Cyclophosphamide and cancer: golden anniversary

Ashkan Emadi, Richard J. Jones and Robert A. Brodsky

Abstract | Cyclophosphamide remains one of the most successful and widely utilized antineoplastic drugs. Moreover, it is also a potent immunosuppressive agent and the most commonly used drug in blood and marrow transplantation (BMT). It was initially synthesized to selectively target cancer cells, although the hypothesized mechanism of tumor specificity (activation by cancer cell phosphamidases) transpired to be irrelevant to its activity. Nevertheless, cyclophosphamide's unique metabolism and inactivation by aldehyde dehydrogenase is responsible for its distinct cytotoxic properties. Differential cellular expression of aldehyde dehydrogenase has an effect on the anticancer therapeutic index and immunosuppressive properties of cyclophosphamide. This Review highlights the chemistry, pharmacology, clinical toxic effects and current clinical applications of cyclophosphamide in cancer and autoimmune disorders. We also discuss the development of high-dose cyclophosphamide for BMT and the treatment of autoimmune diseases.

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Introduction

Cyclophosphamide is one of the most successful anticancer agents ever synthesized. Even today, 50 years after its synthesis, cyclophosphamide is still widely used as a chemotherapeutic agent and in the mobilization and conditioning regimens for blood and marrow transplantation (BMT). Among 1,000 selected compounds and antibiotics tested against 33 tumors, cyclophosphamide was the most effective molecule. The initial clinical trials[1,2] of cyclophosphamide for the treatment of cancer were performed in 1958, and in 1959 it became the eighth cytotoxic anticancer agent approved by the FDA. It is also approved for minimal change disease of the kidney in children (a disease that causes nephrotic syndrome), but despite its widespread use in other autoimmune disorders and BMT, it has never been approved for these indications.

Chemistry and pharmacology

Mechanism of action

Reviewing the chemistry and pharmacology of cyclophosphamide is crucial for understanding its wide therapeutic applicability. Efforts to modify the chemical structure of nitrogen mustard to achieve greater selectivity for cancer cells culminated in the synthesis of cyclophosphamide in 1958.[3,4] The intention was to generate a prodrug in a chemically inactive form that could be converted into the active compound predominantly in cancer cells. The chemical design of cyclophosphamide—substitution of an oxazaphosphorine ring for the methyl group of nitrogen mustard (Figures 1 and 2)—was based on the rationale that some cancer cells express high levels of phosphamidase, which is capable of cleaving the phosphorus–nitrogen (P–N) bond, releasing nitrogen mustard.[3] Thus, cyclophosphamide was one of the first agents rationally designed to selectively target cancer cells. Although cyclophosphamide is in fact a prodrug that requires metabolic activation, the original hypothesis that it would function as targeted anticancer therapy via phosphamidase activation proved to be inaccurate.

The cytotoxic action of nitrogen mustard is closely related to the reactivity of the 2-chloroethyl groups attached to the central nitrogen atom. Under physiological conditions, nitrogen mustards undergo intramolecular cyclizations through elimination of chloride to form a cyclic aziridinium (ethyleneiminium) cation. This highly unstable cation is readily attacked on one of the carbon atoms of the three-member aziridine ring by several nucleophiles, such as DNA guanine residues (Figure 1).[5] This reaction releases the nitrogen of the alkylating agent and makes it available to react with the second 2-chloroethyl side chain, forming a second covalent linkage with another nucleophile, thus interfering with DNA replication by forming intrastrand and interstrand DNA crosslinks (Figure 1).

In contrast to aliphatic (or open chain) nitrogen mustards, cyclophosphamide is an inactive prodrug that requires enzymatic and chemical activation to release active phosphoramide mustard. Hydroxylation on the oxazaphosphorine ring by the hepatic cytochrome P-450 system generates 4-hydroxycyclophosphamide, which coexists with its tautomer, aldophosphamide (Figure 2).

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These unstable transport precursors freely diffuse into cells, where aldophosphamide is decomposed into two compounds, phosphoramide mustard and acrolein.[6] Phosphoramide mustard produces the interstrand and intrastrand DNA crosslinks (Figure 1) responsible for the cytotoxic properties of cyclophosphamide, whereas acrolein is the cause of hemorrhagic cystitis, one of the major toxic effects of cyclophosphamide.

