RESEARCH ARTICLE

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In Vitro FVIII-Encoding Transgenic Mesenchymal Stem Cells Maintain Successful Coagulation in FVIII-Deficient Plasma Mimicking Hemophilia A

İn Vitro FVIII İçeren Transgenik Mezenkimal Kök Hücreler Hemofili A'yı Taklit Eden FVIII İçermeyen Plazmada Başarılı Koagülasyon Sağlamaktadır

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Abstract

Objective: Hemophilia A is an X-linked recessive bleeding disorder caused by a deficiency of plasma coagulation factor VIII (FVIII), and it accounts for about 80%-85% of all cases of hemophilia. Plasma-derived therapies or recombinant FVIII concentrates are used to prevent and treat the bleeding symptoms along with FVIII-mimicking antibodies. Recently, the European Medicines Agency granted conditional marketing approval for the first gene therapy for hemophilia A. The aim of this study was to determine the effectiveness of coagulation in correcting FVIII deficiency with FVIII-secreting transgenic mesenchymal stem cells (MSCs).

Materials and Methods: A lentiviral vector encoding a B domain-deleted FVIII cDNA sequence with CD45R0 truncated (CD45R0t) surface marker was designed to develop a transgenic FVIII-expressing primary cell line by transducing MSCs. The efficacy and functionality of the FVIII secreted from the MSCs was assessed with anti-FVIII ELISA, CD45R0t flow cytometry, FVIII western blot, and mixing test analysis in vitro.

Results: The findings of this study showed that the transgenic MSCs maintained persistent FVIII secretion. There was no significant difference in FVIII secretion over time, suggesting stable FVIII

Öz

Amaç: Hemofili A, pıhtılaşma faktörü VIII'in (FVIII) eksikliğine bağlı gelişen, hemofili hastalarının yaklaşık %80-85'ini oluşturan, X'e bağlı resesif geçiş gösteren bir kanama bozukluğudur. Kanama semptomlarını önlemek ve tedavi etmek için plazma kaynaklı tedaviler ya da rekombinant FVIII konsantreleri ile FVIII'i taklit eden monoklonal antikorlar kullanılmaktadır. Son zamanlarda EMA, hemofili A'nın ilk gen tedavisi için koşullu pazarlama onayı vermiştir. Bu çalışmada, hemofili A hastalığından sorumlu olan FVIII eksikliğini düzeltebilmek amacıyla FVIII salgılayan transgenik mezenkimal kök hücreler (MKH) ile koagülasyon etkinliğinin değerlendirilmesi amaçlanmaktadır.

Gereç ve Yöntemler: CD45R0 truncated (CD45R0t) yüzey belirteci ile B domaini silinmiş FVIII cDNA dizisini kodlayan bir lentiviral vektör, MKH'leri transdükte ederek transgenik FVIII ekspresyonu sağlayan birincil bir hücre hattı geliştirilmiştir. MKH'lerden salgılanan FVIII proteininin etkinliği ve fonksiyonalitesi anti-FVIII ELISA, CD45R0t akım sitometrisi, FVIII western blot ve karışım testi (mixing test) analizleri ile in vitro olarak değerlendirilmiştir.

Bulgular: Çalışmamızda transgenik MKH'lerin kalıcı FVIII sekresyonu sağladığı gösterilmiştir.

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expression from the MSCs. The functionality of the FVIII protein secreted in the MSC supernatant was demonstrated by applying a mixing test in coagulation analysis. In the mixing test analysis, FVIII-deficient human plasma products were mixed with either a saline control or FVIII-secreted MSC supernatant. The mean FVIII level of the saline control group was 0.41±0.03 IU/dL, whereas the mean level was 25.41±33.38 IU/dL in the FVIII-secreting MSC supernatant mixed group ($p<0.01$). The mean activated partial thromboplastin time (aPTT) of the saline control group was 92.69±11.38 s, while in the FVIII-secreting MSC supernatant mixed group, the mean aPTT level decreased to 38.60±13.38 s ($p<0.001$).

