EXPERT REVIEWS

Use of defibrotide in the treatment and prevention of veno-occlusive disease

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Hepatic veno-occlusive disease (VOD) is one of the most important complications of high-dose chemotherapy and stem cell transplantation. VOD is a clinical syndrome characterized by jaundice, hepatic enlargement and fluid retention typically seen by day +30 after transplantation. Severe VOD is complicated by multiorgan failure and a high mortality rate approaching 100%. Defibrotide (DF) is a novel agent with both antithrombotic and fibrinolytic properties that has emerged as an effective therapy for severe VOD. In Phase II studies, treatment of severe VOD has resulted in complete responses of 30–60% and survival past day 100 ranging between 32–50%. A Phase III, historically controlled study of DF for treatment of severe VOD has recently been completed and results are awaited with interest. In addition, DF may be effective prophylaxis for VOD in high-risk patients. This review will focus on a summary of the pharmacology of DF and the clinical evidence for its use in VOD.

Keywords: defibrotide • SCT • sinusoidal obstruction syndrome • veno-occlusive disease

Overview of defibrotide pharmacology

Defibrotide (DF; Gentium, S.p.A, Como, Italy) is a nucleic-acid-derived oligonucleotide that has shown promise in the treatment of hepatic veno-occlusive disease (VOD) and other disorders characterized by microangiopathy. DF was initially developed for the treatment of vascular disorders on the basis of preclinical studies that showed antithrombotic, fibrinolytic, anti-ischemic, anti-inflammatory and antiatherosclerotic properties [1–3]. Its application to VOD will be the focus of this review.

Defibrotide is a first-in-class polydisperse mixture of single-stranded oligonucleotides from the depolymerization of mammalian genomic DNA from either porcine intestinal mucosa or bovine lung [1–3]. DF is composed of clusters of oligonucleotide chains that vary in length with a molecular weight ranging from 15–30 kDa in a Gaussian distribution and a defined ratio of purine to pyrimidine bases of greater than 0.85. The exact base sequences are unknown and unlikely to be determined. DF is available in intravenous and oral forms. The time to C_{max} is almost immediate for the intravenous form following bolus administration with rapid decline and steady-state plasma concentrations achieved at 90–120 min, whereas the C_{max} following oral administration is approximately 30 min [4]. The oral form has a bioavailability of 58–71% due to first-pass hepatic metabolism. The volume of distribution is primarily intravascular. Excretion following intravenous administration is primarily via the kidney, with 66% retrieved in the urine and 25% in the feces. Following oral administration, 40% was excreted in the urine and 52% in the feces. The elimination half-life of the parent drug ranges between 9.45 and 27.1 min. The elimination of the oral form was several hours [5,6]. DF is generally well-tolerated with few side effects.

Defibrotide has multiple effects on endothelial responses and platelet activity. DF exhibits aptameric binding to adenosine A_1 and A_2 receptors on vascular endothelium, key receptors thought to modulate endothelial cell regulation and mitigate response to injury [2]. The antithrombotic and fibrinolytic properties of DF are believed to be due to increased release of tissue plasminogen activator (tPA) and decreased plasminogen activator inhibitor (PAI)-1 and tissue factor (TF) from endothelium [4,7–10]. DF has also been shown to significantly increase thrombomodulin (TM) and upregulate tissue factor pathway inhibitor (TFPI) [10–12]. DF enhances the release of prostacyclin (PGI_2) and

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prostaglandin E_2 (PGE_2), accounting for its anti-ischemic properties via vasodilatory effects on vascular tone [2,4,10,13–17]. DF also has selective activity on the microvasculature rather than macrovasculature [9]. Importantly, DF modulates its endothelial protection without compromising the anti-tumor effects of cytotoxic therapy [18]. Table 1 summarizes the proposed mechanisms of DF on endothelial cells and platelets.

More recently, it has been demonstrated that DF has anti-apoptotic effects on the vasculature as well as inhibitory effects on transendothelial migration of immune effector cells [17–19]. DF has also been shown to downregulate expression of cell adhesion molecules (CAMs) on endothelium contributing to anti-inflammatory effects as well as a potential role in stem cell mobilization [19–22]. DF has been shown to have antiangiogenic properties in certain model systems as a mechanism of its antineoplastic effects [23–25]. Intriguingly, recent *in vitro* studies suggest endothelial stabilization and revascularization through effects on antiangiogenic peptides by DF, pointing to a more complex pharmacodynamic profile in different vascular beds [25]. In a recent publication, DF has been shown to increase the chemosensitivity of tumor cells *in vitro*, pointing to another potential antineoplastic effect that could be used in treating malignancy more broadly [26].

