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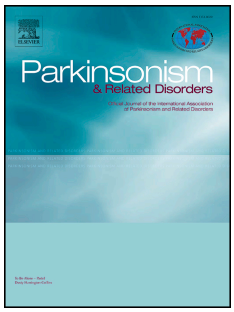

Effects of intracerebral neurotrophic factor application on motor symptoms in Parkinson's disease: A systematic review and meta-analysis

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Effects of intracerebral neurotrophic factor application on motor symptoms in Parkinson's disease: a systematic review and meta-analysis.

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Abbreviations: CI = confidence interval; DA = dopaminergic/dopamine; GDNF = glial cell line-derived neurotrophic factor; NTF = neurotrophic factor; NTN = neurturin; PD = Parkinson's disease; RCT = randomized controlled trial; RR = risk ratio; UPDRS - Unified Parkinson's Disease Rating Scale.

Key words: Parkinson's disease; neurotrophic therapy; clinical trials; systematic review/meta-analysis; neurotrophic factors.

ABSTRACT

Introduction: Neurotrophic factors (NTFs) have been evaluated for neuroprotective effects in Parkinson's disease (PD). However, clinical trials examining the efficacy of intracerebral administration of NTFs on motor symptoms in PD have produced mixed results, and are thus inconclusive. The objective of this systematic review and meta-analysis was to determine the effects of intracerebral NTF application on motor symptoms in people with PD.

Methods: We searched PubMed, MEDLINE, EMBASE, and Cochrane from inception through to March 31 2016 for open-label trials and randomized controlled trials (RCTs) which intracerebrally administered NTFs to PD patients, and which performed motor examination of Unified Parkinson's Disease Rating Scale.

Results: Eight studies with a total of 223 participants were included. Fixed effects analysis revealed that NTF treatment did not significantly reduce motor symptoms in PD patients compared to placebo controls ($P = 0.98$). Combining open-label and RCT data, both treatment with NTFs ($P < 0.001$) and treatment with placebo ($P < 0.05$) significantly improved motor function in PD patients when compared to predicted symptoms in untreated PD controls. Finally, random effects analysis revealed that NTF-treated PD patients were not significantly likely to improve following intracerebral NTF administration ($P = 0.25$).

Conclusion: In conclusion, intracerebral NTF administration does not improve motor symptoms in PD patients, when compared to placebo-treated controls. These findings may guide therapeutic decisions and inform future research on NTFs and their application in PD.

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder, in which nigrostriatal dopaminergic (DA) neurons progressively degenerate to cause debilitating motor symptoms [1-5]. Despite decades of research, there is no disease-modifying therapy for PD [4-6]. Current symptomatic treatments improve quality of life and functional capacity, however their efficacy wears off over time and they cause disabling side-effects [6, 7]. Thus, there is an urgent need to develop new therapies that halt/reverse the neurodegeneration in PD.

Neurotrophic factors (NTFs) are endogenous proteins critical for the development and maintenance of neurons [8]. Several NTFs promote the survival and growth of midbrain DA neurons *in vitro* and *in vivo*, while glial cell line-derived neurotrophic factor (GDNF) and neurturin (NTN) have been used in PD clinical trials [8-10]. These NTFs have been delivered to the PD brain via various delivery methods, to distinct target region(s), in small- and larger-scale clinical trials. While initial open-label trials have demonstrated the feasibility and potential efficacy of NTFs in improving motor symptoms in PD patients, more recent clinical trials have had limited success. Despite this, in principle NTF therapy is still a promising disease-modifying therapy for PD, and remains an area of intense scientific research. To date however, a systematic review of the NTF trials in PD patients has not been published. Thus, the aim of this study was to conduct a systematic review and meta-analysis to quantitatively evaluate the effectiveness of intracranial NTF application in clinical trials on the motor symptoms of people with PD, in comparison to PD patients who did not receive NTF treatment.

METHODS

Study Design and Registration

The present systematic review and meta-analysis adhered to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement [11], and is registered with PROSPERO (registration number CRD42016033889).

Selection Criteria for Studies

Study designs:

Eligible studies included open-label trials and randomized controlled trials (RCTs) which were published in the English language.

Participants:

We included studies which examined people with PD. We did not make exclusions based on PD disease stage, age, gender or medication.