Conclusion: The findings of this in vitro study suggest that the new method presented here is promising as a possible treatment for hemophilia A. Accordingly, a study of FVIII-secreting transgenic MSCs will next be initiated in a FVIII-knockout animal model.

Keywords: Factor VIII, Gene therapy, Hemophilia A, Lentivirus, Mesenchymal stem cells

Zamana bağlı yapılan analizlerde FVIII sekresyonunda anlamlı bir değişim olmadığı, MKH'lerde stabil FVIII ekspresyonu sağladığı gösterilmiştir. Koagülasyon analizlerinde, MKH süpernatanına sekrete olan FVIII proteininin fonksiyonalitesi, karışım testi kullanılarak saptanmıştır. Karışım testi analizlerinde, FVIII içermeyen insan plazma ürünleri salinle (kontrol grubu) ve FVIII-salgılayan MKH süpernatantı (çalışma grubu) ile karıştırılmıştır. Kontrol grubunun ortalama FVIII düzeylerinin 0,41±0,03 IU/dL olduğu, MKH süpernatant ile karıştırılan çalışma grubunda ise ortalama FVIII düzeylerinin 25,41±33,38 IU/dL ($p<0,01$) olduğu gösterilmiştir. Kontrol grubunun ortalama aktive parsiyel tromboplastin zamanı (aPTT) 92,69±11,38 saniye iken, FVIII-salgılayan MKH süpernatantı ile karıştırılan grubun ise ortalama aPTT seviyelerinin 38,60±13,38 saniyeye düştüğü gösterilmiştir ($p<0,001$).

Sonuç: Bu in vitro çalışma, hemofili A'yı tedavi etmek için uygulanabilir umut verici yeni bir yöntem ortaya koymaktadır. Bu veriler ışığında FVIII knock-out hayvan modelinde FVIII salgılayan transgenik MKH çalışması başlatılacaktır.

Anahtar Sözcükler: Faktör VIII, Gen tedavisi, Hemofili A, Lentivirüs, Mezenkimal kök hücre

Introduction

Hemophilia A is an X-linked recessive disorder caused by a deficiency in factor VIII (FVIII), an essential protein for blood coagulation [1]. Hemophilia A occurs in 1 in 5000 male births worldwide [2]. A decrease in FVIII levels caused by the mutation leads to the loss of coagulation activity and bleeding symptoms. The severity of these symptoms is directly correlated with FVIII levels in plasma [3], as low FVIII levels lead to severe bleeding symptoms at all ages [4].

Treatment options for patients with hemophilia A have changed over the years. Currently, hemophilia A is managed by life-long therapy with exogenous FVIII protein, either prophylactically or as on-demand treatment for bleeding episodes [3]. In the last 20 years, gene therapies have also shown promise in the possible treatment of hemophilia A as the molecular and genetic basis of this disease has become more fully understood. Although there is only one licensed gene therapy product for hemophilia A to date, undeniable progress has been achieved [5]. There are preclinical and clinical studies using viral and non-viral gene transfer methods for the treatment of hemophilia A. As of 2022, approximately 10 clinical trials were available for hemophilia A gene therapy using viral vectors [6]. Multiple ongoing clinical trials of gene therapy for hemophilia A involve different serotypes of recombinant adeno-associated viral vectors targeting hepatocytes [7,8,9]. These clinical trials have shown that long-term stable FVIII expression could not be achieved using adeno-associated viral vectors due to previous natural infections with the wild-type adeno-associated virus (AAV) [10]. Preexisting AAV immunity causes a cellular immune response against AAV vectors in the presence of neutralizing antibodies (NAbs) that eliminate the AAV antigen, resulting in ineffective transgene expression [11]. NAbs also prevent the re-administration of AAV-mediated gene therapy [12]. Furthermore, the viral genomes of the AAV vectors remain extrachromosomal in the transduced cells, eventually resulting in the loss of the transgene and transgene expression since the rate of transgene loss is dependent on the turnover rate of the transduced cells [13,14]. Selecting a lentivirus as a viral vector may possibly overcome the challenges faced with AAV vectors. The lower prevalence of HIV infection in the population, resulting in a lower incidence of NAbs against lentiviral vectors, may provide longer-term FVIII expression and allow repeated administration of lentivirus-mediated gene therapy [15]. Furthermore, lentiviruses can be imported more actively into the nuclei of the cells and integrated into the host genome in a stable manner independently of the cell cycle, preventing the loss of gene expression [16].