Defibrotide is associated with limited adverse events in healthy volunteers, primarily allergic-type reactions of flushing, sweating and nausea. These symptoms are generally self-limited and require no specific treatment [1]. In clinical trials of patients treated with DF, adverse drug reactions (ADR) have been observed in less than 9% of patients [1]. Serious ADRs include transient hypotension, gastrointestinal disturbances, hemorrhage (especially when combined with anticoagulants) and allergic reactions [27–30]. Severe ADRs requiring withdrawal of DF therapy are uncommon.

Overview of veno-occlusive disease

Hepatic VOD is one of the most feared and severe organ injury syndromes that can occur after high-dose chemotherapy and hematopoietic stem cell transplantation (SCT). The incidence of VOD varies depending on the patient population and conditioning regimen but has been reported to occur in 8–15% of patients [31–35]. VOD has been reported to occur in other settings including after toxin ingestion, as well as solid organ transplant [36–39].

Veno-occlusive disease is a clinical syndrome of hyperbilirubinemia, painful hepatomegaly and fluid retention, which generally develops within the first 2–3 weeks after SCT [31,40], although later presentations can be seen [41]. Diagnosis is usually made on clinical findings, most often by applying the case definitions of either the Baltimore or Seattle criteria, detailed in Box 1. Clinical trials most commonly specify inclusion criteria by one of these two systems. Although similar, there are important differences that may account for variability in outcomes between trials, with the Baltimore criteria considered diagnostically rigorous and more closely associated with severity. VOD encompasses a broad clinical spectrum, ranging from mild disease requiring no specific therapy to severe disease associated with multiorgan failure (MOF), requiring a critical care level of support and with fatality rates ranging from 80 to 100%. Table 2 summarizes clinical features of patients with VOD according to severity, as defined by the Seattle criteria.

Table 1. Proposed mechanisms of action of defibrotide.

Action	Possible mediators	Ref.
Anti-ischemic	Increased PGI_2 and PGE_2 synthesis and release Increases smooth muscle intracellular cAMP → vasodilation Antagonism of endothelin-1	[1,2,15]
Profibrinolytic	Decreases PAI-1 Increases tPA	[1,9,10]
Antithrombotic	Increased thrombomodulin release → activation of protein C and S Altered TF expression	[9–11]
Antiplatelet	Via PGI_2 and PGE_2 has direct antiplatelet effects Interruption of platelet–endothelial cell interactions Decreased release of Cathepsin G from leukocytes	[1,10,16,17]
Anti-inflammatory	Inhibition of free radical production Decreased Cathepsin G Altered migration of inflammatory cells	[1,17,19]
Antiangiogenesis, endothelial stabilization	Reduction of activation of p70S6 kinase → target in PI3K/AKT/mTOR signaling pathway Prevents differentiation of dendritic cells into endothelial cells	[23–25]
Stem cell mobilization	Enhances mobilization of primitive peripheral blood progenitor cells	[22]

PAI: Plasminogen activator inhibitor; PGE_2: Prostaglandin E_2; PGI_2: Prostacyclin; tPA: Tissue plasminogen activator.

Risk factors for VOD

Several risk factors have been identified for VOD. These risk factors can be broadly divided into pretransplantation patient characteristics and transplant-related factors. Pretransplant patient-related characteristics include older recipient age, female gender, poor performance status, abnormal baseline liver function test (specifically elevated aspartate aminotransferase), advanced malignancy, second myeloablative transplant, prior abdominal radiation, prior gemtuzumab ozogamicin therapy, abnormal baseline pulmonary function (reduced carbon monoxide diffusion capacity) and norethisterone treatment [31,33,42–44].

Transplant-related risk factors for VOD include type of transplant, degree of HLA histoincompatibility and graft-versus-host disease (GVHD) prophylaxis, including sirolimus. The incidence of VOD is lower in autologous SCT and reduced-intensity conditioning transplantation than in myeloablative transplant [33,45]. Conditioning-based factors include total body irradiation (TBI) dose, TBI dose rate and chemotherapy

Box 1. Clinical criteria for case definition of hepatic veno-occlusive disease.

Baltimore criteria [40]**:**

- By day +21:
  - Hyperbilirubinemia (>34.2 μM or >2 μg/dl)
- Plus at least two of the following:
  - Painful hepatomegaly
  - Fluid retention or ascites
  - Sudden weight gain (>5% of baseline weight)

Seattle criteria [31]**:**

- Two or more of the following by day +20:
  - Hyperbilirubinemia (>34.2 μM or >2 μg/dl)
  - Painful hepatomegaly
  - Unexplained weight gain (>2% of baseline weight)

agents used [46–48]. Busulfan-containing regimens have a higher risk of VOD as compared with non-busulfan-containing regimens [46,47,49]. Targeted busulfan dosing has been shown to reduce the incidence of VOD, demonstrating that individual variation in metabolism may explain in part the subsequent development of VOD [50–52]. Similarly, the degree of liver toxicity was correlated with exposure to toxic metabolites of cyclophosphamide [53]. Alternate conditioning regimens have been associated with a lower risk of developing VOD, as well as decreased severity [54]. Transplant with T-cell-depleted allografts has been associated with a lower incidence of VOD [55–57]. A reduced incidence of VOD was also seen with peripheral blood SCT as compared with bone marrow transplant [58]. Sirolimus use has been associated with increased rates of microangiopathy, including thrombotic thrombocytopenic purpura-hemolytic uremic syndrome [49]. Most recently, a threefold increased VOD risk has been seen with its use, which poses an important potential barrier to its incorporation as an effective anti-GVHD agent [49]. With identification of risk factors, strategies aimed at prophylaxis for VOD (including the use of DF) and risk factor modification can be studied more extensively.

