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Expert Opinion on Investigational Drugs

ISSN: 1354-3784 (Print) 1744-7658 (Online) Journal homepage: https://www.tandfonline.com/loi/ieid20

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To cite this article: Stephen J. Ralph, Andrew Weissenberger, Ventzislav Bonev, Liam D. King, Mikaela D. Bonham, Samantha Ferguson, Ashley D. Smith, Adrienne A. Goodman-Jones & Anthony J. Espinet (2020) Phase I/II parallel double-blind randomized controlled clinical trial of perispinal etanercept for chronic stroke: improved mobility and pain alleviation, Expert Opinion on Investigational Drugs, 29:3, 311-326, DOI: 10.1080/13543784.2020.1709822

To link to this article: https://doi.org/10.1080/13543784.2020.1709822

EXPERT OPINION ON INVESTIGATIONAL DRUGS
2020, VOL. 29, NO. 3, 311–326
https://doi.org/10.1080/13543784.2020.1709822

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ORIGINAL RESEARCH

Phase I/II parallel double-blind randomized controlled clinical trial of perispinal etanercept for chronic stroke: improved mobility and pain alleviation

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ABSTRACT

Background: Previous open-label studies showed that chronic post-stroke pain could be abated by treatment with perispinal etanercept, although these benefits were questioned. A randomized double-blind placebo controlled clinical trial was conducted to test perispinal etanercept for chronic post-stroke pain.

Research design and methods: Participants received two treatments, either perispinal etanercept (active) or saline (control). Primary outcomes were the differences in daily pain levels between groups analyzed by SPSS.

Results: On the 0–100 points visual analog scale, perispinal etanercept reduced mean levels for worst and average daily pain from baseline after two treatments by 19.5 – 24 points ($p < 0.05$), and pain alleviation was maintained in the etanercept group, with no significant change in the control group. Thirty percent of etanercept participants had near complete pain abatement after first treatment. Goniometry of the paretic arm showed improved mean shoulder rotation by 55 degrees in active forward flexion for the etanercept group ($p = 0.003$) only.

Conclusions: Perispinal etanercept can provide significant and ongoing benefits for the chronic post-stroke management of pain and greater shoulder flexion by the paretic arm. Effects are rapid and highly significant, supporting direct action on brain function.

Trial registration: ACTRN12615001377527 and Universal Trial Number U1111-1174-3242.

ARTICLE HISTORY
Received 10 December 2019
Accepted 24 December 2019

KEYWORDS
Clinical trial; perispinal etanercept; reduced post-stroke pain; increased flexion

1. Introduction

1.1. Scientific background, clinical relevance, rationale, and objectives

Major impairment after stroke is exacerbated when accompanied by chronic, severely debilitating and intractable long-term pain [1,2]. Strokes in different regions of the brain are often associated with the occurrence of central post-stroke pain (CPSP). CPSP is a neuropathic pain disorder, arising from a combination of both central [3] and peripheral nervous system mechanisms [4]. CPSP is a highly intractable disorder with significant health burdens [1,2], as is stroke disability itself. A recent systematic review of clinical trials for CPSP has shown no beneficial effects for any other experimental treatments [5]. CPSP frequently requires the use of strong analgesics which may result in further significant impairment and reduced quality of daily life. Often, patients also demonstrate clinical features such as depression and have greater risk of suicide [2]. Hence, finding a beneficial therapy is imperative.

The common proinflammatory cytokine, tumor necrosis factor alpha (TNFα) is involved in all phases of stroke, including rehabilitation (reviewed in [6]). Initially, during stroke, TNFα is synthesized and released by astrocytes, microglia, and neurons in response to ischemia and is a major factor in the pathophysiological processes of stroke. TNFα activates the microglia and astrocytes, affects the blood–brain barrier permeability, and can adversely affect synaptic transmission and synaptic plasticity during stroke, including rehabilitation [7]. TNFα has long been implicated as a key factor in the post-stroke neuroinflammatory response with levels in the CSF and plasma [8–10] correlating with severity of symptoms and as a mediator of focal ischemic brain injury [11]. Elevated TNFα levels not only exist in the cerebral spinal fluid in the acute stage [9,12–18] but also in chronic post-stroke patients [19] with increased TNFα expression found in postmortem brain long after the initial stroke episode [20].

TNFα has also been established as an important mediator of neuropathic pain in animal models [21,22] where blocking TNFα alleviated this pain. The role of TNFα in neuropathic pain has recently been extensively reviewed elsewhere [23]. For over two decades, research studies have shown the relief of neuropathic pain, recognized and reported for chronic stroke with the use of the TNFα blocker, etanercept – an agent comprising immunoglobulin fused with the soluble

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Supplemental data for this article can be accessed here.

TNFα receptor domain (reviewed in [24–26]). Studies with tracers also showed that administering large molecules such as etanercept, via injection into the perispinal space, facilitates their uptake into the cerebrospinal venous plexus, thereby providing an effective method for direct delivery into the brain through the choroid plexus [27,28]; reviewed in detail in [29]. Such treatments using etanercept were also shown to alleviate neuropathic pain in rat models [28,30] and in open-label use for human patients with stroke [31,32]. Thus, previous observational studies on 600 patients reported that perispinal etanercept (PSE) therapy provided rapid improvements (within 30–60 min after treatment) in stroke-related disabilities, including pain [32]. It was concluded that perispinal delivery of etanercept was mediating the rapid actions by a direct effect of blocking TNFα in the central nervous system [27–29,31–33]. These reports were met with controversy such that the American Academy of Neurology published a practice advisory in 2016 noting that the evidence to support or refute a benefit of etanercept for treatment of post-stroke disability was insufficient to determine the treatment's effectiveness [34].

Since its first approval by the FDA in 1998, etanercept has been used to treat a spectrum of rheumatoid disorders as a generally well-tolerated drug with a favorable safety profile, even when administered weekly for many years on a chronic basis in elderly patients or children [35–37]; for review of safety profiles, see [38,39]. Etanercept may provide advantages over other TNF blockers by inhibiting both TNFα and TNFβ; has a higher affinity for TNFα than the monoclonal antibody therapies; and in some studies has shown less adverse effects [40,41]. This trial was designed to determine whether the perispinal etanercept injection procedure developed to treat post-stroke patients for pain and other dysfunction, was in fact successful and worthwhile. Furthermore, the methods followed were exactly those approved and used by Dr Tobinick and have been well documented [32,42–49]. Therefore, we undertook the first randomized double-blind clinical trial to determine the effects of two treatments at day 1 and day 14 of perispinal etanercept therapy with primary outcome measures examining the differences from baseline levels in patients with constant daily post-stroke pain to day 30, 2 weeks after the second treatment on trial.

2. Patients and methods

The CONSORT guidelines were followed in the preparation of this publication.

2.1. Description of trial design including allocation ratio

This was a double-blind randomized controlled parallel trial with 1:1 allocation ratio between individuals receiving either etanercept (active) treatment or saline (placebo) control. Approval of the study was obtained from the Griffith University Human Research Ethics committee (MSC/10/14/HREC). Subject applications were clinically evaluated by a neurologist during the screening process for eligibility. The study protocol was fully explained to participants. Informed and written consent was obtained before participation with allocation based on the numerical value assigned upon enrollment into either the etanercept or control group (Figure 1). Trial clinical investigators including the neurologist were involved in the enrollment of participants.

Specific inclusion criteria used for screening were initially based on the following four requirements:

- Aged between 30 and 80 years old encompassing the most frequent age incidence given stroke is uncommon in young adults [50] and to reduce the possibility of comorbidities in older patients.
- Stroke occurring at least 6 months and not more than 15 years prior to screening for this study.
- Chronic neurological impairment, including hemiparesis, following an ischemic stroke in the territory of the middle cerebral arteries (MCA) (including MCA clot or embolus or carotid occlusion causing MCA territory stroke) or basal ganglia form of intracerebral hemorrhage.
- Constant daily pain post-stroke incorporating one or both contralateral limbs and experiencing intractable chronic post-stroke pain with hemiplegic post-stroke shoulder pain and central post-stroke pain. The post-stroke pain is moderate-to-severe in intensity, with a daily average intensity between 4 and 8 inclusive on an 11-point (0–10) vertical Numerical Pain Rating Scale supplemented with a faces pain scale (vNPRS-FPS) [51].
- The post-stroke pain is in an area of the body affected by the stroke.

