have a 50% chance of being haploidentical. Furthermore, the reduced-intensity conditioning regimen allows for inclusion of patients with mild to moderately impaired cardiac, renal, hepatic, or pulmonary function. The major barrier to the success of HLA-haploidentical BMT, heretofore, has been the high incidence of graft rejection and high risk of severe graft-versus-host disease (GVHD); however, animal studies show that alloreactive (host-versus-graft and graft-versus-host) T cells, which are activated a few days after allogeneic transplantation, are exquisitely susceptible to killing by high-CY [33, 34]. Moreover, high-CY has been shown to eradicate HLA alloimmunization to platelets in patients with SAA [35]. Based on these data and the known stem cell sparing effects of high-CY, a phase II trial of reduced-intensity HLA-haploidentical BMT with post-transplantation high-CY (50 mg/kg on days 3 and 4 after transplant) was initiated in patients with high-risk hematologic malignancies. This non-randomized trial demonstrated that high-CY after reduced-intensity haploidentical-related BMT can achieve rates of acute or chronic GVHD similar to that seen with HLA-matched sibling BMT [36]. The median time to neutrophil and platelet recovery was 15 and 24 days, respectively; the overall survival at 1 and 2 years was 46 and 36%, respectively; the incidence of graft failure was 13%; the incidence of grade III or greater acute graft-versus-host disease was 6%; and the incidence of chronic graft-versus-host disease was 5%. Thus, post-transplantation high-CY appears to be effective for facilitating engraftment and mitigating acute and chronic graft-versus-host disease. This expands the pool of potential donors for patients in need of an allogeneic BMT. Unfortunately, the probability of relapse at 2 years in patients with high-risk hematologic malignancies is 58% following reduced-intensity haploidentical-related BMT [36]. However, the use of this approach in non-malignant hematologic diseases (e.g., sickle cell anemia, thalassemia, aplastic anemia, and paroxysmal nocturnal hemoglobinuria) is particularly promising since relapse is unlikely [37]. A recent report of three patients with paroxysmal nocturnal hemoglobinuria (one of whom also had severe sickle cell disease) treated with reduced-intensity HLA-haploidentical BMT with post-transplantation high-CY demonstrates the potential of this approach to cure patients with life-threatening hematologic diseases. Rapid engraftment without graft-versus-host disease occurred in two of the patients, including the patient with sickle cell disease. Both patients were disease free with full donor chimerism and required no immunosuppressive therapy, with follow-up of 1 and 4 years, respectively, at the time of publication [38].

Conclusions

Cyclophosphamide is best known as an anti-cancer agent; however, it has potent immunosuppressive activity and in high doses appears to be able to induce tolerance. Differential expression of ALDH between hematopoietic stem cells (high ALDH levels) and lymphocytes (low ALDH levels) accounts for the differential sensitivity of these cells to high-CY and explains why stem cells are resistant to high-CY. High-Cy has shown great clinical promise for the treatment of severe autoimmune diseases. In SAA, more than 60% of patients achieved durable remissions without the need for any additional drug treatment. High-CY is also showing great promise for the treatment of other severe refractory autoimmune diseases such a multiple sclerosis, myasthenia gravis, and others. Most

recently, high-CY is being used to facilitate engraftment and to mitigate GVHD in the setting of allogenic BMT.

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