Inactivation of cyclophosphamide

The major mechanism of cyclophosphamide detoxification is oxidation of aldophosphamide to carboxyphosphamide by cellular aldehyde dehydrogenase (ALDH).[7] Withdrawal of the hydrogen adjacent to the aldehyde, by a base, is a necessary step for decomposition of aldophosphamide. Conversion of the aldehyde to carboxylic acid by ALDH makes this hydrogen less acidic for removal,[8] subsequently releasing the active phosphoramide mustard. Thus, cellular concentrations of ALDH are serendipitously responsible for many of the differential activities of cyclophosphamide in cells.[9] Carboxyphosphamide and its degradation products are the major metabolites found in urine.[10]

The ALDH family consists of at least 17 distinct enzymes.[11] These NADP-dependent enzymes oxidize aliphatic and aromatic aldehydes to their corresponding carboxylic acids. ALDH1A1 is the isoform mainly responsible for cyclophosphamide detoxification.[12] ALDH1A1 also has an important role in ethanol metabolism, but its major function is the biosynthesis of retinoic acid from retinol (vitamin A).[11] After alcohol dehydrogenase oxidizes retinol to retinaldehyde, ALDH1A1 oxidizes retinaldehyde to retinoic acid. As retinoic acid is essential for cellular growth and differentiation, cells with high proliferative potential—especially liver cells, intestinal mucosa and hematopoietic stem cells[13]—express high levels of ALDH1A1 and as a consequence are relatively resistant to cyclophosphamide (Figure 3). Conversely, cyclophosphamide is cytotoxic to mature hematopoietic progenitors[14] and all lymphocyte subsets,[13,15] which express low levels of ALDH1A1. Thus, while leukopenia is common after cyclophosphamide treatment, no dose of cyclophosphamide has been found to cause irreversible bone marrow aplasia. The dose-limiting toxic effect of cyclophosphamide is hemorrhagic perimyocarditis.[16] High expression of ALDH1A1 in hematopoietic stem cells is also the prime reason that activated congeners of cyclophosphamide, 4-hydroperoxycyclophosphamide and mafosfamide, have been the most widely studied agents for *ex vivo* eradication or purging of occult tumor cells from autologous BMT grafts.[17,18]

Mechanism of resistance

Although ALDH1A1 expression is the major determinant of normal cellular sensitivity to cyclophosphamide and is associated with resistance to cyclophosphamide in tumor cell lines,[19] it has a minor role in the clinical response of cancer cells to cyclophosphamide.[9,12,20] In particular, leukemia and lymphoma specimens from newly diagnosed patients rarely express high levels of ALDH isozymes.[9] Increased cellular levels of glutathione and glutathione S-transferases have been shown to cause cyclophosphamide resistance in tumor cell lines, but the lack of a similar correlation *in vivo* suggests that this is not the major mechanism contributing to cyclophosphamide resistance.[20] The inherent sensitivity of cancer cells to undergo apoptosis following DNA damage is the most important determinant of the clinical sensitivity of cancer cells to cyclophosphamide.[21,22]

Clinical pharmacology

The complex, multistep metabolic process of cyclophosphamide, which involves unstable intermediate compounds, has complicated pharmacokinetic studies. For example, the incidence of both cardiac toxicity and antitumor activity was found to be higher in women with lower cyclophosphamide plasma levels after high-dose administration and autologous BMT for metastatic breast cancer. This observation was postulated to be the

Key points

- Cyclophosphamide is an inactive prodrug that requires enzymatic and chemical activation; the resultant nitrogen mustard produces the interstrand and intrastrand DNA crosslinks that account for its cytotoxic properties
- The major mechanism of cyclophosphamide detoxification involves aldehyde dehydrogenase; cells with high proliferative potential express high levels of aldehyde dehydrogenase and as a consequence are relatively resistant to cyclophosphamide
- Cyclophosphamide, in combination with other antineoplastic agents, is used for the treatment of various cancers, including breast, lymphoid and pediatric malignancies
- Cyclophosphamide is also widely used in bone marrow transplantation 'conditioning' and 'mobilization' regimens, and for the treatment of different autoimmune conditions
- The toxic effects of cyclophosphamide include bone marrow suppression, cardiac and gonadal toxicity, hemorrhagic cystitis and carcinogenesis, with cumulative dose being the principal risk factor

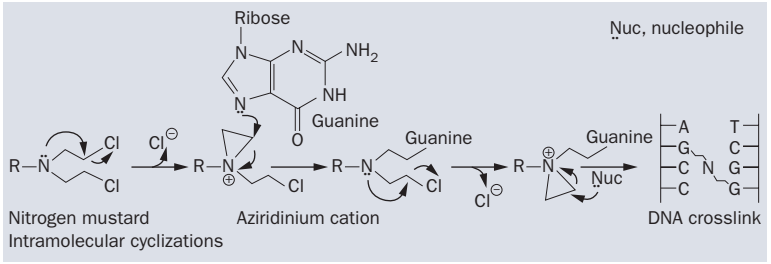

Figure 1 | Formation of aziridinium ion from nitrogen mustard and its DNA alkylating activity. In nitrogen mustard, the nitrogen atom displaces the chloride to result in a strained, three-membered intermediate cation, called aziridinium. This ion reacts easily with nucleophiles. The N-7 position of guanine in DNA is strongly nucleophilic and can be readily alkylated by the aziridium cation. The second chemical transformation of nitrogen mustard provides another aziridinium ion, which, upon reaction with another DNA nucleophile, forms a DNA crosslink.