As aphasia is recognized as less common in patients with right than left sided MCA strokes, this randomized, parallel-group controlled clinical trial was initially aimed at studying the clinical effects of perispinal delivery of etanercept versus saline in a cohort of participants with chronic ischemic stroke in the territory of the right MCA. In addition to pain as a primary outcome measure, shoulder flexion, spasticity, cognition, executive function, hemispatial neglect, and post-stroke depression were examined, some as exploratory outcome measures because it was unknown whether the trial would be adequately powered for these particular endpoints.

Before participating, all participants underwent physical examination and vital signs were recorded. All patients were informed prior to trial that any previous medication used regularly was to be maintained and not altered during the period from 2 weeks before visit 1 on trial until day 30 after visit 1.

2.1.1. Important changes to methods after trial commencement (such as eligibility criteria), with reasons

The initial cohort was based on right MCA stroke but due to the limited numbers of available patients presenting that met the initial criteria, enrollment was widened to expand the age limit to 27 and to include non-aphasic stroke patients with left MCA strokes and right-sided impairment or basal ganglia strokes. Applicants were reviewed on a case by case basis by

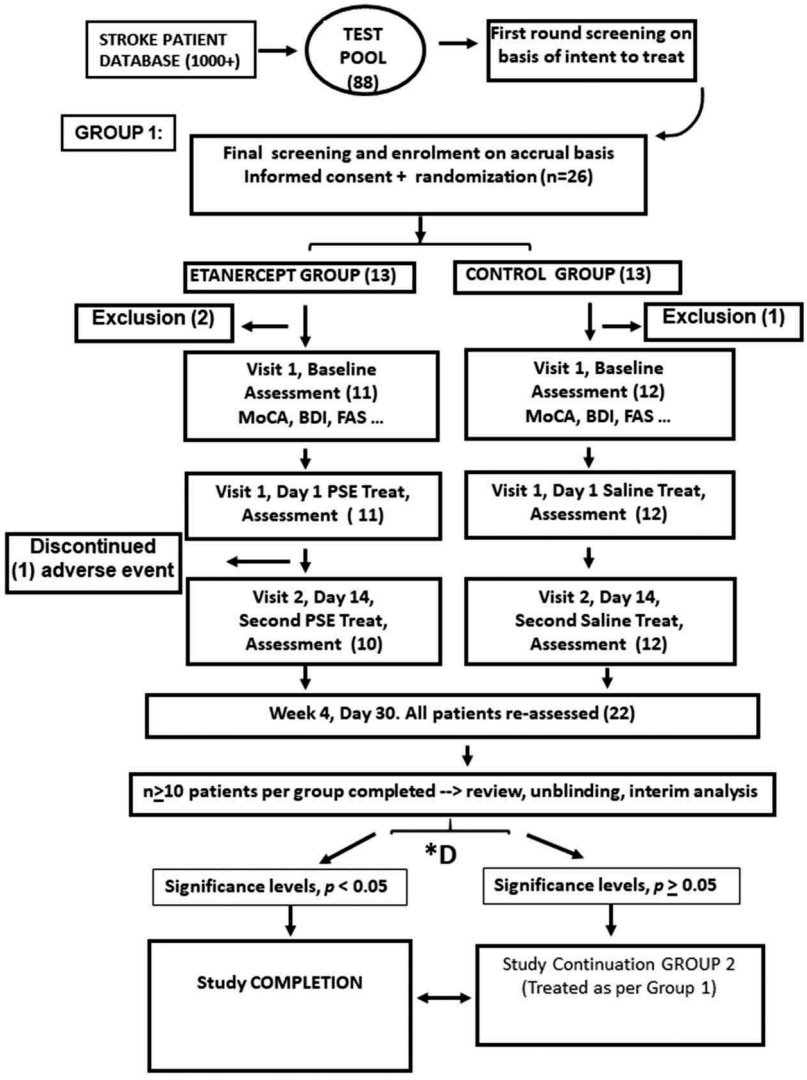

Figure 1. Trial profile. Outline of study protocol and regimen for randomization on enrollment into the etanercept and control group, including numbers of patients in brackets and all dropouts due to exclusion or adverse events. *D: decision point upon trial completion after interim unblinding and primary outcome measures showed significance values for $p < 0.05$.

the neurologist and assessed for their capacity to understand and communicate during verbal testing.

3. Eligibility criteria for participants

3.1. Inclusion criteria

At the time of recruitment, all participants were required to travel to the study site and speak fluent English to facilitate effective communication regarding their pain levels. Participants started a pain diary including listing all medications taken from day −7 prior to visit 1 and were required to score their vNPRS-FPS through to completion at day 30 after visit 1, which were collected. For the complete list of specific inclusion/exclusion criteria, including neurological details of stroke diagnosis, refer to Supplementary Table 1.

3.1.1. Settings and locations where the data were collected

Patient medical histories relating to their stroke and hospital discharge summaries were collected via e-mail or as hard copies for evaluation by the study investigators during enrollment. All data was collected, bound and stored with Case Report Forms for each participating subject at the Clinical Trials Unit, G40 Health Center, Griffith University Gold Coast campus, Southport Queensland.

3.2. Interventions and administering procedure

Two active or control treatments (the first at visit 1/day 1 and the second at visit 2/day 14) on trial were administered with all injections double-blinded to the principal medical investigators and participants. Assessments measuring the responses of participants to treatments were also undertaken in a blinded manner. Treatments were to be halted in the event of an adverse reaction or participant request to discontinue participation. Each etanercept (ENBREL®, Pfizer, USA) single-use injectable dose was prepared by solubilizing with the addition of 1.8 cc of non-bacteriostatic sterile water into a 25 mg lyophilized powdered vial of etanercept (containing 10 mg/mL sucrose, 5.8 mg/mL sodium chloride, 5.3 mg/mL

L-arginine hydrochloride, 2.6 mg/mL sodium phosphate, monobasic, monohydrate, and 0.9 mg/mL sodium phosphate, dibasic, anhydrous). A single dose was administered by injection overlying the spine, as described previously [32,42–49]. Thus, each dose was delivered to the participant in a sitting position with their head bent forward, reaching the chin toward the chest and with the neck horizontal. The injection was given subcutaneously into the posterior cervical interspinous midline (into the interspace midway between C6-C7 or C7-T1 vertebra) using a 27 gauge, half-inch needle, free-hand guided and inserted fully into the skin at an angle near perpendicular to the surface. The injection was quickly followed by Trendelenburg positioning, with participant supine on an inversion table and the head dependent for 4 min at an incline of 45 degrees. This approach is in order to effect entry into the cerebrospinal venous system (CSVS) as a previously validated delivery to the brain via the choroid plexus detected using radioactive or fluorescent-tagged etanercept [27,28]; for an extensive recent review outlining this mechanism of drug delivery to the brain, see [29]).

3.3. Control

The control treatment was sterile saline (suitable for human injection) as a clear colorless solution, with the same appearance as for the etanercept, and prepared in the same type of syringe with the same volume of 1.8 ml as for the test drug. The control was administered using the identical perispinal injection procedure as for the active drug treatment described above.

3.4. Outcome measures

Measures are underlined below.

3.4.1. Primary outcome measures

3.4.1.1. Pain. On the vertical Numerical Pain Rating Scale (vNPRS), patients were asked where they would mark the number between 0 and 10, or 0 and 100 that fits best to their pain intensity. Zero represented 'no pain at all' whereas the upper limit represented 'the worst pain imaginable'. The 11-point vertical Numeric Pain Rating Scale supplemented with a Faces Pain Scale (vNPRS-FPS) [51] was provided to patients in the weeks preceding trial visit 1. This was used to assess their levels of pain and familiarize prospective trial participants with the pain test as a guide for self-reporting daily pain intensities recorded on their pain diary from day −7 before participation on trial, until day 30. Participant pain levels were also assessed by interview using an expanded 0–100 point (1 cm/5 point numeric interval on the vertical visual analog scale with two emoticon face indicators: one for 'no pain' at 0 and one for 'worst pain imaginable' at 100) vNPRS-FPS placed before patients during trial visits 1 and 2, recording both pre- and post-treatment scores, as well as recorded on day 30 by phone interview. Average and Worst levels of Pain: Changes in mean vNPRS-FPS (change in pain intensities on the 0–100 point scale) for values recorded from visit 1 before treatment (the baseline (PRE) values) compared with on the final day of participation on trial (day 30 after visit 1; D30). Participants were asked at visits 1 and 2 on trial before and after treatment to rate their 'average' and 'worst' pain levels considering the last 8 h and the previous week.

