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Ursodiol does not prevent hepatic venoocclusive disease associated with Mylotarg therapy

Ursodiol is variably reported to reduce stem cell transplantation (SCT)-associated hepatic venoocclusive disease (VOD). VOD developed in 10 of 85 (12%) patients receiving Mylotarg-based regimens with ursodiol 300 mg *bid* for 21 days beginning on the day prior to cytotoxic therapy – an incidence seen with these regimens when administered without ursodiol.

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Mylotarg (gemtuzumab ozogamicin, CMA-676) is a semisynthetic derivative of calicheamicin linked to a recombinant anti-CD33 monoclonal antibody.[1] In phase I/II studies, Mylotarg has been associated with an approximately 20% incidence of grade 3 or 4 hyperbilirubinemia and raised liver transaminases.[1] VOD has been associated with Mylotarg therapy, both in patients with or without a prior SCT.[1-9] No therapy has been proven to reduce the incidence of SCT-associated VOD.[10] Ursodiol has been variably reported to reduce VOD associated with SCT.[10] We thus assessed the potential efficacy of ursodiol in reducing the incidence and/or severity of Mylotarg-associated VOD in patients with refractory acute myeloid leukemia (AML) or myelodysplastic syndromes (MDS).

The patient's details are presented in Table 1. VOD was diagnosed according to the standard Seattle criteria.[10] All patients gave written informed consent to participation in Institutional Review Board-approved studies. Patients received the Mylotarg-based regimens shown in Table 2. Patients received ursodiol 300 mg *bid* orally starting on the day prior to the first day of Mylotarg or other cytotoxic therapy and continuing for 21 days. No patient

Table 1. Patients' characteristics.

Characteristics	*% of total (n=85)*	*% without VOD (n=75)*	*% with VOD (n=10)*
Diagnosis			
AML	81	85	50
MDS	19	15	50
Prior therapy			
No	60	63	40
Yes	40	37	60
Age > 60 yrs	52	52	55
PS 2-4	75	75	82
Hg < 10 g/dL	81	80	91
WBC > 20×10^9/L	30	32	18
Plts < 100×10^9/L	79	79	82
AHD	40	39	38
Adverse karyotype (–5, –7, 11q23, +8)	22	21	20
Serum bilirubin (mg/dL)			
Baseline	0.8 (0.4-1.7)	0.8 (0.4-1.4)	0.8 (0.4-1.7)
Maximum elevation	1.9 (0.4-9.6)	1.5 (0.4-1.3)	4.5 (2.6-9.6)
Days to maximum elevation	5 (2-25)	4 (2-8)	15 (4-25)
SGPT (μ/L)			
Baseline	36 (12-239)	36 (12-239)	39 (12-112)
Maximum elevation	118 (12-3966)	106 (12-3966)	280 (52-2370)
Days to maximum elevation	13 (5-27)	13 (5-27)	13 (7-23)

Table 2. Mylotarg regimens (number of patients / number with VOD – disease status).

Mylotarg 9 mg/m^2 IV over 2 hours on day 1 and day 8; (10/1 – previously untreated)
Mylotarg 9 mg/m^2 IV over 2 hours on day 1 and day 8; Interleukin-11 15 μg/kg SQ on days 3 to 28; (10/1 – relapse);
Mylotarg 4.5 mg/m^2 over 2 hours after loading dose of cyclosporine on day 1; Fludarabine 1.5 mg /m^2 IV *bid* on days 2 to 6; Cytarabine 0.5 g/m^2 over 2 hours IV *bid* on days 2 to 6, 4 hours after fludarabine started;
Cyclosporine 6 mg/kg over 4 hours followed by 16 mg/kg CIV on days 1 and 2; (31/2 – previously untreated); (12/3 – relapsed);
Mylotarg 9 mg/m^2 over 2 hours IV on day 1 and day 15; Troxacitabine 4 mg/m^2 IV on days 1 to 5; (2/1 – relapsed);
Mylotarg 9 mg/m^2 over 2 hours IV on day 1; Cytarabine 1 g/m^2 over 2 hours daily on days 1 to 5; Topotecan 1.25 mg/m^2 CIV daily on days 1 to 5; (16/2 – relapsed);
Mylotarg 9 mg/m^2 over 2 hours on day 1; ATRA 22.5 mg/m^2 /day bid on days 1 to 15; (3/0 – relapsed).