Interventions:

We included clinical trial studies in which PD patients received intracranial administration of NTFs. We included studies which administered NTFs to the brain (any region(s)), brain parenchyma and/or ventricular system. Studies administering NTFs peripherally, outside of the central nervous system, were not included as NTFs do not cross the blood-brain barrier. We did not exclude studies based on the method chosen to administer NTFs. We defined NTFs as proteins that are critical for the development and maintenance of neurons in the developing and adult brain, and we excluded any studies which administered molecules, compounds or proteins that did not meet this definition.

Comparators:

Given the selective, yet broad, nature of participants chosen for this review, and the single therapeutic intervention of interest, we solely compared PD patients which had received intracranial NTF administration to control PD patients which did not receive intracranial NTF administration. We did not exclude studies based on the nature of the control treatment.

Outcomes:

The primary outcome measure for this systematic review was the assessment of motor symptoms of PD patients through motor examination using the Unified Parkinson's Disease Rating Scale (UPDRS), in which a decrease in UPDRS score is indicative of improved PD symptoms. Studies which did not assess motor symptoms by use of the UPDRS score were excluded. All response rates were calculated as the mean response of all randomised patients. Improved or disimproved motor symptoms (lower or higher UPDRS score, respectively) served as a dichotomous outcome. When studies reported UPDRS scores at various-time points during a trial, we recorded the mean of those multiple values. Adverse effects that resulted in death at any point during or after the trial, as a direct result of the treatment intervention, were also recorded. Studies were not selected for inclusion or exclusion based on the length of follow-up of outcomes. No secondary outcomes were recorded.

Search Strategy

We searched PubMed, MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trials from inception through to March 31, 2016 using a combination of the following MeSH search terms: Parkinson disease AND nerve growth factors AND clinical trial. To ensure literature saturation, we scanned the reference lists of included studies or relevant reviews identified through the search. We also searched the authors' personal

literature databases to make sure that all relevant material was captured. The literature search was limited to studies in the English language.

Data Collection and Analysis

Selection of Studies:

Two review authors (SH/GO'K) independently screened titles and abstracts of all studies identified through database searches in the citation library. Irrelevant studies were excluded. For the remaining studies which appeared to meet the inclusion criteria, the full text article was uploaded to the citation library, and two authors (SH/GO'K) independently applied the predefined selection criteria. We resolved any disagreement through discussion, and consultation with a third author (AS) when necessary. We recorded the reasons for exclusion.

Data extraction and management:

A form for standardised data extraction was designed and tested before two review authors (DL/AS) independently extracted data, which was subsequently verified by another independent reviewer (SH) to reduce errors and bias in data extraction. Data abstracted included all information of interest e.g. participant details, methodology, intervention details, and relevant patient outcomes. Reviewers resolved any disagreements by discussion, and one arbitrator (SH) adjudicated any unresolved disagreements. One review author (SH) collated and entered all data into Review Manager 5.3 (ReviewManager version 5.3, The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark).

Assessment of risk of bias in included studies:

Two review authors (DL/AS) assessed the risk of bias using the Cochrane risk of bias assessment tool outlined in chapter 8 of the Cochrane Handbook for Systematic Reviews of

Interventions, which classifies studies as having low, high, or unclear risk of bias in the following domains: selection bias, performance bias, detection bias, attrition bias, reporting bias, and carryover effect. Any disagreements were resolved first by discussion and then by consultation with a third author for arbitration (SH). One author (SH) computed graphic representations of potential bias within and across studies using Review Manager 5•3.

Measures of Treatment Effect:

The treatment effect for the primary outcome data was expressed as a pooled risk ratio (RR) with 95% confidence interval (CI). Studies with multiple treatment groups were combined into a single group, while missing data was sought from original authors if deemed necessary. The primary analysis was per individual randomised.

Assessment of heterogeneity:

Clinical heterogeneity was assessed by considering the variability in participant factors between trials (e.g. age) and trial factors (e.g. randomization concealment, blinding of outcome assessment, losses to follow-up, treatment type, co-interventions). We discussed clinical homogeneity, and based on this discussion, we decided whether pooling of data was appropriate. Statistical heterogeneity was tested using the Chi^2 test (significance level: 0.1) and I^2 statistic (0% to 40%: might not be important; 30% to 60%: may represent moderate heterogeneity; 50% to 90%: may represent substantial heterogeneity; 75% to 100%: considerable heterogeneity). If high levels of heterogeneity among the trials existed (I^2 >=50% or P <0.1) the study design and characteristics in the included studies were explored.

