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Cryopreservation of CD34+ Hematopoietic Stem Cells Using Cryofit® DMSO and Its Outcomes

CD34+ Hematopoietik Kök Hücrelerin Cryofit® DMSO Kullanılarak Kriyoprezervasyonu ve Sonuçları

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ABSTRACT

Objective: Autologous hematopoietic stem cell transplantation (auto-SCT) is a key treatment for hematological malignancies and immune disorders. Cryopreservation of CD34+ hematopoietic stem cells (HSCs) ensures transplant success. Dimethyl sulfoxide (DMSO) is a widely used cryoprotectant but can cause infusion-related toxicities. CryoFit® DMSO aims to enhance cell viability while reducing adverse effects. This study evaluates its efficacy and safety in auto-SCT.

Material and Method: A single-center, retrospective study was conducted on 80 patients who underwent auto-SCT with CD34+ HSCs cryopreserved using CryoFit® DMSO. Mobilization was performed using granulocyte colony-stimulating factor (G-CSF) ± chemotherapy and plerixafor when required. CD34+ cells were quantified via flow cytometry before cryopreservation. Post-transplant engraftment, transfusion needs, and infusion-related side effects were assessed. Data analysis was conducted using SPSS 26.0.

Results: The median patient age was 58.5 years (range: 19-75) and 53.8% (n=43) of the cohort sample was female. Multiple myeloma was the most common diagnosis (57.5%). The median collected CD34+ cell count was 5.8 x 106/kg (range: 3.2-14). Post-thaw viability was 98% (range: 90-99.5%). Neutrophil and platelet engraftment occurred at medians of 13 and 17 days, respectively. The median hospitalization duration was 24 days (range: 15-60). Infusion-related adverse effects occurred in 26.3% of patients, primarily nausea/vomiting (15%), all manageable.

Conclusion: CryoFit® DMSO effectively preserves CD34+ HSCs with high post-thaw viability and favorable engraftment. Mild infusion-related toxicities were observed but were transient. The results support its continued use in auto-SCT. Further multicenter studies are required to optimize cryopreservation protocols.

Keywords: Cryopreservation, dimethyl sulfoxide, engraftment, hematopoietic stem cell transplantation, mobilization.

ÖZET

Amaç: Otolog hematopoetik kök hücre nakli (OKHN), hematolojik maligniteler ve immün bozukluklar için önemli bir tedavi yöntemidir. CD34+ hematopoetik kök hücrelerin (HKH) kriyoprezervasyonu, nakil başarısını sağlamak için kritik bir adımdır. Dimetil sülfoksit (DMSO), yaygın olarak kullanılan bir kriyoprotektandır ancak infüzyona bağlı toksisiteler oluşturabilir. CryoFit® DMSO, hücre canlılığını artırmayı ve olumsuz etkileri azaltmayı amaçlamaktadır. Bu çalışma, DMSO ile kriyoprezervasyonun OKHN'de etkinliğini ve güvenliğini değerlendirmektedir.

Gereç ve Yöntem: Bu çalışma, tek merkezli retrospektif bir analiz olup daha önceden CryoFit® DMSO ile kriyoprezervasyon yapılmış kök hücrelerden OKHN olan 80 hasta dahil edildi. Kök hücre mobilizasyonu, granülosit koloni stimüle edici faktör (G-CSF) ± kemoterapi ve gerekli durumlarda plerixafor kullanılarak gerçekleştirilmiştir. CD34+ hücreleri kriyoprezervasyondan önce akış sitometrisi ile sayıldı. Nakil sonrası kök hücre yamanması (engraftment), transfüzyon ihtiyacı ve infüzyona bağlı yan etkiler değerlendirildi. Veri analizi SPSS 26.0 yazılımı ile yapıldı.

Bulgular: Hastaların medyan yaşı 58.5 (aralık: 19-75), ve kohortun %53,8'i kadındı. En yaygın tanı multipl miyelomdu (%57,5). Toplanan CD34+ hücrelerin medyan miktarı 5.8 x 106/kg (aralık: 3.2-14) idi. Çözündürme sonrası hücre canlılığı %98 (aralık: %90-99.5) olarak bulundu. Nötrofil ve trombosit engraftmanı sırasıyla medyan 13 ve 17 gün olarak izlendi. Medyan hastanede kalış süresi 24 gün (aralık: 15-60) idi. Hastaların %26,3'ünde infüzyona bağlı yan etkiler gözlendi, en yaygın olanı bulantı/kusmaydı (%15) ve tüm yan etkiler yönetilebilir düzeydeydi.

