Pre-existing Anti-Adeno-Associated Virus (AAV) Serotype 5 Neutralizing Antibodies (NABs) Titers In Minipig Serum Do Not Reflect Levels Of Anti-AAV5 NABs Titers In Their Cerebrospinal Fluid (CSF)

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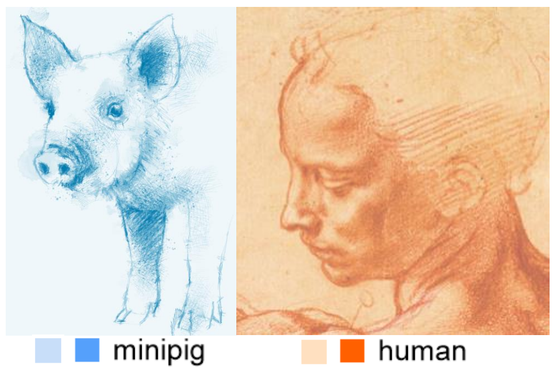
BACKGROUND

- AAV-based therapies generated promising pre-clinical results translating into phase I clinical trials for several neurodegenerative diseases [1].
- Even low levels of anti-AAV2 or anti-AAV8 NABs have been related to a decrease or total impairment of AAV liver transduction in NHPs and humans when delivered intravenously [2, 3].
- We have previously reported that anti-AAV5 NABs titers up to 340 in humans and as high as 1030 in primates did not interfere with the therapeutic efficacy of intravenously administered AAV5 vector [4].
- It has been demonstrated that limited amount of antibodies present in the circulation cross the blood-brain barrier and reach the Central Nervous System (CNS) (~0.6%). In mice, circulating anti-AAV2/1 NABs at levels typically found in the general human population did not prevent AAV2/1-mediated gene delivery to the mouse CNS. However, presence of relatively high levels of circulating anti-AAV2/1 NABs impaired the gene transfer to CNS [5].
- Therefore, it is of importance to determine whether naturally acquired pre-existing systemic immunity to AAV5 (serotype used by uniQure for Huntington's Disease program) would affect the therapeutic efficacy of AAV5 vector delivery to the CNS in larger animal model and humans.

OBJECTIVES

Minipigs are large brain animals that are used as models for proof of concept studies for development of treatment for neurogenerative disorders. Their brain size and structure surely better model the human brain than rodents. Therefore, we investigated the levels of pre-existing anti-AAV5 NABs in serum and in CSF in a cohort of 30 minipigs.

Furthermore, to extrapolate our minipig serum sample screening to humans, we have performed an anti-AAV5 NABs prevalence study in healthy donor serum (n=350).



METHODS

Serum and CSF samples were collected from 30 minipigs (Pigmod, Libechov, Czech Republic). Healthy human donor samples (n=350) were commercially obtained (BioreclamationIVT). All the samples were analyzed for the presence of anti-AAV5 NAB with the use of anti-AAV5 NABs luciferase-based bio-assay. The assay entails incubation of the test samples (sera or CSF) dilution series with an AAV5-based reporter vector that carries the luciferase (LUC) gene. This incubation allows neutralizing antibodies in the test serum to bind to the reporter vector particles. These mixtures are subsequently transferred onto HEK293T cells, where reporter vector particles can transduce cells and mediate expression of luciferase. Anti-AAV5 NABs titer is determined by calculation of the percentage of neutralization for each sample dilution and fitting the neutralization curve with a four-parameter method. Anti-AAV5 NABs titer (IC50) is the dilution at which antibodies inhibit Hek293T cell transduction by AAV5-LUC by 50%.

RESULTS

- Serum samples of minipigs had detectable levels of anti-AAV5 NABs that varied between a titer of 2 (negative) to 256 (positive). However, CSF samples of all the minipigs tested were found negative for the presence of anti-AAV5 NABs. Hence, no detectable anti-AAV5 NABs were detected in CSF of animals that had systemic pre-existing anti-AAV5 NABs titers up to 256 (**Table 1**).