3.4.2. Secondary outcome measures

Average level of Pain: Change in mean vNPRS-FPS scores (0–100 point scale) from baseline compared with day 1 (visit 1 after treatment) and day 14 (visit 2 after treatment), where the subject was rated for his or her 'average pain' over the last 8 h and considering the previous week. Worst level of Pain: Change in mean vNPRS-FPS score (0–100 point scale) from baseline compared with visit 1 and 2 after treatment, where the subject was rated for his or her 'worst pain' over the last 8 h and considering the previous week. Instant Change in Worst Level of Pain: between 30 and 60 minutes before treatment on day 1 (visit 1) to 30–60 min after treatment at visit 1. Shoulder Flexion/Spasticity: mean change in paretic arm by angle of shoulder flexion (active and passive) (measured in degrees of rotation by goniometry) before and after treatment at each visit on day 1 and day 14. Cognition: mean change in Montreal Cognitive Assessment (MOCA) [52] score from baseline on day 1 compared to day 14 (visit 2, after treatment). The Albert's Line Bisection Test is used to detect unilateral visuospatial neglect [53]. Hemispatial Neglect: mean change in Albert's Line Bisection Test from baseline on day 1 to day 14 (visit 2, after treatment). Fatigue Assessment Scale (FAS): mean change in FAS level (out of 50) from baseline to day 30. The FAS has 10-items as statements about different aspects of fatigue, each rated from 1 to 5 (1, never; 2, sometimes; 3, regularly; 4, often; and 5, always) and is a valid and reliable test in stroke with higher scores indicating greater fatigue [54].

3.4.3. Exploratory

The Clock Drawing Test (CDT) is used as a psychometric measure for mild to moderate cognitive impairment [55]. Cognition: mean change in CDT score from day 1 of treatment to day 14 (visit 2, after treatment). Motor Function/Balance: mean change in time to complete the five times Sit-To-Stand test from day 1 to day 14 (visit 2, after treatment). Psychological/behavioral function: mean change in Beck's Depression Inventory (BDI) scores from day 1 to day 14 (visit 2, after treatment). Hemispatial Neglect (Instant change, in participants with hemispatial neglect on day 1): mean change in Albert's Line Bisection Test score from 30 to 60 minutes before treatment on day 1 to 30–60 min after treatment on day 1. Thermosensory analysis: mean change in pain detection and pain thresholds using the TSA II thermosensory device (Medoc Advanced Medical Systems) from day 1 (visit 1, prior to treatment) to day 14 (visit 2 after treatment).

3.4.3.1. Changes to trial outcomes after the trial commenced, with reasons. A Fatigue Assessment Scale (FAS) test, shoulder flexion as well as algometry (ALG) were added as secondary outcome measures to assess changes in patient fatigue levels and sensitivity to pressure. A Force 10 FDX-25 force gauge (Wagner Instruments) in peak mode was used for algometry over the medial area of the lower anterior arm regions, repeated three times on each arm and averaged over triplicate measures in Newtons for analysis. The Medoc

TSA II Quantitative Sensory Thermoanalyser was also included to evaluate the mean of triplicate tests for patient thermal detection and pain sensitivity over the medial forearm [56,57].

3.4.4. Sample size and power estimation

The sample size of 20 patients on trial with 10 completed in each study group (at least 10 control and 10 etanercept participants) was based on the published data from observational studies reported previously [32,43]. The assumption was that the population of participants and their outcomes were normally distributed. Dr David Schoenfeld's Harvard website http://hedwig.mgh.harvard.edu/sample_size/js/js_parallel_quant.html was used for sample size determination. This was validated by the trial biostatistician. For the outcomes used in this study, power estimations were as follows:

3.4.4.1. Power calculation for sample size based on previous reports of analyses by pain test. The values used for this power calculation were taken from those reported [32] (Table 10), obtained after one injection of perispinal etanercept treatment. From this study, the group baseline for the vertical assessment scale (VAS) mean score (±S.D.) was 7.1 (± 2.09) on the 11-point scale [32]. After treatment, the mean score (± S.D.) was 2.3 (± 2.81). Based on this data, a minimum total of 14 patients (7 in each group) were required for enrollment with power to detect a treatment difference = 83% at a two-sided 0.05 significance level. Hence, given the predicted size effect, the study was considered suitably powered with a minimum number of patients at 20 to reach levels of significance.

3.4.5. Interim analyses and stopping guideline

After the first randomized group reached 10 patients on both the control and etanercept groups (total of n = 26 enrolled patients with n = 22 participants completing week 4/day 30 on trial), interim unblinding and analysis of outcomes was triggered.

3.4.6. Method used to generate the random allocation sequence

A computer-based random number generator in blocks of five was used by the pharmacist to establish the trial unblinding code for random assignment of enrolled patients into either group (allowing up to a total of at least 40 patients in each group, if required).

3.4.7. Randomization, blinding and patient replacement procedures

Patients were assigned based on the randomization code if they met all the inclusion criteria and none of the exclusion criteria. Every subject who passed initial screening based on inclusion/exclusion testing was included as intent to treat as they were assessed and entered numerically into the test pool. A final round of screening occurred as patients attended the first treatment, visit 1 at the clinic for validation as suitable for enrollment. A numbered ID was assigned to each participant by the trial pharmacist. Any patient on trial who discontinued or failed to complete the study was replaced and the replacing patient denoted with the next numbered ID in the order of the randomization number code. Allocation was concealed from clinical investigators, assessors, and participants during the trial to ensure double-blinding.

Interventions were prepared by the pharmacist to be identical in appearance and placed inside containers labeled only with patient ID and number on enrollment, with the identification of the intervention sealed inside an unblinding envelope at the bottom of the containers and to be opened only in the event of emergency.

4. Statistical methods used to compare groups for primary and secondary outcomes

For statistical analyses, Statistical Package for Social Sciences (SPSS; Vn25) software program was used, available at Griffith University. The data from all analyses of each individual participant were recorded on their Case Report Form and data from this entered onto spreadsheets using Microsoft Excel and then processed via SPSS for generalized estimating equations with first-order autoregressive relationship as the working correlation matrix. Other analyses included two-tailed paired or independent-samples t-tests, ANCOVA, logistic regression using the general linear model with repeated measures or non-parametric and other suitable tests as required. Univariate analysis of variances (UNIANOVA) was used to provide an estimation of effect size (η^2; eta-squared value) and observed power. Analyses included comparison of outcomes within and between the etanercept and the control groups for differences in median scores using the Wilcoxon signed-rank test for pain scores out of 100 points or mean scores from cognitive function, sensory, motorneuron or other tests, as well as differences from baseline. The data analyses included changes from baseline scores with standard error about the mean or interquartile ranges about the changes in median levels. Assumptions of normality were satisfied using Shapiro–Wilks test, normal Q–Q plots of differences and box-plot outlier analysis. For Mann–Whitney U exact tests, the treatment effects on pain levels (difference between groups) were quantified using the Hodges-Lehmann (HL) estimator from SPSS. This estimator (HLΔ) was used to determine the median of all possible differences in outcomes between each subject in the etanercept group versus each subject in the control group. A non-parametric 95% confidence interval for HLΔ accompanied these estimates and determined the median of differences between the two groups or the location shift in the median. For analysis of thermal perception and pain thresholds, multilevel modeling with the linear-mixed models (LMM) function in SPSS was also used. Bonferroni corrections were applied for multiple comparisons. Where relevant for all the above analyses, p-values <0.05 were considered significant and <0.01 highly significant.

Four participants initially enrolled into the trial were excluded. From the etanercept group, one developed shingles after visit 1, one had complications of severe lower back pain from spinal stenosis considered unrelated to stroke, and one demonstrated delusional features and hence, was unreliable for assessment. One patient was excluded from the control group after starting oxycodone medication the week of visit 1 and on Day 1, no longer experienced any pain. Patients were

recruited over the period from November 2016 through to March 2019.

4.1. Decision for study completion

After interim review of the first cohort (n = 22 participants) showed significance ($p < 0.05$) across both primary (baseline to day 30 change in vNPRS-FPS pain measures on 0–100 scale) and secondary outcomes (pre-post visit 1 change in vNPRS-FPS and shoulder flexion in arc degrees by goniometry), the completion phase was triggered. The study was then stopped early due to the significance of the positive results. Of the completing participants, for each individual, all four data points (baseline; visit 1 after treatment 1; visit 2 after treatment 2; and day 30) were included in the vNPRS analysis and by original assigned groups with n = 10 in the active and n = 12 in the control group used for comparison.