Sonuç: CryoFit® DMSO, CD34+ HKH'ların çözündürme sonrası yüksek canlılık oranları ile etkin bir şekilde korunmasını sağlamıştır. Hafif infüzyona bağlı toksisiteler gözlemlenmiş ancak geçici olmuştur. Bu sonuçlar, CryoFit® DMSO'nun OKHN'de kullanımını desteklemektedir. Kriyoprezervasyon protokollerini optimize etmek için çok merkezli ileri çalışmalar gereklidir.

Anahtar Sözcükler: Dimetil sülfoksit, hematopoetik kök hücre transplantasyonu, kriyoprezervasyon, dimetil sülfoksit, mobilizasyon, engrafman.

Introduction

Autologous transplantation of hematopoietic stem cells (auto-SCT) is a well-established treatment for numerous hematological and non-hematological malignancies, as well as immune disorders (1). The auto-SCT procedure involves multiple critical stages, including mobilization of stem cells into the peripheral bloodstream, CD34+ cell counting, apheresis, cryopreservation, and the re-infusion of thawed cells after intensive therapy (2).

Hematopoietic progenitor stem cells (HSCs) can be obtained from different sources such as peripheral blood, bone marrow, and umbilical cord blood (3). Peripheral blood stem cells (PBSCs) are often preferred due to the less invasive collection method compared to bone marrow. The mobilization process involves administering granulocyte colony-stimulating factor (G-CSF) alone or in combination with other agents over several days. Quantification of CD34+ cells is performed before collection to ensure sufficient cell doses (4).

Although auto-SCT is a well-standardized and relatively safe procedure, various elements influence the capacity of HSCs to efficiently reconstitute hematopoiesis. These include the quantity and viability of progenitor cells, cryopreservation methods, storage conditions, and thawing techniques (1,5). Freshly harvested HSCs require specific handling procedures, sterility, and temperature stability at 2-6°C (1,6,7). The duration between hematopoietic stem cell collection and infusion or cryopreservation can impact cell viability, with the preservation of fresh HSCs typically considered for 1 to 6 days, particularly at lower temperatures (8-10). In cases where myeloma and lymphoma patients planned autologous PBSC transplantation beyond 72 hours post-collection can result in up to 35% loss of stem cells (11,12). However, when immediate transplantation is unfeasible, cryopreservation is necessary. Dimethyl sulfoxide (DMSO) and diluted by substances like human albumin, plasma, or other clinically approved solutions, it is a common cryoprotectant to prevent ice crystal formation and cellular shock. These cryopreserved cells are stored in liquid nitrogen freezers at -140°C to -195°C for up to a decade (13-15). Furthermore, the freezing/thawing procedure can cause cell harm, as indicated by a substantial reduction in total nucleated cells (TNC), CD34+ cells, cell viability, and/or the number of colony-forming units (CFU) in post-thaw specimens compared to fresh PBSC (16-18). Studies have shown that the infusion of fresh PBSC leads to superior outcomes and fewer side effects (10,19). Furthermore, DMSO is thought to be the most hazardous component of the transplant material, causing adverse symptoms such nausea, vomiting, cramps, and headaches in some cases, cardiovascular or neurological reactions during infusion. These reactions may stem from factors like damaged cells post-cryopreservation or an excessively low infusion temperature (20-24).

The HSC cryopreservation is in widespread use among transplant centers, however there is a lack of standardized procedures. DMSO has been standard for a very long period. Now it is available in various forms of vials. These vials are accepted similarly in regular use, since DMSO is a chemical component and for pharmaceutical technology easy to produce. However, their production conditions and last product state are not identical. Here, we evaluated CryoFit® DMSO and its effect on outcomes.

Materials and Methods

Patient Selection

This retrospective, single-center study involved adult patients with hematological malignancies undergoing auto-SCT. Only patients whose HSCs were cryopreserved with CryoFit® DMSO were included. Ethical approval was obtained, and The Declaration of Helsinki was followed when conducting the study. (Approval No: 2023/39-12, Date: 06.12.2023).