Table 1. anti-AAV5 NABs titers in minipig serum and CSF

Animal Number	anti-AAV5 NAB titer	
	Serum	CSF
1	75	<2
2	59	<2
3	113	<2
4	14	<2
5	237	<2
6	2	<2
7	35	<2
8	4	<2
9	256	<2
10	9	<2
11	21	<2
12	5	<2
13	35	<2
14	52	<2
15	4	<2
16	13	<2
17	20	<2
18	6	<2
19	3	<2
20	3	<2
21	5	<2
22	13	<2
23	16	<2
24	27	<2
25	191	<2
26	9	<2
27	20	<2
28	45	<2
29	42	<2
30	144	<2

- In order to gather more information on the prevalence and levels of anti-AAV5 NABs in circulation of humans, 350 serum samples from healthy donors were screened for anti-AAV5 NABs presence and its level. No anti-AAV5 NABs titers were detected in 153 out of 350 donors (43.7%), 197 donors had titers that were above 2 (56.3 %), with mean and median titers of 355 and 41 respectively **(Figure 1)**.
- No anti-AAV5 NABs were detected in CSF of minipigs that had systemic pre-existing anti-AAV5 NABs titers up to 256. Out of 350 analyzed human serum samples from our prevalence study, 304 donors had anti-AAV5 NABs titers lower than 256 (87%) **(Figure 2)**.
- Our previously reported data in non-human primates indicate that anti-AAV5 titers up to 1030 and in humans up to 340 are compatible with efficient systemic AAV5-based gene delivery. 89% of 350 donors had anti-AAV5 NAB titers <340, while 95% of all donors presented NABs titer <1030 **(Figure 2)**.

Figure 1. anti-AAV5 NABs titers distribution in human and minipig serum samples positive for anti-AAV5 NABs

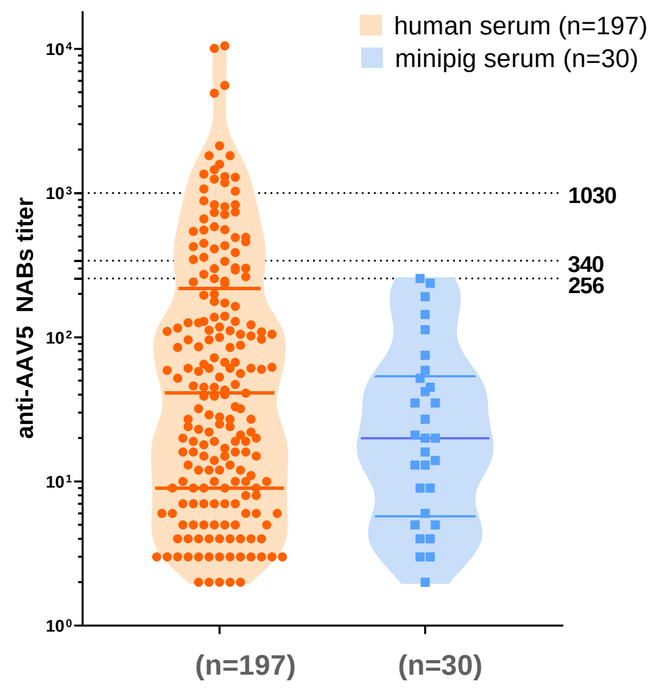
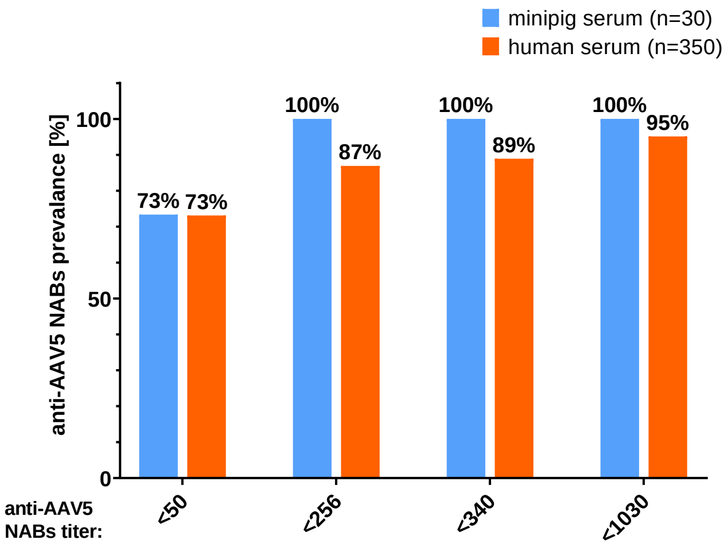


Figure 2. anti-AAV5 NABs prevalence in human and minipig serum samples



CONCLUSION

Anti-AAV5 NABs titer in minipig serum up to 256 does not result in any detectable anti-AAV5 NABs titer in CSF of the same animal.

We have previously reported that anti-AAV5 NABs titers up to 340 in humans and as high as 1030 in primates did not interfere with the therapeutic efficacy of intravenously administered AAV5 vector [5].

Based on the above findings, we conclude that the risk for reduced therapeutic efficacy of intrathecal or intraparenchymal administration of therapeutic AAV5 vectors due to pre-existing neutralizing antibodies against AAV5 is very low.

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