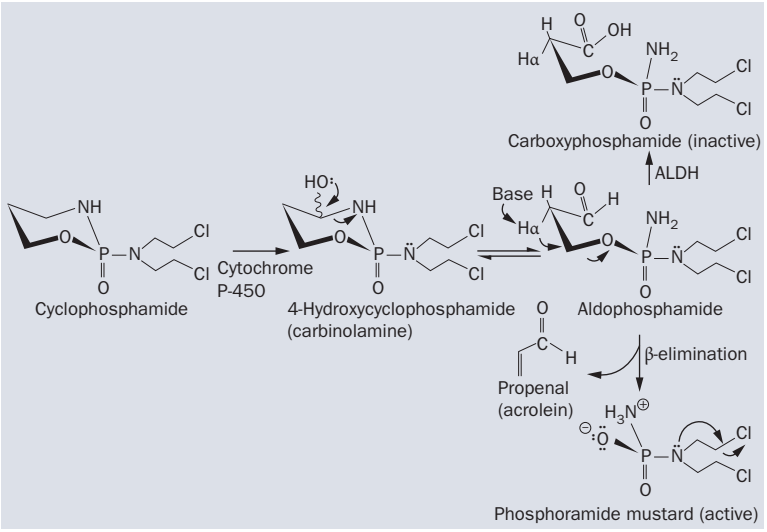

Figure 2 | The metabolic pathway of cyclophosphamide. Cyclophosphamide is activated by the hepatic cytochrome P-450 enzyme system to form 4-hydroxycyclophosphamide, which is in equilibrium with its open ring tautomer, aldophosphamide. These two intermediate metabolites readily diffuse into cells but are not cytotoxic. Depending on the type of the cell, aldophosphamide decomposes to form cytotoxic phosphoramide mustard and the byproduct acrolein by a chemical process called β-elimination. Alternatively, aldophosphamide is oxidized to the inactive metabolite carboxyphosphamide by aldehyde dehydrogenase. This latter reaction occurs in hematopoietic stem cells because they express high levels of aldehyde dehydrogenase.

result of increased conversion of cyclophosphamide to its alkylating metabolites.[23] Support for this hypothesis was derived from data demonstrating an inverse correlation between cyclophosphamide and its metabolites hydroxycyclophosphamide and aldophosphamide after BMT.[24] Others have also found that cyclophosphamide plasma levels are not predictive of toxicity or tumor response[7,25] in contrast to the levels of its active metabolites, 4-hydroxycyclophosphamide and aldophosphamide.[26,27] A potential explanation for these opposing findings is that cyclophosphamide activation can be saturated at concentrations achieved after administration of high doses, leading to an increased renal excretion of the parent drug. The dose of cyclophosphamide that saturates its activation varies among patients, but is in the range used for high-dose clinical indications.[28]

Cyclophosphamide metabolism demonstrates substantial variability between patients,[7,25,29] and can be altered by a variety of drugs, including antiemetics,[30] phenytoin,[31] cyclosporine,[32] and busulfan.[33] Moreover, cyclophosphamide can autoinduce its activation by hepatic cytochrome P-450 (that is, enhancement of self 4-hydroxylation).[34] It has been shown that cytochrome P-450 protein levels were increased by pre-exposure of hepatocytes to cyclophosphamide. The approved product label for cyclophosphamide recommends dose adjustments in patients with renal or hepatic failure; however, most reports suggest that these are not necessary as the major mode of cyclophosphamide detoxification is by tissue ALDH.[35] Indeed, cyclophosphamide has been administered safely without dose adjustment to patients with renal or hepatic failure. Cyclophosphamide levels are undetectable by 12–24 h after administration, even in patients with severe renal failure. Thus, delaying dialysis for at least one day after cyclophosphamide administration will prevent drug elimination in patients with renal failure.[36]

Patients with a creatinine clearance less than 50 ml/min who receive high doses of cyclophosphamide (>750–1,000 mg/m^2) have increased systemic drug exposure.[36] In these patients, MESNA (2-mercaptoethane sulfonate sodium) at a total dose equivalent to 100% of the cyclophosphamide dose should be added to prehydration and posthydration fluids to decrease the risk of hemorrhagic cystitis.

Toxic effects

The dosage, and hence toxicity profile, of cyclophosphamide varies widely depending on the clinical indication. For the purpose of this Review, we somewhat arbitrarily define the dosages of cyclophosphamide as follows: 'low' dose: 1–3 mg/kg (40–120 mg/m^2) usually administered orally daily; 'intermediate' or 'pulse' dose: 15–40 mg/kg (600–1,500 mg/m^2) usually administered intravenously every 3–4 weeks; and 'high' dose: >120 mg/kg (>5,000 mg/m^2) most commonly administered over 2–4 days as conditioning for BMT. Low to intermediate dosages tend to have fewer acute toxic effects; however, prolonged usage (>6 months) may result in substantial chronic toxicity. Conversely, high-dose cyclophosphamide is associated with more acute toxic effects, but seems to mitigate the risk for chronic toxic effects.[37,38]

Hematologic toxic effects

Bone marrow suppression is the most common toxic effect of cyclophosphamide. Neutropenia is dose dependent. Patients treated with low-dose cyclophosphamide should be monitored closely, although they rarely develop significant neutropenia. Leukopenia, thrombocytopenia and anemia are common after high-dose cyclophosphamide administration. Rapid hematologic recovery invariably occurs within 2–3 weeks in patients with normal bone marrow reserve, regardless of the dose.