4.2. All important harms or unintended effects in each group

No serious adverse events were recorded on trial. However, a single adverse event occurred with one patient developing shingles after visit treatment 1. A clinical review of etanercept usage reported herpes viral opportunistic infections as a treatment-emergent adverse event of special interest (AESI) [39], as specified by the Federal Drug Administration, USA and hence is not a serious adverse event. The risk of shingles was previously noted only after long-term therapy with anti-TNFα medication [58] and in Strangfeld et al. (2009), the risk of developing shingles was not increased for those treated with etanercept [41]. Hence, the present case may have resulted from a compromised immune status due to the chronic pain. Consequently, this patient was excluded from further participating on-trial as the results would be complicated by the pain from shingles and potential for post-herpetic neuralgia.

5. Results

Of the 26 CPSP patients enrolled on the trial, 22 completed the protocol. Only the one adverse event of special interest (AESI) [39], due to a case of shingles occurred as a possible treatment-emergent risk. The distribution of the study group and participant baseline characteristics are shown in Table 1. All participants initially demonstrated significant intractable and constant daily CPSP with pain scores at baseline entry between 40 and 80 inclusive on the 0–100 point vNPRS-FPS, with their pain refractory to analgesic medications (including oxycodone or pregabalin). Seven participants in the etanercept and five in the control group had limited shoulder flexion of their paretic arm with active motion ≤75 degrees at baseline. No significant baseline differences between the groups were noted for any of the trial measures by independent samples t-test ($p > 0.05$; Table 1) and no unequal variances by Welch's t-test.

5.1. Primary outcome measures

5.1.1. Significant decline in pain severity of patients receiving perispinal etanercept compared to control

The descriptive statistics for the pain scores out of 100 for each group at baseline, visit 1 (V1) after treatment and at day 30 are reported in Table 2, including the medians and means for the two groups and the interquartile ranges (IQR). The change from baseline in pain intensities (based on the 0–100 point score on the vNPRS-FPS) for the mean values over the four repeated measures of average pain (including baseline (PRE); visit 1 (V1) after treatment 1; visit 2 (V2) after treatment 2; and at day 30 (D30 after treatment 1) scores) for each group were compared (Figure 2(a)).

Analysis by original assigned group of the repeated measures using generalized estimating equations showed a significant group*treatment interaction for worst pain (Wald $\chi^2 = 4.58$; df 1; $p = 0.032$) and for average pain (Wald $\chi^2 = 4.161$; df 1; $p = 0.041$). Post-hoc analysis demonstrated a greater reduction in pain levels for the etanercept compared

Table 1. Baseline characteristics of patients on the stroke trial.

Characteristic mean (±S.E.)	Etanercept 25 mg (n = 10)	Saline control (n = 12)	Mean difference (95%CI), p value (2-tailed)
Age, y	57.3 (4.95)	61.65 (8.66)	−4.35 (−15.3 to 6.6), $p = 0.42$
Weight, kg	77.74 (5.73)	85.85 (4.86)	7.09 (−22.9 to 6.7); $p = 0.266$
Gender M:F	5:5	7:5	
Years since stroke	4.18 (0.72)	4.98 (1.15)	−0.8 (−3.43 to 1.82), $p = 0.52$
Average daily pain (vNPRS-FPS)	68 (3.35)	60.42 (485)	7.58 (−5.18 to 20.35), $p = 0.23$
Worst daily pain	82.5 (3.96)	74.58 (3.45)	7.92 (−3.0 to 18.83), $p = 0.15$
Active Shoulder Flexion/ROM[a]	55 (15.69)	84.11 (13.26)	−29.11 (−73 to 14.8), $p = 0.179$
Passive Shoulder Flexion/ROM[a]	103 (10.25)	116.11 (12.35)	−13.11 (−46.73 to 20.51), $p = 0.42$
MOCA	21.8 (2.33)	25.5 (0.77)	−3.66 (−8.6 to 1.29), $p = 0.138$
BDI	17.6 (3.32)	20.8 (3.36)	−3.23 (−13.18 to 6.72), $p = 0.51$
FAS	31.4 (1.54)	34.8 (1.62)	−3.43 (−8.16 to 1.29), $p = 0.145$
STS	19.5 (2.7)	24.4 (3.5)	−4.88 (−14.4 to 4.6); $p = 0.298$
CDT	N/A	N/A	N/A
Albert's Line Bisection Test/	N/A	N/A	N/A

S.E, standard error; y, years; vNPRS-FPS, vertical Numeric Pain Rating Scale with a Faces Pain Scale (0–100); MOCA, Montreal Cognitive Assessment test; BDI, Beck's Depression Inventory; STS, 5 times Sit-to-Stand/seconds; FAS, fatigue assessment score/50. CDT, Clock Drawing Test; N/A, not applicable. [a]Values shown for patients with restricted (<180 degree) shoulder flexion/rotation of movement (ROM) in arc degrees at baseline only for the paretic/hemiplegic arm (n = 10 in etanercept group, n = 9 in control group).

Table 2. Nonparametric related-samples tests and descriptive statistics for pain scores comparing within-group responses.

A. Descriptive Statistics

GROUP /(Numbers)		Mean	S. D.	Min.	Max.	Percentiles 25th	50th (Median)	75th
1. Etanercept (10)	V1_PRE_Av_Pain	68.00	10.593	50	80	58.75	70.00	76.25
	V1_POS_Av_Pain	44.00	31.429	0	90	15.00	50.00	71.25
	D30_Av_Pain	44.10	28.862	0	80	22.25	50.00	65.00
	V1_PRE_Worst_Pain	82.50	12.528	60	100	72.50	87.50	90.00
	V1_POS_Worst_Pain	49.00	35.182	0	90	3.75	60.00	78.75
	D30_Worst_Pain	63.00	24.967	0	90	57.50	70.00	80.00
2. Control (12)	V1_PRE_Av_Pain	60.42	16.714	30	85	50.00	62.50	70.00
	V1_POS_Av_Pain	57.08	24.258	0	95	50.00	57.50	72.50
	D30_Av_Pain	57.50	25.540	0	95	42.50	60.00	77.50
	V1_PRE_Worst_Pain	74.58	11.958	50	90	66.25	77.50	85.00
	V1_POS_Worst_Pain	61.25	25.595	0	100	52.50	60.00	75.00
	D30_Worst_Pain	72.08	16.440	40	95	60.00	75.00	87.50

B. Related-Samples Wilcoxon Signed-Rank Test

Related-Samples:	V1PRE-D30 Average Pain		V1PRE-D30 Worst Pain		V1PRE-V1POST Average Pain		V1PRE-V1POST Worst Pain	
Group:	Etanercept	Control	Etanercept	Control	Etanercept	Control	Etanercept	Control
Total Numbers	10	12	10	12	10	12	10	12
Test Statistic	2.000	12.500	3.500	23.000	3.000	26.500	0.000	13.000
Standard Error	7.133	5.809	9.747	9.740	7.141	11.164	7.133	11.186
Standardized Test Statistic	-2.243	-0.258	-2.462	-0.462	-2.100	-0.582	-2.524	-1.788
Asymptotic Sig.(2-sided test)	0.025	0.796	0.014	0.644	0.036	0.560	0.012	0.074
Exact Sig. (2-sided test)	0.023	0.859	0.012	0.687	0.039	0.584	0.008	0.079

S.D., Standard Deviation; V1, visit 1; PRE – before treatment, POS – after treatment; D30, day 30. Min., minimum; Max., Maximum. Percentile scores are shown out of 0–100 on the vNPRS-FPS to indicate interquartile ranges; Sig., significance.

