**Abstract**

Defibrotide is a naturally derived product prepared by controlled depolymerization of porcine intestinal tissue DNA resulting in a polydisperse collection of predominantly single-stranded oligodeoxyribonucleotides. Defibrotide is currently indicated in Europe for the treatment of severe hepatic veno-occlusive disease [VOD, also known as sinusoidal obstruction syndrome (SOS)] in hematopoietic stem-cell transplantation (HSCT) therapy. In the present studies, experiments were conducted to determine induction of CYP1A2, CYP2B6, CYP3A4, and UGT1A1; inhibition of UGT1A1 and UGT2B7; transporter interactions; plasma protein binding and blood-to-plasma partitioning. Defibrotide (50, 250 and 500 µg/mL) did not show induction of CYP1A2, CYP2B6, CYP3A4 nor UGT1A1 mRNA or activities in hepatocytes from three individual donors. Defibrotide (1, 2, 5, 13, 32, 80, 200 and 500 µg/mL) did not show inhibition of UGT1A1 mediated glucuronidation of estradiol (10 µM) or UGT2B7 mediated glucuronidation of morphine (500 µM). Defibrotide was not a substrate of the human uptake transporters OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3 or the efflux transporters P-gp and BCRP. In addition, defibrotide did not show inhibition of the transporters tested at concentrations up to 500 µg/mL. Protein binding of defibrotide to mouse, rat, dog and human plasma was determined using the ultrafiltration method. Defibrotide showed high protein binding in mouse (average of 92.3%), rat (average of 93.1%), rabbit (average of 95.6%), dog (average of 95.9%) and human (average of 93.2%) plasma. The protein binding appeared to be concentration-dependent for defibrotide (up to 200 µg/mL) in mouse plasma but not in other species. Defibrotide showed relatively low association with blood cells. The blood cell association was ≤14.1% in mouse, ≤9.16% in rat, ≤36.4% in rabbit, ≤13.9% in dog, ≤12.7% in Human Subject 1, ≤23.6% in Human Subject 2 and ≤22.4% in Human Subject 3. These results indicate that metabolism- or transporter-based drug-drug interactions are unlikely to occur with defibrotide.

**Introduction**

Defibrotide is a naturally derived product prepared by controlled depolymerization of porcine intestinal tissue DNA resulting in a polydisperse collection of predominantly single-stranded oligodeoxyribonucleotides. Defibrotide is currently indicated in Europe for the treatment of severe hepatic veno-occlusive disease [VOD, also known as sinusoidal obstruction syndrome (SOS)] in hematopoietic stem-cell transplantation (HSCT) therapy. In the present studies, experiments were conducted to determine induction of CYP1A2, CYP2B6, CYP3A4 and UGT1A1; inhibition of UGT1A1 and UGT2B7; transporter interactions; plasma protein binding and blood-to-plasma partitioning for defibrotide. The results of these experiments were used to evaluate the potential of drug-drug pharmacokinetic-based interactions (DDI) for defibrotide.

**Method**

**CYP Induction**

Defibrotide (50, 250 and 500 µg/mL) was incubated with hepatocytes from three individual human donors for 72 hours with medium changes every 24 hours. The mRNA levels of CYP1A2, CYP2B6, CYP3A4 and UGT1A1 were determined using quantitative polymerase chain reaction (qPCR). The activities of CYP1A2, CYP2B6, CYP3A4/5 and UGT1A1 were measured after incubating selective CYP substrates phenacetin (100 µM), bupropion (500 µM), testosterone (250 µM) and estradiol (100 µM), respectively, with hepatocytes for 1 hour. Known CYP enzyme inducers of CYP1A2 (omeprazole, 50 µM), CYP2B6 (phenobarbital, 1000 µM), CYP3A4 (rifampicin, 20 µM) and UGT1A1 (3-methylcholanthrene, 5 µM) and non-inducer (flumazenil, 20 µM) were also included as controls.

**Inhibition of UGT1A1 and UGT2B7**

UGT1A1 mediated glucuronidation of estradiol (10 µM) and UGT2B7 mediated glucuronidation of morphine (500 µM) were determined. Known inhibitors of UGT1A1 and UGT2B7 were also included as positive controls.

**Transporter Interactions**

Defibrotide (15 and 100 µg/mL) was incubated with OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3 or vector control at 37°C for 5 and/or 15 minutes. Uptake of defibrotide was determined with a fluorescence detection method. Uptake of known substrates Na^+/-para-aminobenzoate (1 µM) by OAT1, 3β-hydroxyestrone-3-sulfate (1 µM) by OAT3, 14C-tetraethylammonium (1 µM) by OCT1, 14C-estradiol-17β-D-glucuronide (0.5 µM) by OATP1B1 and 3β-hydroxycholesteryl octanoate (1 µM) by OATP1B3 was performed in the absence and presence of defibrotide (50 and 500 µg/mL). Known inhibitors of uptake transporters (probenecid at 200 µM for OAT1 and OAT3, quinidine at 256 µM for OCT1 and OCT2 and cyclosporine A at 10 µM for OATP1B1 and OATP1B3) were also included as positive controls. In addition, defibrotide (15 and 100 µg/mL) was incubated with Caco-2 cells for 2 hours and the efflux ratio was determined. Efflux ratios of P-gp substrate 3H-digoxin (1 µM) and BCRP substrate 3H-estrone-3-sulfate (0.1 µM) were assessed in the absence and presence of defibrotide (50 and 500 µg/mL). Known P-gp and BCRP inhibitors (zosuquidar at 2 µM and Ko143 at 1 µM, respectively) were also included.

**Protein Binding and Blood-to-Plasma Partitioning**

Protein binding of defibrotide (5, 10, 50, 200 and 500 µg/mL) to mouse, rat, rabbit, dog and human plasma was determined using the ultrafiltration method. Blood-to-plasma partitioning was also assessed for defibrotide (10, 50, 200 and 500 µg/mL).

**Results**

- Defibrotide did not show induction of CYP1A2, CYP2B6, CYP3A4 nor UGT1A1 mRNA or activities in hepatocytes from three individual donors. Effect of defibrotide on CYP3A4 mRNA level and enzyme activity is presented in Figures 1 and 2, respectively.
- Defibrotide did not show inhibition of UGT1A1 or UGT2B7. Results are presented in Figures 3 and 4, respectively.
- Defibrotide was not a substrate of the human uptake transporters OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3 or the efflux transporters P-gp and BCRP. In addition, defibrotide did not show inhibition of the transporters tested at concentrations up to 500 µg/mL.
- Defibrotide showed high protein binding in mouse (average of 92.3%), rat (average of 93.1%), rabbit (average of 95.6%), dog (average of 95.9%) and human (average of 93.2%) plasma. The protein binding appeared to be concentration-dependent for defibrotide (up to 200 µg/mL) in mouse plasma but not in other species. Results are presented in Table 1.
- Defibrotide showed relatively low association with blood cells. The blood cell association was ≤14.1% in mouse, ≤9.16% in rat, ≤36.4% in rabbit, ≤13.9% in dog, ≤12.7% in Human Subject 1, ≤23.6% in Human Subject 2 and ≤22.4% in Human Subject 3. These results indicate that metabolism- or transporter-based drug-drug interactions are unlikely to occur with defibrotide.

**Summary**

These studies indicate that metabolism- or transporter-based drug-drug interactions are unlikely to occur with defibrotide.