Transferrin receptor binding BBB-shuttle facilitates brain delivery of a therapeutic Aβ-antibody: A mouse whole-brain 3D light sheet imaging study

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Background & Aim

Amyloid β (Aβ)-directed antibodies, including aducanumab, have recently been approved for the treatment of early-stage Alzheimer's disease (AD). Improved CNS access of Aβ-directed antibodies may potentially increase therapeutic benefits while also reducing dose-dependent adverse effects of this drug class. Using a high-throughput light sheet fluorescence microscopy (LSFM) pipeline, the present study aimed to 3D map and quantify whole-brain distribution of a blood-brain barrier (BBB) shuttle-enhanced Aβ antibody in a transgenic mouse model of AD.

Methods

See Fig. 1 for study outline. 7-11 month-old transgenic mice expressing disease mutant forms of human APP and PSEN1 (AD mice, C57BL/6NTac.CBA-Tg(Thy1-PSEN1*M146V,-APP*Swe)10Arte) received a single infusion (50 nmol/kg, i.v.) of Aducanumab biosimilar (AduBS; n=4), Aducanumab biosimilar combined with anti-mouse transferrin receptor (TfR) BBB-shuttle (AduBS-BBB; n=4), or Control hIgG (n=6). Mice were terminated 48h after the infusion. Other ARTE10 mice were administered (10 or 50 nmol/kg, i.p.) AduBS (n=8 or 10), AduBS-BBB (n=9), or Control IgG (50 nmol/kg, n=8) once weekly for 12 weeks. Intact hemispheres were stained with an antibody against hIgG, cleared and LSFM-scanned for AI-based automated anatomical mapping and quantification of compound distribution (fluorescence intensity in 840 individual brain regions) using a custom mouse brain atlas.

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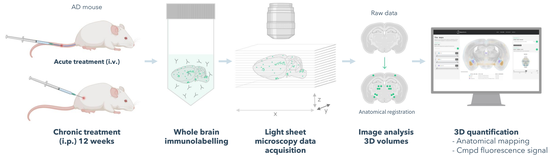
1 Automated whole-brain imaging pipeline



Study 1	Group	Treatment	Number of animals	Termination
Acute treatment (i.v. infusion)	1	Control hIgG	6	48h post-dosing
	2	AduBS 50 nmol/kg	4	
	3	AduBS-BBB 50 nmol/kg	4	

Study 2	Group	Treatment	Number of animals	Termination
Chronic treatment (i.p., once weekly for 12 weeks)	1	Control hIgG	8	72h post-dosing
	2	AduBS 10 nmol/kg	8	
	3	AduBS 50 nmol/kg	10	
	4	AduBS-BBB 10 nmol/kg	9	

Figure 1. Automated quantitative 3D light sheet fluorescence microscopy imaging workflow. The pipeline was applied to evaluate whole-brain distribution of Aducanumab biosimilar (AduBS) and Aducanumab biosimilar combined with anti-mouse transferrin receptor (TfR) BBB-shuttle (AduBS-BBB) upon acute and chronic dosing in a transgenic mutant hAPP/PS1 mouse model of Alzheimer's disease (AD mouse).

2 BBB shuttle-enhanced brain delivery: Acute dosing

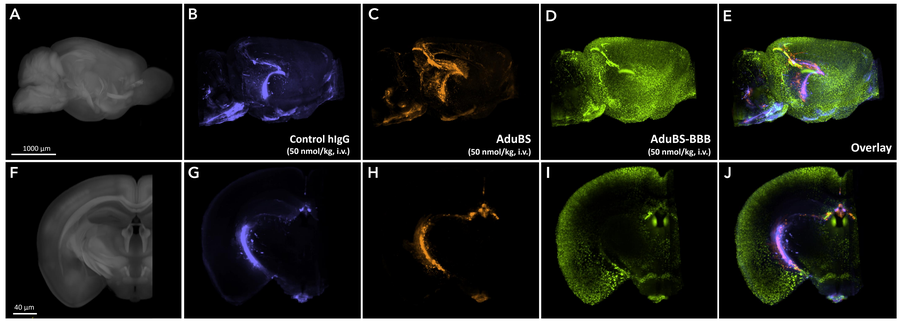


Figure 2. BBB-shuttle enhanced brain delivery of Aducanumab biosimilar in AD mice following acute dosing in AD mice. 3D LSFM-imaged whole-hemisphere. While Control hIgG and Aducanumab biosimilar (AduBS) predominantly distribute to the choroid plexus, AduBS fused with anti-mouse TfR BBB-shuttle (AduBS-BBB) demonstrates markedly enhanced brain delivery upon acute administration. Sagittal (A-E) and coronal view (F-J). **(A, F)** Custom mouse brain atlas. **(B, G)** Control IgG. **(C, H)** AduBS. **(D, I)** AduBS-BBB. **(E, J)** Overlay.