The current study differs from ongoing hemophilia A gene therapies in both the structure of the gene construct and the introduction of the designed gene with carrier cells in the form of mesenchymal stem cells (MSCs). In the gene design of this study, the B domain was deleted to reduce the size of the transgene, similar to previous studies [17]. However, unlike previous studies, CD45R0 was added to the construct without its intracellular portion, to be expressed extracellularly, in order to trace the functional gene. Also unlike previous studies, a non-viral EF-1 alpha (EF-1α) promoter was included in the gene construct instead of the liver-specific promoter in order to express the functional gene. For this purpose, a lentiviral vector with an EF-1α promoter, B domain-deleted FVIII cDNA sequence, and CD45R0 truncated surface marker (EF1α-FVIII.CC-CD45R0t) was designed.

Materials and Methods

Construction of the Lentiviral Vector

The envelope pCMV-VSV-G plasmid (Addgene #8454; a gift from Bob Weinberg) and the packaging psPAX2 plasmid (Addgene #12260; a gift from Didier Trono) were obtained from Addgene. The lentiviral vector EF1α-FVIII.CC-CD45R0t encoding the B domain-deleted FVIII gene in pHIVEGFP was synthesized by GenScript. The lentiviral construct was synthesized as previously stated [18]. The quantity and purity of the plasmids were confirmed by nanodrop analysis and DNA purity analysis was performed by gel electrophoresis method.

Production and Concentration of Lentiviral Vectors

The production and concentration of Lv-EF1α-FVIII.CC-CD45R0t were performed as previously described [18].

Vector Titration and Calculation of Number of Infectious Units per Milliliter (IFU/mL)

Umbilical cord MSCs (donated to the Acıbadem Labcell Cellular Therapy Center) were suspended as 10,000 cells in 100 µL of MSC NutriStem® XF Supplement (#05-201-1U, Biological Industries) including 1% pen/strep. MSCs in 100 µL of medium was plated in 96-well plates from A to G. The wells were adjusted to have 10 µL, 3 µL, 1 µL, 0.3 µL, 0.1 µL, 0.03 µL, and 0.01 µL of FVIII-LV solution in each 50 µL of the medium, respectively. Subsequently, 50 µL of virus dilution from each concentration was transferred to the MSC-cultured wells, the total volume was adjusted to 150 µL, and cells were incubated for 4 days. Flow cytometry was performed using CytoFLEX (Beckman Coulter) flow cytometry to determine the CD45R0 expression with the anti-CD45R0 antibody (Miltenyi Biotec). Following viral titration analysis, the number of infectious units (IFU) was calculated as the dilution coefficient corresponding to the lowest CD45R0t expression higher than 10% as measured by flow cytometry. Following the titer assay, viruses were stored at -80 °C. Quality control tests of FVIII-Lv were performed, including sterility and purity. Sterility was tested with aerobic-anaerobic (BACTEC), endotoxin (Charles River Laboratories), and mycoplasma analysis (Mycoplasma species 500 PCR kit and GeneAmp PCR System 2700, Applied Biosystems).

Lentiviral Transduction of MSCs

The transduction process was evaluated using three different transduction agents. Protamine sulfate (40 µg/mL, Sigma), Vectofusin-1 (10 µg/mL, Miltenyi MACS), and protamine sulfate + Vectofusin-1 were used to determine the efficiency of the FVIII lentiviral transduction of MSCs. MSCs were suspended as 10,000 cells in 100 µL of MSC NutriStem® XF Supplement (Biological Industries) including 1% pen/strep. MSCs in 100 µL of medium were plated in 96-well plates from A to D. These plates were incubated at 37 °C for 24 h for MSC adherence. The wells were adjusted to multiplicity of infection (MOI) levels of 10 MOI, 5 MOI, 3 MOI, and 1 MOI, and the transduction process was initiated with Vectofusin-1, protamine sulfate, and protamine sulfate + Vectofusin-1, with plates subsequently incubated for 4 days. CytoFLEX (Beckman Coulter) flow cytometry was applied to assess CD45R0 expression with the anti-CD45R0 antibody (Miltenyi Biotec).

