Triptolide T10 enhances AAV-mediated gene transfer in mice striatum

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Abstract: Adeno-associated virus (AAV) mediated gene transfer has been demonstrated to be an effective approach for treating Parkinson’s disease (PD). Triptolide T10 is a monomeric compound isolated from Tripterygium wilfordii Hook.f., a traditional Chinese herb for anti-inflammatory medications. In the present study, we co-administered T10 with recombinant AAV2 in SH-SY5Y human neuroblastoma cells and in the striatum of C57BL/6 mice, and then evaluated the AAV-mediated gene expression levels. The results have shown that T10 significantly augmented the expression of AAV-mediated gene in a dose-dependent fashion without detectable cytotoxicity. As growing evidence indicated that inflammation contributed to the progression of PD, and the anti-inflammatory effect of T10 was shown in our previous studies, our data of T10 to enhance AAV transduction suggest that T10 might be potentially used as a facilitating reagent for the AAV gene therapy applications in neurodegenerative diseases.

Tripterygium wilfordii Hook.f. (TWHF) is a native woody vine in southeast of China, Korea and Japan. TWHF has a long history as one of the traditional herbal medicine for the treatments of swelling, fever, chills, sores, joint pain, and inflammation. Since the 1960s, TWHF has been prescribed to autoimmune disease patients as an allopathic medicine in the cases of rheumatoid arthritis (RA) and other inflammatory disorders, including chronic nephritis, hepatitis, systemic lupus erythematosus, ankylosing spondylitis, cancers, and a variety of skin conditions. Triptolide T10 has been identified as the major components in TWHF to exert immuno-suppressive and anti-inflammatory effects. Previous studies have shown that triptolides significantly improved the survival of TH-ir neurons in the SNpc in LPS-induced PD models by reducing the productions of inflammatory cytokines like TNF-α and IL-1β.

Adeno-associated virus (AAV) was emerged as a classical vector for gene replacement therapies and has been used for treating PD in the past decade. Aside from the potential to persistently express the transgene following integration, AAV as a nonpathogenic virus is able to efficiently transduce a wide range of host cells without severe immune responses. In PD-related clinical trials, the result of non-detectable vector-associated adverse events in more than 30 subjects injected with AAV appeared to be extremely encouraging.

Recent studies have shown that the CNS inflammatory responses, especially when involving microglia, significantly contributed to the onset and the progression of PD. As T10 was reported to suppress the production of microglia-derived inflammatory factors, it was suggested as a potential supplementary medicine for PD therapies. Therefore, how T10 in coexistence with AAV vectors might affect the AAV-mediated gene expression became an interesting question that has never been addressed. In this study, we investigate the AAV transduction in neurons in the presence of T10 and found that the transduction efficiency of recombinant AAV type 2 (AAV2) was significantly enhanced within the dose range of T10 anti-inflammatory activities. Meanwhile, no detectable cytotoxicity was observed. The data have provided the first evidence to facilitate AAV-mediated gene therapy with an herbal medicine compound. Our findings suggested that T10 might be used as a potent adjuvant for the gene therapy of neurodegenerative diseases. It will be an attractive strategy to further reduce the risk and cost in clinical application by the advantage of combining the neuroprotective and anti-inflammatory effect with the increase of the AAV-mediated transgene expression with T10 co-administration.

Adult male C57BL/6 mice with 18–20 g body weight were used for in vivo studies. The animals were maintained on a 12/12 h light/dark cycle at constant temperature and humidity, and provided with food and water ad libitum. The experimental procedures in this study were approved by the Committee on Animal Care.
were aliquoted into 100 U/ml penicillin-streptomycin and 10% NCS.

3. RESULTS

3.1. AAV2 mediated transgene expression in vivo

3.1.1. Dose–response curve

The AAV2 transduction in response to different doses of T10 administration at 24 h resulted in a comparable augmentative effect with the co-administration group (Fig. 1C). AV2.luc infections only showed a mild effect, whereas a T10 re-administration at 24 h caused much more significant luciferase expression (Fig. 1D).

3.1.2. Dose–response effect on cell viability

The AAV2 vectors were diluted with PBS and supplemented without or with T10. The mice were stereotaxically injected with normal saline or with T10 for 24 h. The injection sites were then rinsed with 100 ml PBS per well and immediately applied to mice, we first repeated the T10 dose–response experiments. The samples were then collected and subjected to mice, we first repeated the T10 dose–response experiments.