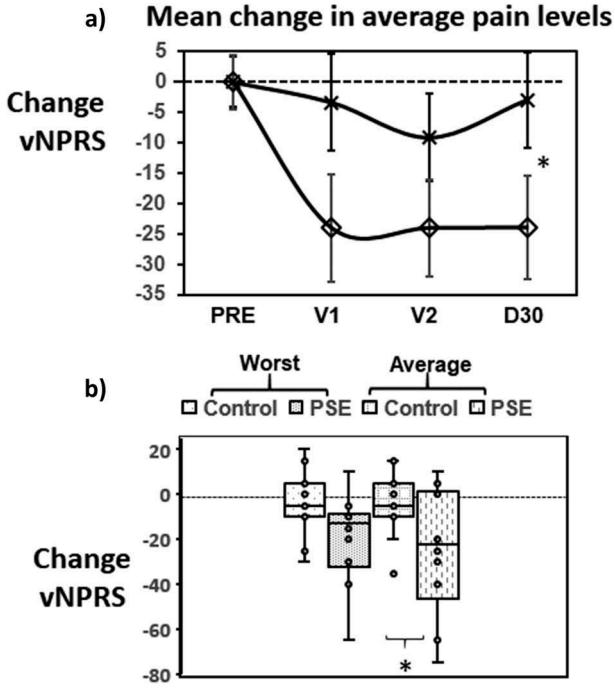

Figure 2. (a) Pain levels are rapidly decreased following PSE treatment. Mean changes in score levels of average pain on the vNPRS-FPS (0–100 points scale) comparing between the saline control (×) and etanercept (◊)-treated groups ± S.E. PRE: baseline; V1: Visit 1 after treatment 1; V2: Visit 2 after treatment 2; D30: day 30 after Visit 1. (b) Box-plot analysis of differences in median of worst and average pain levels comparing between baseline and day 30. Control group compared to perispinal etanercept (PSE) treatment group with medians, quartile ranges, and outliers for changes in pain levels as shown on 0-100 point scale. * $p < 0.05$.

to the control group over the trial period with a mean decline (change on 0–100 point scale ± S.E.) in average and worst daily pain levels from baseline to day 30 within the etanercept group of 24 ± 9 and 19.5 ± 6 points, respectively (Figure 2(a, b)). The decline in pain levels from baseline to day 30 within the etanercept group for both the average and worst daily pain levels were significant (two-tailed paired t-test: $t_{(9)} = 2.63$; $p = 0.027$; $t_{(9)} = 2.94$; $p = 0.017$, respectively).

Mann–Whitney U tests showed a significant reduction in both average and worst pain scores comparing the differences in vNPRS-FPS (on the 0–100 point score) from baseline to day 30 after the two perispinal etanercept treatments. From Figure 2(b), the values from the Mann–Whitney U test were for average pain: U = 29.5; $p = 0.04$, HLΔ between group medians = 15; 95% CI = 0–40; and for worst pain: U = 26; $p = 0.023$, HLΔ between group medians = 15; 95% CI = 5–30. Table 2 also shows the results from the related-samples Wilcoxon Signed-Rank test summary with the levels of significance for the median of differences within groups from baseline. Again, the median of differences (two-sided test) between baseline and day 30 for worst ($p = 0.014$) and average ($p = 0.025$) pain scores from the etanercept group were significantly different, whereas the pain values for the control group (p > 0.5) were not significantly altered.

In order to obtain an estimate of the power of the study for detecting the change in pain levels, UNIANOVA was applied and the comparison between groups for changes in mean vNPRS-FPS scores out of 100 points from baseline to day 30 after treatment were significant (for worst pain: $p = 0.037$, $\eta^2 = 0.20$, observed power of 56% and for average pain, $p = 0.045$, $\eta^2 = 0.19$, observed power of 53%). At the individual level, 4 of the 10 patients in the etanercept group showed no or limited effects on pain, whereas 3 others had rapid and complete or almost complete resolution of their pain levels directly (by 30 min) post-treatment during visit 1.

Within the control group, there was no significant decrease in the median values for the average or worst daily pain levels (which each decreased by only 2.5 points on the 0–100 point scale) comparing baseline to day 30 (D30; by related-samples Wilcoxon Signed Rank two-sided tests, p > 0.5 and by paired t-tests: average pain, $t_{(11)} = 0.52$; $p > 0.5$ and worst pain, $t_{(11)} = 0.59$; p > 0.5). Categorically, one participant in the control group demonstrated a response for average pain during the trial (Figure 2(b); outlier).

5.2. Secondary outcome measures

1) Instant differences in worst and average pain.

For the secondary outcome measures, within the etanercept group a significant difference was detected in the immediate effects from baseline at visit 1 compared to directly after treatment (within 30–60 min) on the 0–100 point scale for the change in worst and average pain levels (paired t-test: $t_{(9)} = 3.24$; $p = 0.01$ and $t = 2.59_{(9)}$; p = 0.029, respectively). Post-hoc nonparametric tests demonstrated that for this instant pain reduction, the median decrease (on the 0–100 point vNPRS-FPS) for worst pain was by 27.5 points (related-samples Wilcoxon signed-rank test; p = 0.012) and the mean ± S.E. decreased by 33.5 ± 10.4 points (out of 100) within the etanercept group (Table 2; Figure 2). By comparison, within the control group, the median of the differences in worst pain was 17.5 points (related-samples Wilcoxon signed-rank test; p > 0.05) and the mean change was by 13.33 ± 6.7 points, which was non-significant (paired t-test: $t_{(11)} = 1.98$; $p > 0.05$). Pain scores in the control group also returned to baseline levels by day 30, indicating no significant effects overall on the pain levels within the saline control group (Figure 2).

2) Improvements in functional mobility.

At baseline, the limited extent of shoulder flexion by the paretic arm by all participants (comparing arc degrees of ROM across the entire cohort) was highly correlated comparing the active and passive movements (Pearson's correlation: r = 0.898; $p < 0.01$). Analysis of repeated measures using generalized estimating equations for changes in mean active shoulder flexion range of motion (ROM/degrees of arc) by the paretic arm demonstrated a highly significant group*treatment interaction over the course of the trial (Wald $\chi^2 = 8.625$, df 1; $p = 0.003$; Figure 3(a)).

Post-hoc analysis showed successive improvements occurred within the etanercept-treated group for their active ROM when compared to baseline, initially shoulder flexion improving by a change in mean (± S.E.) of 30 ± 7.3 arc degrees after treatment 1 within 30–60 min (paired t-test: $t_{(9)} = 4.07$; p = 0.003), which increased to 55 ± 12 arc degrees after treatment 2 (paired t-test: $t_{(9)} = 4.54$; p = 0.001) (Figure 3(a)). UNIANOVA demonstrated a significant difference between

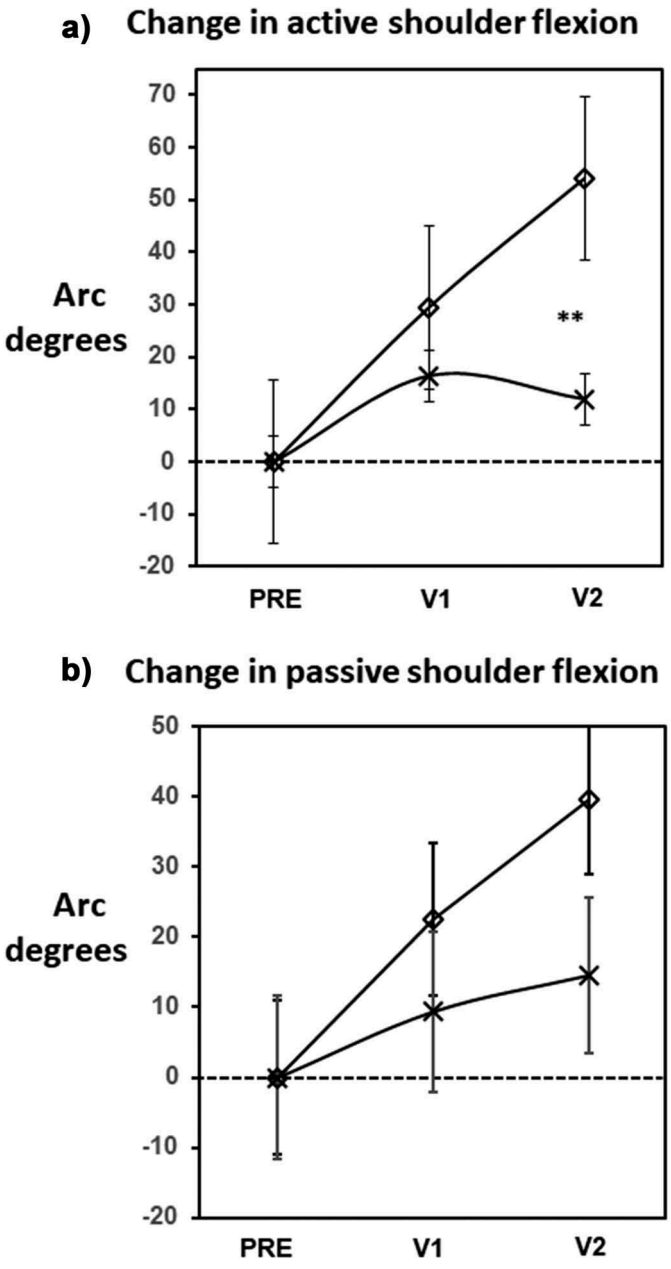

Figure 3. Rapid and marked improvement in active shoulder flexion by paretic arm after PSE treatment. Goniometry for mean changes in rotated angle (arc degrees) of (a) active and (b) passive shoulder flexion from baseline, comparing between the control (×) and etanercept (◊)-treated groups (± S.E.). PRE: baseline; V1: Visit 1 after treatment 1; V2: Visit 2 after treatment. ** $p < 0.01$.

groups in arc degrees of shoulder flexion from baseline to after treatment at visit 2 ($p = 0.011$, $\eta^2 = 0.28$, observed power of 76%). Categorically, 9 out of the 10 patients from the etanercept group with restricted paretic arm mobility had improved shoulder flexion of their paretic arm, such that 6 of these 10 showed marked improvements in active shoulder flexion by an increase ≥60 arc degrees, 3 of the 10 fully regaining 180 degrees of flexion. Again, as with the changes seen with the reduced pain levels, this improvement was noted to begin immediately (by 30 min) after the first treatment during visit 1 (Figure 3(a)).

