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Adenoviruses for better brain function

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Abstract. Using adenoviruses for therapy became well accepted due to some vaccines against COVID-19 pandemic. Actually, not the native viruses, but a recombinant form lacking fertility—a so called vector—is used with the aim to transfer genetic materials to adult cells. Decades of research preceded this development. Although the most important field of utilization is the vaccines, but neurological and even psychiatric disorders may also benefit from viral vectors. Neurons seem to be especially convenient target for viral vectors as they are considered as non-dividing cells. Extra genetic information provided by the viral vectors is not integrated into the genome rather remains in the cytoplasm as plasmid. During repeated cell divisions the cell loses this extra information, it is “diluted” and finally disappears. It is not the case in the slow dividing cells including neurons. For preclinical research purposes canine adenovirus 2 is widely used. It is a retrograde transporter therefore especially good in marking specific pathways between remote brain areas and providing a useful tool for functional brain mapping. Therapeutic usages include restoration of missing enzymes or parts of sensory systems and treatment of cancer. All in all, at present most of the studies on brain utilizing adenoviral vectors are still in preclinical phase and focus on studying mechanisms. However, we should be aware of this important topic, which may be an everyday therapy in the future.

Keywords: adenovirus, vaccine, cancer, Parkinson’s disease, mechanisms.

Introduction

For decades viruses were considered as harmful, pathogen agents. However, recently using adenoviruses for therapy became well accepted due to some vaccines against COVID-19 (like Gamaleya (Sputnik V, Gam-COVID-Vak), AstraZeneca, Johnson & Johnson). However, people may still be concerned with safety issues. Especially the long-term consequences seem to be problematic as there was not enough time for proper, long-term clinical trials with these vaccines. To soothe the feeling of uncertainty that arose due to this question here we briefly summarised decades of research results on adenoviral vectors with the main focus on the nervous system.

Why viruses?

In the debate of nature or nurture genes always were considered substantial. Nowadays for the

development of disorders the Engel’s biopsychosocial model (Engel 1978) (also known as three hit theory (Daskalakis et al. 2013)) is well accepted, with major genetic contribution to health and disease. Using knockout animals (especially mice) to reveal the role of specific genes was a big breakthrough. However, lifelong deficiency leaves much room for development of alternative routes and compensation. Moreover, it cannot be really used for therapy. Therefore, genetic engineering of adult cells was utmost need. Since the discovery of viruses we know that they can reprogram adult cells adding their own genetic information to them. The development of gene technology allows us to modify these viruses removing genes responsible for replication and disease and replacing them with useful genetic information.

Adenoviruses (Ad) are widespread (there are more than 200 types) and although several (more than 90) types are capable of infecting humans, most of the infections are asymptomatic or mild

upper respiratory symptoms, conjunctivitis or gastroenteritis (Junyent, Kremer 2015). Only neonates or those with immunodeficiency may develop fatal symptoms. Adenoviruses can carry up to 30kb genetic information, which are relatively big and sufficient for a lot of purposes.

Why the brain?

Our skull is like a sealed can, the material injected into it will most likely not come out. Therefore, using viral vectors in the brain seems to be rather safe, making these kinds of experimentations attractive. However, this feature may also be a limitation of clinical treatments for neurological and psychiatric disorders, as viral vectors need to be delivered directly to the brain. Moreover, since they are non-reproducing viruses (lacking genes necessary to replications), they can only act on a small area of the brain, where they are injected.

Another attractive feature of our neurons for gene transfer is that they do not (i. e., only to a small extent) divide. Recombinant adenoviruses are unlikely (only with 10^{-5} – 10^{-7} /cell frequency) to be incorporated into the genome of the host (Stephen et al. 2010). The genetic information of the vector will be present in the cell as an extrachromosomal element, which will be “diluted” and maybe even lost during serial divisions. Although from the 80s it is widely accepted that there is a neurogenesis in our brain (Goldman, Nottebohm 1983; Nottebohm 1985), it only takes place in special areas (subventricular) (Kumar et al. 2019) and rather slowly. Therefore, neurons seem to be an especially ideal target for viral vector treatments. It was already confirmed that permanent phenotypic changes (at least for a year) can be induced in neurons by a single injection of viral vectors (Bienemann et al. 2003; Geddes et al. 1997; Ideno et al. 2003). These studies utilized the vasopressin-deficient Brattleboro rats having diabetes insipidus thereby drinking and urinating a lot. This feature makes it very convenient for studying the effectiveness of a gene therapy as only a drop in water consumption should be measured.