3.1.3. Luciferase activity

The luciferase activities was observed with a maximum about 30 minutes after repetitive measurements at different time post treatments. The statistical evaluation was performed using an unpaired two-tailed Student’s t-test (two group data set comparisons) and one-way ANOVA followed by Newman–Keuls post hoc test (for multi-group comparison). For all the results in this study, the differences with a value of p < 0.05 were considered as significant.

3.2. AAV2 transduction in response to different doses of T10 treatments

The AV2.luc-infected cells were exposed to increasing concentrations (0, 6.25, 12.5, 25, 50, 100, 200 nM) of T10 for 24 h, a dose–dependent increase of the luciferase activities was observed with a maximum about 30 minutes after repetitive measurements at different time post treatments.

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ment was observed. The optimized concentrations were also at about 50 nM. Interestingly, the fold-increase of the luciferase activity was larger than that in SH-SY5Y cells and reached to 8 folds (Supp. Fig. 2).

For a qualitative evaluation of T10 on AAV2 transductions in vivo, AV2.EGFP was stereotaxically injected without or with 5 μM T10 into the mice striatum, and the EGFP fluorescence was examined at 1 week post-infection with the tissue sections. The significant increase of AAV-mediated EGFP expression following T10 treatments was observed as reflected by both the increase of the number of EGFP-positive cells and the fluorescent intensity of individual cells (Fig. 2A, B). To obtain more quantitative measurements, AV2.luc was used to inject the mice striatum for viral infections without or with T10. We have found a significant increase of the luciferase activity to nearly 6 folds with the treatment of 50 μM T10. The minimal dose for the AAV transduction promoting activity of T10 in vivo appeared to be above 0.5 μM (Fig. 2C). For long-term experiments, AV2.luc without or with 5 μM T10 were injected to the mice striatum and the increases of luciferase activities of 5 and 10 folds were detected at 2 and 6 weeks respectively, as compared to those of AV2.luc alone injections (Fig. 2D, E).

Whether T10 caused cytotoxic responses in vivo is a critical concern especially for clinical applications. We performed TUNEL staining with the striatum sections from the mice at 24 h after receiving injections of 5 μM T10. As a neurotoxin known to induce apoptosis, 1-methyl-4-phenylpyridinium ion (MPP+) was used as the positive experimental controls. No appreciable apoptotic effect was detected in the T10-injected samples (Fig. 3A, B). We also performed Rotarod tests on T10 injected mice for behavior changes. Only the MPP+ treated group has shown a profound reduction of motor performance, while the T10-treated mice exerted a similar locomotor ability with the mice injected with N.S. (Fig. 3C). For long-term locomotor behavioral tests, T10 injected mice were compared with the mice with MPTP treatments. The mice injected i.p. four times (2-h intervals over 1 day) with 20 mg/kg MPTP/HCl were used as the positive controls. The locomotory activities of mice were evaluated at 1, 3 and 7 days after the injections of N.S., T10 or MPTP by Rotarod assays. The results showed that only MPTP induced a reduction of locomotor ability, and no change was observed between the T10 and N.S. groups (Fig. 3D).

The medical applications of TWHF in far-eastern countries have a history of thousand years. Modern researches have revealed that T10 is one of the potent components with the immuno-suppressive and anti-inflammatory effects [6]. In recent PD-related studies, T10 has been demonstrated to increase the number of TH-ir neurons through the inhibition of inflammatory cytokine productions of TNF-α and IL-1β in the SNpc, particularly in LPS-induced PD models [15]. AAV is a well-developed vector for the gene therapy of neurodegenerative diseases with superb safety and tolerability, and the results of its application in several PD clinical trials appeared to be very encouraging [3, 7, 10]. In the present study, we have found that under the appropriate dose ranges without causing notable cytotoxicities, T10 will not only serve as a neuroprotective and anti-inflammatory reagent, but will also be able to overcome the frustration of unsatisfactory transduction efficiency in AAV-based gene therapy. At least, the amount of AAV used could be reduced to decrease the clinical cost and the potential adverse effect when co-administrated with T10. Therefore, our discovery of T10 co-administration with AAV2 significantly enhancing the AAV-mediated gene expression will potentially lead to a strategy to merge the benefit of anti-inflammatory and AAV transduction promoting activities of T10 in future clinical applications.

