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The Emerging Role of Gemcitabine in Conditioning Regimens for Hematopoietic Stem Cell Transplantation

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The specific combination for conditioning regimens in hematopoietic stem cell transplantation continues to be a premier area of focus in research. Although conditioning regimens have significantly evolved over time, obstacles continue to persist, including regimen-related toxicities, graft-versus-host disease, and disease relapse. Gemcitabine (2',2'-difluoro 2'-deoxycytidine, dFdC) is a pyrimidine nucleoside analog that distinguishes itself from other agents in the class by possessing a favorable pharmacokinetic and cytotoxic profile, while maintaining acceptable toxicities. Given the desirable properties, gemcitabine has garnered much attention and been assessed in several conditioning regimens. In this article, we review the pharmacology of gemcitabine with other nucleoside analogs and report the findings of pivotal trials conducted in both autologous and allogeneic transplantation. The positive results suggest a potential future role for gemcitabine and necessitate the need to conduct studies to further define its role.

INTRODUCTION

The use of hematopoietic stem cell transplantation (HSCT) has evolved over the past 50 years and now represents a potentially curative therapy for patients worldwide with various forms of malignant and nonmalignant diseases. Although the risks associated with HSCT have been significantly reduced, obstacles remain with regimen-related toxicities (RRT), graft-versus-host disease (GVHD), and disease relapse. Because of the impact on these various areas, refining HSCT conditioning regimens continues to be a premier area of focus. Research has been dedicated to finding the specific combination of chemotherapy agents, total body irradiation, and targeted therapies to optimize disease control, while also limiting treatment-related toxicities and GVHD. Advances, such as optimizing the systemic exposure of certain drugs through prospective pharmacokinetic-based monitoring, have aided in reducing toxicities. Incorporating novel agents into conditioning regimens has helped improve overall antitumor activity [1-3]. For example, nucleoside analogs have become a focus of interest, given their broad therapeutic activity and mild extramedullary toxicities. Although fludarabine already has an established role in allogeneic HSCT, other agents within the class are also showing promise. Recently, clinical studies have successfully incorporated gemcitabine into HSCT conditioning regimens. In this review, we will discuss the potential role of gemcitabine in HSCT and summarize the available clinical data.

GEMCITABINE PHARMACOLOGY

Gemcitabine (2',2'-difluoro 2'-deoxycytidine, dFdC) is a pyrimidine nucleoside analog [4,5]. Although mechanistically similar, gemcitabine distinguishes itself structurally from cytarabine by a fluorine group substituted at position 2' on the furanose ring (Figure 1). Gemcitabine, like other nucleoside analogs, requires cellular uptake via nucleoside transporters and intracellular phosphorylation for activation. Cellular uptake of the highly lipophilic gemcitabine molecule across the cell membrane involves specific nucleoside transport proteins through both active processes and facilitated diffusion. Specifically, 2 types of human nucleoside transporters have been identified: equilibrative (sodium independent) and concentrative (sodium dependent). Although both transporters are involved in the cellular uptake of gemcitabine, the bidirectional equilibrative carriers, such as human ENT1, have been identified as the primary transport [4,6,7].

Upon cellular uptake, gemcitabine undergoes phosphorylation by deoxycytidine kinase (dCK) to an intermediate metabolite gemcitabine monophosphate. The presence of dCK is rate limiting in gemcitabine activation [8,9]. Although at a much reduced substrate affinity compared with dCK, thymidine kinase 2 is also thought to play a minor role

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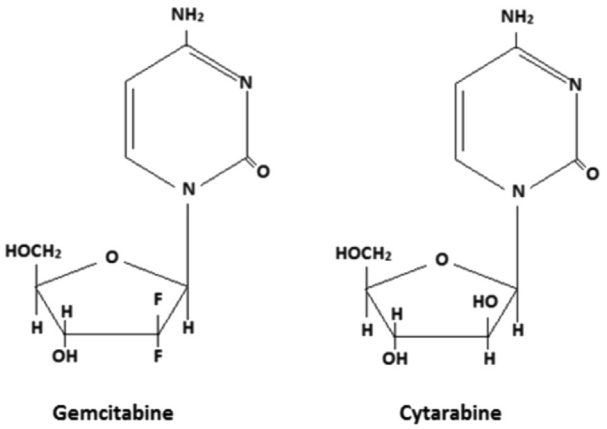

Figure 1. Nucleoside Analog Structures.

in phosphorylating gemcitabine [4]. The monophosphate metabolite is then further converted by other nucleotide kinases to the active metabolites gemcitabine diphosphate (dFdCDP) and, most importantly, triphosphate (dFdCTP). It is dFdCTP that is subsequently incorporated into DNA, resulting in DNA synthesis inhibition (Figure 2) [6-9].

Although the major mechanism through which gemcitabine exerts its activity is inhibition of DNA synthesis, it also exhibits other cytotoxic mechanisms. Other mechanisms include direct inhibition of DNA polymerase, resulting in termination of DNA chain elongation; inhibition of ribonucleotide reductase (RNR), leading to reduced competing deoxyribonucleotide pools necessary for DNA synthesis; and incorporation into RNA resulting in direct apoptosis [4,5,8,9]. Of these, the inhibition of DNA polymerase and RNA incorporation are largely attributed to dFdCTP. In contrast, dFdCDP is thought to inhibit RNR and the subsequent reduction in deoxyribonucleotides, particularly deoxycytidine triphosphate [8,9]. Furthermore, it has been suggested that gemcitabine may also contain cytotoxic activity by inducing topoisomerase I-mediated DNA strand breaks [4].

