Rapid Improvement in Jaundice Using Transdermal Isosorbide Tape as a Nitric Oxide Donor in Two Adult Patients with Transplantation-associated Microangiopathy Related to Graft-versus-host Disease

Yuki Hiroshima¹, Keijiro Sato¹, Toshimitsu Ueki¹, Masahiko Sumi¹, Mayumi Ueno¹, Naoaki Ichikawa¹, Hidetoshi Satomi² and Hikaru Kobayashi¹

Abstract:
Two adult patients with acute leukemia developed transplantation-associated microangiopathy (TAM) related to graft-versus-host disease (GVHD). Both patients were resistant to standard therapy for TAM and GVHD, which led to markedly elevated serum total bilirubin levels of 47.5 and 10.6 mg/dL, respectively. Transdermal isosorbide tape as a nitric oxide donor was applied to Patients 1 and 2 on post-transplantation days 60 and 66, respectively, which rapidly improved their jaundice after 1 day. This is the first report to describe the efficacy of transdermal isosorbide tape for adult patients with jaundice associated with TAM related to GVHD.

Key words: transplantation-associated microangiopathy (TAM), liver dysfunction, nitric oxide donor (NO donor), transdermal isosorbide tape, graft-versus-host disease (GVHD), endothelial cell injury

(Intern Med 61: 1225-1230, 2022) (DOI: 10.2169/internalmedicine.7789-21)

Introduction

Transplantation-associated microangiopathy (TAM) is a severe complication of hematopoietic stem cell transplantation (HSCT). The endothelial cell injury associated with radiation therapy, anti-cancer drugs, immunosuppressants, graft-versus-host disease (GVHD), and infection are involved in the pathogenesis of TAM (1-3). Excess hemolysis in TAM induces the consumption of nitric oxide (NO) in the microcirculation, as plasma free hemoglobin exhibits a high affinity for endothelial-derived NO (2, 4). A deficiency in NO, which exhibits potent vasodilatory and platelet antiaggregatory activities, may result in the formation of platelet microthrombi in various organs, including the kidneys, central nervous system, heart vessels, and liver, ultimately resulting in exacerbation of TAM (2, 4-7).

Previous studies have reported the effects of NO donors administered to two pediatric patients with microcirculation disorders derived from endothelial injury in the context of HSCT; one patient had hemolytic uremic syndrome (HUS), and the other had sinusoidal obstruction syndrome/veno-occlusive disease (SOS/VOD) (8, 9). However, the utility of NO donors in the treatment of adults with microcirculation disorders derived from endothelial injury in the context of HSCT has not yet been investigated.

We herein report two adult cases of the successful use of transdermal isosorbide tape as an NO donor for liver dysfunction related to TAM. The application of transdermal isosorbide tape attenuated jaundice and hemolysis and reduced the elevated percentage of schizocytes, with a rapid improvement being observed in jaundice.

Case Reports

Case 1
A 57-year-old man presented to our hospital with acute myeloid leukemia with myelodysplasia-related changes and complex chromosomal abnormalities. He underwent emer-

¹The Department of Hematology, Nagano Red Cross Hospital, Japan and ²The Department of Pathology, Nagano Red Cross Hospital, Japan
Received: April 17, 2021; Accepted: August 2, 2021; Advance Publication by J-STAGE: September 25, 2021
Correspondence to Dr. Yuki Hiroshima, yh6@hotmail.co.jp
gant umbilical cord blood transplantation. Conditioning included fludarabine (25 mg/m²/day, 5 days), melphalan (70 mg/m²/day, 2 days), and total body irradiation (total 4 Gy). The infused nucleated and CD34-positive cell numbers were 3.03×10⁷ and 0.49×10⁵ cells/kg, respectively. He received tacrolimus for GVHD prophylaxis and ursodeoxycholic acid for SOS/VOD prophylaxis. Nine days after transplantation, the patient exhibited signs of pre-engraftment immune reaction (PIR) with hemophagocytic syndrome (HPS); therefore, the administration of prednisolone (PSL; 2 mg/kg; 120 mg/day) was initiated. PIR gradually improved, and the PSL was tapered to 5 mg/day on day 24. Jaundice, watery and bloody diarrhea, ileus, eruption, and elevated liver enzymes were observed on day 26. Neutrophil engraftment was achieved on day 38, and a skin biopsy revealed GVHD. The patient was diagnosed with acute GVHD grade IV (skin, stage 3; liver, stage 4; gut, stage 4). Treatments with mycophenolate mofetil (MMF) and increased PSL were ineffective.