Safety

The first question that arises in everyone is how safe these vectors are, since they are viruses. Although for research purposes canine pathogens are used extensively, some vaccines contain adenoviruses specific for chimpanzees (AstraZeneca), while some contain even human pathogens (Sputnik V, Johnson & Johnson). Be aware, that these are not native, but recombinant viruses unable to replicate and induce diseases.

Using as a vaccine it is important that these virus vectors more or less activate our immune system (Lee et al. 2017). In all cases, the efficiency of gene transfer is impaired if the recipient’s organism has previously been infected, i. e., it already has an antibody against the vector. 90% of people are protected against some human adenovirus (mainly against Ad5 serotype), but some other serotypes (for example Ad26) are uncommon in Europe. Accordingly, repeated administration of the same serotype may be problematic due to the appearance of neutralizing antibodies (see different serotypes of Sputnik V vaccine, rAd26 and rAd5). For modification of the brain function, the immunogenic properties of the adenoviral vectors are not favorable. Therefore, non-human pathogen viruses should be used.

Indeed, in neurological research the most commonly used adenovirus is the canine adenovirus type 2 (CAV-2), which is a non-human pathogen (Junyent, Kremer 2015). Its attenuated version was used to vaccinate dogs since the 80s (against the more infectious variants e. g. CAV-1) (Curtis et al. 1978), making it the best known non-human adenovirus. Further CAV-2 developments began with the idea that humans are not immune to it, meaning gene transfer can be more efficient with them (Bru et al. 2010). This idea has also been demonstrated for both humoral and cellular immune responses, but unfortunately some cross-reactivity with antibodies to human adenoviruses may occur in vivo.

Experimentally administered CAV-2 primarily infects neural elements despite various route of administration (intramuscular, intranasal, intracerebral). This makes it an important element of nervous system research and therapies. Although CAV-2 administration can activate the innate immune response not only in host dogs, but also in humans or experimental mice and rats, it is relatively non-immunogenic and does not interfere with neuronal homeostasis. Adenoviruses enter cells through the well-known coxsackievirus and adenovirus receptors (CARs), which have at least two splice variants and two corresponding isoforms in humans. In addition to the epithelium (see primary respiratory tract infection), such receptors are widely present in the nervous system, but are also found in the heart (Junyent, Kremer 2015). Because CAR is expressed also in humans, adenoviruses are the most effective means of introducing genes into us (Lee et al. 2017).

As we interfere with the genetic material (the adenovirus is a double-stranded DNA virus), we also reprogram the cell cycle. Thus, even cancerous degeneration may occur. Indeed, for example, natural herpes viruses are responsible for

a number of cancers, and administration of some retroviral vectors has in some cases been genotoxic (incorporated into chromosomes and damaged them), specifically causing leukemia (Stephen et al. 2010). However, with genetic modification, artificial herpes viruses can also acquire oncolytic properties and thus be excellent for use in anti-cancer therapy (Yura 2017). That is, the carcinogenic effect of vectors depends largely on the genes it introduces. Adenovirus vectors, also provided with tumor-specific promoters, may selectively attack and kill cancer cells (“tumor-specific adenoviral vectors”) (Jooss, Chirmule 2003). That is, the cytolytic (cell-killing) property of viruses is particularly useful in this case. But sequences that inhibit DNA synthesis can also be introduced into tumor cells using viral vectors (Huang et al. 2007).

Another important note is that only those segments of DNA that help the adenovirus to enter the host cell and express the delivered genes remain intact in the recombinant viral vector (hence they were also called “gutless” viruses). Thus, the vectors do not carry the pathological characteristics of the original virus and the specific disease(s) cannot be developed.

