Laboratory for Pharmaceutical Biology, KULeuven, Belgium

Biochemical characterization of derivatives of MA-T12D11, a TAFI neutralizing antibody

Develter J, Declerck PJ, Gils A

Introduction

- Thrombin Activatable Fibrinolysis Inhibitor (TAFI) provides a link between the coagulation and the fibrinolytic cascade.[1]
- TAFI can be activated to TAFIa by the thrombin-thrombomodulin complex (T/TM).
- TAFIa has carboxypeptidase activity and exerts an antifibrinolytic effect.

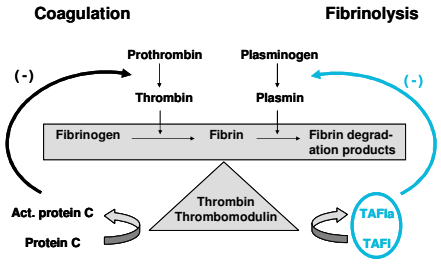

Figure 1 Scheme of blood coagulation and fibrinolysis

- TAFI/TAFIa is a risk factor for thrombosis and related diseases.[2]
- TAFI inhibitors can be a powerful tool in thrombosis models.
- Already known inhibitors of TAFI(a) are not selective or powerful enough:
  - –Chelating agents (e.g. EDTA)
  - –Reduction of disulfide bridges (e.g. DTT)
  - –Arginine and lysine analogs (e.g. GEMSA)
  - –Naturally occurring inhibitors (e.g. Carboxypeptidase inhibitor or CPI)
- Monoclonal Antibodies (MA) towards human TAFI were raised in our lab.
- MA-T12D11 inhibits the activation of TAFI by T/TM.

Objective

To characterize the biochemical properties of antibody fragments derived from MA-T12D11.

Materials & Methods

- Fab-T12D11 was generated by papain digestion of MA-T12D11, followed by protein A purification.
- The scFv-T12D11 fragment was constructed by isolation of the cDNA of MA-T12D11 producing hybridomas, followed by amplification and assembling of V_H and V_L with a $(Gly_4Ser)_3$ linker.
- Affinity between TAFI and the antibody (fragments) was measured with surface plasmon resonance technique.
- The inhibitory effect of MA (derivatives) was tested in a chromogenic assay by activating TAFI with T/TM. Resudual TAFIa activity was determined using a chromogenic assay in which hippuryl-arginine is used as a substrate.
- The profibrinolytic effect of MA (derivatives) was investigated with an *in vitro* clot lysis assay in which $CaCl_2$ was used to start clot formation and tPA to initiate clot lysis.

Results

- The amino acid sequence of the V_H and V_L region of MA-T12D11 is shown in Figure 2. Based on this sequence, a model of scFv-T12D11 was obtained using the AbM software (Figure 3).

- **V_H:**
QVQLQQSGAELVKPGSSVKISCKASGYTFT*DHAIH*WVKQKPE
QGLEWIG*YISPGNGDVKYNEKFKG*KATLTADKSSSTAYMQLN
SLTSEDSAVYFCHH*GNWAAWFAY*WGQGTTVTVSS

- **V_L:**
DIVLTQSPASLAVSLGQRATISC*KASQSVDYDGDDYLN*WYQQ
RPGQPPKLLIY*AASNLES*GIPARFSGSGSGTDFTLNIHPVEEE
DAATYYC*HQSNEDPFT*FGSGTKLEIKR

Figure 2: V_H and V_L chain of MA-T12D11 (CDR regions of V_H and V_L in red and blue, respectively)

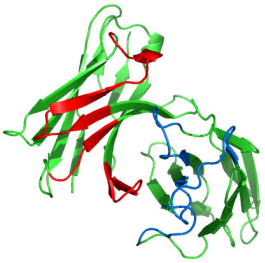

Figure 3: Model of scFv-T12D11, obtained by modeling with AbM software (WAM server)

- The rate constants for association (k_a) and dissociation (k_d) and the affinity constant (K_A) as well as the inhibitory effect of the antibody fragments on TAFI activity was compared to the parental MA-T12D11.