A significant group*treatment interaction was also demonstrated for changes in mean passive flexion ROM for the paretic arm (Figure 3(b); Wald $\chi^2 = 4.861$, df 1; $p = 0.027$). Changes from baseline comparing the active to passive flexion ROM within the etanercept-treated group were strongly correlated (Pearson's correlation, $r = 0.805$; $p < 0.01$), indicating that the etanercept effect was improving both aspects of mobility for the paretic arm. No significant effect on either active or passive shoulder flexion by the paretic arm was detected within the 9 out of 10 in the control group with restricted mobility (Figure 3; paired t-test, $t_{(8)} = 0.828$; $p > 0.5$ for active; $t_{(8)} = -1.076$; $p = 0.313$ for passive). It should be noted that one of the patients in the control group (the outlier in Figure 1(b)), regained complete 180-degree flexion during the trial.

No significant relationship was detected when comparing the decreased pain levels and increased shoulder flexion of the paretic arm in the etanercept group ($r = 0.001$, $p > 0.05$). Categorically, although the majority of patients in the etanercept group showed improvements in shoulder flexion for their paretic arm, one patient in this group had significant pain reduction, but without any accompanying changes in arm mobility, whereas another four from this group, whilst showing limited changes in their pain levels, had greatly improved shoulder flexion.

5.3. Pressure pain sensitivity

Analysis by algometry (ALG) measured in Newtons across all participants for baseline responses of the forearms to applied pressure showed significantly lower levels detected as painful by the paretic versus the unaffected arm (paired t-test: $t_{(21)} = 2.3$; $p = 0.03$). Repeated measures from baseline (PRE), visit 1 (V1) and visit 2 (V2) after treatment were compared for changes between groups (Figure 4(a,b) for the paretic and unaffected arm, respectively).

During the trial, a mean increase in the applied pressure (Newtons, N) was required to induce pain from the paretic arm of the control group, whereas a decrease was demonstrated for the etanercept group (Figure 4(a)) and analysis by generalized estimating equations demonstrated that the group*treatment interaction was significant ($p = 0.02$). Post hoc analysis demonstrated that the mean pressure pain threshold (in Newtons) for the paretic arm within the control group at visit 2 after treatment was significantly greater than the mean level at baseline (paired t-test: $t_{(11)} = 2.53$; $p = 0.028$). Within the etanercept group, analysis of the unaffected arm also showed a decrease in the pressure pain threshold at visit 2 after treatment compared to baseline, although this was not significant (paired t-test: $t_{(9)} = 1.58$; $p = 0.147$; Figure 4(b)).

5.4. Other secondary measures

No significant effects were detected for the between-group differences in fatigue (FAS), depression (BDI) or sit-to-stand (STS) measures over the course of the trial (Table 3).

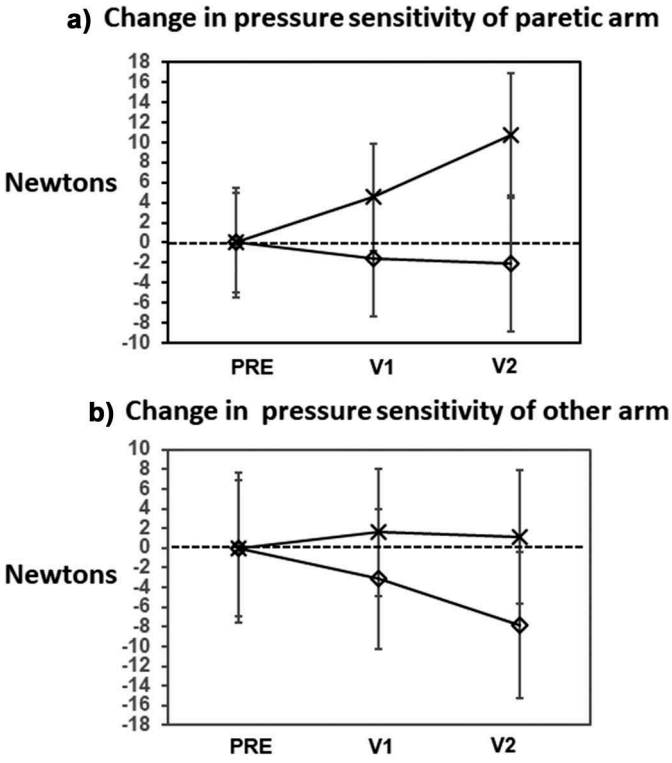

Figure 4. Change in pressure sensitivity by algometry. Comparison of etanercept versus control treatment groups for sensitivity to applied pressure by algometry on the (a) paretic/hemiplegic arm versus (b) unaffected arm. Mean change shown for the control (×) and etanercept (◊)-treated groups (± S.E.). PRE: baseline; V1: Visit 1 after treatment 1; V2: Visit 2 after treatment. $p > 0.05$. Scale for applied pressure measured in Newtons (N).

Impairment from stroke in parameters including MOCA, STS and BDI were noted to be insufficient in our study groups in order to enable detection of any changes and hence, these parameters were not further assessed. The change in fatigue levels (FAS; out of 50) from baseline for the two patient groups showed a similar trending improvement based on the slopes of the graphs (Figure 5).

5.5. Thermosensory analysis

Approximately 40% of patients with CPSP reportedly experience hypoalgesia [59]. Consistent with this observation, 7 of the 10 etanercept and 5 of the 12 control group (totaling n = 12 out of the 22 participants on trial) showed thermal pain insensitivity at baseline with extensive thermal hypoalgesia displayed by the paretic arm (Supplementary Table 2). Using the thermosensory analyzer, reduced sensitivity to perceive stimuli as either hot ($p < 0.001$) or cold ($p < 0.001$), as well as lower hot or cold pain thresholds (both $p < 0.001$) were detected when comparing the paretic to the unaffected arm across all the participants at baseline.

Apart from a trend toward an increase in cold pain threshold over time in the etanercept group (Figure 6; generalized estimating equations: group*treatment interaction, p = 0.053), no other significant differences were apparent in thermal detection or pain thresholds between the active and control groups. Etanercept within-group bivariate analysis showed a significant correlation (Pearson's r = 0.730; $p = 0.016$) in the magnitude of change from baseline to visit 2 (after treatment) comparing the decrease in % pain levels (by the 0–100 point vNPRS-FPS) with the increase in cold pain detection. No significant correlation was detected within the control group (r = 0.045, $p > 0.5$).

In summary, these results demonstrate that there was a rapid (by 30–60-min post-treatment) pain alleviation which was maintained over time, together with marked improvement in both active and passive mobility of the paretic arm following two doses of etanercept treatment.

6. Discussion

This is the first double-blinded randomized controlled trial in CPSP demonstrating the significant effects of two doses of perispinal etanercept in reducing pain and improving mobility. The significant reduction in pain remained evident 30 days after trial enrollment and was not only statistically significant but exceeded the minimal clinically important difference or MCID [60] indicating the clinical relevance of such findings. The effects were demonstrated to occur rapidly (starting within 30–60 min) after the first treatment and were also complemented by improvements in mobility indicating a role for perispinal etanercept in improving the overall quality of life in CPSP.

Improvements in mechanical and thermal pain sensitivities also showed some interesting trends. Changes in functional sit-to-stand times or psychological measures were not significantly different between the two groups. Caution should be observed when interpreting the results of this trial. Although the overall responses for the etanercept group were clearly apparent with the observed lowering in mean pain levels reaching significance, universal improvement in the primary outcome was not achieved. Four of the 10 etanercept group showed no or limited effects on their

Table 3. Secondary Outcomes: differences in test scores at Visit 2 after treatment compared with baseline.

