
DEFIBROTIDE FOR THE TREATMENT AND PREVENTION OF HEPATIC VENO-OCCLUSIVE DISEASE

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Hepatic veno-occlusive disease (VOD) is a serious complication of stem cell transplantation (SCT) and is characterized by weight gain, hepatomegaly, hyperbilirubinemia and ascites. The pathogenesis of VOD is still incompletely understood but involves damage to the sinusoidal endothelium, resulting in endothelial injury. This process leads to concomitant progressive hepatocellular dysfunction and subsequent fluid retention and renal impairment. Whilst the spectrum of illness is broad, VOD in its severe form is typically associated with multi-organ failure (MOF) and high mortality. A number of possible strategies for the prevention and/or treatment of VOD have been investigated. The most promising agent to date is defibrotide, a novel polydeoxyribonucleotide with fibrinolytic properties and no major bleeding risk. Several clinical trials have shown that patients benefit from defibrotide treatment, and positive results have also been observed with defibrotide used as prophylaxis.

Introduction

Hepatic veno-occlusive disease (VOD) is a potentially fatal complication that usually arises within the first weeks of hematopoietic stem cell transplantation (SCT) with a peak around day +12 post-transplant. VOD is usually fatal in its severest form and is typically characterized by fluid retention, weight gain, hepatomegaly, ascites and hyperbilirubinemia, with no other identifiable cause for liver dysfunction (1-3). Two sets of clinical criteria are used for the diagnosis of VOD. The Seattle criteria require the presence of at least two of the major

clinical features (jaundice, painful hepatomegaly or ascites and/or unexplained weight gain) within 30 days of transplantation (1,3). The Baltimore criteria require a blood bilirubin level over 2.0 mg/dL in addition to the presence of two or more major clinical characteristics (hepatomegaly, ascites or $\geq 5\%$ weight gain) within 21 days post-transplantation (4). VOD is thought to result from endothelial cell injury, followed by hepatocellular toxicity, and typically follows high-dose chemotherapy and radiation used as part of conditioning for stem cell transplantation. (1,3,5).

Pathogenesis of VOD

The development of VOD is thought to begin with damage to sinusoidal endothelial cells most often caused by pre-SCT conditioning (2,6). Sinusoidal obstruction is prominent histopathologically, and thus VOD is also described as sinusoidal obstruction syndrome (SOS) (7,8). Damage to the endothelium triggers a prothrombotic phenotype at the endothelial cell surface, resulting in endothelial activation and a variety of downstream effects (8,9). Cellular debris and fibrin-related aggregates block the small pores that perforate the endothelial lining; venous outflow is obstructed, and the resulting intrahepatic portal hypertension causes the main clinical manifestations of VOD. In addition, other features specific to the transplant setting, such as stem cell source (autologous versus allogeneic), and the effects these on endothelial cells may further add to the pathogenicity of VOD (9-12).

Reduced levels of hepatic nitric oxide (NO) have been detected as a consequence of the endothelial

damage and vascular obstruction that occurs (13). Furthermore it has been shown that NO plays a key role in reducing hepatocyte damage and increasing hepatic microcirculation, suggesting that NO is important in the abrogation of ischemia-reperfusion injury (14).

Several markers of endothelial injury (e.g., plasminogen activator inhibitor type-1 (PAI-1) and soluble thrombomodulin) are upregulated in patients with VOD (15,16). PAI-1 is an important inhibitor of fibrinolysis (17) and has been identified as being both a diagnostic and prognostic biomarker for VOD (18). Another biomarker associated with elevated VOD risk following BMT is transforming growth factor- β (TGF- β), a cytokine directly involved in fibrogenesis (19,20,21).

Incidence and risk factors for VOD

SCT is frequently offered with a variety of malignant and nonmalignant conditions (22). SCT in children is associated with a particularly high risk for VOD. The incidence following SCT ranges from 11–60%(23-29,30), giving a mean incidence in children of approximately 25% compared with an incidence of VOD in adults of 13.7% (7). In part this increased incidence in children is due to certain malignant and inherited diseases that are associated with a substantially increased risk of VOD during SCT including neuroblastoma (29), familial hemophagocytic lymphohistiocytosis (HLH, Griscelli syndrome) (30) and osteopetrosis (28).

Additional independent risk factors for VOD were identified (24). Younger age (<6.7 years) was associated with an increased incidence of VOD compared with age \geq 6.7 years (17% vs 4%, respectively; $p = 0.001$) (24). In this study, busulfan-containing conditioning regimens were associated with an increased risk of VOD compared with other conditioning regimens (25% vs 13%, respectively; $p < 0.001$). This observation was confirmed by other investigators where busulfan conditioning was shown to be a significant risk factor for the development of VOD (31) ($P = 0.001$), (23), with an incidence of hepatic VOD ranging from 22% to 32% (32,33). In addition, the incidence of VOD was less in patients receiving heparin prophylaxis (6%), compared with those either receiving pentoxifylline, prostaglandin E1, or no prophylaxis at all (21%; $p = 0.001$) (24).

