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Research Paper

Therapeutic efficacy of regulable GDNF expression for Huntington's and Parkinson's disease by a high-induction, background-free “GeneSwitch” vector

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ABSTRACT

Gene therapy is currently an irreversible approach, without possibilities to fine-tune or halt the expression of a therapeutic gene product. Especially when expressing neurotrophic factors to treat neurodegenerative disorders, options to regulate transgene expression levels might be beneficial. We thus developed an advanced single-genome inducible AAV vector for expression of GDNF, under control of the approved small molecule drug mifepristone. In the rat brain, GDNF expression can be induced over a wide range up to three hundred-fold over endogenous background, and completely returns to baseline within 3–4 weeks. When applied with appropriate serotype and titre, the vector is absolutely free of any non-induced background expression. In the BACHD model of Huntington's disease we demonstrate that the vector can be kept in a continuous ON-state for extended periods of time. In a model of Parkinson's disease we demonstrate that repeated short-term expression of GDNF restores motor capabilities in 6-OHDA-lesioned rats. We also report on sex-dependent pharmacodynamics of mifepristone in the rodent brain. Taken together, we show that wide-range and high-level induction, background-free, fully reversible and therapeutically active GDNF expression can be achieved under tight pharmacological control by this novel AAV - “Gene Switch” vector.

1. Introduction

Gene therapy in its current layout is an irreversible treatment: a curative gene is introduced into patient's tissues by means of a viral or non-viral vector and produces its gene product (a protein or regulatory nucleic acid) from the time of vector application onward. In case of unwanted side effects or sufficient therapeutic success, transgene expression cannot be stopped nor can the level of expression be modified according to individual patient's needs.

Thus, gene therapy systems allowing for external control over the expressed transgene by pharmacological means would be highly beneficial under certain circumstances. Several such gene transfer systems are available, and especially tetracycline-controlled gene expression has been developed into very advanced systems (Das et al., 2016), but none has advanced into clinical applicability yet (Manfredsson et al., 2012). This unfortunate fact at least partially depends on the finding that the bacteria-derived Tet-operator provoked substantial immune responses when exploited in non-human primate muscle, although it worked almost perfectly in immune restricted compartments like the retina (Favre et al., 2002; Stieger et al., 2006; Le Guiner et al., 2014). Another highly promising inducible gene therapy concept, based solely on human components and thereby by default preventing immune issues, did not advance to application in patients due to unavailability of clinically approved analogues to its small molecule drug, rapamycin, a

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strong immunosuppressant (Rivera et al., 2005). Furthermore, rapamycin crosses the blood-brain-barrier inefficiently, requiring clinically non-acceptable dosages for regulation of transgene expression in the brain (Hadaczek et al., 2011).

In order to drive forward development of alternative regulated gene therapy vectors, we recently adopted the "GeneSwitch" system to AAV vectors, where regulated GDNF expression is under control of the clinically approved synthetic steroid mifepristone (Mfp) (Tereshchenko et al., 2014; Maddalena et al., 2013). In this system, Mfp application activates the GeneSwitch (GS) protein by dimerization, resulting in transgene expression from the responsive minimal UAS-TATA promoter (Ye et al., 2002). GS is a fusion of a short fragment of the yeast Gal4-DNA binding domain with a truncated human progesterone receptor and the human p65 transactivator. As it does not contain bacterial or viral components, it is anticipated to be less immunogenic than the Tet repressor and thus might become clinically exploitable.

Our earlier adaptions of the GS system to AAV vectors had to rely on a dual vector layout in order to achieve relatively low background expression of GDNF. Furthermore, the rate of induction of GDNF expression was only moderate, and the promoters used to drive GS expression were active in both neurons and glia. Here we show that an advanced single vector design allowed for high-level GDNF induction in the absence of any non-induced background expression. We also demonstrate a very good dose response for this system in the rat brain, allowing us to achieve a wide variety of GDNF levels by different Mfp dosing regimens. Importantly, we found gender-specific pharmacodynamics of Mfp in the rat brain. We further demonstrate that the vector can be kept in the ON-state for prolonged periods of time and shows biological effects in a mouse model of Huntington's disease. Finally, we show that in an established rat model of Parkinson's disease GDNF expression from this AAV-GS-GDNF vector restored motor capabilities.

2. Results

2.1. Design of a single-vector layout for Mfp-induced GDNF expression

It was the first aim of our present study to design an improved vector layout allowing for incorporation of all necessary elements into a single AAV vector genome, with high rate of induction and without any detectable background expression in absence of Mfp. Fig. 1A shows some of the iteratively tested vector constructs, differing mainly in the orientation of the two expression cassettes to each other. In all these constructs GS expression is driven by the strictly neuron-specific human synapsin 1 gene (hSyn) promoter (Kügler, 2016). Vectors were evaluated for GDNF expression in absence and presence of the inducer Mfp, by injecting the respective recombinant AAV-5 viruses unilaterally into the striatum, application of Mfp or solvent at 3 weeks after vector injection, and collection of striatal tissue for GDNF-ELISA at 7 days after the first Mfp application (Fig. 1B). Levels of GDNF achieved by the various vectors are shown in Fig. 1C.