Jaundice developed, and bilirubin increased to 19.0 mg/dL. Computed tomography (CT) revealed atrophy of the liver, which suggested circulatory failure in the liver. The patient was diagnosed with TAM based on the gradual increase in the percentage of schizocytes to >4% as well as prolonged thrombocytopenia, a persistent increase in lactate dehydrogenase (LDH), an increased requirement for the transfusion of red blood cells, and a decreased serum haptoglobin concentration. The ADAMTS-13 activity was 34%, and the anti-ADAMTS-13 antibody titer was <0.5 Bethesda units/mL.

On day 54, tacrolimus was discontinued because of TAM. The patient received MMF, PSL (120 mg/day), recombinant human-soluble thrombomodulin (rTM), and fresh-frozen plasma (FFP) daily to treat GVHD and TAM. However, these treatments did not attenuate the jaundice, thrombocytopenia, or bloody diarrhea, and increases were observed in total bilirubin and LDH levels. On day 60, the serum total and direct bilirubin levels increased to 47.5 and 37.8 mg/dL, respectively, and transdermal isosorbide tape (40 mg/day; Yamanouchi Pharmaceutical, Tokyo, Japan) was applied. The serum total bilirubin and LDH levels started to decrease the next day. Platelet consumption improved, and the amount of platelets that needed to be transfused decreased by 50%. On day 74, the serum bilirubin levels and percentage of schizocytes decreased to 25.8 mg/dL and 3%, respectively, despite the re-initiation of tacrolimus (Fig. 1). Skin GVHD remained stage 3, but gut GVHD improved to stage 2 on day 74. Although TAM improved, leukemia recurred on day 64. The patient ultimately died on day 77 due to the exacerbation of acute myeloid leukemia.

An autopsy revealed the histological features of microthrombi formation in the kidneys, the vascular lumens
Figure 2. Pathological findings of the autopsy. A: Hematoxylin and Eosin (H&E) staining of the kidney ×400. Vascular lumens were occluded by platelet thrombi (black arrows), and the colliquation of capillary loops was noted in the glomeruli. B: H&E staining of the small intestine ×200. Thrombi were found in the lumen (black arrow). C: AZAN staining of the liver ×100. D: AZAN staining of the liver ×400. A slight increase in fibrotic components and luminal stenosis were suspected around the central lobular vein.

were occluded by platelet thrombi, and the colliquation of capillary loops was observed in the glomeruli (Fig. 2A). Vasodilation, edema, bleeding, hemosiderin deposits, and iron-bearing cells were detected under the small intestinal epithelium. These changes were consistent with chronic-stage ischemic changes. Dilated vessels were found in the intestinal submucosa. The vessel wall was thickened with hyaline degeneration, and the lumen was meandering and expanding. At some sites, thrombi were detected in the lumen. Changes in the small intestine suggested ischemic circulatory disorders associated with microangiopathy and their healing and recovery stages (Fig. 2B). In the liver, the majority of these changes were frequent findings at the end of acute or chronic liver failure with sepsis. However, in a small part of the liver, slight increases in fibrotic components and luminal stenosis were suspected around the central lobular vein (Fig. 2C, D). These liver changes suggested the complication of central hepatic vein occlusion associated with HSCT.

Case 2

A 23-year-old man presented to our hospital with mixed-phenotype acute leukemia, B/myeloid, not otherwise specified. He underwent bone marrow transplantation from his human leukocyte antigen full-match sister. Conditioning included 12 Gy total body irradiation and granulocyte colony-stimulating factor (G-CSF) combined with high-dose cytarabine (2 g/m², every 12 hours for two days) and cyclophosphamide (60 mg/kg/day, two days). The number of infused nucleated cells was 2.2×10⁹ cells/kg. He received cyclosporin and short-term methotrexate for GVHD prophylaxis and ursodeoxycholic acid for SOS/VOD prophylaxis.

Elevated liver enzymes were detected on day 17. Neutrophil engraftment was achieved on day 22, and the patient showed signs of acute skin and liver GVHD. The administration of MMF at 2,000 mg/day was initiated on day 27. Jaundice and liver dysfunction developed on day 31, and serum chemistry showed aspartate transaminase (AST) 96 IU/L, alanine transaminase (ALT) 164 IU/L, and total bilirubin (T.bil) 2.2 mg/dL. PSL 0.5 mg/kg (25 mg/day) was started for acute hepatic GVHD grade III. However, the jaundice worsened, and the T.bil level was 6.5 mg/dL on day 34. A skin biopsy on day 34 revealed GVHD. The patient was diagnosed with acute GVHD grade III (skin, stage 2; liver, stage 3; gut, stage 0). PSL was increased to 2 mg/kg (90 mg/day) on day 34. Serum chemistry peaked at AST 73 IU/
Figure 3. Clinical course of Patient 2. aGVHD: acute graft-versus-host disease, ALT: alanine aminotransferase, BMT: bone marrow transplantation, CMV: cytomegalovirus, CyA: cyclosporine A, GCV: ganciclovir, LDH: lactate dehydrogenase, sMTX: short-term methotrexate, T.bil: total bilirubin

L, ALT 400 IU/L, and T.bil 12.7 mg/dL on day 44 but then gradually decreased.