Data synthesis:

Each outcome was combined and calculated using the statistical software RevMan 5.3, according to the statistical guidelines referenced in the current version of the Cochrane Handbook for Systematic Reviews of Interventions. The Mantel-Haenszel method was used for the fixed effect model if tests of heterogeneity were not significant. When statistical heterogeneity was observed (I^2 >=50% or P <0.1), the random effects model was chosen. No subgroup or subset analyses were performed within our participant group.

Sensitivity analysis:

The process of undertaking a meta-analysis involves making decisions about inclusion criteria. We used sensitivity analysis to explain high levels of clinical heterogeneity, and to assess the impact on the overall treatment effect of inclusion of trials which did not report an intention to treat analysis, had high rates of participant attrition, and/or had other missing data.

Assessment of meta-bias(es):

In order to determine whether publication/selection bias was present, we determined whether the protocol of the trial was published before recruitment of patients to the study was started. We then evaluated whether selective reporting of outcomes was present (outcome reporting bias) by comparing outcomes reported in the protocol and the published report. We compared the fixed effect estimate against the random effects model to assess the possible presence of small sample bias.

Quality of the evidence:

We used the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach to assess the quality of evidence [12]. An initially assumed ‘high quality’

of evidence was downgraded for meeting any of the following criteria: (1) risk of bias, (2) heterogeneity, (3) indirectness, (4) imprecision, and (5) publication/selection bias.

RESULTS

Search Results

The search strategy yielded 244 records, of which 233 trials were excluded based on our exclusion criteria and 11 were retrieved in full text and assessed for eligibility [13-23]. Among the 11 potentially eligible studies, 2 full-text articles were excluded; one for being an abstract of an ongoing study [22], and the other because the NTFs were co-applied with a cell transplant [20]. Two full-text articles were part of the same study [14, 21], and were thus combined for the meta-analysis. Eight studies were included in the systematic review and meta-analysis (Figure 1A) [13-19, 21, 23].

Study Characteristics

The eight eligible studies included a total of 223 participants. Participants included men and women between the ages of 35 and 75 years old who had moderate-to-severe PD for at least 5 years. Five of the studies only included PD patients who had a good response to Levodopa, and motor complications that could not be satisfied with medical therapy [13, 14, 16, 17, 19, 21]. All studies excluded patients which had medical conditions that may have compromised the study, e.g. dementia, abnormal Parkinsonism, and previous neurosurgery. Four trials administered GDNF [14, 15, 18, 21, 23], and four trials administered NTN [13, 16, 17, 19]. Five trials intracerebrally applied the chosen NTF to the putamen [14-17, 21, 23], one to the ventricular system [18], and two to both putamen and substantia nigra [13, 19]. Four trials directly infused the NTF [14, 15, 18, 21, 23], while four trials used viral vectors to deliver the NTF [13, 16, 17, 19].

Risk of bias in included studies

Figure 1B summarises the scores for the risk of bias assessment. Four of the included studies were RCTs [15, 16, 18, 19] and four were open-label trials [13, 14, 17, 21, 23]. The four RCTs were double-blinded, but the four open-label trials scored high risk for selection, performance, and detection biases [13, 14, 17, 21, 23]. Two of the RCTs scored high risk for attrition bias due to participant drop-out without explanation [16, 19]. One study scored unclear risk for other bias as the intended number of patients to be investigated was not completed due to an early end to the study [18].

Effects of interventions

Fixed effects analysis of the four RCT [15, 16, 18, 19] revealed a pooled risk ratio (RR) of 0.99 (95% confidence interval (CI) = 0.47–2.06), indicating that the overall effect did not significantly favour NTF treatment over placebo control in the reduction of motor symptoms (decrease in UPDRS score) in people with PD (P = 0.98) (Figure 2). The risk ratio for one of the RCTs [18] was not estimable as no improvements in motor symptoms were reported for either group. Heterogeneity between the included studies did not exceed that expected by chance (P = 0.59; I^2 = 0%), implying that the results across the included studies were statistically homogeneous.