3 Quantitative analysis: Acute dosing

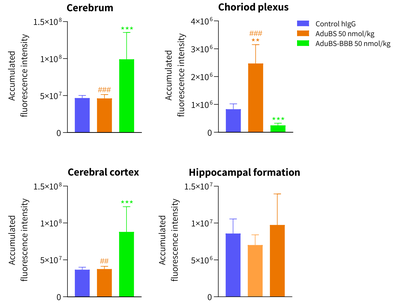


Figure 3. BBB-shuttle enhances brain delivery of Aducanumab biosimilar following acute dosing in AD mice . Quantitative 3D LSFM analysis. Accumulated fluorescence intensity (arbitrary units) in selected major brain regions. **p<0.01, ***p<0.001 vs. Control hIgG 50 nmol/kg; ##p<0.01, ###p<0.001 vs AduBS-BBB 10 nmol/kg (Dunnett's test negative binomial generalized linear model).

4 BBB shuttle-enhanced brain delivery: Chronic dosing

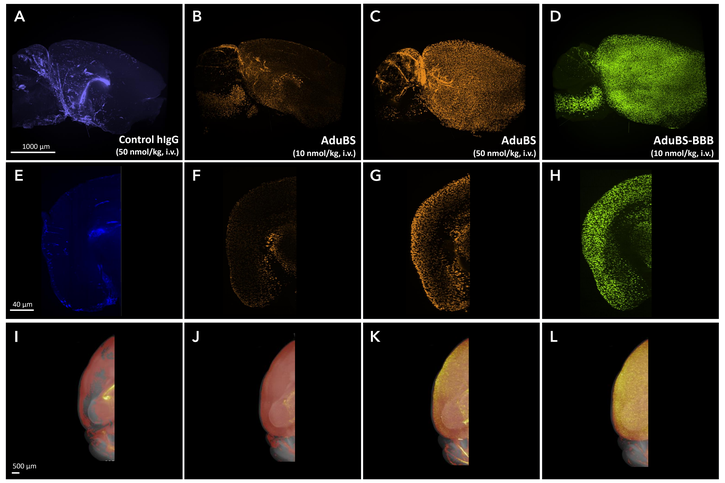


Figure 4. BBB-shuttle enhanced brain delivery of Aducanumab biosimilar following chronic dosing in AD mice. 3D LSFM-imaged hemisphere. Control hIgG is predominantly localized to the choroid plexus. AduBS (10 and 50 nmol/kg) shows dose-dependent brain delivery. Compared to AduBS, AduBS-BBB (10 nmol/kg) demonstrates enhanced brain delivery. See Figure 5 for quantitative analysis of LSFM-imaged brain hemispheres. Sagittal (A-D) and coronal (E-H) view. Transverse view of group-averaged compound distribution (I-L). **(A, E, I)** Control hIgG. **(B, F, J)** AduBS 10 nmol/kg. **(C, G, K)** AduBS 50 nmol/kg. **(D, H, L)** AduBS-BBB 10 nmol/kg.

5 Quantitative analysis: Chronic dosing

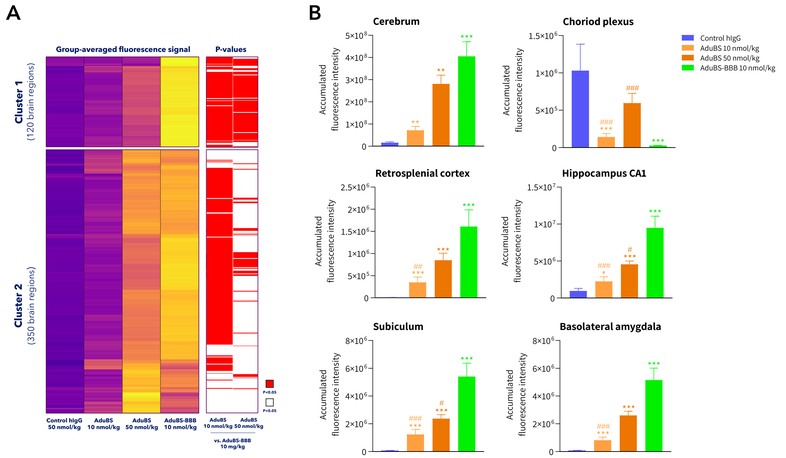


Figure 5. BBB-shuttle enhances brain delivery of Aducanumab biosimilar in a substantial number of brain regions following chronic dosing in AD mice. Quantitative 3D LSFM analysis. **(A)** Heatmap of a total of 470 individual brain regions with significantly increased fluorescence signal in AduBS (50 nmol/kg) or AduBS-BBB (10 nmolkg) treated groups relative to Control hIgG (50 nmol/kg). **(B)** Accumulated fluorescence intensity (arbitrary units) in selected brain regions. **p<0.01, ***p<0.001 vs. Control hIgG 50 nmol/kg; #p<0.05, ##p<0.01, ###p<0.001 vs AduBS-BBB 10 nmol/kg (Dunnett's test negative binomial generalized linear model).

Conclusion

- TfR BBB-shuttle enhances brain delivery of Aducanumab biosimilar (AduBS) in a transgenic mouse model of Alzheimer's disease
- AduBS-BBB distributed to brain areas with severe Aβ plaque load, notably in the cerebral cortex
- In a chronic dosing regimen, similar brain delivery efficiency was achieved using a five times lower dose of AduBS-BBB compared to AduBS
- Quantitative 3D LSFM imaging is ideal for mapping and quantifying distribution of therapeutic antibodies in the intact mouse brain at micrometre resolution

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