These experiments demonstrated, that in case of a head-to-head orientation of GS to GDNF expression cassettes, i.e. when hSyn and inducible promoter are directly side-by-side, background expression of GDNF in absence of Mfp was not significantly lower than GDNF expression after induction by Mfp: the GS-GDNF-HtH construct expressed 650 ± 192 pg GDNF/mg tissue in absence of Mfp, and 1094 ± 879 pg GDNF/mg tissue after induction by Mfp. Isolation of both promoters from each other by incorporation of one or three synthetic transcription blocker sequences reduced background expression somewhat, allowing for a roughly 10-fold induction of GDNF expression: the GS-GDNF-HtH-TB construct expressed 174 ± 25 pg GDNF/mg tissue in absence of Mfp, and 868 ± 552 pg GDNF/mg tissue after induction by Mfp. However, non-induced GDNF levels were still about 30-fold higher as compared to 5–6 pg GDNF/mg tissue in non-treated control brains, or in brains injected with a GS-EGFP vector in absence or presence of Mfp (construct GS-EGFP-HtT in Fig. 1A).

This situation changed completely by flipping the GS expression cassette around, so that GS and GDNF were in a head-to-tail configuration (construct GS-GDNF-HtT in Fig. 1A). Now, non-induced GDNF levels were found to be at 10 ± 4 pg/mg tissue, while Mfp-induced GDNF levels reached 1942 ± 793 pg/mg tissue, i.e. an almost 200-fold induction above non-induced background and almost 400-fold above endogenous background levels. Induced levels of GDNF achieved by the novel one-vector construct were at least in the same order of magnitude as from a constitutive vector designed for maximum expression. They were also about one order of magnitude higher than those recently achieved by the best suited two-vector approach (Tereshchenko et al., 2014; Maddalena et al., 2013). All these experiments were carried out with a vector titre of 3×10^9 vg/striatum.

2.2. Fine-tuning: influences of AAV titre and serotype

Given the significant background expression seen with certain constructs, we next sought to establish Mfp-induced GDNF expression, which is absolutely free from any leaky, non-induced GDNF production, meaning that without Mfp application only the endogenous 5–6 pg GDNF per mg of striatal tissue are detectable. To achieve this, we tested titres of 1×10^9 and 3×10^9 vg of AAV-5-GS-GDNF and AAV-1/2 GS-GDNF vectors, both in the head-to-tail (HtT) configuration, which was used in all following experiments. The mosaic AAV-1/2 serotype was chosen as an alternative to AAV-5, as this serotype allows for similar tissue penetrance as AAV-5, but uses different primary receptors and is purified by heparin affinity chromatography, while AAV-5 was purified by AVB sepharose chromatography. These experiments should also allow for a correlation between vector titre and GDNF levels.

A side-by-side comparison of 3×10^9 and 1×10^9 vg/striatum of AAV-5-GS-GDNF revealed that: i) at 7 days after Mfp induction the 3-fold higher vector titre resulted in 1.5-fold increased levels of GDNF (1942 ± 793 pg/mg tissue versus 1298 ± 450 pg/mg tissue, $p < 0.05$, power (1-ß err prob) = 0.55); ii) at 28 days after Mfp induction GDNF levels have declined close to endogenous background with both 3×10^9 and 1×10^9 vg/striatum, but were still higher than endogenous GDNF levels ($p < 0.05$; power (1-ß err prob) = 0.6); iii) at 7 days non-induced GDNF levels were significantly higher than endogenous background with 3×10^9 vg/striatum (10.4 ± 4 pg/mg tissue versus 5.5 ± 1 pg/mg tissue; $p < 0.01$; power (1-ß err prob) = 0.83), but after application of 1×10^9 vg/striatum GDNF levels were indistinguishable from background (5.3 ± 1.6 pg/mg tissue versus 5.5 ± 1 pg/mg tissue). Thus, with minor modifications of AAV-5 vector titre, we achieved a 260-fold induction of GDNF levels over endogenous background, while without Mfp application absolutely no GDNF above endogenous background was detectable (Fig. 2A, B).

Since in these animal groups GDNF levels did not completely return to endogenous background levels at 28 days after Mfp application, we performed another animal group with striatal injection of 1×10^9 vg of AAV-5-GS-GDNF, and followed the decay of GDNF in more detail and over longer time (Fig. 2C). These results demonstrate that GDNF levels drop rapidly after withdrawal of Mfp application, and completely decay to endogenous background between 28 and 42 days after Mfp application. Thus, the novel one-vector GS-GDNF system proves to be fully reversible in terms of induction of GDNF expression.

The rate of induction and the levels of non-induced GDNF levels could only partially be repeated with the same vector genome packed into an AAV-1/2 capsid, indicating that the choice of AAV serotype has some influence on the performance of the GeneSwitch system (Fig. 2D). An AAV-1/2 vector constitutively expressing GDNF resulted in GDNF levels of 9917 ± 3102 pg/mg tissue, which is significantly more GDNF as compared to 5013 ± 1102 pg/mg tissue achieved with an AAV-5 at the same titre of 3×10^9 vg/striatum ($p < 0.001$; power (1-ß err prob) = 0.95). Application of 3×10^9 vg of AAV-1/2 GS-GDNF resulted in 1012 ± 553 pg GDNF/mg tissue, which is significantly less GDNF as compared to 1942 ± 793 pg/mg tissue achieved with the equivalent