Pathophysiology of VOD

Veno-occlusive disease is caused by a toxic insult to the sinusoidal endothelial cells and hepatocytes in zone three of the liver, typically caused by the high-dose chemotherapy. Initial reports of VOD occurred prior to the use of SCT and in fact were caused by ingestion of pyrrolizidine alkaloids in cereals or herbal teas in otherwise healthy individuals [36–39,59]. Monkeys who receive monocrotaline demonstrate hepatic endothelial damage within 6–12 h [60]. Zone three hepatocytes contain both high concentrations of cytochrome P450 enzymes and glutathione *S*-transferase enzyme, both of which are important in the metabolism of chemotherapeutic agents. Preliminary studies of genetic polymorphisms in SCT patients have variably suggested a possible association between a mutation of glutathione *S*-transferase synthesis and increased VOD risk [61–64]. Depletion of glutathione can result in hepatocyte necrosis, while administration of glutathione mono-8-diester can protect hepatocytes in experimental models [65–67]. This evidence points to the toxic nature of chemotherapeutic agents as key initiators of VOD.

In response to the toxic injury of chemotherapy, marked elevations in markers of endothelial injury have been observed. Increases in local secretion of TF and PAI-1, as well as dysregulated TM and TFPI secretion, account for a local prothrombotic state in the liver [12,68–71]. The importance of PAI-1 in the pathophysiology of VOD has been demonstrated by its role as both an independent diagnostic and prognostic marker for VOD [12,68,69,71–73]. PAI-1 production is triggered by the release of TGF-β from activated platelets [74]. Elevated TGF-β has been associated with the development of both liver and pulmonary fibrosis following transplantation [75]. In a murine model of VOD, PAI-1 knockout mice were protected from hepatic dysfunction and venular thromboses, as opposed to wild-type mice. Wild-type mice given a PAI-1 inhibitor, tiplaxtinin, were protected from nitric oxide-induced injury [76]. Other abnormalities include upregulation of CAMs, including P- and E-selectins, a dysregulated cytokine environment, an increase of extracellular matrix proteins including procollagen type 3, and an increase in von Willebrand factor (vWF) release, as well as thrombomodulin [77–87].

The histopathology of VOD reveals a disrupted cobblestone appearance of the sinusoidal endothelium of the liver due to subendothelial edema and cell damage, which leads to sinusoidal obstruction. Microthromboses, expression of factor VIII/vWF complexes, and fibrin deposition can be demonstrated within the hepatic venular walls [88–91]. Progressive sinusoidal obstruct is a hallmark of VOD and has led to the use of the term "sinusoidal obstruction syndrome" as either an alternate or complementary term for the syndrome [92]. This hepatic venule obstruction results in dilatation of the sinusoids with subsequent hepatic necrosis. Collagen is deposited both within the hepatic venules as well as abluminally. Eventually, this process progresses to central vein occlusion with extensive hepatic fibrosis, mimicking end-stage liver disease or cirrhosis (Figure 1A & B).

Table 2. Clinical features of patients with hepatic veno-occlusive disease of the liver according to severity of disease by Seattle criteria.

Severity	Weight gain (% increase)	Maximum total serum bilirubin before day 20 (μg/dl)	Patients with peripheral edema (%)	Patients with ascites (%)	Platelet transfusion requirements to day 20	Day 100 mortality (all causes; %)
Mild	7.0 (±3.5%)	4.73 (±2.9)	23	5	53.8 (±27.6)	3
Moderate	10.1 (±5.3%)	7.95 (±6.6)	70	16	83.6 (±5.0)	20
Severe	15.5 (±9.2)	26.15 (±15.3)	85	48	118.3 (±51.8)	98

Data from [31].