Hemorrhagic cystitis associated with BK virus and JC virus was observed on day 41, and the cytomegalovirus antigenemia assay became positive. Ganciclovir was used from days 42 to 46 and from days 57 to 76. Due to viral cystitis, tapering of PSL was initiated on day 48. Between days 58 and 66, T.bil remained in the range of 10.6 to 9.0 mg/dL but did not decrease further. On day 63, 0.7% schizocytes appeared in the peripheral blood, and the LDH level gradually increased. On day 66, MMF was increased to 3,000 mg/day, and transdermal isosorbide tape (40 mg/day; Yamanouchi Pharmaceutical) was applied because of TAM. A reduction was noted in the serum T.bil level the day after the application of transdermal isosorbide tape, and ALT, reticulocytes, and the percentage of schizocytes subsequently decreased. On day 113, the serum T.bil level was 1.3 mg/dL, and the percentage of schizocytes decreased to 0% despite the tapering of PSL to 25 mg/day and changing tacrolimus to oral administration. The elevated LDH, alkaline phosphatase (ALP), and gamma-glutamyltransferase (G-GTP) levels did not decrease because of the prolonged progression of GVHD with a gradual decrease in PSL on day 120 (Fig. 3).

On day 121, bone marrow aspiration showed hematological complete remission. The patient was discharged on day 137. On day 261, the LDH and ALT levels had decreased to 206 IL/L and 82 IU/L, respectively. On day 323, LDH plateaued at 170 IL/L, and ALT normalized to 32 IU/L with oral tacrolimus, PSL, MMF, and transdermal isosorbide tape.

The patient has remained in complete remission for seven years since his bone marrow transplantation. Liver GVHD has not relapsed with the continued administration of oral tacrolimus and a low dose of steroids.

Discussion

NO plays a major role in vascular hemostasis and is a critical regulator of basal and stress-mediated smooth muscle relaxation and vasomotor tone, endothelial adhesion molecule expression, and platelet activation and aggregation (1-7, 10). In Patients 1 and 2, the serum bilirubin levels rapidly decreased after the application of transdermal isosorbide tape, with more gradual reductions noted in the percentage of schizocytes and platelet consumption. The autopsy of Patient 1 showed microangiopathy in the kidneys and small intestine and suggested SOS/VOD in the liver. A liver biopsy was not possible in Patient 2, so the exact pathology was unknown; however, by day 63 after HSCT, immunosuppressive therapy had not shown sufficient efficacy, and the percentage of schizocytes was increased. Previous studies have suggested that alloimmunity contributes to the pathogenesis of endothelial complications (3, 11, 12). The vascular endothelium is a target tissue exposed to immune-mediated injury, such as that by antibodies and T lymphocytes, in GVHD (11, 12). Vascular endothelial cells are activated by inflammatory cytokines secreted by T lymphocytes, while they also express antigen-presenting and adhesion
molecules, become procoagulant, and are sensitized to pro-apoptotic signals (11). In Patient 2, alloimmunity may have induced vascular endothelial damage, exacerbated the pathology of TAM, contributed to the prolongation of cholestasis liver dysfunction, and increased the percentage of schizocytes.

Kajiume et al. reported a rapid and marked improvement in jaundice after the administration of isosorbide in their two pediatric cases of HUS and VOD after HSCT (3, 9), which is consistent with the present results. NO induces the relaxation of the sphincter of Oddi (13). Thus, decreased NO due to vascular endothelial damage may have promoted the contraction of the sphincter of Oddi, which exacerbated jaundice, whereas NO from the transdermal isosorbide tape may have relaxed the sphincter of Oddi and thus contributed to the rapid attenuation of cholestasis. On a postmortem pathological study, SOS/VOD was seen partly in the liver in Patient 1; however, the majority of the changes were findings at the end of acute or chronic liver failure with sepsis. In Patient 2, hepatic GVHD was considered to be the main etiology of hyperbilirubinemia by a clinical analysis. The present cases suggest that the use of an NO donor improved jaundice due to the relaxation of the sphincter of Oddi caused by supplementation of consumed NO in the short term and suppressed the progression of TAM by vasodilatory and platelet anti-aggregatory activities in the medium to long term. In each case, the rapid improvement of jaundice was shown immediately after the administration of the NO donor, and the percentage of schizocytes, platelet consumption, and liver GVHD improved slowly. These clinical courses indicate that NO supplementation suppressed the progression of the jaundice and organ damage caused by TAM, while the poor pathological condition due to liver failure with sepsis, SOS/VOD or hepatic GVHD still remained. The current SOS/VOD and liver GVHD severity classification includes bilirubin levels (14, 15). If the consumption of NO due to vascular endothelial damage contributes to obstructive jaundice in SOS/VOD or liver GVHD, the administration of NO donors may clarify the actual liver damage of SOS/VOD or liver GVHD.