To analyse data from all eight trials, both open-label and RCTs, included for analysis in this review, PD patients who received a treatment (either intracerebral NTF application or placebo control) were compared to a predicted untreated control. The ‘predicted untreated PD control’ was the corresponding predicted outcome if the PD patient had not received treatment in the trial. Given the progressive neurodegenerative nature of PD [4, 24] and the fact that moderate-to-severe PD patients were included in these trials, all predicted controls

had no improvement in UPDRS score. In this meta-analysis, NTF treatment significantly improved motor symptoms in PD patients when compared to the predicted untreated PD control (RR = 11.00; 95% CI = 3.85–31.45; P < 0.001) (Figure 3A). Similarly, PD patients treated with a placebo had a significant improvement in UPDRS scores when compared to the predicted untreated PD control (RR = 7.67; 95% CI = 1.46–40.39; P < 0.05) (Figure 3B). Heterogeneity between the included studies for both meta-analyses did not exceed that expected by chance (P = 0.97; P = 0.61; I^2 = 0%), implying that the results across the included studies were statistically homogeneous.

Finally, in order to assess the likelihood of motor symptoms improving in PD patients that had received intracerebral NTF administration, we compared the post-intervention UPDRS scores to baseline scores of NTF-treated patients. Due to statistical heterogeneity (P < 0.1; I^2 = 86%), the random effects model was chosen for analysis and revealed that NTF-treated PD patients were not significantly likely to have improved UPDRS scores following intracerebral NTF administration, when compared to the likelihood of no improvement (RR = 0.47; 95% CI = 0.13–1.71; P = 0.25) (Figure 4). None of the eight studies that were evaluated reported severe adverse events that caused death as a result of the intervention.

Quality of evidence across studies

Using the GRADE criteria, we characterized the quality of evidence presented in the primary meta-analysis (Figure 2) as low. An initially-assumed high level of evidence was downgraded to moderate because less than 75% of the included studies were at low risk of bias across all domains. Furthermore, the imprecision of our primary meta-analysis (21 events and 190 participants) resulted in a further downgrade of the quality of evidence to low. Despite the risk of bias and imprecision, our primary meta-analysis exhibited homogeneity and directedness (i.e. all participants were patients with PD), and it was free from selection bias

across included studies. The quality of evidence in the alternative meta-analyses (Figure 3, 4) was classified as very low as, in addition to the above downgrades, further downgrade(s) were made as these meta-analyses had selection bias and/or were indirect (predicted untreated controls) due to the inclusion of open-label trials.

DISCUSSION

The aim of this systematic review and meta-analysis was to evaluate the effectiveness of intracranial NTF application on the motor symptoms of people with PD. Our meta-analysis has found that intracranial NTF application does not significantly improve motor symptoms in people with PD, compared to placebo control-treated PD patients. The fixed effects analysis revealed a pooled RR of 0.99 (95% CI = 0.47–2.06), indicating an overall effect which did not significantly favour NTF treatment (P = 0.59). This finding was obtained from pooled analysis of four RCTs composed of 190 patients. However, it is important to stress that this should be interpreted with caution, due to the limited number of RCTs available for analysis. In addition to this, each of the four RCTs had a different treatment design, either in the NTF used, brain region(s) targeted and/or delivery method. Furthermore, using the GRADE criteria, the quality of evidence of this meta-analysis was characterized as low (further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate). In support of the pooled meta-analysis, lack of efficacy of NTFs in double-blind RCTs was reported in each of the four RCTs [15, 16, 18, 19].

It is important to consider potential reasons for the lack of effectiveness of GDNF and NTN in these RCTs. A number of possibilities have been proposed. The first is that the lack of effectiveness can be primarily attributed to the late timing of the therapeutic intervention. There is robust evidence from the authors of the reviewed trials that retrograde transport of

