DETERMINATION OF DRUG-TO-ANTIBODY RATIO OF ANTIBODY-DRUG CONJUGATES BY LBA-LC-MS

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Abstract

The in vivo drug-to-antibody ratio (DAR) value and drug load distribution on an ADC are crucial to the drug efficacy and safety. The evaluation of ADC stability in systemic circulation is usually achieved by characterization of free payload release rate and DAR value. During linker payload screening stage, the payload release rate showed the extent of linker cleavage in plasma, and the change of DAR value gave more information of deconjugation. The commonly used detection methods for DAR value were UV/Vis spectroscopy[2], hydrophobic interaction chromatography (HIC)[3], RP-HPLC[4], et al. Those methods were more compatible with samples with simple components instead of biological samples. There were many challenges in the analysis of DAR value by LC-MS in biological samples.

A highly specific and sensitive LBA-LC-MS method was successfully developed for the DAR value detection of Trastuzumab deruxtecan (T-DXD) in biological samples. The T-DXD was enriched by immunocapture with biotinylated antigen from human plasma and by anti-human IgG Ab from other nonclinical species plasma. After eluting the ADC from the immunocapture beads, 20 mM TECP was add to the elution; a Thermo QE Plus was used to detect the intensity of LC and HC. The intensity of the top three glycosylation HCs were summarized. The average DAR was calculated based on the summarized intensity. The results showed that the DAR values of T-DXD in mouse plasma sample after Day 0, 3 and 7 day incubation were 7.9, 6.8 and 3.6, respectively. The method had been used to evaluate both in vitro and in vivo ADC plasma stability. The workflow of the method can also apply to both conventional conjugated ADCs and site-specific conjugated ADCs, which could help researchers understand stability of ADC much better.

Objectives

To evaluate the systemic circulation stability of ADC, a highly specific LBA-LC-MS method was established to characterize the DAR value of ADCs in biological samples. And an ELISA and a LC-MS/MS method were established to quantify the total antibody and free payload as complementary tools.

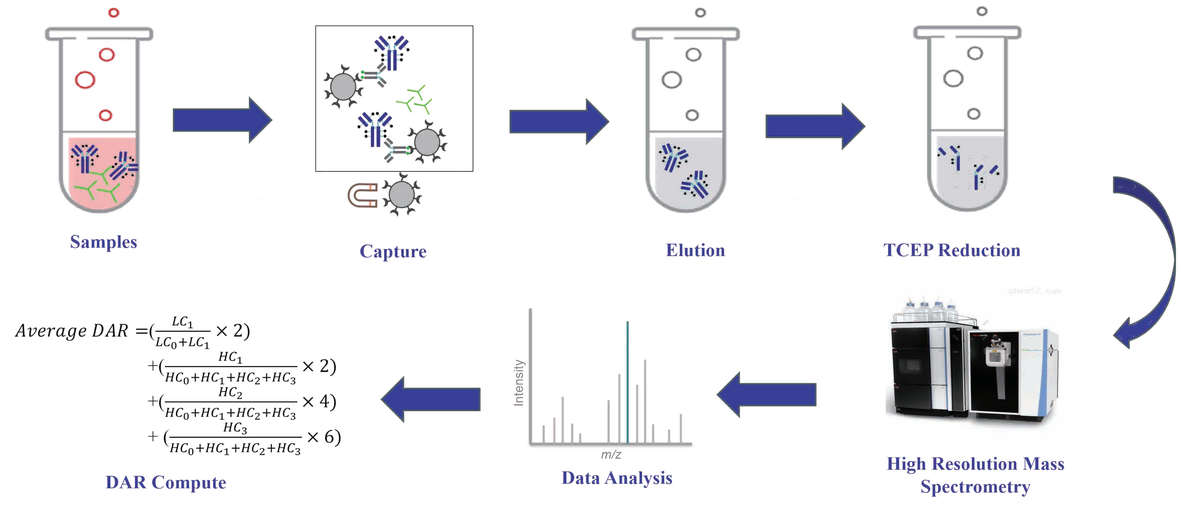

Figure 1. Analysis of ADC drugs in biological samples of DAR value flow chart

Methods

Stability Sample preparation: Dilute T-DXD into mouse plasma to yield a final solution of 100 μg/mL, incubate at 37℃±2℃ for 0 h, 72 h and 168 h.

In vivo administration experiment: 3.00 mg/kg of T-DXD was administered intravenously to mouse, and plasma samples were collected for 5 min, 7 h, 1 day, 3 days, 7 days, 14 days and 21 days after administration.

DAR value detection: T-DXD was captured by biotinylated antigen, eluted the bound ADC with acid, reduced the ADC to light chain (LC) and heavy chain (HC) with TCEP, and the intensity of LC and HC was detected by Thermo QE Plus. The mean DAR values were calculated using the intensity of LCs and the top three glycosylation HCs.

Total antibody quantification: HER2 ECD was used to immunocapture T-DXD, the total antibody was quantified using goat anti-human IgG Fc antibody.

Free payload detection: the LC-MS/MS method was used to determine the concentration of free payload.

