Crossing barriers, delivery of llama antibody fragments into the brain

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SUMMARY

Alzheimer's disease (AD) is the main contributor to the syndrome dementia, one of the major health care concerns in the developed, as well as the aging developing, world. AD is characterized by progressive cognitive impairment and characteristic pathological hallmarks. These hallmarks include aggregates of the protein tau in neurons and of the peptide amyloid beta (Aβ) in plaques outside the neurons as well as around the blood vessels in the brain. Currently there is no drug available that can prevent or cure AD. One of the reasons of the difficulty in developing a drug based cure, is the presence of a very strictly regulated barrier between the brain and the circulation; the blood-brain barrier. Potential therapeutics need not only be effective; they also need to be delivered into the brain, crossing this barrier. Recent clinical trials mainly focus on the use of antibodies that bind the Aβ peptide, aiming to reduce the amount of aggregates either through prevention of build-up or reduction of existing plaque burden. In this thesis we have been focussing on the development of a special type of antibody, the llama heavy chain only antibody fragment VHH. As described in detail in chapters 1 and 6, llamas and other members of the camelid family have an additional group of antibodies that differ from the universal antibody; these camelid antibodies lack the light chain. The VHH is the part of this special antibody that binds the antigen. VHH have a number of benefits: for example, they are 10 times smaller than conventional full length antibodies, they are easier to produce, more stable in terms of temperature and pH and can be modified to fit a wide range of applications. One such application is the detection and prevention of protein aggregation by certain selected VHH. This makes VHH interesting candidates for various therapeutic angles in AD research. The first aim of this thesis is to determine the potential of our previously selected VHH in the fight against AD. The second aim is to find ways in getting VHH into the brain, across the blood-brain barrier.

Chapter 1 contains a general introduction to AD as well as a basic explanation of the selection, generation and application of VHH. Next, chapter 2 focusses on the use of VHH to intervene in the production of the Aβ peptide. The proof of principle was demonstrated using VHH selected against the function of the beta-secretase BACE1. One VHH, VHH-B3a, was shown to inhibit the activity of the secretase by 50%, which in turn results in a reduction in production of Aβ. This inhibition was shown in an *in vitro* assay, and then confirmed in a cellular assay. The effect of the inhibition, i.e. reduction of Aβ production, was finally demonstrated in a living mouse model for AD. The results of VHH-B3a are promising, and the therapeutic value of this reduction should be confirmed in behavioural studies. However, to get VHH-B3a into the brain, a crude method of direct injection into the cerebral ventricles was chosen. This method is effective, as shown in chapter 2, but is not ideal as a long term therapeutic application. Although

VHH-B3a has great potential as a BACE1 inhibitor, no VHH is of use without minimally invasive, yet efficient delivery across the blood-brain barrier. For this reason, chapters 3, 4, and 5 all focus on various methods of delivery. The VHH selected to test the delivery methods is VHH-pa2H, a VHH selected previously against Aβ aggregations. VHH-pa2H has a very high affinity for Aβ aggregates.

In chapter 3 we tested the ability to deliver the VHH as a cargo in liposomes that are targeted to a protein present on the blood-brain barrier. We tested two different types of liposomes; EYPC and DMPC. The use of DMPC did not increase the delivery of the VHH to the brain. However, encapsulation in EYPC did increase the delivery of VHH-pa2H into the brain, and the VHH accumulated in the brains of the mouse models that present Aβ plaques onto which VHH-pa2H can bind. To follow the presence of the VHH in the brain, we used a radioactive particle that we bound to the VHH after it was encapsulated in the liposome. This allowed us not only to follow only the VHH, rather than the liposomes which happens more commonly; it also allowed to us to make the liposomal encapsulated VHH in advance and attach the radioactive label just before use. This increases the shelf-life of the VHH-liposome construct which can be crucial in case of therapeutic use of a radioactively labelled VHH. Brain delivery of VHH in EYPC liposomes seems to be a possibility that can be employed for VHH-pa2H and undoubtedly other VHH.

We then went on to determine if it is possible to let the VHH cross the blood-brain barrier without the help of liposomes. One of the down sides of being small is that the VHH are rapidly cleared from the blood after injection. We therefore reasoned that if the VHH is able to interact with the barrier for a longer period, it might be able to cross the barrier via an active process. To allow more time for interaction, we attached the tail of a conventional antibody to the VHH, as described in chapter 4. Adding the tail indeed increased the time that the VHH spend in the blood significantly. However, not more of the VHH with the tail was delivered into brain compared to the VHH without the tail. We therefore concluded that although the addition was successful, more time in the blood alone did not help to let VHH-pa2H cross the barrier.

Finally, in chapter 5 we made use of the fact that the genetic information of the VHH is contained is such a small piece of DNA, that we can easily package this in a small viral particle, called Adeno Associated Virus, or AAV. We injected the AAV into the brain of the mouse models, allowing them to continuously produce the VHH themselves right in the brain, exploring a form of gene therapy. Using a special signal sequence, the VHH is secreted from the neuronal cells that are producing them, allowing it to bind the Aβ plaques that are in the brain but outside the cells. Additionally, we attached a special fluorescent protein to the VHH, allowing us to track the secreted VHH in real time using an *in vivo* microscopy technique. By giving the

viral treatment right after birth, it seems that VHH-pa2H may reduce the total plaque burden over time; however, this effect is not seen when given to older animals. However, the number of very young animals treated with the VHH is very limited and should be increased to make a definitive conclusion. In the older animals, expression of the VHH in the brain of the living mice is easily seen using the *in vivo* microscope. Only VHH-pa2H and not the control VHH, are able to bind the amyloid plaques. All in all, viral delivery of VHH, using the AAV particles, is an excellent and efficient way to deliver the antibody fragments into the brain. Future research should focus on the optimization of delivery of VHH via AAV, using for example slightly different versions of AAV, or finding the optimal age for injection. It should also focus on the long term benefits of delivering VHH, such as the anti-amyloid VHH-pa2H or BACE1 inhibiting VHH-B3a, to the brain of the mouse models for AD. VHH such as these hold great promise in the fight against neurodegenerative diseases like AD and viral delivery of VHH seems to be an outstanding method to ensure the crucial presence of VHH on the brain side of the blood brain barrier in this fight.