A single nigral injection of human ENGRAILED-1 induces long-lasting behavior benefit in an experimental primate PD model

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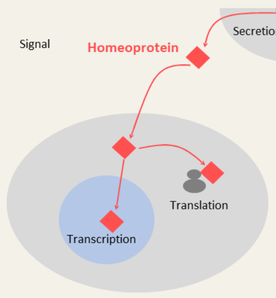
Objective

Evaluate the efficacy of human ENGRAILED-1 (hEN1), locally administered in the Substantia Nigra (SNc), in a recognized and chronic MPTP macaque model.

Background

Homeoproteins are transcription factors with unique intercellular transfer features.[1,2]
The homeoprotein EN1 plays a major role in the development of mesencephalic dopaminergic (DA) neurons at early embryonic stages, and in the survival of midbrain DA neurons in adult mice.[1]

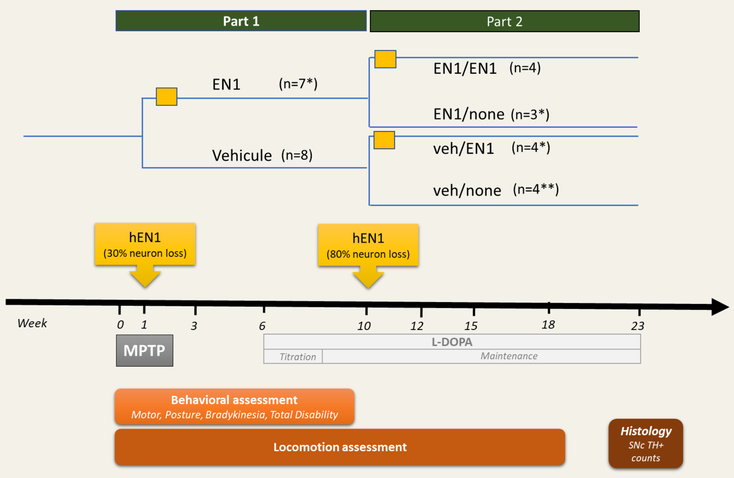


Mice lacking one allele of En1 ($En1^{+/-}$) progressively develop PD-like features. This includes motor and non-motor symptoms, mitochondrial deficits, autophagic disturbances, retrograde axonal degeneration (SNc>VTA), neuroinflammation as well as progressive loss of nigral dopaminergic neurons.[3,4,5]

EN1 protects mDA neurons at the mitochondrial (translational regulation) and at the nuclear level (transcriptional and epigenetic regulation).[6,7]
Intranigral administration of a single dose of hEN1 prevents SNc dopaminergic neurons degeneration in the $En1^{+/-}$ mice as in other induced (6-OHDA, MPTP, α-syn) mouse PD models.

Study Design & Methods

This was an assessment-blinded, placebo-controlled, parallel group study divided into 2 parts:



**: animal dead during study.*

Sixteen macaques were quasi-randomized to either control or hEN1, based on body weight. All animals received daily MPTP for 12 days, as previously described.[8]

- **Part 1**: on day 6, animals underwent surgery for bilateral nigral stereotaxic injection of hEN1 (6µg in 20µL/ hemisphere) or vehicle.
- **Part 2**: At week 10, half of the animals in each group received an additional hEN1 stereotaxic injection dose of 6 µg (in 20 µL) or 12 µg (in 40 µL) for naïve animals, leading to 4 groups.

An average maintenance L-DOPA dose (50% of the averaged optimal dose determined from week 6 to 9 in animals from vehicle group), was administered to all monkeys from week 9 on.