Stem Cell Mobilization, Collection, and Cryopreservation

Patients received G-CSF alone or in combination with chemotherapy or plerixafor when required. CD34+ cells were quantified using flow cytometry before cryopreservation. Using flow cytometry, cell viability (7-aminoactinomycin D [7-AAD]) and viable CD45+ and CD34+ cells were measured in accordance with the ISHAGE recommendations (25). When the absolute number of CD34+ cells in the blood exceeded 10/µl, the collection process started. Apheresis was performed using a continuous flow blood cell separator, processing 2-2.5 times the total blood volume per session. The targeted CD34+ cell

dose was 2-4 × 10^6/kg. If the initial collection was insufficient, additional mobilization and apheresis were performed. Cryopreservation was performed using a cryoprotective solution containing 5% dimethyl sulfoxide (DMSO), hydroxyethyl starch (HES), and autologous plasma. Cell concentrations were adjusted to approximately 100–200 × 10^6 nucleated cells/mL in autologous plasma. Prior to the addition of DMSO, the cell suspension was cooled on ice to minimize thermal shock. DMSO was added slowly with gentle mixing to prevent localized overheating and osmotic injury. Depending on clinical workflow, products were either cryopreserved immediately or stored at 4°C for up to 24 hours before freezing. Freezing was performed using a controlled-rate freezing protocol, with an initial cooling rate of 1–2°C per minute to −40°C, followed by rapid descent to −80°C.

Conditioning Regimens, Engraftment, and Side Effects Monitoring

Conditioning regimens varied according to disease type, with BEAM (carmustine, etoposide, cytarabine, melphalan) used for lymphoma patients and melphalan for myeloma patients. Neutrophil engraftment was defined as the first day with an absolute neutrophil count (ANC) >500/mm^3 or 1000/mm^3 for three consecutive days without growth factor support. Platelet engraftment was achieved when platelet counts exceeded 20.000/mm^3 or 50.000/mm^3. G-CSF support was administered from day +4 post-transplantation. Blood transfusions were provided when necessary. Any adverse effects occurring within 24 hours of HSC infusion were recorded.

Statistical Analysis

Data analysis was performed using SPSS version 26.0. Categorical variables were presented in frequency tables, while numerical variables were expressed as mean ± standard deviation or median and range, depending on distribution.

Results

A total of 80 patients participated in the study. The median age was 58.5 years (range: 19–75), and 53.8% (n=43) of the cohort sample was female. The majority of patients (57.5%, n=46) were diagnosed with multiple myeloma, followed by non-Hodgkin lymphoma (31.3%, n=25) and Hodgkin lymphoma (11.3%, n=9). Most patients (60%, n=48) had received one line of chemotherapy prior to transplantation, while 30% (n=24) had two lines and 10% (n=8) had three or more. Radiotherapy was administered to 10% (n=8) of the patients. The median time of the stem collection to the transplant was 1 (1-6) months. The median time from diagnosis to transplantation was seven months (range: 3–25). Additional patient characteristics are presented in Table I.

Table I. Patient Characteristics

	All donors (n:80)
Median Patient Age	58.5 (19-75)
Gender (Male/Female)	37 (% 46.3) / 43 (%53.8)
Diagnosis	
Multiple Myeloma	46 (57.5%)
Non- Hodgkin Lymphoma	25 (31.3%)
Hodgkin Lymphoma	9 (11.3%)
Previous Treatments	
1 line chemotherapy	48 (60%)
2 lines chemotherapy	24 (30%)
≥ 3 lines chemotherapy	8 (10%)
RT	8 (10%)
Median Duration from Diagnosis to Transplant (months)	7 (3-25)

RT: Radiotherapy

All patients received a conditioning regimen prior to transplantation. The most commonly used regimen was melphalan (MEL) in 57.5% of cases (n=46), followed by BEAM (40%, n=32) and BEAC (2.5%, n=2). The median harvested CD34+ cell count was 5.8 × 10^6/kg (range: 3.2–14), with a high median viability of 98% (range: 90–99.5). The median duration of hospitalization was 24 days (range: 15–60). Neutrophil engraftment occurred at a median of 13 days (range: 7–19), while platelet engraftment occurred at a median of 17 days (range: 12–50). The median number of erythrocyte transfusions required was 0 units (range: 0–6), and the median platelet transfusion requirement was 2 units (up to 12). Further transplant characteristics are detailed in Table II.

Infusion-related side effects were observed in 26.3% (n=21) of patients. The most frequent complication was nausea and vomiting, affecting 15% (n=12) of the cohort. Other side effects included hypotension (5%, n=4), hypertension (3.8%, n=3), chills (2.5%, n=2), arrhythmia (2.5%, n=2), chest pain (1.3%, n=1), and various other mild symptoms in 6.3% (n=5). All side

effects were grade 1–2 in severity and manageable; no life-threatening adverse events were recorded. Complete side effect profiles are presented in Table III.