FVIII Secretion Analysis

Quantitative measurement of FVIII was performed with a human FVIII enzyme-linked immunosorbent assay (ELISA) kit (#ab272771, Abcam) [19]. Transduced MSCs were used for FVIII expression analyses in a dose-dependent manner, collected on day 3 and day 10. The experiment was performed independently four times. All reagents, samples, and standards were prepared according to the manufacturer's instructions (#ab272771, Abcam). After 100 µL of FVIII-transduced MSC supernatant was added to the wells of the plate included in the ELISA kit, the plate was gently shaken at 300 rpm for 30 min, 300 µL of wash buffer was added to the wells, and the excess wash buffer was removed. Next, 100 µL of the primary antibody was added to each well and the plate was gently shaken at 300 rpm for 30 min, and then 300 µL of wash buffer was added to the wells and the excess wash buffer was removed. In the next step, 100 µL of streptavidin-HRP was added to each well. The plate was shaken at 300 rpm for 30 min, and then 300 µL of wash buffer was added to the wells and the excess wash buffer was removed. Next, 100 µL of TMB substrate solution was added to each well and the plate was shaken for 2-10 min. Finally, 50 µL of HCl stop solution was added. All wells were evaluated with the Omega ELISA reader (BMG LABTECH) at an absorbance value of 450 nm.

Coagulation and Mixing Test Analysis

Mixing tests were performed to investigate the activated partial thromboplastin time (aPTT). MSCs were suspended as 10,000 cells in 100 µL of MSC NutriStem® XF Supplement (#05-201-1U, Biological Industries) including 1% pen/strep. MSCs in 100 µL of medium were plated in 96-well plates and plates were incubated at 37 °C for 24 h. The wells were adjusted to 1 MOI FVIII-Lv and the transduction process was initiated with protamine sulfate (40 µg/mL, Sigma). Transduced MSCs were incubated at 37 °C for 7 days. Supernatants of the incubated cells were collected and mixed with FVIII-deficient human plasma product (DiaPharma) at a 1:1 ratio and labeled as test plasma. FVIII-deficient human plasma product (DiaPharma) was also mixed at a 1:1 ratio with saline and labeled as control plasma. The Dade® Actin® FS Activated PTT Reagent kit (#B4218-20, Siemens) was used to perform aPTT and FVIII analyses for the samples obtained from the mixing tests. All reagents, samples, and standards were prepared according to the manufacturer's instructions and the analyses were performed using coagulometer principles.

Western Blot Analysis of FVIII Expression

FVIII-expressing transduced MSCs were centrifuged, and the supernatant was collected and resuspended with MSC NutriStem® XF Supplement mixture (#05-201-1U, Biological Industries). MSCs were incubated at 37 °C for 7 days. Using 50 µL of a lysis buffer (pH 8.8) and 0.2-mm stainless immobilized beads, the cells were lysed. The cell-free fraction was collected after centrifugation. The protein concentration of this fraction was measured by modified Bradford assay (#CB-P005K, Bio-Rad). Proteins were then loaded onto 12% acrylamide gel (8 cm x10 cm) and separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The blots were then probed with the Immune Booster system (Takara) using a FVIII antibody (Santa Cruz) and HRP-conjugated anti-mouse secondary antibody at a 1:1000 dilution ratio. A chemiluminescent detection system was used for signal generation.