Cardiac toxic effects

Cardiotoxicity is the dose-limiting toxic effect of cyclophosphamide, and is observed only after administration of high doses.[16] The cardiac manifestations that result from high-dose cyclophosphamide are heterogeneous and range from innocuous to fatal. The most severe form is hemorrhagic necrotic perimyocarditis, with a reported incidence of <1–9% after the most commonly used high doses of cyclophosphamide (60 mg/kg daily × 2 days or 50 mg/kg daily × 4 days).[39,40] However, in most transplant centers the rate of hemorrhagic myocarditis is

less than 0.1%. This clinical syndrome occurs abruptly within days of drug infusion and is fatal. Perimyocarditis is manifested by severe congestive heart failure accompanied by electrocardiographic findings of diffuse voltage loss, cardiomegaly, pleural and pericardial effusions. Postmortem findings reveal hemorrhagic cardiac necrosis.

Transient cardiac toxicity attributed to cyclophosphamide occurs in up to 45% of BMT recipients.[41] This usually manifests as a subclinical depression of left ventricular function. Mild arrhythmias and small pleural and pericardial effusions can also occur. Some patients can develop symptoms of congestive heart failure; however, if electrocardiogram voltage is maintained, these symptoms are usually reversible. Although pretreatment cardiac evaluation cannot predict which patients will develop hemorrhagic myocarditis, it does predict for high incidence of reversible cardiac toxicity.[41]

Gonadal toxic effects

Gonadal failure is a major complication of cyclophosphamide administration, especially in females. The patient's age at treatment, the cumulative dose, and the administration schedule are major determinants for this adverse effect.[42,43] The risk for sustained amenorrhea in patients with lupus receiving monthly intermediate-dose cyclophosphamide is 12% for women under 25 years of age, and greater than 50% for women over 30 years of age.[43] The risk for ovarian failure following high-dose cyclophosphamide administration seems to be less than that of intermediate-dose. No ovarian failure following allogeneic BMT for aplastic anemia was observed after conditioning with high-dose cyclophosphamide in women less than 26 years of age, although it was occasionally noted in older women.[44]

A protective effect of testosterone and triptorelin against cyclophosphamide-induced gonadal damage has been reported in men and women with various forms of kidney disease.[45] The usefulness of cryopreservation of eggs, embryos or ovaries remains uncertain for women seeking to preserve their reproductive potential. Storing sperm before chemotherapy is widely practiced and technically successful.[46]

Bladder toxic effects

Hemorrhagic cystitis is the most common form of cyclophosphamide bladder toxicity,[47] but bladder fibrosis and transitional or squamous-cell carcinoma can also occur. Hemorrhagic cystitis can occur early or late after cyclophosphamide administration. Early onset disease, in the first few days after cyclophosphamide administration, seems to be caused by acrolein (Figure 2).[48] Vigorous hydration, forced diuresis and MESNA, which interacts with acrolein to form nontoxic adducts, can prevent acute hemorrhagic cystitis by limiting uroepithelial exposure to acrolein.[49] Hemorrhagic cystitis can develop weeks to months after treatment in 20–25% of patients who receive high-dose cyclophosphamide.