It can also be reassuring from a security point of view that decades (since the early 1990s) experience

was gained on therapeutic usage of adenovirus vectors (Junyent, Kremer 2015; Lee et al. 2017). That is, the claims that they are non-toxic and the dosages and routes of administration considered to be the safest are well-developed and well-founded (Junyent, Kremer 2015; Lee et al. 2017).

CAV-2 as one of the best vectors in neuroscience

One of the goals of preclinical research is to better understand physiological and pathological processes. In this respect, CAV-2 is “the best friend of neuroscientists” (Junyent, Kremer 2015).

CAV-2 is retrogradely transported along the axons to the cell body of neurons. Most likely, this can be explained by the fact that its receptor, CAR, occurs primarily at the axon terminal, in the presynaptic fraction (Planul, Dalkara 2017). It is transported in endosomes, intracellular organelles, which provide protection even during long transport (Bru et al. 2010). This feature can be used to study the relationships between brain areas and provide a tool for manipulating these connections. For example, we may inject a CAV-2 vector containing a recombinase enzyme (Cre) to the target area (near the axon) (Fig. 1). At the same time with the help

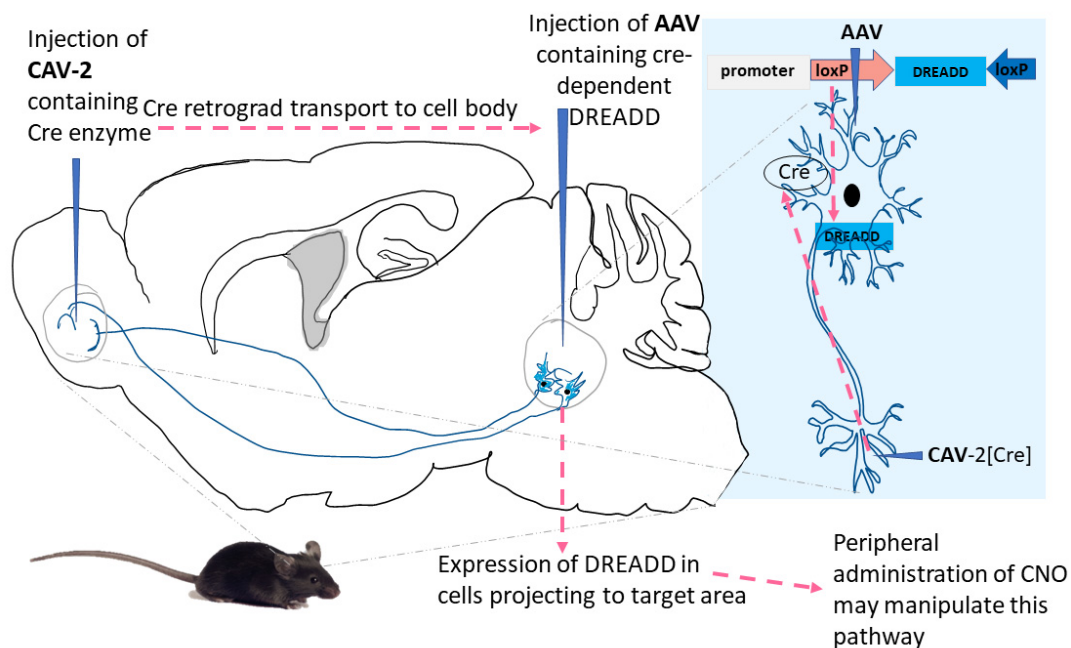


Fig. 1. Manipulating neuronal pathway with the help of an adenoviral vector. CAV-2 (canin adenovirus serotype 2) transfer Cre recombinase enzyme from the axon terminals to the cell bodies. Neurons sending axons to this area will contain this enzyme and—only!—in these cells a DREADD (designer receptor exclusively activated by designer drug) receptor will be expressed which was delivered here with the help of a direct injection of a AAV (adenoassociated virus vector) between two loxP locuses (recognition sites of the Cre enzyme). After viral injection at least 4 weeks have to be pass before all these genetic modifications occur. Finally, during tests this specific pathway may be manipulated (stimulated or inhibited depending of the nature of DREADD) by peripheral (mostly intraperitoneal, or per os with the drinking water) administration of clozapine-N-oxide, the arteficial specific ligand of these receptors.