Results

Construction of the FVIII-Lv Gene and Process Optimization of Lentivirus Production

The construct used in this study was designed as a second-generation lentiviral vector that codes for a human B domain-deleted FVIII cDNA sequence with CD45R0t surface marker and EF-1α promoter. FVIII expression in the MSCs was determined upon transduction using the CD45R0 antibody. In the testing of CD45R0 expression on MSCs, the cells were transduced dose-dependently with the FVIII-encoding lentivirus (Figure 1). It was found that the CD45R0 expression increased as the concentration of the virus in the transduced MSCs increased.

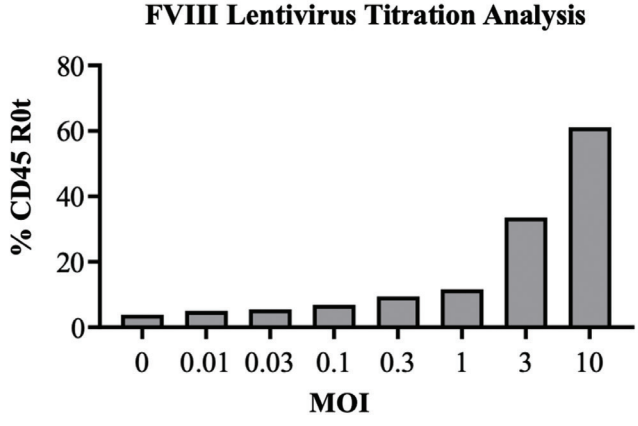

Figure 1. Titration of factor VIII (FVIII)-encoding lentivirus on mesenchymal stem cells (MSCs) using CD45R0t expression. The plot shows CD45R0 expression of MSCs transduced with FVIII-encoding lentivirus in a dose-dependent manner on day 4 of transduction.

MOI: Multiplicity of infection.

To optimize the transduction process and the efficacy of the FVIII-encoding lentivirus, different transfection reagents including protamine sulfate, Vectofusin-1, and protamine sulfate + Vectofusin-1 were tested. All three transduction reagents were compared according to CD45R0 expression on MSCs in a dose-dependent manner. There was no statistically significant difference in the transduction efficacy of the three different reagents (p=0.181) (Figure 2). However, protamine sulfate was preferred due to cost-effectiveness. All transduction procedures were performed in the presence of protamine sulfate.

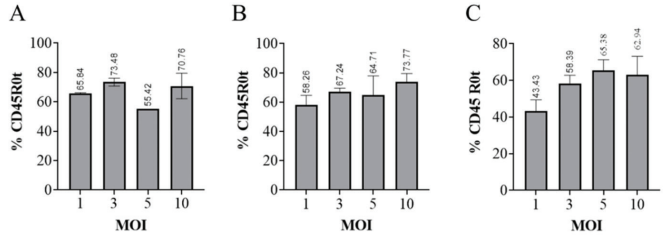

Figure 2. Transduction efficacy of factor VIII (FVIII)-encoding lentivirus using protamine sulfate, Vectofusin-1, and protamine sulfate + Vectofusin-1. Bar graphs show titers of FVIII-encoding lentivirus transduced with different transduction reagents including **(A)** only protamine sulfate, **(B)** only Vectofusin-1, and **(C)** protamine sulfate and Vectofusin-1 in a dose-dependent manner. Means and standard deviation ranges of three independent experiments are shown.

MOI: Multiplicity of infection. *: p<0.05.

FVIII Lentivirus Persistence and Expression Analysis

FVIII persistence and expression analyses of transgenic MSCs were performed on days 0, 3, and 10. The data showed that CD45R0t-expressing MSC levels remained relatively stable from day 0 to day 10 (p=0.779). Lentiviral transduction did not show any statistically significant differences in CD45R0t expression over time (Figure 3), indicating stable gene expression.