GDNF/NTN to the substantia nigra is impaired in patients with moderate-to-severe PD; the very low levels of DA in the striata of patients after >5 years of PD indicate that the disease has progressed significantly [19, 25-28]. Indeed, there is a profound loss of DA axons in the striata of PD patients at one year post diagnosis, and the innervating nigrostriatal DA axons are largely depleted by 4 years post diagnosis [26], highlighting the need for early intervention in PD. Such impaired axonal transport would severely reduce the amount of NTF that can reach the nigral DA cell bodies, and thus limit their neuroprotective effect [29]. Moreover, a post-hoc analysis has indicated that NTN has greater therapeutic benefits in PD patients evaluated <5 years after diagnosis [19]. Taken together, these findings suggest that if future trials are to take place, the recruitment of patients at an earlier disease stage, when the integrity of the nigrostriatal system is not so severely compromised, would be important. A second reason that has been proposed for the lack of efficacy of NTFs in PD clinical trials is that there may be inadequate bioavailability of NTFs in the PD striatum following their intracerebral administration [25, 27, 28, 30]. It has been reported that NTN has a poor diffusion profile *in vivo,* tipified by the fact that intrastriatal AAV2-NTN viral delivery resulted in NTN protein expression in only 15% of the striatal volume, with no NTN detected in the SN [28]. Despite this, the exact dose of NTFs applied, as well as the rapid internalisation of NTFs within neurons, is an important consideration when assessing NTF bioavailability in the PD brain [30, 31]. It is possible that limitations in the bioavailability and retrograde transport of NTFs in the brains of PD advanced patients impedes their neurotrophic effects on the remaining nigrostriatal DA neurons.

A third potential explanation for the lack of effectiveness of GDNF and NTN in the RCTs comes from studies in animal models of PD, which have shown that these NTFs may not be able to signal in the PD brain. α-synuclein, the protein which pathologically accumulates in PD [4], has recently been shown to downregulate the expression of the

GDNF/NTN receptor, RET, in the α-synuclein rat model of PD, in which GDNF has failed to demonstrate neurotrophic effects [32, 33]. Indeed, the authors of the latest NTN trial [19] stated that *"better results might be achieved with other trophic factors that are not RET dependent"*. For example GDF5, which like GDNF/NTN is a member of the transforming growth factor β superfamily, is a RET-independent DA neurotrophic factor that signals via BMP receptors and Smad signalling to elicit neurotrophic effects in midbrain DA neurons *in vitro* and *in vivo* [8, 34, 35]. Moreover, other studies have shown that employing a combination of NTFs may be more beneficial than administering a single factor [36]. Improving our understanding of the mechanisms of NTF action in the most disease-relevant models, and developing solutions to the potential issues of bioavailability, delivery and patient selection, will be critical for advancing this field.

Although no intervention-related deaths were identified in the studies included in this systematic review, concerns were raised with regards to less severe adverse effects, such as headaches, immune responses and other illnesses, which were not systematically reviewed herein. However, such milder adverse effects reported in the reviewed trials indicate that this neurosurgical procedure can be a burden to patients, which should be considered before the pursuit of further trials of this therapeutic approach in people with PD. We can conclude from these trials that intracranial application of NTFs is not a life-threatening therapeutic intervention.

To analyse data from all eight trials, both open-label and RCT, trial PD patients were compared to a predicted untreated control. The predicted untreated control were presumed to have no improvement in UPDRS score due to the progressive neurodegenerative nature of PD [4, 24], and because the patients that were included in these trials all exhibited moderate-to-severe PD. In this meta-analysis, NTF treatment had a significant effect on motor symptoms when compared to the predicted untreated PD controls (RR = 11.00; 95% CI =

3.85–31.45; P < 0.001). However, it is important to note that this effect did not take into account any potential placebo effect. This finding was obtained from pooled analysis of eight trials composed of 149 patients. Using the same criteria for meta-analysis, placebo-treated PD patients were compared to the predicted untreated controls. In this meta-analysis, placebo control treatment also had a significant effect on motor symptoms when compared to the predicted untreated control (RR = 7.67; 95% CI = 1.46–40.39; P < 0.05). This finding was obtained from pooled analysis of four RCTs composed of 74 patients. Placebo-induced improvements on motor symptoms of PD patients have been reported in other clinical trials previously, with placebo treatment also being shown to increase DA release in the striatum of PD patients [37-40]. Thus, a significant dopamine-mediated placebo effect in PD patients may affect measurements of the effectiveness of novel treatments in RCTs. The findings of these meta-analyses should be interpreted with caution due to the low quality of evidence, the nature of the controls, and the well-documented significant placebo effect that PD patients experience in clinical trials.