Behavioral assessments were carried out at baseline and every 3 weeks (in OFF-state) as previously described.[9]
At week 23, monkeys were euthanized, and brains collected for stereological counting of TH+ neurons in SNc.[10]

References

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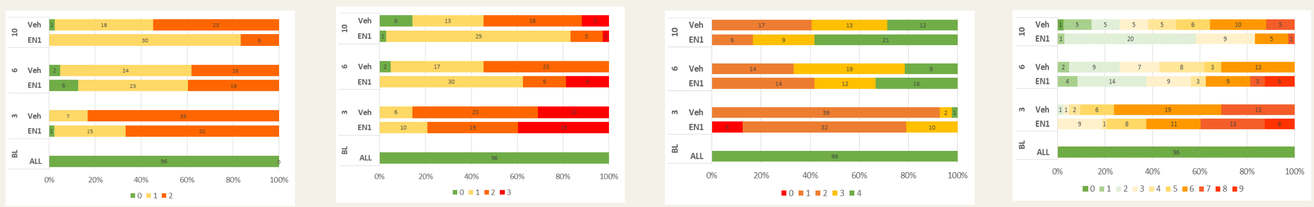
Results

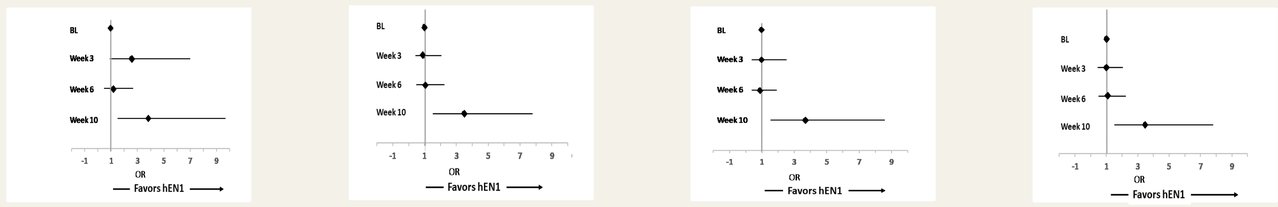
hEN1 improves behavioral endpoints
Significant effect from week 10

Data



Assessments by an observer blinded to treatment allocation on video-recorded material (10 min every 30 min for 3 h).[11] Scores as described[12]: Posture (0 to 2), Bradykinesia (0 to 3), with high score indicating more severe symptoms and motor (0 to 4) where a higher score indicated less impairment. Total disability score = [(4 – motor score) + bradykinesia + posture]. Numbers in bars indicate number of evaluations.

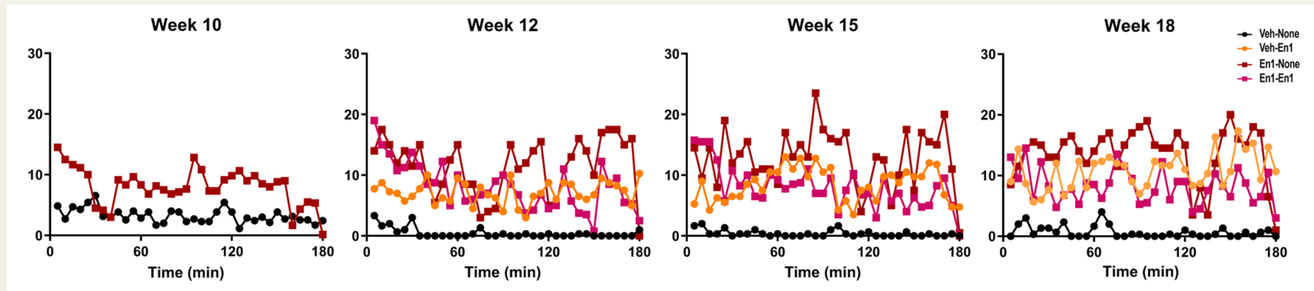
Statistical Analysis



Statistical analyses used cumulative multinomial logit models using Odds Ratio (OR). OR describes the odds that an event under a certain group is more likely than in the other. As this is a ratio, a value of 1 implies that there is no difference between groups. A Significant difference is considered when OR > 1 if the confidence intervals do not include 1. As cumulative multinomial models require a minimum number of data points per group to be reliable, only Part 1 could be analyzed by this method (week 3 to 10).