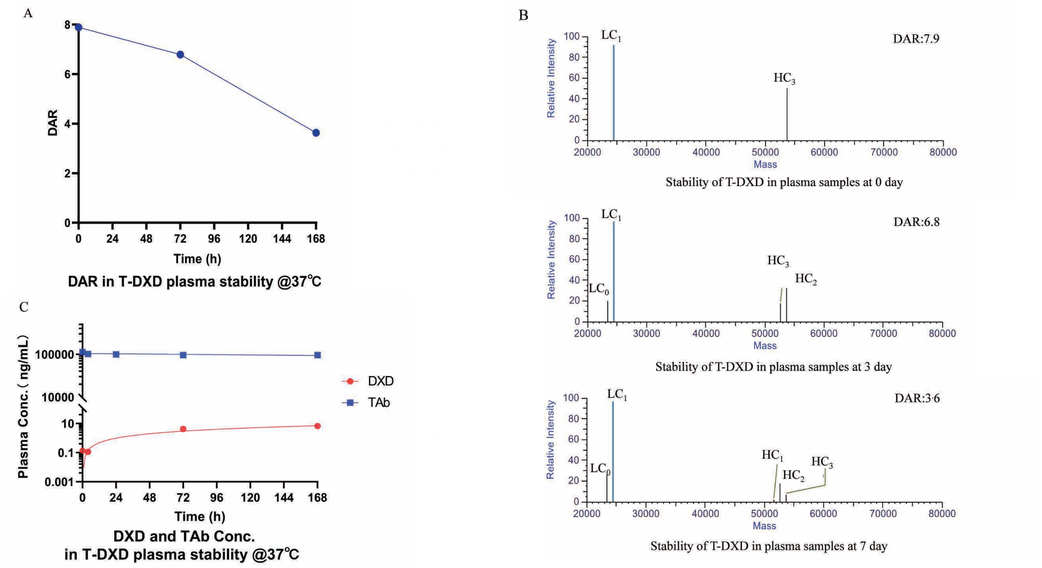

Figure 2. The stability of T-DXD in plasma samples at different time

Results

The in vitro stability of T-DXD was evaluated by measurement of the DAR using LBA-LC-MS, following in vitro incubation of the ADC with mouse plasma for 168 h (Figure 2A). Following the plasma incubation, a time-dependent decrease in the average DAR was observed, decreasing from 7.9 to 3.6. And the increased LC0, HC1 and HC2 intensity could account for the decreased DAR value (Figure 2B).

The free payload and the total antibody were also quantified. The amount of total antibody remained at similar level (from 111.5 ng/mL to 95.4 ng/mL). And the concentration of the free payload increased mildly from 0.13 ng/mL to 6.62 ng/mL, which could not account for the loss of average DAR and further evaluate the circulation stability of ADC (Figure 2C).

The DAR change of T-DXD after intravenous administration to mouse was characterized to evaluate the in vivo stability. Similarly, the average DAR decrease with the time, from 8.0 to 0.05 in 21 days. And in a complementary method, the total antibody was also quantified, which decrease from 42.5 μg/mL to 2.7 μg/mL in 14 days. Besides, the amount of T-DXD total antibody was below the limit of the quantification level in 21 days.

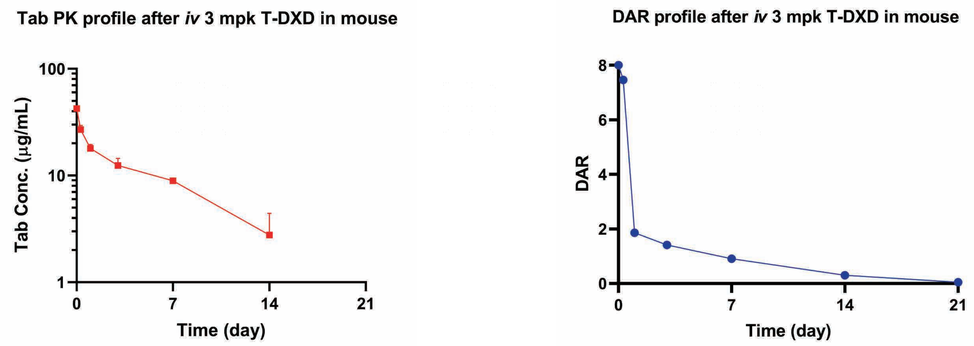

Figure 3. Tab PK and DAR profile after iv 3 mpk T-DXD in mouse (all HR-MS based assay)

Conclusions

We have successfully established a highly specific LBA-LC-MS method and applied it to the detection of DAR in biological samples. This method could be used to evaluate the DAR change in vitro and in vivo for ADCs conjugated to cysteine, including conventional conjugated ADCs and site-specific conjugated ADCs.

References

1. Qin, Qiu, and Likun Gong. "Current Analytical Strategies for Antibody–Drug Conjugates in Biomatrices." Molecules 27.19 (2022): 6299.
2. Wei, Cong, et al. "Where did the linker-payload go? A quantitative investigation on the destination of the released linker-payload from an antibody-drug conjugate with a maleimide linker in plasma." Analytical chemistry 88.9 (2016): 4979-4986.