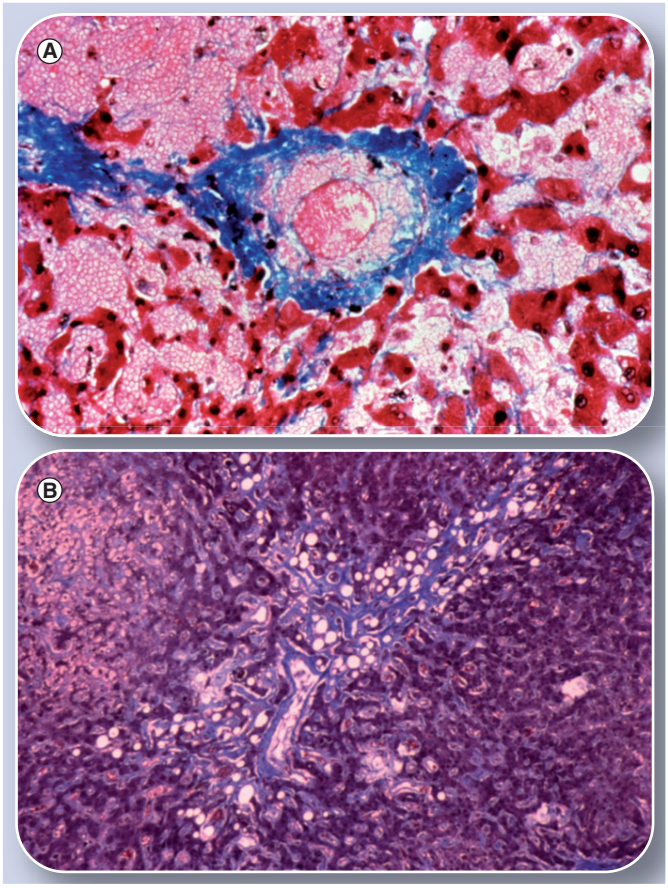

Figure 1. (A) This terminal hepatic venule is not fibrotic but does demonstrate striking subendothelial edema with narrowing of the lumen. The detached sinusoidal endothelium surrounding results in stasis and plugging of the pores, which drain the sinusoids into the venules by fibrin (demonstrable by immunostaining). Elevated intrasinusoidal pressures and ischemia lead to hemorrhagic necrosis with disrupted necrotic hepatocyte liver cords. **(B)** Liver biopsy in a patient with prior Mylotarg® exposure and severe hepatic veno-occlusive disease: sinusoidal obstruction is prominent.
Reproduced with permission from [90].

Clinically, patients with VOD may have a broad clinical spectrum of disease severity [31,93]. Patients with mild disease have laboratory evidence of VOD, but do not require specific treatment. Patients with moderate disease require symptomatic treatment with pain medications and diuresis for fluid management. Severe disease is defined by MOF, with associated ascites and portal hypertension, typically demonstrated radiologically with portal flow reversal [94,95], although ultrasound may lag behind clinical symptoms early in the development of VOD. Transvenous liver biopsy and wedged hepatic pressure gradient measurement (WHVPG) remain gold standards of diagnosis [96]. The ability to perform liver biopsy may be limited in patients with coagulopathy or other bleeding diathesis. Generally, the transjugular approach is preferred for this reason. A WHVPG of greater than 10 mm of mercury has a specificity of 91% and positive predictive value of 86%, but a modest sensitivity of 52% [97]. The combination of clinical and radiographic findings are used to diagnose VOD.

Patients with severe VOD generally die of the complications of MOF rather than hepatic failure *per se* [31,93]. Patients die of hepatorenal syndrome with renal failure and hemodynamic compromise, infections leading to sepsis and complications of long-term mechanical ventilation. Supportive therapy for severe disease includes diuresis, paracentesis, renal replacement therapy, oxygen support including mechanical ventilation, prevention and treatment of infection, and blood product support. Despite these supportive therapies, the outcome of severe VOD is dismal with mortality approaching 100% [31,32,40,98]. A model predicting the development of severe VOD was developed by Bearman [32]. Studies of the treatment of VOD have generally included patients at high-risk for fatal outcome by the Bearman model.

Attempts to improve treatment of VOD beyond supportive care have primarily included prevention strategies and treatment of established VOD with thrombolytic therapy and/or anticoagulation. Prevention strategies that have been reported include the use of prophylactic ursodiol, supplemental glutathione or other antioxidants, and prophylactic heparins and fresh frozen plasma supplementation [66,99–108]. Of these, the use of ursodiol has demonstrated an impressive reduction in the incidence of VOD in a recently performed meta-analysis (RR = 0.58) [103], but results across trials have been variable. The use of anticoagulation to prevent VOD has been limited by the high risk of bleeding in this population. Interestingly, low-molecular-weight-heparins (LMWHs) have been shown to reduce the incidence of VOD (4 vs 11–22%) without a significant increase in major bleeding [108], although subsequent studies have failed to duplicate this result definitively. Pharmacologic treatment of established VOD has included several case series of thrombolytic therapy with and without heparin, reviewed recently in Ho *et al.* [109]. The best studies showed a response rate of approximately 30% in the larger series [110–115], but prohibitive toxicity from life-threatening hemorrhage. Novel therapies aimed at altering the natural history of VOD are thus needed.

