In Vitro Metabolic Stability Evaluation of ADCs in Plasma and Whole Blood

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Abstract

Antibody-drug conjugates (ADCs) are typically designed to selectively target tumor cells to improve cell-killing efficiency and reduce toxicity in healthy tissues. For efficacy and safety concerns, they must be stable in circulation to carry the payload to the site of the targeted cell. Therefore, understanding the release of small molecule cytotoxic drugs from ADC in plasma or whole blood *in vitro* should help identify factors affecting *in vivo* payload release[1]. In a previous study[2], lithium heparin was used as an anticoagulant rather than EDTA-K_2 so as not to inhibit any enzymes in the biological matrix. Unfrozen plasma was also used in the ADCs study, but there was no experimental data to illustrate this choice. In our study, the effects of corresponding experimental conditions were explored.

Objective

The objective of this study was to establish an *in vitro* method to determine the released concentration of payload from ADC after incubations with the plasma or whole blood.

Method

The ADC DS8201 (Enhertu) and payload Dxd were purchased from Daiichi Sankyo and MCE. The ADC Brentuximab vedotin (Adcetris) and payload MMAE were purchased from Takada and Bide.

Matrix

- **Plasma and Whole Blood**[3]
  - Frozen plasma was purchased from BioIVT.
  - Fresh plasma and whole blood were prepared in-house.
  - Anticoagulant: **EDTA-K_2 or heparin**.

Incubation

- The ADC stock solution (20 mg/mL) was spiked to the blank matrix to get a final concentration of 0.1 mg/mL.
- Samples of 100 µL were incubated in a CO_2 incubator for 0, 1, 3, 5, 7, 14 and 21 days.

Analysis

- Free payload was quantitated with calibration curve by LC-MS/MS

Results

Table 1. Effect of incubation time on plasma enzyme activity and plasma stability of 5 commercial compounds which are metabolized by hydrolase and peptidase (2 different runs, with duplicate incubations in each run)

	$T_{1/2}$ (min)				
Species	Mouse	Rat	Dog	Monkey	Human
Compound Name	Propantheline	Enalapril	Bisacodyl	Procaine	Propantheline
Fresh plasma	26.0	16.6	5.6	5.3	16.0
Plasma incubated for 21 days	20.8	11.2	8.1	5.2	13.1

	$T_{1/2}$ (min)				
Species	Mouse	Rat	Dog	Monkey	Human
Compound Name	Somatostatin Acetate				
Fresh plasma	5.7	4.5	4.6	7.3	4.8
Plasma incubated for 21 days	8.4	6.3	4.1	8.5	4.4

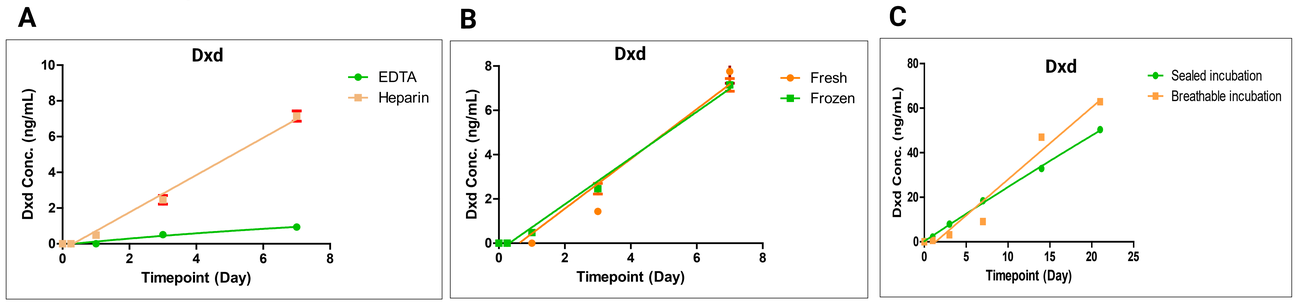

Figure 1. Comparison of the release of Dxd from DS8201 in mouse plasma (Mean +/- error bar of three runs) (A) Frozen plasma, with EDTA-K_2 and heparin as anticoagulants (B) Fresh and frozen heparin anticoagulated plasma (C) Sealed and breathable incubation conditions.