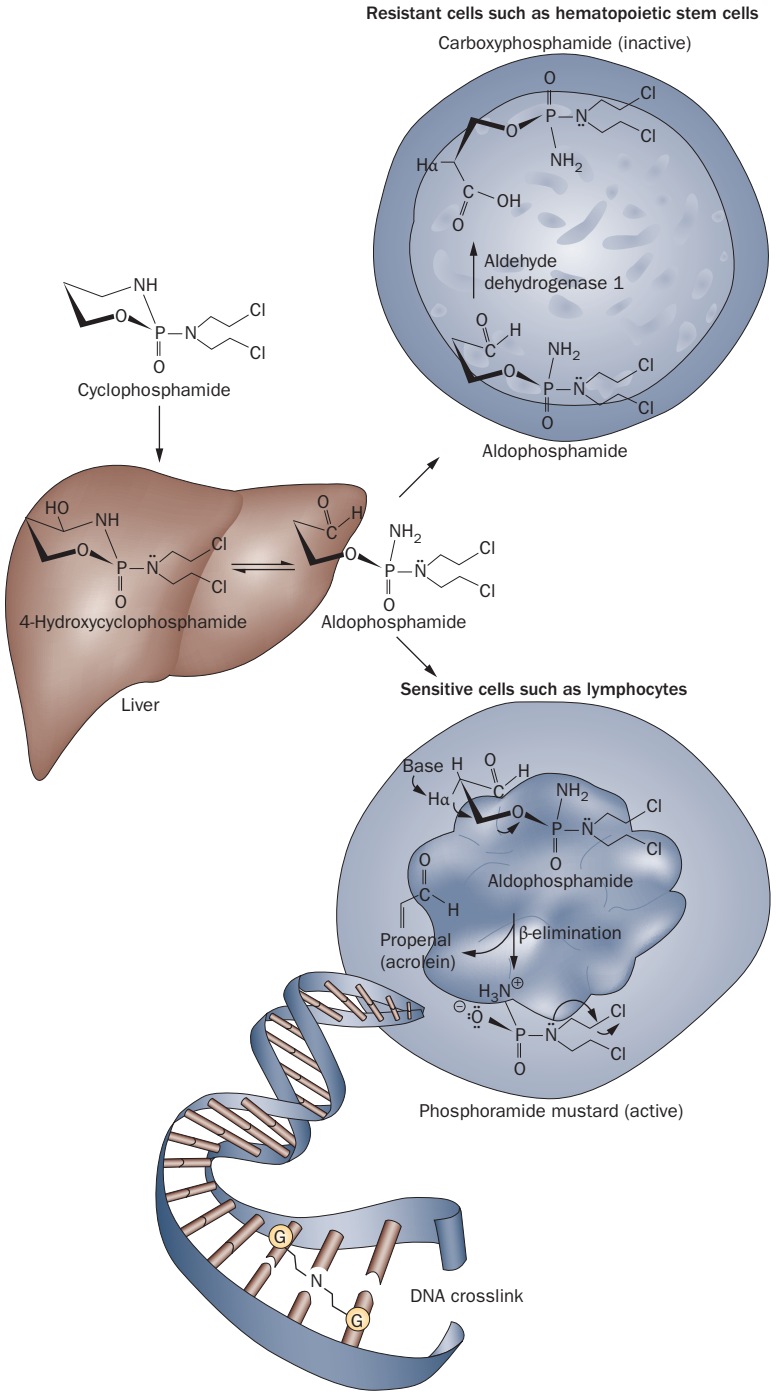

Figure 3 | Differential sensitivities between hematopoietic stem cells and lymphocytes to cyclophosphamide's cytotoxic effect. After intravenous or oral administration, cyclophosphamide is rapidly distributed in the body. In the liver, it is converted to 4-hydroxycyclophosphamide, which stays in equilibrium with aldophosphamide. 4-hydroxycyclophosphamide and aldophosphamide readily cross the cell membranes by passive diffusion. In cells with high levels of aldehyde dehydrogenase 1 (for example, hematopoietic stem cells), aldophosphamide is irreversibly converted to carboxyphosphamide. Carboxyphosphamide does not decompose to phosphoramide mustard and therefore lacks alkylating and cytotoxic activity. In the absence of a high concentration of aldehyde dehydrogenase (for example, lymphocytes), aldophosphamide spontaneously liberates phosphoramide mustard and acrolein. Phosphoramide mustard is a bifunctional DNA alkylating molecule and forms interstrand DNA crosslinks primarily at the guanine (G) sites.

Most cases of late hemorrhagic cystitis have a viral etiology, usually secondary to the BK polyoma virus[50,51] or adenovirus.[52] More than 90% of adults are seropositive for the BK virus, which is a ubiquitous double-stranded DNA virus.[53] Following primary infection, usually in childhood, the virus persists indefinitely in the urinary tract and is reactivated and excreted in the urine during periods of impaired immunity. Late hemorrhagic cystitis that occurs after BMT, irrespective of high-dose cyclophosphamide conditioning, indicates that virus reactivation secondary to immunosuppression has a major role in the development of this condition.[54–56] In addition, chronic low-dose cyclophosphamide has been associated with late-onset hemorrhagic cystitis,[57] which has also been attributed to the BK virus.

Most cases of cyclophosphamide-induced bladder cancer have been reported in patients who received the drug orally for more than 1 year. A cumulative dose of more than 20 g is the principal risk factor, with a median interval from treatment to diagnosis of bladder cancer of 7 years.[58] Patients treated with long-term cyclophosphamide should be followed indefinitely with routine urinalysis for microscopic hematuria and cystoscopy if red blood cells are present.[59]

Carcinogenicity of cyclophosphamide

Cyclophosphamide is carcinogenic. In addition to bladder cancer, secondary acute leukemia (often preceded by myelodysplastic syndrome) and skin cancer are the most common malignancies after cyclophosphamide therapy. The probability of acquiring a therapy-related malignancy is proportional to the length of drug exposure and cumulative dose. Therapy-related leukemia occurs in roughly 2% of patients treated with chronic cyclophosphamide, primarily in patients who have received the drug for more than 1 year.[60]

Although therapy-related leukemia has been reported after high-dose cyclophosphamide as part of the conditioning regimen for BMT, most evidence suggests that the disease results from infused hematopoietic progenitors previously damaged by the conventional-dose cytotoxic therapy provided before BMT.[61] Therapy-related leukemia from alkylating agents often displays abnormalities in chromosomes 5 and 7, or complex cytogenetic abnormalities,[60] and usually occurs between 3–10 years after treatment.