Finally, in order to determine the probability of improved motor symptoms in PD patients that received intracerebral NTF application, UPDRS scores post-intervention were compared to those at baseline of NTF-treated patients. This finding was obtained from pooled analysis of eight trials composed of 149 patients, and the quality of evidence of this meta-analysis was characterized as very low according to GRADE criteria. The pooled random effects analysis revealed that PD patients were not significantly likely to have improved UPDRS scores following intracerebral NTF application, when compared to the likelihood of no improvement (RR = 0.47; 95% CI = 0.13–1.71; P = 0.25). This finding may not be surprising considering the advanced disease stage of the PD patients included in these trials. Such information could be useful in informing treatment decisions for people with PD, and in deciding on inclusion criteria for the recruitment of PD patients for future clinical trials.

CONCLUSIONS

The current evidence indicates that intracerebral NTF application does not improve motor symptoms for patients with PD compared to placebo controls. However, it is important to note that the conclusions of this meta-analysis are based on a small number of studies that are, by their nature, characterised by a small sample size, which increases the probability of a type II error (concluding that the treatment is not effective when in reality it is). The evidence also indicates that intracranial NTF administration is not a life-threatening procedure. Despite this, the ineffectiveness of GDNF and NTN in the RCTs to date suggests that NTF therapy may not warrant further clinical trials using its current intervention strategy and trial design. It also highlights a critical need for continuing preclinical research, with the aim of developing approaches for harnessing the survival- and growth-promoting actions of NTFs into a more targeted, robust, and less invasive, therapeutic strategy. These findings may guide therapeutic decisions and clinical trial design, and inform future research.

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AUTHORS ROLES

AS is the guarantor. AS and SH are corresponding authors. SH, AS and GOK drafted the manuscript. All authors contributed to the development of the selection criteria, the risk of bias assessment strategy and data extraction criteria. SH, GOK and AS developed the search strategy. AS and DL both separately implemented the data extraction plan, and all authors contributed to the review of inclusions/exclusions and resolutions of disagreements (see methods for further details on this). SH provided statistical expertise and performed statistical analysis. SH, AS and GOK provided expertise on neurotrophic factor therapy for Parkinson's disease. SH, AS and GOK constructed and approved the final manuscript.

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FIGURE LEGENDS

Figure 1: Study Flow Diagram and Risk of Bias Summary

Figure 1: Study Flow Diagram and Risk of Bias Summary: (A) The search and selection procedure that was used for this systematic review and meta-analysis (adapted from [11]). (B) Review authors' judgements about each risk of bias item for each included study.

Figure 2: Effect of NTF Treatment on UPDRS Score Compared to Placebo Control

Figure 2: Effect of NTF Treatment on UPDRS Score Compared to Placebo Control: Forest Plot of Comparison 1: Intracranial NTF administration compared to placebo control. Outcome 1,1: Improved UPDRS score (post-intervention). Meta-analysis of 4 RCTs composed of 190 patients.

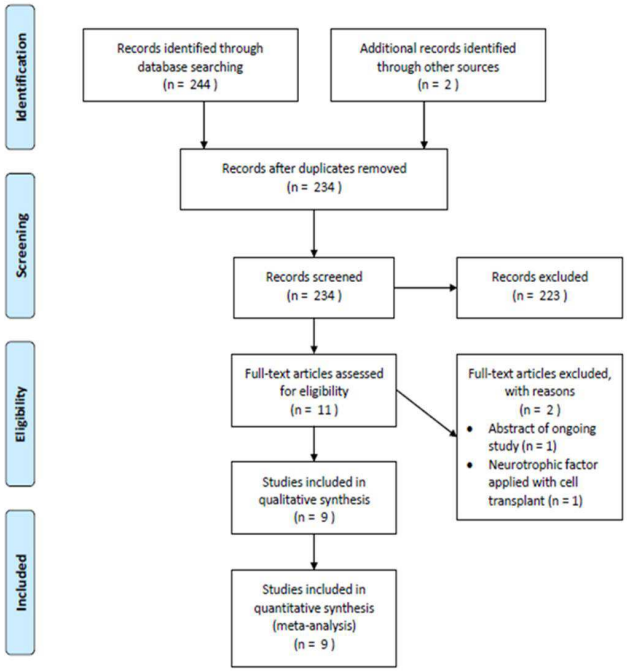
Figure 3: Effect of NTF Treatment or Placebo Control on UPDRS Score Compared to Predicted Control