CIV = continuous intravenous infusion; ATRA = all trans retinoic acid.

received aluminum hydroxide-based antacids.

Severe VOD developed after the first Mylotarg therapy in 10 of 85 (12%) patients who were treated with Mylotarg-based regimens and ursodiol. The ten patients (5 with AML, 5 with MDS) who developed VOD included 5 patients who had received no prior cytotoxic therapy, 1 of whom had received single-agent Mylotarg 9 mg/m^2 on a day 1 and 9 regimen. In each case, the clinical syndrome was very similar with rapid onset of weight gain, hepatic pain and jaundice and finally evident ascites. Alternative causes of these symptoms, such as acute hepatitis, progressive systemic or abdominal sepsis, pancreatitis, cholangitis, and bowel obstruction were not detected in patients at the time of the diagnosis of VOD. Three of 10 patients had confirmatory histology on liver biopsy while eight had results of radiological studies, including CT scan and/or ultrasound,[10] which were supportive of the diagnosis of VOD.

These was no relationship between Mylotarg-associated VOD and age, gender, diagnosis (AML versus MDS), baseline liver or renal function tests, history of hepatitis or alcoholism, prior or concurrent non-steroid anti-inflammatory drug intake, type or amount of concomitant antimicrobial agents, type or amount of prior cytotoxic therapy, presence or absence of infusion reactions to Mylotarg or the use of steroids to reduce such infusion reactions. VOD developed in 10 of 85 (12%) patients who were treated with Mylotarg-based regimens and ursodiol — a similar incidence to that reported in a prior cohort of 119 patients with AML or MDS who received these Mylotarg-based regimens without ursodiol.[1] The latter cohort consisted of 61 previously untreated patients and 56 with relapsed disease, of whom 14 (12%) developed VOD. Four (29%) of these 14 patients with VOD had received no prior cytotoxic therapy, including 2 patients who received single-agent Mylotarg therapy.[1] The 12% incidence of VOD associated with Mylotarg-based regimens, given with or without ursodiol, in 204 patients with AML or MDS at MD Anderson Cancer Center (MDACC) is very similar to that reported by various investigators at the 2001 American Society of Hematology meeting — of 112 patients who had received a variety of Mylotarg-based regimens, 12 (11%) developed VOD.[4,6-9]

Mylotarg targets CD33 expressing cells via a humanized anti-CD33 antibody (hP67.6), which is linked to N-acetyl-γ calicheamicin DMH, a potent enediyne antitumor antibiotic. Calicheamicin metabolites are detectable in the serum and urine of patients receiving Mylotarg therapy.[2] VOD has not been reported in association with treatment with other anti-CD33 antibodies. Thus calicheamicin seems the most likely cause of Mylotarg-associated VOD, possibly by causing damage to hepatic sinusoidal endothelial cells.[10] Potentially effective modalities for the prevention of VOD e.g. heparin, or for the treatment of VOD, e.g. recombinant human tissue plasminogen activator or defibrotide, warrant investigation in future studies of Mylotarg-based regimens.[10]

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Inhibition of fluid phase and clot-bound thrombin by the thrombin exosite I ligand LU 58463: comparison with heparin and hirudin

Heparin inhibits clot-bound thrombin less efficiently than fluid phase thrombin. We compared the ability of heparin, a non-selective indirect thrombin inhibitor, r-hirudin, a selective bifunctional thrombin inhibitor, and LU 58463, a non-catalytic exosite I thrombin inhibitor, to inhibit fluid-phase and clot-bound thrombin. The thrombin inhibitor LU 58463 was more effective than heparin and as effective as hirudin in inhibiting clot-bound thrombin.

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Human α-thrombin has three key functional domains. The catalytic center is the site where substrates are cleaved. The non-catalytic substrate-binding domains, known as anion binding exosite I and II, are involved in fibrin(ogen) and heparin binding, respectively.[1] Thrombin is continuously incorporated into the growing clot by binding to fibrin(ogen) through the anion binding exosite I, so that clot-bound thrombin remains active.[2] Heparin catalyses thrombin inactivation through the formation of thrombin-antithrombin (AT) complex, while hirudin directly inhibits thrombin by binding to both the catalytic site and the anion binding exosite I. When bound to fibrin, thrombin is resistant to inactivation by the AT-heparin complex but not by hirudin.This difference could explain the improved ability of hirudin over heparin in preventing the extension of venous thrombosis,[3] or re-thrombosis after thrombolysis.[4,5] LU 58463 is a non-catalytic exosite I thrombin inhibitor derived from the C-terminus of hirudin $_{56\text{-}65}$.[6,7]