Other toxic effects

Nausea and vomiting are common adverse effects of cyclophosphamide administration and are most prominent with intermediate and high dosages. Prophylaxis with 5-hydroxytryptamine 3 (5-HT3) receptor antagonists, such as ondansetron or dolasetron, can usually mitigate these adverse effects. Reversible alopecia is also common, especially with high dosages of cyclophosphamide. Diarrhea is uncommon with oral cyclophosphamide administration, but may occur following high-dose therapy. Mild to moderate hyponatremia, attributed to the syndrome of inappropriate antidiuretic hormone (SIADH), and central pontine myelinolysis have also been associated with cyclophosphamide therapy.[62]

Clinical activity

Cyclophosphamide is one of the few drugs with a broad indication for cancer. Although it is effective as a single agent in malignancies, it is usually used in combination with other antineoplastic agents. Even though it has been substituted by newer agents (such as platinums, taxanes and targeted therapies) for the treatment of many solid tumors, it is quite active for many of these indications, and there often remains limited evidence for the superiority of the newer approaches.

Lymphomas

Cyclophosphamide-based therapy is used extensively for lymphomas and is often curative for aggressive non-Hodgkin lymphoma, with Burkitt lymphoma being particularly sensitive. Although modern therapeutic regimens employ intensive cyclophosphamide-based combination chemotherapy,[63] in the 1960s durable complete remissions were reported following a single course of cyclophosphamide.[64] R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) remains the most commonly employed regimen for aggressive non-Hodgkin lymphoma, with cure rates of 30–40%. Many newer cyclophosphamide-based multidrug combinations have been developed for aggressive non-Hodgkin lymphoma, but none have proven to be superior to CHOP.[65] Myeloablative therapy, usually including high-dose cyclophosphamide, followed by BMT is the most effective treatment for relapsed aggressive non-Hodgkin lymphoma.

Indolent lymphomas typically occur in adults over 50 years of age and these patients have a median survival of 8–12 years, with follicular lymphomas comprising the majority of cases. Other distinct entities include small lymphocytic lymphoma (SLL) or chronic lymphocytic leukemia (CLL), lymphoplasmacytoid lymphoma, marginal zone lymphoma, and mycosis fungoides. Cyclophosphamide alone or in combination with other agents remains a standard of care for these lymphomas. Other therapeutic options include watchful waiting, other alkylating agents, purine nucleoside analogs, and monoclonal antibodies such as rituximab or alemtuzumab. The FCR (fludarabine, cyclophosphamide and rituximab) regimen induces high complete remission rates in previously untreated[66] patients with SLL or CLL, as well as those with relapsed and refractory[67] disease. Substitution of fludarabine with pentostatin in the FCR regimen is also safe and effective in patients with CLL.[68] Attempts to eliminate cyclophosphamide from chemotherapy regimens by increasing the dose of purine nucleosides have not been successful, demonstrating that cyclophosphamide remains an important component of therapy in patients with CLL.[69] Cyclophosphamide, in combination with multiple other antineoplastic agents, is also used in the management of acute lymphocytic leukemia in children and adults.[70]

Solid tumors

Breast cancer

Cyclophosphamide, in combination with other agents, has been the mainstay of adjuvant and metastatic breast cancer chemotherapy regimens such as CMF (cyclophosphamide, methotrexate, 5-fluorouracil) and FEC (5-fluorouracil, epirubicin, cyclophosphamide) for decades. AC (doxorubicin, cyclophosphamide) was demonstrated to be equivalent to 6 months of classic CMF in two separate National Surgical Adjuvant Breast and Bowel Project (NSABP) studies.[71,72] The addition of a sequential taxane to this adjuvant regimen further improved outcomes and has since become the standard of care for human epidermal growth factor receptor 2 (HER2)-negative early-stage breast cancer.[73] The primary aim of this study was to determine whether four cycles of adjuvant paclitaxel after four cycles of adjuvant AC could prolong disease-free survival and overall survival compared with four cycles of AC alone in patients with resected breast cancer and histologically positive axillary nodes. In total, 3,060 patients were randomly assigned to AC ($n = 1,529$) or AC followed by paclitaxel ($n = 1,531$). Patients with estrogen-receptor-positive or progesterone-receptor-positive tumors also received tamoxifen for 5 years. Postlumpectomy radiotherapy was mandated. The median follow-up was 64.6 months. The study showed that the addition of paclitaxel to AC significantly reduced the hazard for disease-free survival by 17% (relative risk [RR] 0.83; 95% CI 0.72–0.95; $P = 0.006$). The 5-year disease-free survival was 76% for patients who received AC followed by paclitaxel compared with 72% for those who received AC. Improvement in overall survival was small and not statistically significant (RR 0.93; 95% CI 0.78–1.12; $P = 0.46$). The 5-year overall survival was 85% for both groups. Toxicity with the AC and paclitaxel regimen was acceptable for the adjuvant setting. More-recently, the less cardiotoxic docetaxel and cyclophosphamide regimen has gained preference for the treatment of this population of patients. Docetaxel and cyclophosphamide improves disease-free survival compared to AC and is well tolerated in elderly patients.[74] The cyclophosphamide-containing regimen TAC (docetaxel, doxorubicin, cyclosphosphamide) can also be used in the adjuvant setting but requires the use of granulocyte colony-stimulating factor (G-CSF) or granulocyte–macrophage colony-stimulating factor (GM-CSF) support.[75]