Figure 3: Effect of NTF Treatment or Placebo Control on UPDRS Score Compared to Predicted Control: (A) Forest Plot of Comparison 2: Intracranial NTF administration compared to predicted control. Outcome 2.1: Improved UPDRS score (post-intervention). Meta-analysis of 8 trials composed of 149 patients. (B) Forest Plot of Comparison 3: Placebo control compared to predicted control. Outcome 3.1: Improved UPDRS score (post-intervention). Meta-analysis of 4 RCTs composed of 74 patients.

Figure 4: Comparison of Improvement vs. No Improvement in UPDRS Score following NTF Intervention

Figure 4: Comparison of Improvement vs. No Improvement in UPDRS Score following NTF Intervention: Forest Plot of Comparison 4: Improvement vs. No Improvement following NTF Intervention. Outcome 4.1: UPDRS score (post-intervention). Meta-analysis of 8 trials composed of 149 patients.

(A)



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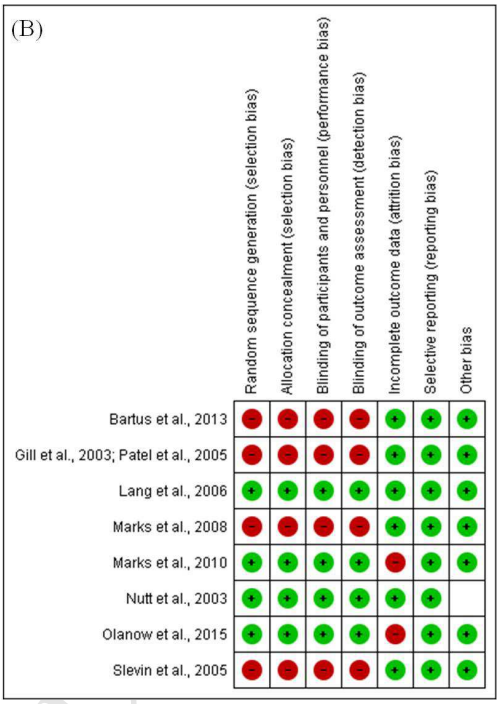
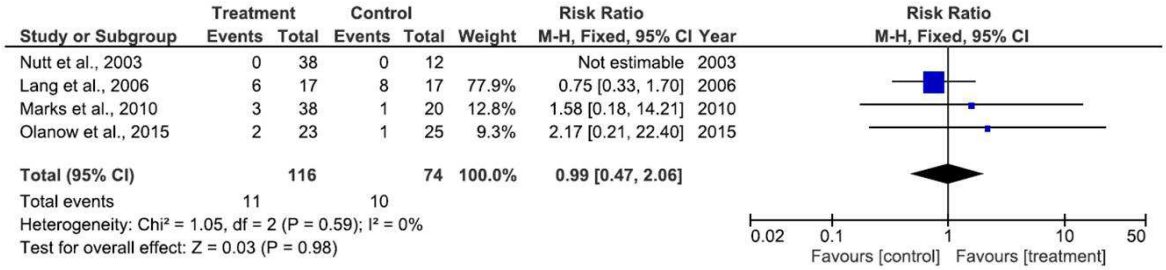
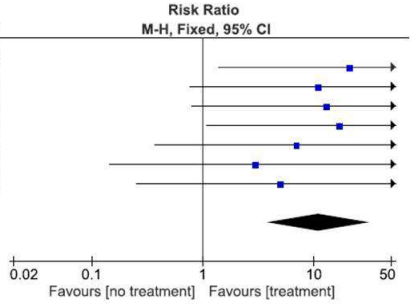




(A)