Gemtuzumab ozogamicin (GO) is a monoclonal antibody was withdrawn from the U.S. market in

2010 following reports of increased incidence of VOD in AML patients in the absence of SCT as well as during the early post-transplantation period in patients who had been previously treated with the agent. Time from GO treatment to transplantation appeared to be important with respect to the development of VOD (34).

Prevention of VOD

A number of possible strategies for the prevention of VOD in children have been investigated, including lipo-prostaglandin E1, danaparoid prophylaxis, and a combined prophylactic regimen of heparin, glutamine and ursodiol (35). A retrospective review of 188 children who received a combined prophylactic regimen of intravenous (IV) heparin, oral glutamine and ursodiol prior to hematopoietic SCT reported a low incidence of VOD with this approach (one case in 188 patients), which again would require confirmation in a larger prospective randomized clinical trial (36).

Treatment of VOD

Current management of established VOD focuses on supportive care, which includes reduction of fluid overload with diuresis, paracentesis, correction of any bleeding diathesis, management of infection, adequate analgesia and in cases of multi-organ failure, hemodialysis and ventilator support (37). Studies investigating systemic anticoagulants and/or thrombolytics as treatment options for VOD have no survival benefit and have a risk of severe and often life threatening bleeding complications (38). One of the more promising agents used for the treatment of VOD has been recombinant t-PA with a response of up to 30%, but t-PA is limited by severe hemorrhagic complications (39).

Defibrotide

Defibrotide is a novel agent in development for the treatment of VOD after SCT. A number of recent clinical trials, described below, have evaluated its efficacy and tolerability in this setting.

Mechanism of action of defibrotide

Defibrotide is an adenosine receptor agonist with fibrinolytic and other pleiotropic properties (8,40). Defibrotide acts initially at the endothelium and minimal systemic bleeding risk (41,42).

Defibrotide acts primarily as an anti-thrombotic, conferred in part through interactions with the plasmin pathway (6,8). Defibrotide increases t-PA

expression in microvascular cells, which is responsible for activation of plasminogen to plasmin and leads to increased plasmin levels and microscopic thrombolysis (42). Defibrotide reduces PAI-1 levels, leading to an increase in active plasmin (42). Defibrotide increases plasmin activity by binding to the enzyme directly but has no effect on activation of plasminogen to plasmin (41). Defibrotide in contrast to t-PA seems to act locally, rather than systemically and this could be the reason for the benign toxicity profile (43).

The anti-thrombotic action of defibrotide at the endothelial cell surface is also achieved through inhibition of tissue factor (TF). It was found that when microvascular endothelial cells were cultured with sera from patients who had undergone autologous SCT there was a marked increase in deposition of TF in the extracellular matrix with pro-thrombotic effects in the liver microvasculature as well as effects on fibrogenesis (44). However, defibrotide was found to not only inhibit this process (44), but also cause release of Tissue Factor Pathway Inhibitor (TFPI) from the endothelial cell surface (45), further abrogating endothelial injury and its downstream effects. In addition sera from patients post-autologous SCT, when added to endothelial cells, stimulated expression of von Willebrand Factor (vWF), a key element of cell surface coagulopathy and platelet aggregation (44). Intriguingly, this effect was markedly and specifically reduced with defibrotide (44).

A number of early clinical studies with defibrotide in the setting of established VOD after SCT suggested not only that various selectins were markedly elevated in VOD patients but also that defibrotide treatment decreased these levels in responding patients (46,47). Intercellular adhesion molecule-1 (ICAM-1) expression is associated with inflammation in the blood vessel wall (48). Defibrotide was found to block this increase in ICAM-1, and significantly reduced the inflammatory response in this endothelial cell model (44).

Defibrotide has been reported to promote endothelial cell proliferation, and has stimulated tubular morphogenesis of endothelial cells cultured in 3D collagen gels (45). These in-vitro findings further support the role of defibrotide in contributing to the recovery of liver injury following vascular occlusion, such as in VOD.

Acute graft versus host disease (GvHD) following SCT is a major cause of mortality and morbidity.

Elevated expression of the heparanase gene has been identified as a risk factor for acute GvHD, as well as other factors, inducing increased activity of adhesion molecules (49). Defibrotide was found to suppress heparanase expression (50), which could, in turn, reduce the risk of acute GvHD, as well as favorably influence the expression of adhesion molecules, as described above. The role of defibrotide in decreasing the risk of GVHD described below suggest it may be beneficial.