Ovarian cancer

In two prospective, randomized trials,[76,77] paclitaxel in combination with cisplatin produced a greater survival benefit compared with cyclophosphamide and cisplatin in patients with advanced ovarian cancer. The standard treatment for women with advanced ovarian cancer is cytoreductive surgery, followed by cycles of paclitaxel and carboplatin. However, a three-armed, randomized trial from The International Collaborative Ovarian Neoplasm Group showed that single-agent carboplatin as well as CAP (cyclophosphamide, doxorubicin and cisplatin) are as effective as paclitaxel and carboplatin as first-line treatment for women with ovarian cancer.[78] Cyclophosphamide in combination with vincristine and dactinomycin (VAC) is used as an alternative regimen for the treatment of ovarian germ-cell tumors[79] and for patients with ovarian cancer who have residual or recurrent disease after multiple chemotherapy regimens.

Bone and soft tissue sarcomas

Cyclophosphamide is the cornerstone of curative chemotherapy regimens for numerous newly diagnosed and recurrent pediatric malignancies. Combinations of carboplatin and etoposide, and vincristine, cyclophosphamide and doxorubicin (CAdO), showed 5-year overall and event-free survival rates of over 90% in infants with initial localized and unresectable neuroblastoma as well as allowance for subsequent surgical excision.[80] Cyclophosphamide in combination chemotherapy regimens as an adjunct to surgery and radiation has also been used in a variety of rare cancers such as retinoblastoma, Wilms tumor, rhabdomyosarcoma and Ewing sarcoma.

BMT conditioning regimens

Preliminary preclinical and clinical BMT conditioning regimens used total body irradiation (TBI) as a single modality for refractory leukemias and lymphomas because of its dual anticancer and immunosuppressive properties. Toxicity concerns and limited access to facilities that could provide TBI led to the development of BMT conditioning regimens that did not involve TBI. Cyclophosphamide possessed these dual anticancer and immunosuppressive properties, and, therefore, was used as a replacement for TBI.[81] Animal studies showed high-dose cyclophosphamide to be a potent immunosuppressant; however, in contrast to TBI, doses sufficient for allogeneic engraftment were not myeloablative.[81] On the basis of these preclinical data, trials with high-dose cyclophosphamide as conditioning for patients undergoing allogeneic BMT for refractory leukemias and lymphomas were initiated. The initial dose was 60 mg/kg daily for 4 days, but after one of the first four treated patients died of hemorrhagic myocarditis, the dose was decreased to 50 mg/kg daily for 4 days. Fatal hemorrhagic myocarditis has not been reported for this dose of cyclophosphamide when used as a single agent.[16] In 1972, Thomas *et al.*[82] reported the first successful human allogeneic BMT in a patient with aplastic anemia when high-dose cyclophosphamide was used as a conditioning agent. High-dose cyclophosphamide remains the most commonly used BMT conditioning regimen for aplastic anemia.[83] Owing to high relapse rates, cyclophosphamide or TBI are not used as single agents for BMT in malignant diseases.[84] High-dose cyclophosphamide in combination with other agents, especially busulfan or TBI, proved to be quite effective against high-risk hematologic malignancies, with many of these patients achieving cure. Hence, these are the most commonly used conditioning regimens

for myeloablative BMT. More-recently, intermediate-dose cyclophosphamide, especially in combination with fludarabine, has become a standard conditioning regimen for nonmyeloablative allogeneic BMT.

Mobilization regimens

The use of peripheral-blood progenitor cells has largely replaced bone marrow transplantation in autologous BMT. Although it does not produce an overall survival advantage, autologous peripheral-blood administration is associated with more rapid engraftment, approximately 5 days sooner compared with BMT.[85] For instance, in one study, absolute neutrophil counts exceeded 500 per cubic millimeter 5 days earlier in patients assigned to receive peripheral-blood cells compared with those who received BMT.[85] This is because high numbers of hematopoietic progenitor cells that generate rapid early engraftment can be collected from 'mobilized' peripheral blood.[86] Peripheral-blood progenitor cells are collected by leukopheresis after mobilization to increase the amount of circulating progenitors. A number of mobilization regimens are active, including G-CSF. However, regimens that include high-dose cyclophosphamide (2–7 g/m^2) with G-CSF seem to mobilize the highest number of hematopoietic progenitors and cyclophosphamide's cytotoxic properties help to treat the malignancy.[87]