of another viral vector (mostly adeno-associated virus vector, AAV) a specific ligand-sensitive artificial receptor (designer receptor exclusively activated by designer drug; DREADD) between two gene locus (loxP) will be delivered to the cell bodies. The CAV-2 will transfer the Cre from the target area to the cell body where it will recognize the loxP locus and consequently the DREADD sequence will be converted into a correct reading direction and will be expressed. Thus, only neurons projecting to this specific area will express DREADD and can be manipulated (stimulated or inhibited depending on the type of DREADD) with its specific artificial ligand (clozapine-N-oxide). This method can be used for *functional mapping of neural networks*. By inserting a fluorescent signaling protein into the AAV in place of the artificial receptor and even labeling the cell body inputs with another rabies virus vector, we can map the inputs and outputs of the same neuron at the same time (TRIO: Tracing the Relationship Between Input and Output) (Planul, Dalkara 2017). This technique takes advantage of the specific properties of adeno-, AAV and rabies viral vectors (e. g. antero- or retrograde propagation).

Another possible field of application is the study of the *etiology and progression of diseases*, for example by animal models created with the help of viral vectors. Parkinson's disease is a highly researched disease, not only because of its relative high prevalence, but also because of its well-known, defined brain disease (characterized by the selective destruction of dopaminergic cells in the substantia nigra). In this case, for example, CAV-2 vectors can be used to model the course of the disease by introducing a putative protein, a mutant gene, that leads to neuronal death (Junyent, Kremer 2015; Planul, Dalkara 2017).

Virus vectors for therapy: Preclinical research

Perhaps an even more important preclinical research direction is the search for possible therapies. Because viral vectors can be used to deliver genetic information into cells, their obvious potential use is to correct gene defects with gene therapy, i. e., to use genetic material as a medicine.

Due to the easy accessibility of the *eye*, it is at the forefront of the viral vector research (Planul, Dalkara 2017). However, most cells of the eye cannot be easily reached by adenoviruses, only by the much smaller (approximately 20nm vs. 100nm adenovirus) AAVs (beyond synthetic vectors). AAVs are also popular vectors because they have no known pathological properties and are unable to proliferate on their own, even in their native form

(i. e., without recombination). Because of all this, they can be used with greater safety and fewer side effects (e. g., almost without activating the immune system). In their case, however, the size of the genetic material to be introduced will be limited (about 5kb for AAV, while for adenoviruses it can be up to 30kb) (Del Rio et al. 2019). Therefore, adenovirus vectors still have their own field of application (Lam et al. 2014).

Although vision is our most important sensory organ, *hearing* loss affects also hundreds of thousands of people worldwide (Richardson, Atkinson 2015). A common cause of deafness is the destruction of sensory cells (hair cells) in the cochlea, which can be replaced—at least in part—by spontaneous differentiation of supporting or reserve cells. This regeneration is initiated by a transcription factor (Atoh-1), which can also be introduced into the inner ear using adenovirus vectors. In addition to direct injection, this vector also efficiently passes through the round window, which can be aided by the addition of hyaluronic acid. However, regeneration is only possible for a limited time because then the supporting cells are also killed. In guinea pigs, it was shown that 7 days after a noise exposure, enough hair cells remained for an adenovirus vector to deliver Atoh-1 gene and restore hearing. Human adenovirus vectors were used in these experiments, of which Ad28 appeared to be the most effective. Clinical phase 1 safety studies are currently underway into this direction (Safety, tolerability, and efficacy... 2019).