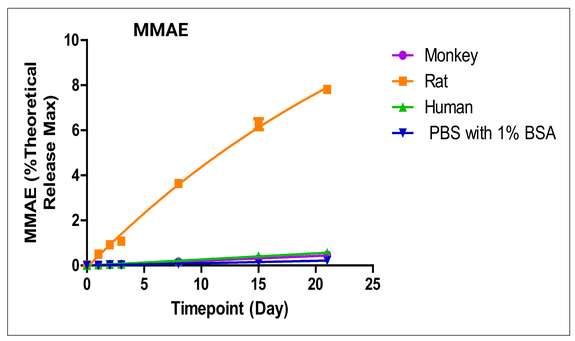

Figure 2 Release of MMAE after incubating Brentuximab vedotin with plasma or PBS with 1% BSA for up to 21 Days.
The released concentration of MMAE in all but rat plasma was within 3-fold of those reported in the literature[4].

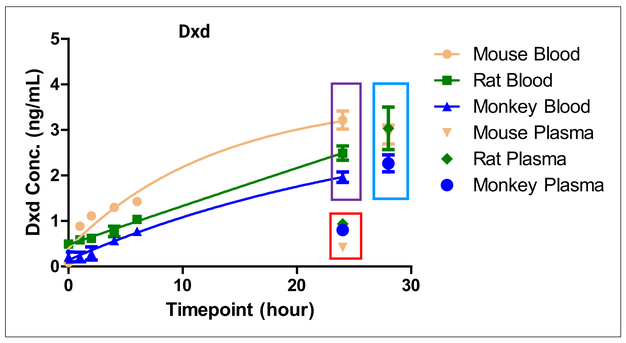

Figure 3 Release of Dxd from DS8201 after incubations of DS8201 with whole blood for up to 24 h. Dxd released in whole blood at 24 h was higher than that in plasma and was similar to that in plasma after 72 h (data points inside the red rectangle: 24 h in plasma, data points inside the purple rectangle: 24 h in blood, data points inside the blue rectangle: 72 h in plasma).

Table 2. Concentrations of released Dxd after incubating DS8201 with plasma for 21 days

Compound ID	Incubation Conc.	Dxd Concentration (ng/mL)				Source
		Mouse	Rat	Monkey	Human	
DS8201	100 µg/mL	54.3±7.4	27.9±7.1	14.9±2.4	34.8±10.6	In-house
		29.0	42.8	85.8	37.4	Literature[5]

- The released concentration of Dxd in mouse plasma was approximately twice of the reported in the literature, while in rats, it was about half. The released concentration in humans closely approximated the values cited in the literature.
- The significantly reduced release of Dxd observed in our in-house monkey plasma study, as compared to values in the literature, could be attributed to different sources of plasma.
- In general, the ±3-fold range of payload concentration was acceptable based on the relative error of this assay.

Conclusion

1. The plasma with heparin sodium as the anticoagulant was more suitable than EDTA-K_2 anticoagulated plasma since EDTA-K_2 could inhibit the activities of enzymes. There was no clear difference between fresh and frozen plasma, sealed and breathable conditions during incubations.
2. The released concentration of Dxd in whole blood was higher than that in plasma at the same timepoint (such as 24 h), but it was similar to that in plasma at 72 h.
3. The released concentration of MMAE after incubations of Brentuximab vedotin with human and monkey plasma and the released concentration of Dxd after incubation of DS8201 with mouse, rat and human plasma were consistent with those in the literature. Differences in certain species were observed, possibly due to different sources of plasma.

References

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