hEN1 improves locomotor activity
EN1-Veh = EN1-EN1 > Veh-EN1 >> Veh-none

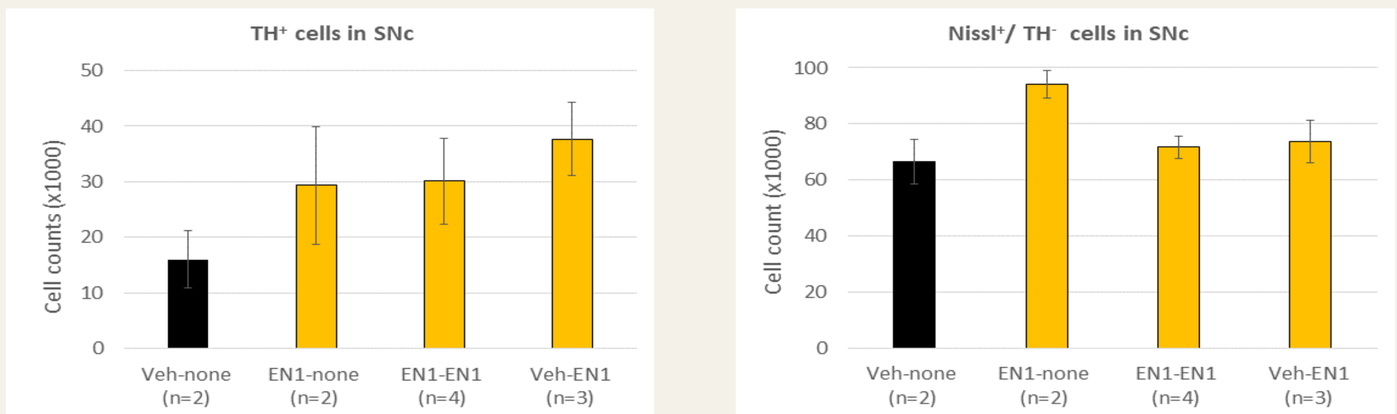
Part 1 | Part 2



Comparison		Estimate	SD	t Value	Pr > |t|		Estimate	SD	t Value	Pr > |t|		Estimate	SD	t Value	Pr > |t|		Estimate	SD	t Value	Pr > |t|	
EN1	Veh	2.23	1.25	1,78	0.076	-															
EN1-none	**Veh-none**	6.66	1.53	4.35	**<0.0001**	***	5.55	1.49	3.72	**0.0002**	***	4.71	1.46	3.23	**0.001**	**
EN1-EN1	**Veh-none**	5.34	1.34	3.98	**<0.0001**	***	4.27	1.30	3.28	**0.001**	***	3.76	1.26	2.98	**0.003**	**
Veh-EN1	**Veh-none**	3.78	1.38	2.74	**0.006**	**	2.85	1.34	2.13	**0.03**	*	2.05	1.30	1.58	0.11	.
EN1-none	**Veh-EN1**	2.87	1.36	2.11	**0.03**	*	2.70	1.36	1.98	**0.05**	*	2.66	1.36	1.96	0.05	.
EN1-EN1	EN1-none	-1.32	1.32	-1.00	0.32	-	-1.28	1.32	-0.97	0.33	-	-0.96	1.32	-0.72	0.47	.
EN1-EN1	Veh-EN1	1.56	1.09	1.42	0.16	-	1.42	1.09	1.30	0.20	-	1.71	1.09	1.56	0.12	.

Locomotor activity was quantitatively determined as movement amount observed every 5 min for 3 h, using computer-based activity monitors. P <0.05 significant.

hEN1 protects from MPTP-induced neurodegeneration



Analysis done at week 23. Unbiased stereological analysis was used to estimate the number of tyrosine hydroxylase (TH)-immunopositive neurons (TH+) and TH-immunonegative/Nissl-positive cells. Mean estimated number of neurons ± SEM calculated for each group.

Conclusion

- ❑ Nigral injection of hEN1 up to 12 µg/ hemisphere was well tolerated; no signs of local toxicity and/or specific inflammation observed at week 23.

- ❑ A single hEN1 intranigral injection 6 days after initiation of a chronic MPTP intoxication in macaques protects from DA neurons degeneration as shown by:
  - Improved behavior tests detectable at week 10
  - Improved and long-lasting locomotor activity, detected from week10 and up to week18
  - A trend in increased number of TH+ cells in the SNc measured at week 23

- ❑ Treating early (day 6; pre-symptomatic phase, 30% of DA neurons loss) induces a longer protection than treating late (week 10; symptomatic phase, 80% of DA neuron loss).

These data support further evaluation of hEN1 as a therapeutic candidate for Parkinson disease.