Enzyme defects such as cerebral mucopolysaccharidosis have also been considered. Mucopolysaccharidosis is a rare, autosomal recessive disease group that results from a lack of catabolism of glucosaminoglycans and often involves the nervous system (Del Rio et al. 2019). Because of the extensive changes in the brain, vectors need to be administered to multiple sites or genes need to be delivered neonatally. Indeed, gene therapy with CAV-2 has been shown to be effective in many cases in both mouse and dog models (Junyent, Kremer 2015). Because the immune response (which is an advantage when used as a vaccine, but for gene therapy it might be a disadvantage) may limit the gene transfer efficiency of the CAV-2 vector, transient immunosuppression may be required. Combination (CAV-2 + immunosuppression) therapy has also been shown to increase efficacy in mice (Del Rio et al. 2019).

Even in the case of *stroke*, it was possible to reduce the harmful effects of oxidative stress using CAV-2 vector—containing several genes—in a rat model of hypertension (Ord et al. 2013).

Virus vectors for therapy: Clinical trials

Most clinical gene transfer experiments have been based on adenovectors (Stephen et al. 2010). Human adenovirus vectors have been shown to be effective primarily in *vaccination* and *cancer therapy* due to their immune activating properties. The first adenovirus-based drug, Gendicine, was registered in China in 2003 to treat cancer patients carrying the p53 mutation. (P53 is the central coordinator of apoptosis in tumor cells, but in normal cells it is the “genome guardian”.) According to information from more than 30,000 patients, adenovirus vector therapy in combination with chemotherapy and radiotherapy has been shown to be more effective than standard therapies alone.

However, AAV-based genetic modifications are the most popular treatment for ophthalmic and neurological diseases (Li, Samulski 2020). Most of the treatment options for neurodegenerative diseases, including Parkinson’s disease, utilize various neurotrophic factors (substances that promote nerve cell growth) into the substantia nigra (Del Rio et al. 2019; Junyent, Kremer 2015). Unfortunately, most clinical trials have been ineffective, probably because such trials can only be performed in the final stages of the disease, when the amount of “reserve” cells that could still be stimulated by these factors is small. In any case, further neurological studies in all directions from genetic Huntington’s disease through Alzheimer’s disease to amyotrophic lateral sclerosis are ongoing (Chen et al. 2020). Recently the first gene therapy utilizing AAV9 was approved to treat children less than two years of age with spinal muscular atrophy (SMA) (Zolgensma). One-time intravenous administration can correct genetic problems and ensure better and longer life for these children.

In the brain, adenovirus vectors—in accordance with their general use—have been used primarily for the treatment of *tumors*, which have also been the subject of several phase 1 clinical trials (Vecil, Lang 2003). One possible theoretical solution is to introduce proliferating viruses into the tumor using their cell-killing (cytolytic) properties. Because

many viral particles will be released upon lysis of each cell, they can be used to treat tumors of a large enough size. But it can also be a limitation, as it is difficult to prevent them from spreading in the tissue. Modern genetic engineering has succeeded in creating mutants (such as ONYX-015) that are able to reproduce in tumorous, but not in normal cells. Some replication-selective virus (conditionally replicative adenovirus; CRAAd) variants have already passed the safety filter of a phase 1 clinical trial. The most promising seems to be a combination of gene and viral therapy, i. e., when the gene region encoding the p53 protein is delivered to tumor tissue using well-prevalent CRAs. Clinical testing of this is yet to come. CAV-2 as a vector is also expected to become more widespread, as with their help larger genetic information can be introduced into the nervous system, retrograde transport is also possible (note: peripheral administration) and in human it has relative low immunogenicity (From brain gene transfer... 2016).

Conclusion

Viral vectors have been an important tool in research for decades and have been tested in several clinical conditions. Adenoviruses have so far been considered primarily in vaccines and in cancers, but their wider use in the nervous system is also underway. Perhaps the main barrier to this is that we still do not fully understand the normal and pathological functions of the brain, so we do not really know what we should be influencing. In any case, long-term treatment of wide variety of diseases (including neurological and psychiatric conditions) with viral vectors may become more common in the future.

Conflict of interest

The author declares that there is no conflict of interest, either existing or potential.

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