GEMCITABINE COMPARED WITH OTHER NUCLEOSIDE ANALOGS

Two major factors determine the clinical activity of nucleoside analogs: the substrate specificity for activating nucleoside kinases and the expression of these enzymes within the tumor tissues. The content of dCK is several-fold higher in lymphocytes than in other epithelial cells. The affinity of dCK is higher for gemcitabine compared with the other nucleoside analogs, including fludarabine, cytarabine, and cladribine. This may explain the broader range of clinical activity seen with gemcitabine compared with the other nucleoside analogs [8,10-12].

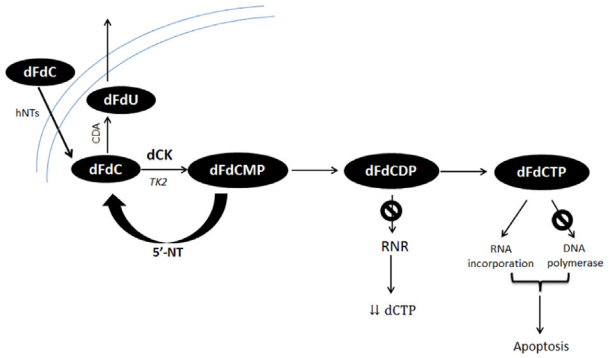

Figure 2. Intracellular Metabolism of Gemcitabine [6-9]. hNTs indicates human nucleoside transporters; TK2, thymidine kinase 2; dFdCMP, gemcitabine monophosphate; CDA, deoxycitidine deaminase; dFdU, 2'2'-difluorodeoxyuridine; dCTP, deoxycytidine triphosphate.

Collectively, the nucleoside analogs share a similar cytotoxic mechanism of inhibiting DNA polymerase at the analog insertion site upon intracellular activation by dCK phosphorylation (Table 1) [4,10,13-19]. Compared with cytarabine and fludarabine, gemcitabine undergoes greater activation to dFdCDP and dFdCTP because of its higher affinity for dCK [9,14]. In addition, gemcitabine possesses an additional cytotoxic mechanism of inhibiting RNR, the major source of deoxynucleotides normally required for DNA synthesis and repair [9]. Although the active metabolites of fludarabine and clofarabine also possess RNR inhibition activity, only dFdCDP results in an irreversible inhibition (Table 2) [9,14,15]. Specifically, in situ assays have identified the active metabolite dFdCDP as inducing the subsequent cellular depletion of deoxynucleotides, resulting in self-potentiation by preferentially incorporating gemcitabine as dFdCTP into DNA [9,10]. Furthermore, gemcitabine exhibits the unique ability of "masked" chain termination. This occurs after dFdCTP DNA incorporation and includes the addition of a single deoxynucleotide by DNA polymerase, predominantly to the 3' end of the extending DNA strand. This specific type of incorporation leads to the masking of the gemcitabine nucleotide from normal DNA polymerase 3' to 5' proof-reading exonuclease activity that normally removes mismatched base pairs [9,10]. This masking phenomenon is unique to gemcitabine, as none of the other nucleoside analog possess a similar mechanism that may help prevent normal DNA polymerase proofreading activity [9,14].

From a pharmacokinetic (PK) perspective, gemcitabine also exhibits favorable attributes that further enhance its mechanisms of action. Similar to clofarabine triphosphate (Cl-F-ara-ATP), dFdCTP has a slow cellular elimination half-life exhibiting both monophasic and biphasic properties (Table 3). At higher cellular concentrations ($\geq$ 100 μM), dFdCTP exhibits more biphasic elimination with a prolonged terminal half-life of 15 to 24 hours as compared with monophasic elimination of 4 to 6 hours in lower cellular concentrations [4,10,13-19]. This unique property of gemcitabine aids in the self-potentiating mechanism by reducing the elimination and further promoting the accumulation of the active metabolite. Specifically, dFdCTP is thought to reduce elimination by blocking deoxycytidylate monophosphate deaminase, its key catabolic enzyme. By blocking deoxycytidylate monophosphate deaminase, the elimination half-life changes from a monophasic to biphasic elimination, resulting in higher cellular dFdCTP concentrations, further enhancing the cytotoxic activity of gemcitabine [14].

Parental gemcitabine at high intracellular levels acts as a substrate inhibitor of dCK, thus explaining that levels of dFdCTP peak with intracellular gemcitabine levels of around 20 μmol/L [8,10,11]. In addition to inherent PK advantages, infusion times of gemcitabine have a direct effect on the activity of gemcitabine. Prolonged infusions at a fixed-dosed rate (FDR) of 10 mg/m^2/minute avoid the saturation of dCK activity by maintaining extracellular gemcitabine concentrations below 15 to 20 μM [20-22]. This FDR strategy has resulted in increased concentration-time curves of dFdCTP in leukemic cells as compared with the standard infusion times [8,23]. Furthermore, DNA synthesis remains suppressed up to 24 hours after the initiation of the gemcitabine infusion [24].