Treatment of VOD: rationale for defibrotide

The pathophysiology and biochemical abnormalities outlined above offer several points for potential intervention. Targeting the response to endothelial cell injury is the central strategy for the use of DF in VOD (Figure 2). DF specifically reduces soluble TF, increases tPA, decreases PGE_2 and PGI_2, and decreases expression of CAMs preventing effective immune migration, producing antithrombotic, fibrinolytic, anti-ischemic and anti-inflammatory effects [1,2,77–79]. Prior attempts at the use of other anticoagulants including heparin, antithrombin (AT)III and profibrinolytics including tPA, have not demonstrated clear benefit in the treatment of VOD and have been limited by significant attributable toxicity, including the risk of serious hemorrhage. The pleiotropic effects of DF on sinusoidal endothelium, together with its relative safety, provided the primary rationale for its testing in VOD.

Clinical trials with defibrotide

The use of DF in VOD was attractive because of its activity in syndromes of endothelial dysfunction, specifically peripheral vascular disease and thrombotic microangiopathy [116,117], as well

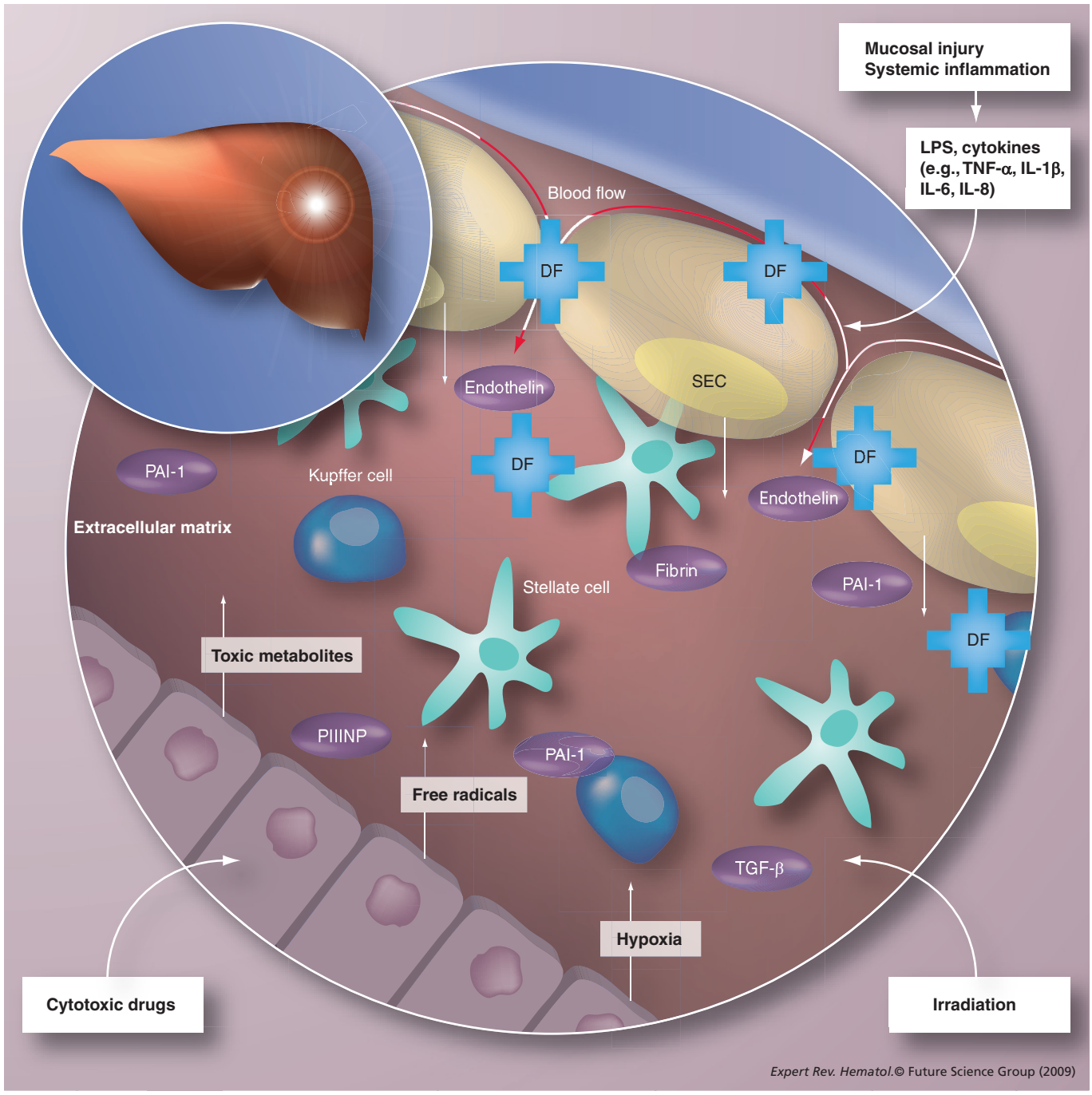

Figure 2. Mechanism of action of defibrotide in hepatic veno-occlusive disease.
DF: Defibrotide; ECM: Extracellular matrix; LPS: Lipopolysaccharide; PAI-1: Plasminogen activator inhibitor-1; PIIINP: Procollagen type 3; SEC: Sinusoidal endothelial cell.

as its relative lack of significant systemic anticoagulant effects. Table 3 summarizes the results of published studies using DF to treat severe VOD.