Cyclophosphamide for autoimmune diseases

Low-dose cyclophosphamide has been used effectively in a variety of autoimmune disorders. High-dose cyclophosphamide followed by autologous BMT has also been used in patients with refractory severe autoimmune diseases, including systemic lupus erythematosus,[88] rheumatoid arthritis,[89] scleroderma,[90] and multiple sclerosis.[91] The rationale for this approach is based on a variety of autoimmune animal models demonstrating marked improvement or complete eradication of autoimmune disease following syngeneic BMT. In addition, there are case reports of allogeneic BMT (performed chiefly for a malignancy) in which a concurrent autoimmune disease was eradicated.[92] However, allogeneic BMT is not generally recommended for treating autoimmune disorders because of its substantial toxicity. Since 1996, The European Group for Blood and Marrow Transplantation (EBMT) and European League Against Rheumatism (EULAR) Autoimmune Disease Working Party in collaboration with networks in the US have treated approximately 1,000 patients with autoimmune diseases with high-dose cyclophosphamide (with or without antithymocyte globulin) and autologous BMT.[93] Although long-term remissions have been observed in all diseases studied, relapses have been common.

The therapeutic potency of autologous transplant for autoimmune conditions is almost completely derived from the immunosuppressive properties of the conditioning regimen. Stem cell infusion is primarily a rescue procedure for shortening hemotopoietic recovery. A major concern with this approach is the potential of reinfusing autoreactive effector cells with the autograft. There are compelling data that high-dose cyclophosphamide without autologous transplant can salvage patients with a variety of refractory severe autoimmune disorders.[94,95] The advantage of high-dose cyclophosphamide alone is that it would shorten the duration of the procedure by several weeks, reduce the cost of the procedure by up to 50%, and eliminate the potential for reinfusing autoreactive lymphocytes with the autograft.

The impetus for this approach arose from studies in aplastic anemia, which is an autoimmune disease directed against hematopoietic stem cells. Although most allogeneic grafts in patients with aplastic anemia persist indefinitely after immunosuppression with high-dose cyclophosphamide, complete autologous hematologic recovery occurs in up to 20% of patients in some series.[96] These data suggest that cyclophosphamide alone, particularly in high doses, may be beneficial to patients with aplastic anemia. Indeed, several studies have confirmed that high-dose cyclophosphamide without BMT can induce durable remissions in most patients with acquired severe aplastic anemia.[97] Although it is not universally accepted that high-dose cyclophosphamide should be used as first-line therapy, its activity in aplastic anemia highlights the drug's unique pharmacology. Hematopoietic stem cells, reduced but still present in aplastic anemia, are resistant to cyclophosphamide as a consequence of their high ALDH expression (Figure 3). Eradication of the autoimmune effectors,[98] which have low levels of ALDH, allows for a gradual return of normal hematopoiesis. In contrast to aplastic anemia, where the marrow reserve is limited,[97] the duration of aplasia after high-dose cyclophosphamide in other autoimmune diseases is brief, usually 7 to 14 days.[94,99–102] Whether the use of high-dose cyclophosphamide without autologous BMT can reduce the risk of relapse associated with autologous BMT for autoimmunity, without increasing toxicity, is under investigation.

High-dose cyclophosphamide for alloimmunity

Animal studies have shown that alloreactive (host-versus-graft and graft-versus-host) T cells, which are activated a few days after allogeneic transplantation, become exquisitely susceptible to killing by high-dose cyclophosphamide.[103] Indeed, administration of high-dose cyclophosphamide after BMT was shown to inhibit both graft rejection and graft-versus-host disease (GVHD) in animal models.[104] On the basis of these data and owing to its stem-cell-sparing effects, high-dose cyclophosphamide (50 mg/kg on days 3 and 4 after transplant) has been found to be an effective method for clinical GVHD prophylaxis. In a phase II clinical trial, high-dose cyclophosphamide after nonmyeloablative haploidentical-related BMT was shown to be associated with rates of acute or chronic GVHD similar to those seen with matched allogeneic BMT.[105] These results are encouraging and emphasize the need for future randomized studies.

Conclusions

Fifty years after its synthesis, cyclophosphamide continues to be used for a wide array of diseases, including solid tumors, hematologic malignancies, autoimmune disorders, stem-cell mobilization, and as a conditioning regimen for BMT. Its therapeutic index in cancer and its potent immunosuppressive properties are due to differential cellular expression of ALDH. More recently, high-dose cyclophosphamide post-BMT is being used to mitigate GVHD. The toxicity of cyclophosphamide is highly predictable and is a function of dose and duration of therapy.

Review criteria

Our comprehensive search included electronic and manual searching. We searched MEDLINE® for articles published from 1950 through July 2009, abstracts of international meetings 2000–2009 (American Society of Hematology [ASH], American Society of Clinical Oncology [ASCO], American Association for Cancer Research [AACR], European Hematology Association [EHA] and European Society for Medical Oncology [ESMO]) to identify primary literature on the cyclophosphamide-related basic and clinical research.

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