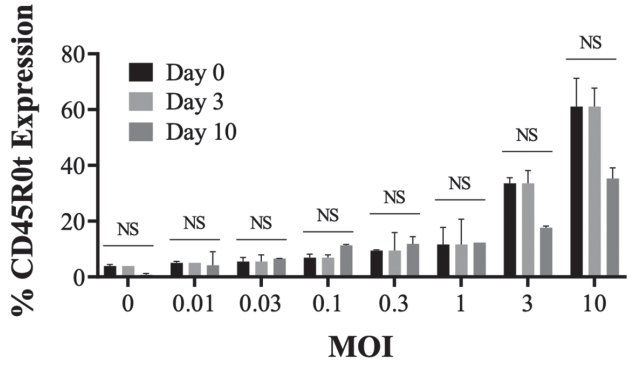

Figure 3. Factor VIII (FVIII) lentivirus persistence and expression analysis. Bar graph shows CD45R0t expression on transgenic mesenchymal stem cells (MSCs) in a dose-dependent manner on days 0, 3, and 10. Means and standard deviation ranges of three independent experiments are shown.

MOI: Multiplicity of infection.*: p<0.05; NS: not significant.

Quantitative Measurement Analysis of Factor VIII Antigen

Following confirmation of the gene expression of transduced MSCs using the CD45R0 surface marker (flow cytometry), the quantitative FVIII antigen level of the transduced MSC supernatant was demonstrated by ELISA in a dose-dependent manner. The average normal plasma level of FVIII protein was defined as 1.0 IU/mL and it ranges between 0.4 and 1.8 IU/mL [19]. The FVIII antigen level of MSCs transduced with 0.1 MOI FVIII-encoding lentivirus was 0.265 IU/mL, and that of MSCs transduced with 10 MOI FVIII-encoding lentivirus was 0.670 IU/mL. The FVIII antigen level in the transduced MSC supernatant gradually increased with the rise of the FVIII-encoding lentivirus concentration on day 3 and day 10 ($p<0.001$) (Figure 4). There was no significant difference between FVIII protein levels on days 3 and 10 ($p>0.05$) (Figure 4). This also showed that MSCs transduced with the FVIII-encoding lentivirus secreted the FVIII antigen from day 3 to day 10 in a stable manner.

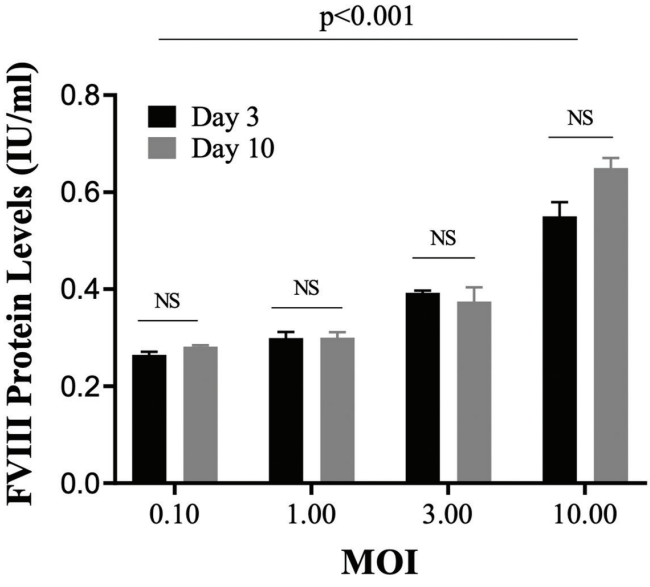

Figure 4. Quantitative analysis of factor VIII (FVIII) antigen in transduced mesenchymal stem cell (MSC) supernatant. Bar graph shows mean FVIII antigen levels of transduced MSC supernatant according to enzyme-linked immunosorbent assay results. Means and standard deviation ranges of three independent experiments are shown.

MOI: Multiplicity of infection; *: $p<0.05$; NS: not significant.

Activated Partial Thromboplastin Time and FVIII Level Analysis (Mixing Test)

The coagulation effectiveness of FVIII-secreting MSC supernatant was evaluated by mixing test analysis. The normal reference range of aPTT is 26-35 s, and an increase in this level is associated with delayed coagulation. The mean aPTT of the saline control group was 92.69±11.38 s, while the mean aPTT of the FVIII-secreting MSC supernatant mixed treatment group was decreased to 38.60±13.38 s, showing that the elevated blood clotting time had been lowered in a statistically significant manner ($p<0.001$) (Figure 5). Thus, the results proved that secreted FVIII protein from the transduced MSCs led to successful coagulation in a FVIII-deficient human plasma product mimicking hemophilia A.