The initial compassionate-use study that suggested the potential efficacy of DF was conducted from 1995–1997 [118]. A total of 19 consecutive patients with established severe VOD and a predicted risk of fatal outcome of greater than 40% by the Bearman model [32] or the presence of MOF were enrolled in this trial. DF was administered intravenously every 6 h with a dose range of 5–60 µg/kg/day. Patient age ranged from 4–58 years (median: 35 years) with four patients aged under 18 years. The median bilirubin at time of initiation of DF was 22.3 µg/dl. All patients had evidence of MOF with oxygen dependence in 14 patients (including patients on mechanical ventilation), renal insufficiency in 12 patients (five on dialysis) and encephalopathy in eight patients. Response to therapy required resolution

Table 3. Defibrotide for the treatment of hepatic veno-occlusive disease.

Author	Patients (n)	DF dose (µg/kg/day)	CR rate (%)	Day +100 survival (%)	Patient characteristics and comments	Ref.
Richardson *et al.*	19	5–60	42	32	High-risk patients only: all had MOF	[118]
Chopra *et al.*	40	10–40	55 (overall) 36 (high risk)	43	28/40 patients considered high risk	[119]
Richardson *et al.*	88	10–60	36	35	All patients high risk by Bearman model and/or MOF	[120]
Corbacioglu *et al.*	45	10–110	76 (overall) 50 (high risk)	64 (overall) 36 (high risk)	22/45 pediatric patients considered high risk with MOF	[121]
Bulley *et al.*	14	11–40	64*	79	Pediatric patients only, risk not specified	[28]
Richardson *et al.*	150	25 (arm A) 40 (arm B)	46	41	All high risk by Bearman model and/or MOF No significant CR or OS difference between doses	[27]
Haussman *et al.*	14	60	100	93	All patients received prophylactic antithrombin III infusions If VOD, then received DF plus antithrombin III Compared with historical controls	[30]

*CR rate not reported, nine out of 14 (64%) discontinued DF due to clinical improvement.
CR: Complete response; DF: Defibrotide; MOF: Multiorgan failure; OS: Overall survival.
Adapted from [109].

of hyperbilirubinemia and improvement in VOD-related organ toxicity: out of 19 patients, 42% had complete resolution of VOD with six out of 19 (32%) surviving past day +100. No toxicity or attributable bleeding was observed with the active dose noted to be 25 mg/kg/day. Survival of similar untreated patients would have been expected to be less than 10% by day +100 [31–33]. The favorable outcome together with the encouraging tolerability of DF seen in this setting prompted enthusiasm for the further study of DF for severe VOD/MOF.

In a multicenter European compassionate trial [119], 40 patients with established VOD defined by either Baltimore or Seattle criteria were treated with 10–40 mg/kg/day of DF. In total, 28 out of 40 patients (70%) had either MOF or a predicted risk of severe VOD of greater than 40% by the Bearman model. Patient age ranged from 1–64 years (median: 30 years). Median bilirubin at onset was 140.2 µmol/l (8.2 µg/dl), six patients had received prior tPA or unfractionated heparin/LMWH as treatment of VOD, and 20 had received heparin prophylaxis. In total, 22 out of 40 patients (55%) achieved a complete response with normalization of serum bilirubin. Overall survival at day +100 was 17 out of 40 (43%). The complete response rate of the patients with severe VOD was 36% (10 out of 28). These results confirmed the benefit of DF seen in the initial North American study and suggested that further trials were warranted.

Given these promising results, an expanded compassionate-use study involving a consortium of eight US transplant centers enrolled an additional 69 patients for a total of 88 patients evaluable for response as well as toxicity [120]. Patients were included if they met Baltimore criteria or if they had two features of VOD with a diagnostic liver biopsy. In addition, patients had to have a predicted risk of severe VOD of greater than 30% by the Bearman model or MOF. The complete response rate was 36% (32 out of 88) with day +100 survival of 35% (31 out of 88), in this cohort of patients with severe VOD who were all high risk or had MOF at enrollment. Most responses were seen at doses of DF between 20–40 mg/kg/day. No worsening of bleeding or additional toxicity was observed. Among responders, no chronic liver dysfunction or recurrent VOD after successful treatment was observed. Predictors of survival included patients receiving autologous transplant as opposed to allogeneic transplant, those with nonmalignant diagnosis or solid tumor versus hematologic malignancy and those who received prior tPA/heparin, although the numbers for this particular part of the analysis were small. Busulfan-based conditioning predicted for worse outcome, as did encephalopathy and more advanced MOF. Biochemical markers of response were also evaluated, including change in bilirubin, creatinine and PAI-1 levels. Reduction in serum creatinine was predictive of response by multivariate analysis, whereas bilirubin was not, suggesting that reversal of the renovascular pathophysiology contributed to long-term response. Median reduction of PAI-1 was also noted to be associated with better outcome, supporting a role for this marker in this setting.