We compare the ability of heparin, r-hirudin and LU 58463 to inhibit fluid-phase and clot-bound thrombin. In the fluid-phase system 50 μL of human α-thrombin (final concentration 0.4 nM) were incubated with 450 μL aliquots of citrated plasma for 60 minutes at 37°C in the presence of increasing concentrations of thrombin inhibitors. In the clot-bound system fibrin clots were formed around siliconized polystyrene coated wire hooks by the addition of $CaCl_2$ (final concentration 25 mM) to 450 μL aliquots of fresh citrated plasma. After washing in TBS, fibrin clots were incubated in 1 mL aliquots of fresh citrated plasma for 60 minutes at 37°C in the presence of increasing concentrations of thrombin inhibitors. The generation of FPA was used as an index of thrombin activity.

The three agents were tested over a wide range of concentrations: r-hirudin from 0.1 to 1000 nM; LU 58463 from 0.1 to 2500 nM; unfractionated heparin from 0.025 to 5 anti-Xa U/mL. The ratio of the concentrations of the three inhibitors able to achieve

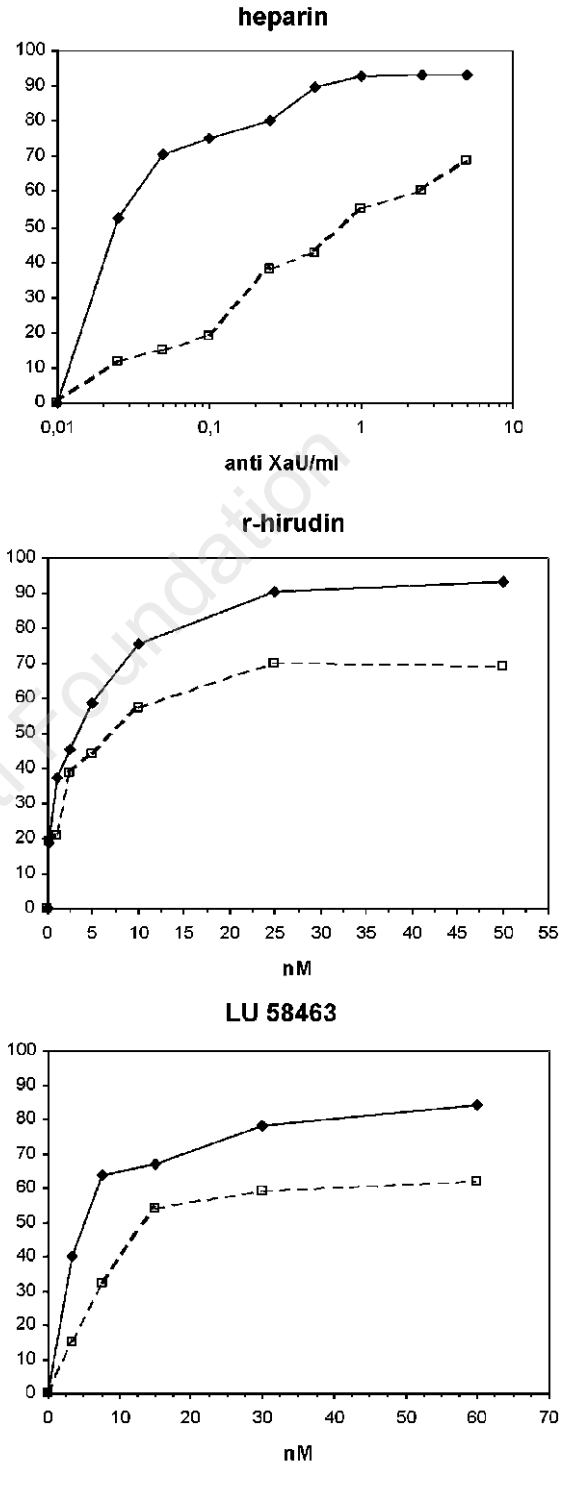

Figure 1. Percentage of inhibition of FPA generation induced by heparin, r-hirudin and LU 58463 in fluid phase and clot-bound thrombin.
***—* fluid phase thrombin. *– – –* clot-bound thrombin.**