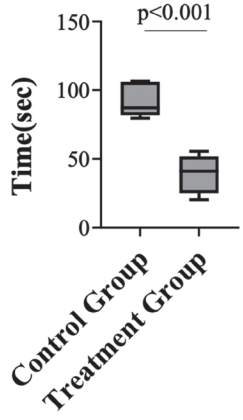

Figure 5. Analysis of the activated partial thromboplastin time (aPTT) of factor VIII-secreting mesenchymal stem cell supernatant as a measure of coagulation effectiveness.

The FVIII levels of the saline control group and the FVIII-secreting MSC supernatant mixed group were evaluated after the mixing test. While the mean FVIII level of the saline control was 0.41±0.03 IU/dL, the mean FVIII level of the FVIII-secreting MSC supernatant mixed treatment was 25.41±33.38 IU/dL (Figure 6). A significant difference was found between the groups in terms of FVIII levels, showing that the FVIII protein secreted inthe MSC supernatant successfully increased the FVIII levels in a FVIII-deficient human plasma product mimicking hemophilia A ($p<0.01$).

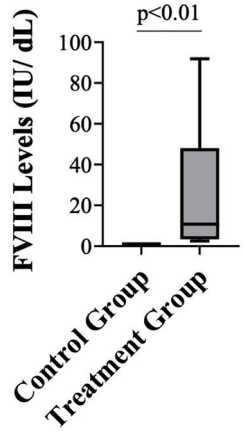

Figure 6. Factor VIII (FVIII) analysis for the FVIII-secreting mesenchymal stem cell supernatant treatment group and saline control group.

To characterize the FVIII protein secreted from the MSCs, western blot analyses were performed on day 7. The B domain-deleted FVIII sample showed fragments of 35-55 kDa. The presence of FVIII protein was confirmed for all samples in 20-40 s (Figure 7).

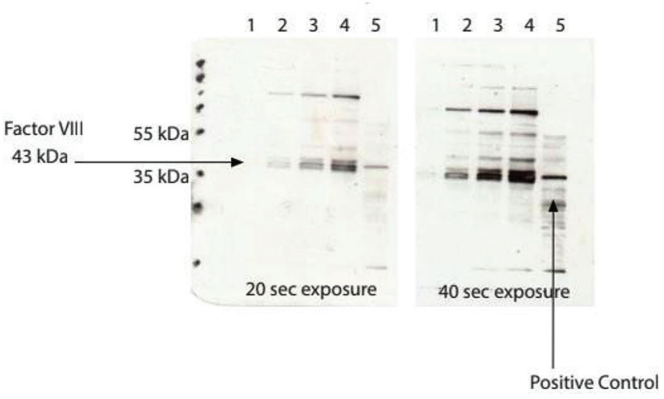

Figure 7. Western blot analysis using anti-factor VIII (FVIII) antibodies against FVIII-encoding lentivirus-transduced mesenchymal stem cells.

Discussion

Hemophilia A is an X-linked recessive disorder caused by a deficiency of functional plasma coagulation by FVIII [20]. Currently, treatment for hemophilia A is based on replacement of the missing or low coagulation factor. With the help of bispecific antibodies, aptamers, and/or innovative RNA technologies, various groups have tried to overcome the challenges in the treatment of hemophilia A [21]. Gene therapies provide a way of possibly curing hemophilia A and there are currently several gene therapy products whose clinical trials are ongoing. Recently, valoctocogene roxaparvovec, a hemophilia A gene therapy produced under the brand name of Roctavian, was announced to have received conditional marketing authorization [5]. However, the present study is the first study reporting that lentiviral vector-expressing FVIII-transduced umbilical cord-derived MSCs can successfully maintain systemic coagulation in an in vitro model mimicking hemophilia A.