Fig. 1. T10 augments AAV2 transduction in SH-SY5Y cells. (A) Increasing doses of T10 were co-administered with AV2.luc (MOI = 10^5 v.g./cell) in SH-SY5Y cells, the relative luciferase activities were determined at 24 h post-infections. (B) The effects of T10 and doxorubicin co-administration with AV2.luc (MOI = 1 × 10^4 v.g./cell) were compared in SH-SY5Y cells at 24 h post-infections. (C) The effect of T10 24 h pre-treatment in SH-SY5Y cells then removed for AV2.luc infection was compared to that of T10 re-administration at 24 h after AV2.luc infection. (D) The MTT results of the SH-SY5Y cells treated with different concentrations of T10 for 24 h. Data from 6 independent samples were shown as mean ± SEM with **p < 0.01 as compared to the untreated group.
Fig. 2. T10 enhances AAV2 transduction in the mice striatum. (A) C57BL/6 mice were injected with $3 \times 10^9$ v.g AV2.EGFP to the striatum without or with 5 μM T10. The EGFP expression was evaluated at 1 week post-injections. Scale bar = 200 μm. (B) The quantification of EGFP fluorescent intensities of (A). (C) A total of $3 \times 10^9$ AV2.luc was injected into mice striatum with the absence or presence of various doses of T10, luciferase assays were performed at 1 week post-injections. Data from 3 independent samples were shown as mean ± SEM with ***p < 0.001. AV2.luc of $3 \times 10^9$ particles were injected with the absence or presence of 5 μM T10 into mice striatum, luciferase assays were performed at 2 (D) and 6 weeks (E). Data from 3 independent samples were shown as mean ± SEM with *p < 0.05 and ***p < 0.001.

Fig. 3. T10 treatments do not lead to detectable cellular or neurological toxicities in vivo. (A) The TUNEL quantification of the stained striatum sections from the mice injected with 5 μM T10 for 24h. MPP+ treatments at 8 μg/injection site were used as the positive control for apoptosis inductions. Data from 3 independent samples were shown as mean ± SEM with ***p < 0.001. (B) Representative photos from the samples in (A). Scale bar = 200 μm. (C) The latency measures of the Rotarod performance of mice injected with N.S., 5 μM T10 or MPTP for 24h. (D) A time course comparison of the Rotarod latency results of the mice injected with N.S., 5 μM T10 and MPTP for 1–7 days.
Although the fact that T10 augments AAV2 transduction is very encouraging, the underlining mechanism remains unknown. First of all, the fact that the increase of AAV-mediated gene expression started to decline above 100 nM of T10 treatment without significant changes in MTT index (Fig. 1A and D) indicates that cell stress responses may not be likely to be involved. In addition, T10 pre-treatment dose not appear to be sufficient to induce AAV2 transduction activation and T10 re-administration fails to further promote the transgene expression, as shown in Fig. 1C. These data suggested that the interaction between T10 and AAV2 may be occurred during the processes of viral trafficking [1]. Although many proteasome inhibitors, such as doxorubicin, could also augment AAV2 transduction by promoting the viral nuclear accumulation [14], it showed lower efficiency to augment AAV2 transduction, by T10 and proteasome inhibitors, appeared to be distinct. From the clinical point of view, the T10 pre-treatment and re-administration test indicated that the co-administration of T10 and AAV was a optimize condition to augment AAV transduction, and whether the patients were pre- or post-prescribed TWHF-derived medicines would not likely to affect the protocols for AAV-mediated gene therapies.

The continued activation of glial cells during the progression of neurodegenerative diseases is a potential factor that might lead to the clearance of AAV transduced neurons and compromise the therapeutic effect. Our previous studies have shown that 10–50 nM T10 was able to inhibited microglia activation [5] and to improve the TH-ir neuron survival in LPS-induced rats [15]. The doses for T10 to reduce the LPS-induced nitrite accumulation and the TNF-α and IL-1β release from LPS-activated microglia at 5 μg/kg [16] were correlated with the 10–50 nM T10 treatment to enhance the AAV transduction. These results have suggested a seemingly beneficial effect when considering T10 in vivo applications where the glial cells existed and being responsive at the viral injection sites. Whether the glial cells are partially responsible for the better augmentation of AAV transduction of T10 in vivo especially for long terms remained to be further investigated.

Previously reported T10 safety doses implied that the 5 μM T10 used in this study should not lead to cellular toxic effect. This was confirmed by the results of MTT and TUNEL assays both in vitro and in vivo. Thus, our results might lead to a new application of T10 to increase AAV transduction for the development of the complementary therapeutics for the treatment of neurodegenerative disorder.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2010.05.046.

References