In order to establish the optimal dosing of DF for future trials, a large multicenter, randomized Phase II study was conducted and enrolled 151 patients to either 25 mg/kg/day (arm A) or 40 mg/kg/day (arm B) [99]. Similar to the former trials, patients were eligible if they met Baltimore criteria and had severe VOD either by a 30% or greater risk by the Bearman model and/or the presence of MOF with 99% of patients having evidence of MOF at enrollment. In total, 141 patients were evaluable for response, and 65 patients achieved a complete response (46%), with 62 surviving to day +100 (41%). No significant difference in response rate or survival was seen between the two arms, although there was a trend towards improved survival in the pediatric population with lower dose DF and a statistically

Table 4. Defibrotide as prophylaxis for hepatic veno-occlusive disease.

Author	Patients (n)	DF dose/duration	VOD incidence	Historical control VOD incidence	Comment	Ref.
Chalandon *et al.*	52	10–25 mg/kg/day (day 0–20)	0/52 (0%) Baltimore criteria	10/52 (19%)	Low-dose heparin 5–10 K IU per day IVCI concurrently with DF	[125]
Dignan *et al.*	58	10 mg/kg/day (day 1–21)	0/58 (0%) Baltimore criteria	NA	Majority 37/58 (64%) received reduced intensity regimen 42 out of 58 received alemtuzumab	[126]
Corbacioglu *et al.*	9	40 mg/kg/day (median) from conditioning until day +30	1/9 (11%) Seattle criteria, moderate severity	7/11 (63.6%), 3 severe, 4 mild/moderate	All children receiving SCT for infantile osteopetrosis	[123]
Versluys *et al.*	5	10–20 mg/kg/day conditioning until day +30	0/5 (0%)	NA	All patients received gemtuzumab ozogamicin within 2 months of SCT	[122]

DF: Defibrotide; IVIC: Inferior vena cava interruption; NA: Not available; SCT: Stem cell transplantation; VOD: Veno-occlusive disease.
Adapted from [109].

nonsignificant trend for less grade 3 and 4 side effects of bleeding and hypotension. Consequently, the 25 mg/kg/day (arm A) dose has been selected for future trials. A pivotal Phase III trial of at least 80 patients matched with historical controls is underway at 35 transplant centers across North America and Israel to definitively show the role of DF in the treatment of severe VOD [100].

In the pediatric population, at least three reports have documented efficacy and safety of DF for treatment of VOD. A retrospective study of 45 pediatric patients aged 0.2–20 years (median: 8.2 years) demonstrated a 76% complete response with a survival rate of 64% at day 100 [121]. Among the patients with severe VOD, 50% achieved a complete response and 36% survived past day +100. Patients who achieved a complete response were treated with higher doses of DF than nonresponders (45 vs 27 mg/kg/day). The authors conclude that higher doses may be beneficial in the pediatric population. The average delay from diagnosis of VOD to start of DF was 1 day in the complete response group and 5.5 days in the nonresponder group ($p < 0.01$), suggesting that earlier initiation of DF impacted the response. A report of 14 children treated with DF in Canada documented a 79% day +100 survival rate [28]. None of the children in this series received prophylaxis with heparin or ursodiol. In a third trial, a large prospective case series of 162 pediatric patients with and without ATIII prophylaxis who developed VOD were all treated with DF [30]. The incidence of VOD was similar in both the ATIII prophylaxis group as well as the control group (18 vs 15%, respectively), showing limited value of ATIII prophylaxis. However, 13 out of 14 patients in the combined DF plus ATIII group (93%) survived past day 100 compared with six out of 13 (46%) in the DF alone treated patients (OR: 16.4; $p = 0.006$), although given the small numbers of patients, this finding requires validation in larger trials. This trial demonstrated the safety of this strategy as well as raising the possibility of improved efficacy with combination therapy. These reports suggest that DF is a potentially safe and effective therapy in children with VOD.

DF as prophylaxis for VOD

As DF was shown to be effective in treatment of established VOD, the question of whether prophylaxis of VOD would be beneficial in high-risk populations has been addressed. This strategy has been reported in four separate series. Table 4 summarizes the results of these trials.