Different genetic designs and viral vectors are used in hemophilia A gene therapies. It is worth noting that the first gene therapy product, Roctavian, was developed by BioMarin Pharmaceutical, while SB-525 was developed by Sangamo Biosciences [9] and SPK-8011 was developed by Spark Therapeutics [8,22], the latter of which are in phase III clinical trials. All of them use AAV as the viral vector. Gene therapies developed using the AAV vector have certain drawbacks in terms of maintaining stable levels of FVIII expression over time.

The findings of this study confirm that lentiviral vector-based transduction of MSCs can potentially overcome the loss of expression that occurs in AAV vectors by facilitating stable FVIII expression. Although previous studies demonstrated that lentiviral vectors can effectively transduce MSCs with protamine sulfate [23], in the current study the transduction process of the MSCs was optimized by comparing the transduction efficiency of three different transduction reagents. Although Vectofusin-1 showed transduction efficiency similar to that of protamine sulfate, the latter was chosen due to economic considerations.

As in other hemophilia A gene therapy products, the FVIII gene in this study had a B domain-deleted sequence (B domain is not present in the active form of the FVIII). The findings of this study showed that MSCs transduced with that B domain-deleted FVIII gene secreted active FVIII protein and were effective in correcting the coagulation defect of a FVIII-deficient human plasma product used as a hemophilia A model.

Liver-specific promoters, as the primary physiological site of FVIII synthesis, have commonly been included as promoters in gene constructs with different serotypes of AAV vectors in a number of in vitro hemophilia A studies [7,8,9]. However, those studies also showed that the use of a liver-specific promoter may be associated with liver toxicity and inhibits the vector's ability to infect different target cells, thereby resulting in low levels of FVIII expression. Furthermore, Picanco et al. [24] compared the effects of lentiviral-mediated viral and non-viral promoters on FVIII expression levels and concluded that viral cytomegalovirus and the non-viral EF-1α promoter achieved higher levels of FVIII expression. Hence, in this study, a non-viral EF-1α promoter was preferred due to its safe nature and it resulted in high levels of FVIII expression. This shows that, unlike other FVIII gene designs [7,8,9], the transduction of different target cells could be possible with the EF-1α promoter. Furthermore, unlike gene constructs with liver-specific promoters, it is possible that specific organ toxicity could be avoided by not applying the lentivirus directly but rather through MCS transduction. Doering et al. [25] also reported that lentiviral vectors can transduce hematopoietic stem cells and FVIII-encoding CD34+ hematopoietic stem cells can express FVIII following differentiation into the blood cell lineages.

The findings of this study demonstrate that transgenic MSCs can maintain stable and functional levels of FVIII expression without the need for differentiation and without hypercoagulability. The product proposed here has the potential to be used as a systemic gene therapy as well as to control local bleeding symptoms. Furthermore, with the help of the CD45R0t surface marker, transduced MSCs can be selected, isolated, and traced in vivo.

Conclusion

This study has reported the results of a lentiviral vector encoding a B domain-deleted FVIII cDNA sequence with the CD45R0t surface marker designed for the treatment of hemophilia A. The gene construct provided effective and stable gene transfer and it was found to be successful as a result of in vitro and ex vivo efficacy and safety analyses. Thus, this study has demonstrated a promising new method that could be applied to treat hemophilia A. Accordingly, a FVIII-secreting transgenic MSC study will be initiated in a FVIII-knockout animal model as the next step of the research process.

Ethics

Ethics Committee Approval: In vitro study.

Authorship Contributions

Concept: E.O, C.H., C.T.; Gene Design: C.H., C.T.; C.H., R.D.T., G.S.K., S.A., and D.C. completed, and analyzed preclinical experiments. M.K. performed Western blot analysis. U.S., O.S.G, M.E., D.D.K., B.Y., and K.Y. performed quality control analysis. C.H., C.T., E.O. and E.S.A. contributed to interpretation of the data. C.H., C.T., and E.O. wrote the manuscript.

Conflict of Interest: C.H., C.T., and E.O. are inventors of patent application (pending) including "Methods for the Production of Gene Products Suitable for Use in the Treatment of Hemophilia A" (2020/18990) at the Turkish Patent and Trademark Office. No other authors have a competing interests except for these authors.

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