Study or Subgroup	Treated Events	Total	Predicted Untreated Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI	Year
Nutt et al., 2003	0	38	0	38		Not estimable	2003
Slevin et al., 2005	10	10	0	10	14.3%	21.00 [1.40, 315.98]	2005
Gill et al., 2003; Patel et al., 2005	5	5	0	5	14.3%	11.00 [0.77, 158.01]	2005
Lang et al., 2006	6	17	0	17	14.3%	13.00 [0.79, 214.05]	2006
Marks et al., 2008	8	12	0	12	14.3%	17.00 [1.09, 265.02]	2008
Marks et al., 2010	3	38	0	38	14.3%	7.00 [0.37, 131.06]	2010
Bartus et al., 2013	1	6	0	6	14.3%	3.00 [0.15, 61.74]	2013
Olanow et al., 2015	2	23	0	23	14.3%	5.00 [0.25, 98.75]	2015
Total (95% CI)		**149**		**149**	**100.0%**	**11.00 [3.85, 31.45]**	
Total events	35		0				

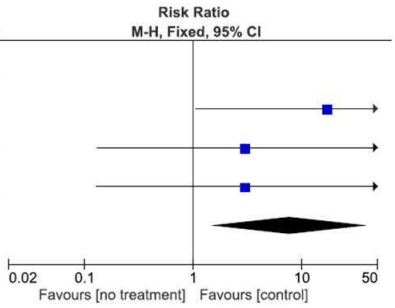
Heterogeneity: Chi² = 1.40, df = 6 (P = 0.97); I² = 0%
Test for overall effect: Z = 4.47 (P < 0.00001)



(B)

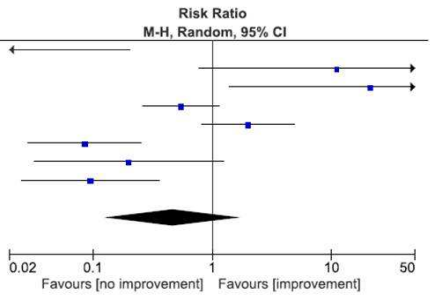
Study or Subgroup	Control Events	Total	Predicted Untreated Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI	Year
Nutt et al., 2003	0	12	0	12		Not estimable	2003
Gill et al., 2003; Patel et al., 2005	0	0	0	0		Not estimable	2005
Slevin et al., 2005	0	0	0	0		Not estimable	2005
Lang et al., 2006	8	17	0	17	33.3%	17.00 [1.06, 273.02]	2006
Marks et al., 2008	0	0	0	0		Not estimable	2008
Marks et al., 2010	1	20	0	20	33.3%	3.00 [0.13, 69.52]	2010
Bartus et al., 2013	0	0	0	0		Not estimable	2013
Olanow et al., 2015	1	25	0	25	33.3%	3.00 [0.13, 70.30]	2015
Total (95% CI)		**74**		**74**	**100.0%**	**7.67 [1.46, 40.39]**	
Total events	10		0				

Heterogeneity: Chi² = 1.00, df = 2 (P = 0.61); I² = 0%
Test for overall effect: Z = 2.40 (P = 0.02)



Study or Subgroup	Improvement Events	Improvement Total	No improvement Events	No improvement Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Nutt et al., 2003	0	38	38	38	9.4%	0.01 [0.00, 0.20]	2003
Gill et al., 2003; Patel et al., 2005	5	5	0	5	9.6%	11.00 [0.77, 158.01]	2005
Slevin et al., 2005	10	10	0	10	9.5%	21.00 [1.40, 315.98]	2005
Lang et al., 2006	6	17	11	17	15.5%	0.55 [0.26, 1.14]	2006
Marks et al., 2008	8	12	4	12	15.1%	2.00 [0.82, 4.89]	2008
Marks et al., 2010	3	38	35	38	14.6%	0.09 [0.03, 0.25]	2010
Bartus et al., 2013	1	6	5	6	12.3%	0.20 [0.03, 1.24]	2013
Olanow et al., 2015	2	23	21	23	13.9%	0.10 [0.03, 0.36]	2015
Total (95% CI)		**149**		**149**	**100.0%**	**0.47 [0.13, 1.71]**	
Total events	35		114				

Heterogeneity: Tau² = 2.68; Chi² = 48.52, df = 7 (P < 0.00001); I² = 86%
Test for overall effect: Z = 1.15 (P = 0.25)



HIGHLIGHTS

- Intracranial neurotrophic factor application did not reduce motor symptoms in PD
- NTFs and placebo improved UPDRS scores in PD over predicted scores in untreated PD
- PD motor symptoms are not likely to improve after intracranial application of NTFs
- Further studies are critical to advance the therapeutic application of NTFs for PD