	Group Statistics		Independent samples t-test for equality of the means	
Assessment/Out of total score	Etanercept (n = 10) (SE)	Saline control (n = 12) (SE)	Mean Difference (95% CI)	p value (two-tailed)
BDI/63	−3 .8 (1.23)	−6.67 (2.46)	2.87 (−2.96 to 8.7)	0.31
MOCA/30	−0.6 (1.79)	0.55 (0.9) (n = 11)	0·56 (−5.22 to 2.93)	0.56
FAS/50[a]	−4.3 (1.35)	−5.8 (1.82)	0.78 (−4.12 to 5.69)	0.74
STS/seconds	−9.2 (2.37)	−9.04 (2.64)	0.16 (−7.71 to 7.4)	0.97
ALG AFF ARM/N	−2.1 (3.9)	10.7 (4.23)	−12.8 (−25 to −0.6)	0.04
ALG GOOD ARM/N	−7.81 (4.91)	1.12 (5.89)	−8.93 (−25.35 to 7.5)	0.27

[a]At day 30 compared to baseline. Abbreviations: ALG: Algometry of paretic/hemiplegic (affected; AFF) versus good arms measured in Newtons (N); BDI: Beck's Depression Inventory; MOCA: Montreal Cognitive Assessment; FAS: Fatigue Assessment Score; STS: Five times Sit-To-Stand.

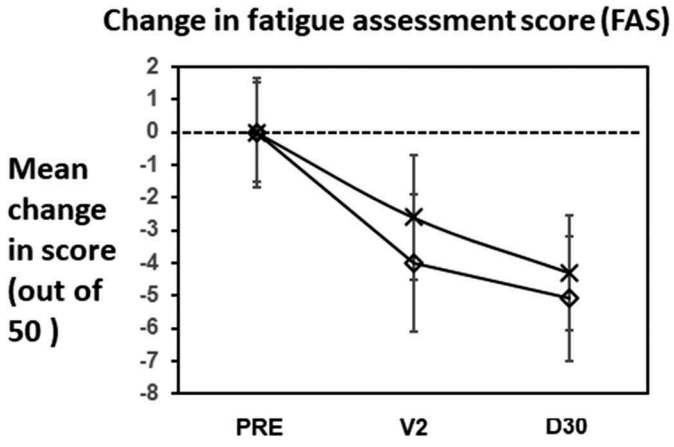

Figure 5. Similar change in Fatigue Assessment Scores (FAS). Both control (×) and etanercept (◊) groups (± S.E.) showed improvement in mean fatigue levels (lower values out of total 50) over the trial period. PRE: baseline; V2: Visit 2 after treatment 2; D30: day 30 after Visit 1. $p > 0.05$.

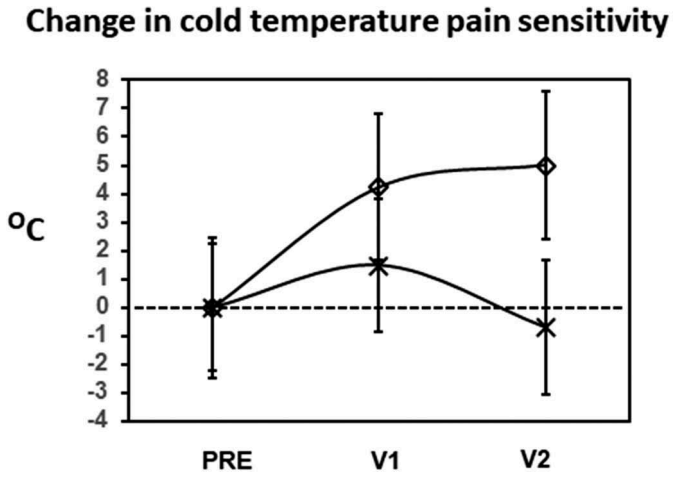

Figure 6. Quantitative thermosensory analysis. Change in recorded temperature (^{0}C) for cold pain sensitivity of control (×) versus etanercept (◊) groups (± S.E.) during trial. PRE: baseline; V1: Visit 1 after treatment; V2: Visit 2 after treatment. $p > 0.05$.

pain levels. However, in contrast, 3 in 10 of the etanercept group showed complete or almost complete resolution of CPSP after either the first or second of the two perispinal treatments. These results indicate that etanercept demonstrates variable responses on CPSP at the individual level. At present, the possible reasons for the significant reduction in pain levels for several of the patients from the perispinal etanercept group, but not by all patients in this group, is not clear. Possible reasons may relate to the extent and outcome of the post-stroke reorganization in the central nervous system and factors including lesion size, extent and severity of the stroke damage, and time elapsed since stroke. Further studies will be required to resolve these points.

Although the processes associated with the reduction in constant levels of pain in the stroke patients are not readily apparent, trends toward a normalization of mechanical and thermal sensitivities were also demonstrated. A previous study of CPSP treatment using deep brain stimulation reported similar improvements in pain associated with normalization of deficits in somatosensory perception, including thermal sensitivity [61]. The results for algometry and thermosensory analysis of CPSP patients obtained in the present study suggest that the changes in the etanercept group were trending toward normalization of somatosensory function occurring with increased thermal and pressure pain sensitivity, in line with the other trial outcomes of improved neuromuscular function.

Typically, normal pain thresholds are approximately 45ºC and 10ºC for hot and cold (Medoc Advanced Medical Systems, Ltd) respectively. Based on the Medoc thresholds and from comparing thermal responses of the paretic to the unaffected arm, almost half of the patients in each group demonstrated thermal hypoalgesia at baseline. Notably, the two patients with the strongest pain reduction in the etanercept group also showed the greatest normalization in their thermal and mechanical pain sensitivity, consistent with the increasing sensitivity associated with returning or restoration of neurosensory function. This was further confirmed by bivariate correlation when comparing the magnitude of the reduction in CPSP levels with the increase in thermal sensitivity to cold temperature-related pain responses.

6.1. The role of TNFα in central post-stroke pain

The data from this trial further adds to the mounting evidence supporting a direct role within the central nervous system for TNFα involvement in pain modulation [62] and specifically for neuropathic pain conditions [23] such as CPSP. The present outcomes from this randomized clinical trial also support the previously reported observational data that showed positive clinical responses to TNFα inhibition in patients with chronic stroke and associated improvements in moderate to severe disability [32,33,43]. Elevated TNFα levels have not only been demonstrated in the cerebral spinal fluid of acute stage [9,12–18] but also chronic post-stroke patients [19], as well as in patients with clinical depression [63], traumatic brain injury [19,64], multiple sclerosis, dementia and probably a host of other neurological disorders, as reviewed in [7,42,45]. This raises the specter of TNFα having a major role not only in the stroke penumbra and acute phase of damage, but also impacting on the ensuing global inflammatory aspects affecting the wider range of normal brain function [65]. The improvement in clinical outcomes in our study for CPSP patients after treatment with a TNFα inhibitor further implicates the role of TNFα in this condition as well.

The underlying mechanism(s) at the molecular level responsible for the improvement in clinical outcomes in our study are challenging to define. TNFα has been recently shown to induce the increased expression of the TRP family of calcium-channel-related thermal nociceptors [66] and this action of TNFα would increase pain sensitivity via nociceptor sensitization [66,67]. TNFα receptor signaling is also required for development and function of primary nociceptors in sensory neurons [68]. The alleviation of CPSP in the present study

supports this mechanism as possibly underlying changes in the central nervous system in response to etanercept actions shown here and reported elsewhere [28,69]. However, the improvements in somatosensory perception in both arms demonstrated after treatment with etanercept are more difficult to explain. Despite the reduction in CPSP in the group administered perispinal etanercept, our study did not demonstrate a clear improvement in thermal sensory thresholds, although there was a trend toward normalization of cold pain thresholds in the etanercept group which correlated significantly with the decrease in CPSP within this group. Hence, the gain in responsiveness to both peripheral pressure and thermal stimuli suggest a normalization/restoration was also occurring in somatosensory perception by the central nervous system, along with the reduced CPSP. The small sample size likely precluded detecting more highly significant differences in thermal and pressure sensitivity. TNFα has a plethora of other roles in the central nervous system for regulating neuronal function, synaptic plasticity and neurotransmitter activity related to pain [70–73] which may also underlie mechanisms associated with the persistence of CPSP and the effects of anti-TNFα detected here.