In previous reports, the transdermal isosorbide tape dose was 20 mg/day for a 9-year-old girl with HUS after HSCT (8) and 20 mg/day for a 5-year-old girl with SOS/VOD (9). In the present report, both patients were adult men. Transdermal isosorbide tape was applied with 40 mg/day, and the effects were similar to those reported in the previous reports. A dose of 40 mg/day with transdermal isosorbide tape is standard for adult ischemic heart disease. However, the optimal dose for TAM has not been established. Further studies are needed concerning the optimal dose for TAM in both children and adults.

One limitation associated with the present and previous reports is that transdermal isosorbide tape for endothelial injury in the context of HSCT may have positive-results bias. It will be necessary to comprehensively analyze all cases in which transdermal isosorbide tape was applied for endothelial injury after HSCT. A further prospective study is also needed.

In conclusion, the present results indicate that NO is an effective treatment option for adult cases of TAM and GVHD-related liver dysfunction with marked jaundice, as evidenced by the rapid decrease in serum bilirubin levels. Although the precise etiology of severe cholestasis observed in our cases is unclear, NO donation may have played an important role in improving jaundice. Furthermore, the decreased percentage of schizocytes indicated that the administration of an NO donor continuously prevented hemolysis by regulating basal and stress-mediated vascular smooth muscle relaxation and vasomotor tone. Transdermal isosorbide tape is a simple method for supplying NO molecules. The pharmacokinetics and efficacy of NO donors in TAM need to be investigated in a larger study population in the future.

This case report was approved by the institutional review board of Nagano Red Cross Hospital and conforms to the guidelines of the Declaration of Helsinki.

The authors state that they have no Conflict of Interest (COI).

References

1. Batts ED, Lazarus HM. Diagnosis and treatment of transplantation-associated thrombotic microangiopathy: real progress or are we still waiting? Bone Marrow Transplant 40: 709-719, 2007.
2. Goldberg RJ, Nakagawa T, Johnson RJ, Thurman JM. The role of endothelial cell injury in thrombotic microangiopathy. Am J Kidney Dis 56: 1168-1174, 2010.
3. Carreras E, Diaz-Ricart M. The role of the endothelium in the short-term complications of hematopoietic SCT. Bone Marrow Transplant 46: 1495-1502, 2011.
4. Thachil J. Nitric oxide in transplantation-related thrombotic microangiopathy. Bone Marrow Transplant 43: 513-514, 2009.
5. Khosla J, Yeh AC, Spitzer TR, Dey BR. Hematopoietic stem cell transplant-associated thrombotic microangiopathy: current paradigm and novel therapies. Bone Marrow Transplant 53: 129-137, 2018.
6. Hollenberg NK. Organ systems dependent on nitric oxide and the potential for nitric oxide-targeted therapies in related diseases. J Clin Hypertens: 8: 63-73, 2006.
7. Laskin BL, Geobel J, Davies SM, Jodele S. Small vessels, big trouble in the kidneys and beyond: hematopoietic stem cell transplantation-associated thrombotic microangiopathy. Blood 118: 1452-1462, 2011.
8. Kajiume T, Nagita A, Yoshimi S, Kobayashi K, Kataoka N. A case of hemolytic uremic syndrome improved with nitric oxide. Bone Marrow Transplant 25: 109-110, 2000.
9. Kajiume T, Yoshimi S, Nagita A, Kobayashi K, Kataoka N. Application of nitric oxide for a case of veno-occlusive disease after peripheral blood stem cell transplantation. Pediatr Hematol Oncol 17: 601-604, 2000.
10. Rother RP, Bell L, Hillmen P, Gladwin MT. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. JAMA 293: 1653-1662, 2005.
11. Biedermann BC. Vascular endothelium and graft-versus-host disease. Best Pract Res Clin Haematol 21: 129-138, 2008.
12. Cooke KR, Jannin A, Ho V. The contribution of endothelial activa-
1225-1230, 2022

Society for Blood and Marrow Transplantation. Bone Marrow Transplantation. Bone Marrow Transplant 51: 906-912, 2016.

15. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet 2: 1550-61, 2009.

The Internal Medicine is an Open Access journal distributed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License. To view the details of this license, please visit (https://creativecommons.org/licenses/by-nc-nd/4.0/).

© 2022 The Japanese Society of Internal Medicine

Intern Med 61: 1225-1230, 2022