The first published report described the outcome of five children with recent gemtuzumab ozogamicin (Mylotarg®; Wyeth, PA) treatment prior to SCT who received prophylactic DF at a dose of 10–20 mg/kg/day [122]. No patient developed VOD, suggesting that DF may be an effective preventative therapy in this high-risk group. In another high-risk pediatric population with malignant infantile osteopetrosis, nine patients received prophylactic DF from the day of conditioning to day +30 [123]. One patient (one out of nine, 11%) developed VOD compared with 64% (seven out of 11) in historical controls. These results demonstrate that DF in high-risk populations may be beneficial and have prompted a large ongoing randomized European Group for Blood and Marrow Transplantation (EBMT) study in high-risk allogeneic pediatric patients. Preliminary results are encouraging, with at least a 40% reduction of VOD seen and excellent tolerability, although more mature results are awaited [124].

Two studies have reported the results of prophylactic DF in a general adult transplant population without known risk factors for VOD. Chalandon and colleagues treated 52 consecutive SCT patients with prophylactic DF at a dose of 10–25 mg/kg/day, in addition to prophylactic heparin and compared outcomes with historical controls [125]. No patient in the treatment group developed VOD, versus 19% (ten out of 52) in the control group. This translated into a statistically significant increase in event-free survival at day +100 (65 vs 44%; $p = 0.02$) as well as a trend towards improved overall survival and decreased transplant-related mortality. These results were replicated in a study of 58 patients treated with prophylactic DF alone [126]. No patients developed VOD by Baltimore criteria, although the majority of patients (37 out of 58) received reduced-intensity transplants. These positive results suggest that a randomized trial of prophylactic DF in adults is warranted.

Conclusion

Defibrotide is a first-in-class oligonucleotide with novel mechanisms of action in reversing microangiopathy through effects on vascular endothelium, platelet biology, the coagulation cascade, the inflammatory process and angiogenesis. These diverse actions on vascular biology are best reflected by DF's emerging role as a treatment for VOD in the setting of high-dose chemotherapy and SCT. Recent clinical trials have demonstrated its efficacy in the setting of severe VOD, an otherwise fatal syndrome. The expansion of DF usage in the preventative setting is under investigation and offers the greatest opportunity for reducing the incidence and severity of VOD. Currently, there are no effective alternatives to DF therapy for severe VOD.

Expert commentary

The future of DF for the treatment and prevention of VOD is promising. The current North American/Israeli Phase III trial utilizing a novel historical control methodology will hopefully confirm the efficacy of DF in the treatment of severe VOD with MOF, although stringent enrollment criteria for severe VOD and MOF, defined diagnostically by the Baltimore criteria, may limit application to patients who present with late or atypical VOD. However, results in other studies do suggest that DF use in later onset of disease is helpful. Nonetheless, defining clearly which populations should be treated with DF as well as the timing and duration of DF will be important in the future. Most importantly, the benefit of DF as prophylaxis suggests that future studies should examine not only treatment of established VOD but prevention of VOD with DF, especially in high-risk patients. In fact, recent results from a large international pediatric trial led by the EBMT are particularly promising and support the role of DF in VOD prevention [124].

The novel mechanisms recently described with DF in preclinical studies showing modulation of angiogenesis as well as endothelial cell stabilization, revascularization of damaged endothelium and augmentation of stem cell mobilization are important areas to study. These results suggest a broader role for DF in SCT and oncology as a whole, with intriguing prospects for this first-in-class oligonucleotide as a novel therapeutic agent, targeting activated endothelium and the stroma it subserves.

Five-year view

Defibrotide and the development of more potent n-mers as derivative oligomers contribute an exciting new area of therapeutics in this class. The incorporation of second-generation oligmers may offer a more effective alternative to DF in the future, but this remains to be seen. The optimal use of DF will continue to be defined through clinical trials, including studies for the treatment and prevention of VOD. The indication for the use of DF may have broader application to other microangiopathies and related disorders, subject of course to the appropriate prospective clinical studies.

Financial & competing interests disclosure

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Key issues

- Defibrotide (DF) is a novel, first-in-class oligonucleotide with anti-thrombotic, fibrinolytic, anti-ischemic, anti-inflammatory and anti-atherosclerotic properties through effects on activated endothelium.
- Hepatic veno-occlusive disease (VOD) is a clinical syndrome of hyperbilirubinemia, painful hepatomegaly and fluid retention that occurs in the setting of stem cell transplantation (SCT) in between 8 and 15% of patients.
- VOD includes a broad spectrum of disease severity, ranging from mild disease not requiring treatment to severe disease resulting in multiorgan failure (MOF) and death.
- DF has been shown to produce complete responses of 30–60% and survival beyond day 100 post-SCT ranging from 32–50% in the treatment of severe VOD/MOF.
- The optimal use of DF is under investigation in several clinical trials, with especially encouraging results seen in the prevention of VOD.

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