Table 1: Binding parameters (k_a, k_d and K_A) of MA-T12D11 and its derivatives for binding to TAFI

T12D11	MA	Fab	scFv
k_a(1/Ms)	$1.3 \pm 0.32 \times 10^6$	$1.2 \pm 0.38 \times 10^6$	$5.4 \pm 2.8 \times 10^5$
k_d(1/s)	$2.5 \pm 0.28 \times 10^{-4}$	$4.3 \pm 0.33 \times 10^{-4}$	$3.2 \pm 0.46 \times 10^{-4}$
K_A(1/M)	$5.4 \pm 1.5 \times 10^9$	$2.8 \pm 1.0 \times 10^9$	$1.7 \pm 0.75 \times 10^9$

The results represent mean ± SD (n ≥ 3)

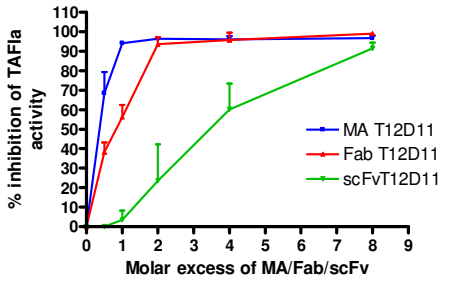

Figure 4: % inhibition of TAFIa activity by MA/Fab/scFv
TAFI was activated with T/TM in presence of MA, Fab or scFv. The results represent mean ± SD (n ≥ 3).

- –Fab-T12D11 and scFv-T12D11 show a similar maximum inhibitory effect as MA-T12D11 i.e. 99.0 ± 0.72 % and 91.4 ± 3.0 % (Fab and scFv, respectively) vs. 96.7 ± 1.3 % for MA.
- –The inhibitory effect of Fab-T12D11 and especially that of scFv-T12D11 shows a shift towards higher concentrations, in accordance to a slightly decreased affinity towards TAFI (table 1).

- Effect of MA-T12D11 and Fab-T12D11 in clot lysis. This effect is compared to that of CPI (a non-specific TAFIa inhibitor).

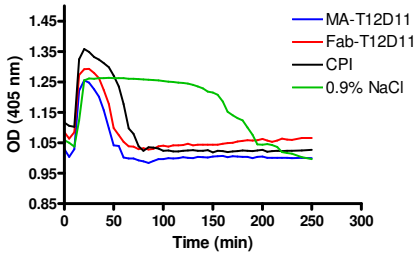

Figure 5: Profibrinolytic effect of MA, Fab and CPI during *in vitro* clot lysis
Clot formation and dissolution is shown in presence of 8-fold molar excess of MA-T12D11 (•), 8-fold molar excess of Fab-T12D11 (•), 45-fold molar excess of CPI (•) and 0,9% NaCl (negative control, •). All concentrations are final concentrations.(n = 3). Note: concentration of scFv-T12D11 was too low to test it in a clot lysis assay.

- –MA, Fab and CPI shorten clot lysis time significantly (i.e. 28.3 ± 2.9 min, 29.2 ± 10 min and 34.3 ± 1.2 min, respectively) vs. 137 ± 25.2 min for the negative control, demonstrating the powerful effect of both the MA and Fab fragment.

Conclusions

- scFv-T12D11 as well as Fab-T12D11 have similar affinity constants as the parental MA.
- Fab-T12D11 has similar functional effects in the chromogenic assay and in the clot lysis.
- scFv-T12D11 has a similar functional effect in the chromogenic assay.

1 Bajzar, Thrombin activatable fibrinolysis inhibitor and an antifibrinolytic pathway. Arterioscler Thromb Vasc Biol. 2000; 20: 2511-8. Review
2 Leurs and Hendriks, Carboxypeptidase U (TAFIa): a metallocarboxypeptidase with a distinct role in haemostasis and a possible risk factor for thrombotic disease. Thromb Haemost. 2005; 94: 471-87. Review