Clinical assessment of defibrotide in children

Defibrotide is available in parenteral and oral formulations and does not interfere with most chemotherapeutics in vitro (50). In a retrospective study from the USA, and the first report on the use of defibrotide in this setting, 19 patients (including six patients <20 years old) with severe VOD after SCT (diagnosed according to Baltimore criteria with multi-organ failure (MOF) and/or greater than 30% risk by the Bearman model) were treated with defibrotide on a compassionate-use basis (51). Resolution of VOD (defined as a bilirubin <2 mg/dL and improvement in other symptoms, including MOF) was observed in 8 patients (42%), with 6 patients surviving for longer than 100 days. There was a trend towards a higher proportion of younger patients (<20 years old) with complete responses, compared with older patients (4 out of 6 (67%), compared with 4 out of 13 patients ≥20 years old (31%) (51).

In a more recent phase II, multicenter, randomized, dose-finding trial, 48 pediatric and 101 adult patients with severe VOD were randomized to receive either 25 mg/kg/day or 40 mg/kg/day of defibrotide (55). Patients were eligible if they had ≥30% chance of developing severe VOD according to the Bearman prognostic model (53), or if they had MOF. Overall CR was reported in 57% of pediatric patients compared with 40% of adults; the D+100 survival rate was 52% in children compared with 37% in adults, and manageable toxicity, again suggesting that defibrotide therapy is particularly effective in children, with encouraging activity also seen in adults (52).

A phase III, historically-controlled clinical trial of defibrotide in 102 patients with severe VOD has recently been completed (54). All patients met Baltimore criteria of VOD within 21 days of SCT and had to have developed MOF (defined as significant renal and/or lung dysfunction including dialysis and ventilator-dependence) within 28 days of

transplantation. This study included 58 pediatric patients, 44 in the defibrotide group and 14 in the HC group. Subgroup analysis of pediatric patients revealed that 100-day CR was achieved in 36% of patients receiving defibrotide compared with 7% of patients in the HC group ($p = 0.04$). In the overall patient population, hemorrhagic adverse events were similar between the two groups (65% in the defibrotide arm, 69% in the HC): 18% of patients in the defibrotide arm experienced a drug-related toxicity that led to defibrotide discontinuation, but overall, the drug was well tolerated (54).

Defibrotide as prophylaxis for VOD

The promising results of defibrotide in treating VOD have resulted in this agent being evaluated as prophylaxis for VOD following SCT. A number of studies investigating the use of prophylactic defibrotide in children with a range of hematologic disorders have reported reduced incidence of VOD as well as a favorable toxicity profile.

A large prospective, randomized, phase II/III multicenter study has recently evaluated defibrotide as prophylaxis (55): 356 pediatric patients were randomly assigned to either defibrotide or the control arm following SCT. VOD incidence by 30 days was assessed according to Seattle criteria, as well as morbidity and overall mortality. Preliminary data showed that defibrotide prophylaxis was associated with a significant reduction in VOD incidence, compared with controls (Intent-to-treat population, 12% versus 20%, $p=0.05$) (55). This trial also showed that the incidence of severe VOD (including MOF) was substantially higher (60% versus 32%) in patients who fulfilled the Baltimore criteria. These data corroborate therefore the observation made by Coppell et al. (7) who showed that the Baltimore criteria reflect a population with rather advanced VOD. In the subgroup of patients who fulfilled the Baltimore criteria (mandatory hyperbilirubinemia $>2\text{mg/dl}$) the incidence of VOD

in the defibrotide prophylaxis arm was reduced from 13% to 7%. The trial confirmed earlier single center observations (23), that development of VOD led to a four-fold higher transplant-related mortality, compared to patients with no VOD (25% versus 6%; p -value not reported) (55). These data were further corroborated by the unexpectedly high (20%) mortality of patients with so-called mild and moderate VOD.

In a retrospective study, a high incidence of VOD was observed after SCT in children with malignant infantile osteopetrosis; seven of 11 children (64%) transplanted with no prophylaxis between 1996 and 2000 were diagnosed with VOD according to Seattle criteria (including three with severe VOD) (28). Nine further patients transplanted between 2001 and 2005 received defibrotide prophylaxis; only one patient was diagnosed with moderate VOD (11%). Similar to the previous study, no adverse events with defibrotide were reported, and prophylactic defibrotide did not adversely influence the incidence of infections or GvHD.

Conclusions

VOD is a potentially devastating complication that can occur following SCT, and severe VOD is almost always fatal (1,2). Therefore, preventing or treating VOD is crucial in lowering transplant related mortality. Current standard treatment remains best supportive care. The prognosis of VOD appears to have been considerably improved by the use of defibrotide with minimal or no toxicity. In addition, the drug may have a role in decreasing GVHD through its anti-inflammatory actions. Ongoing and future research on defibrotide alone or in combination with other agents (either as treatment or prophylaxis) will hopefully further optimize both the therapy of established disease and its prevention, and so improve outcome for pediatric patients undergoing SCT.

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