Table II. Transplant Characteristics

	All donors (n:80)
Conditioning Regimens	
MEL	46 (57.5%)
BEAM	32 (40%)
BEAC	2 (2.5%)
Median Duration from Diagnosis to Transplant (months)	7 (3-25)
Median Duration of Hospitalization (days)	24 (15-60)
Median Neutrophile Engraftment (days)	13 (7-19)
Median Platelet Engraftment (days)	17 (12-50)
Median Erythrocyte Transfusion (units)	0 (0-6)
Median Platelet Transfusion (units)	2 (12)
Median Viability	98 (90-99.5)
Median collected CD34+ cell count ($x10^6$/kg cell)	5.8 (3.2-14)

BEAM: Carmustine, Etoposide, Cytarabine, Melphalan, BEAC: Carmustine, Etoposide, Cytarabine, Cyclophosphamide, MEL: Melphalan.

Discussion

This study aimed to assess the outcomes of cryopreservation of CD34+ HSCs using CryoFit® DMSO and its associated clinical implications. The findings provide insights into the effectiveness and safety of this approach in the context of auto-SCT.

Table III. Side Effects

Infusion-related complications	All donors (n:80, 100%)
Nausea-Vomiting	12 (15)
Chills	2 (2.5)
Chest pain	1 (1.3)
Hypotension	4 (5)
Hypertension	3 (3.8)
Arrhythmia	2 (2.5)
Others	5 (6.3)
Any Side Effect	21 (26.3)

The overall success of auto-SCT relies heavily on the viability and functionality of the cryopreserved stem cells. In this study, the high post-thaw viability of CD34+ cells, with a median viability of 98%, suggests that the CryoFit® DMSO formulation maintains cellular integrity effectively during the freezing and thawing processes. This is consistent with earlier reports, which highlights the critical role of cryoprotectants like DMSO in preventing ice crystal formation and cellular damage during cryopreservation (26).

According to the American Society for Blood and Marrow Transplantation, a target dosage of 3–5×106 CD34+ cells/kg has been suggested (27). Moreover, the observed median CD34+ cell yield (5.8 x 106/kg) surpasses the recommended minimum threshold for successful engraftment, further underscoring the efficacy of the cryopreservation protocol used. Engraftment outcomes in the study were favorable, with a median neutrophil engraftment time of 13 days and platelet engraftment at 17 days. The transfusion requirements were also within acceptable range. These results align with previous reports on autologous transplants utilizing cryopreserved cells (5,12). The engraftment kinetics observed can be attributed to the high viability of CD34+ cells and the individualized conditioning regimens, which were tailored to optimize transplant success. However, as with most cryopreservation methods, the use of DMSO is not without its limitations. Side effects such as nausea, hypo-hypertension, and other infusion-related reactions were documented, although they were manageable and transient in most cases. These effects are consistent with known DMSO-associated toxicities reported in prior studies (28,29). This highlights the need for continued exploration of alternative cryopreservation agents or DMSO formulations that minimize toxicity without compromising cell viability.

An additional finding worth noting is the significant variability in engraftment times and transfusion requirements among patients. This variability could stem from differences in patient characteristics, underlying diseases, or prior treatment regimens. For instance, the myeloma patients in this cohort received Melphalan as the conditioning regimen, which might explain the relatively short time to engraftment compared to other conditioning regimens like BEAM used for lymphoma patients. Further subgroup analyses could help elucidate the impact of these factors on transplantation outcomes.

This study has some limitations. First, it was in a retrospective single-center setting which can limit the generalizability. Second, the lack of a comparator group using alternative cryopreservation methods

restricts the ability to directly assess the relative advantages of CryoFit® DMSO. Future multicenter, prospective studies are required to validate these results and further refine cryopreservation protocols to improve clinical outcomes.

In conclusion, CryoFit® DMSO demonstrated robust efficacy in preserving CD34+ HSCs with high post-thaw viability and favorable engraftment outcomes. While DMSO-related toxicities remain a concern, their transient nature and manageability suggest that CryoFit® DMSO is a reliable cryoprotectant for auto-SCT. Further advancements in cryopreservation techniques hold promise for enhancing the safety and efficacy of SCT.

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