6.2. TNFα and neuromuscular function

In view of the above neurosensory aspects of TNFα, understanding TNFα's role in control of neuromuscular function is limited. An unprecedented finding of this trial was that the majority (90%) of patients within the etanercept group showed significant rapid enhancements in both active and passive shoulder flexion ROM by their paretic arm. This change in movement was irrespective of effects on their pain levels, with three out of the 10 patients in the etanercept group regaining their full use and complete 180 degrees of active shoulder flexion. This result indicates that a relaxation in the extent of spasticity was likely occurring in the arm muscles of patients treated with perispinal etanercept, improving both their active and passive control of movement. TNFα has previously been demonstrated to be associated with muscle atrophy, particularly during cachexia in diseases such as cancer [74], and increased TNFα levels have been linked with skeletal muscle loss/atrophy as a common sequelae associated with individuals with chronic stroke symptoms [75], as well as sarcopenia [76]. The rapid improvement (after first treatment) in active shoulder flexion provides evidence that such sequelae may be reversible and points to TNFα also being involved in regulating neuromuscular function. Hence, when the results with loss of pain, changes in sensory (thermal and pressure) perception and improved mobility are considered together, occurring almost immediately following first treatment (with no significant changes in the control group), the data provide the first confirmatory supportive evidence from a randomized parallel double blinded clinical trial for rapid and wide-ranging benefits achievable by treating stroke patients with perispinal etanercept.

It would be remiss not to discuss the risks from etanercept for potential adverse effects. The risk profile for serious infections with etanercept is similar to that observed with the other TNFα blockers (reviewed in [38]) and in some cases, maybe lower [40]. Among patients with autoimmune diseases, compared to treatment with nonbiologic regimens, initiating anti-TNFα has not been associated with greater risk of serious adverse events (defined as requiring hospitalization for serious infections) [77]. Consistent data from observational studies also suggest that the rate of serious infections was mainly increased over the first 6–12 months of ongoing use (reviewed in [78]). Millions of doses, with many at 50 mg being twice the presently tested dose (25 mg), are commonly being applied in much higher dosage numbers (often up to 50–100 doses per year per patient) over many years of chronic use for the treatment of autoimmune diseases. Hence, the short-term use of two 25 mg doses presently administered over a 2-week period for perispinal etanercept treatment has a relatively low-risk side-effect profile and is well tolerated. It would also seem to offer advantages over the current use of sedative drugs such as opioids or gabapentanoids for treatment of pain in stroke patients [79,80].

Some discussion is warranted regarding the observed power of this study as a guide and reference in relation to future trials. *A posteriori* power analysis from this single study showed that the small sample size was sufficient, particularly based on the goniometry change in ROM by shoulder flexion at 76%, with a high level of significance. On the other hand, the primary outcome measure of pain analysis showed a 53–-56% power with a lower level of significance with this power estimate below the level predicted *a priori*. These findings suggest that the size of the cohorts used was close to the minimum sample size for statistical significance and would likely improve with increased numbers. A limitation of the present study was that it did not improve all the secondary outcomes of the trial, most likely because of the small sample size (total n = 22) which precluded sufficient power to detect statistically significant responses for some of these measures. In addition, these findings may prove to be of value as a guide for proceeding with caution when considering the design and planning for future-related trials in that the improvements in BDI, STS and FAS tests in both groups (Table 3) indicated that the control group may have experienced a placebo effect within these secondary parameters, possibly reflecting the control group participants' positive beliefs in obtaining some benefit on trial. Alternatively, this could also have been an effect from the saline control acting as a dilution factor after injection into the cerebrospinal venous system or may reflect the relatively short duration of the study with measures spanning over a four-week period. In this regard, placebo effects have been previously reported to occur in some pain trials [81,82]. A small effect was noted with a trend toward lower pain levels detected after first treatment within the control group, but this was not significant. A possible reason for this would be the self-realization of the control patients during the trial that they were not obtaining any obvious improvement.

Several questions arise from this study with the intent of optimizing patient outcomes in the longer term, particularly relating to the underlying causes for the variable response rates, with some but not all our stroke patients showing dramatic improvements after perispinal etanercept. With further

studies, it may be possible to expand on the application of perispinal etanercept therapy by identifying more precisely the determining factors for those patients who show significant responses, as well as the longevity of their effects. Our study's focus was on chronic stroke patients (average of 4 to 5 years since stroke) and the primary endpoint was 30 days after the first treatment. Thus, the role of anti-TNFα in earlier stages of stroke, or the longevity of treatment effects cannot be determined from our data, nor can we address the possibility of greater improvements that might be obtained with further perispinal etanercept treatments. Hence, dose optimization clearly needs to be established, including determining the best regimen, the dosing level to be delivered via the cerebrospinal venous system, the optimal timing of stage of stroke and the intervals between successive treatments. Larger trials would also allow the underlying mechanisms to be further evaluated, with our current trial being underpowered to detect changes in thermal or pressure sensitivity/pain, albeit with a trend toward normalization.

Despite the relatively small sample size, the effect size and power of this study was sufficiently adequate for the primary outcome measure of pain, as was predicted based on the previously reported open-label results in stroke using the vertical pain assessment score [32]. Hence, together with the presently documented outcomes from small total participant numbers, our results bode well for any follow-up trials. It is advisable that follow-up studies consider the possibility of the short-term placebo effects as seen with some of the secondary exploratory measures examined here. These effects would likely dissipate over long-term evaluation as patients in the control group undergo the self-realization that they are on the saline treatment, given a lack of significant effects on their pain levels, or on their functional mobility.

7. Conclusions

This randomized, double-blinded, controlled parallel trial design significantly improved health outcomes in CPSP, particularly for reducing average and worst daily pain levels after treatment with perispinal etanercept. Improvements were also obtained with secondary outcome measures of pain and functional mobility. The reduced pain severity has provided significant ongoing benefits for some patients, at least over the medium term offering several months of reprieve (noted from follow-up post-trial) and points to a key role-played by TNFα in the manifestation of CPSP.

Putting our findings into the wider evidential context, etanercept has been shown to improve neurological outcomes in six different experimental animal models of stroke (reviewed in [29]). The supportive evidence from animal models together with the findings from this randomized clinical trial and the favorable outcomes from open-label use in over a thousand stroke patients during the past 9 years [32,43]; reviewed in [29]) and the recent case report of immediate resolution of hemispatial neglect and CPSP after perispinal etanercept [31] should arguably justify the availability of perispinal etanercept therapy for chronic stroke being assigned a higher priority. Furthermore, encouragement should be offered promoting further studies to be undertaken so that this treatment gains wider recognition and the acceptance required by the regulatory authorities. The above results *in toto* provide solid evidential support for the efficacy of perispinal etanercept therapy in improving outcomes with chronic stroke. It also emphasizes the need for further studies to identify in detail how to better exploit this information for alleviating the suffering experienced by stroke victims and to avoid the presently used and often ineffective drugs currently in clinical practice with their higher associated health risks including noted features of sedation and dizziness [83, 84].

Author contributions

All authors agree to be accountable for all aspects of the work and were involved in the conception, design and implementation of the trial. The SJ Ralph and AD Smith analyzed and interpreted the data and prepared the manuscript. All authors were involved in revising the content and final approval of the version to be published.

Acknowledgments

The investigators acknowledge the support of all patients who participated in this study. Also, they thank Dr Coralie Graham, Director, SRTF for support. The support of Dr Ed Tobinick for training in perispinal delivery and input into the design of the study protocol for the trial is also acknowledged. No pharmaceutical companies were involved with this trial.

Funding

This work was supported and sponsored predominantly by a grant from the Stroke Recovery Trial Fund (SRTF); Horizon Accounting, 21 Russell Street, Toowoomba, Queensland 4350, and some smaller donations from the public. The funders had no role in the study, including either participation, design, data collection and analysis, decision to publish, or preparation of the manuscript.

Declaration of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Data sharing statement

The data collected for the study, including participant data and a data dictionary can be made available to other investigator researchers who provide a methodologically sound and approved proposal. Individual participant data that underlie the results reported in this article, after de-identification (text, tables, figures, and appendices) can be made available beginning 3 months and ending 36 months following article publication. Proposals should be directed to the corresponding author to gain access and requests must meet with investigator approval and a signed data access agreement must be in place.

Reviewer Disclosures

Peer reviewers on this manuscript have no relevant financial relationships or otherwise to disclose.

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EXPERT REVIEW OF NEUROTHERAPEUTICS
https://doi.org/10.1080/14737175.2020.1727742

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KEY PAPER EVALUATION

Randomized controlled trial validating the use of perispinal etanercept to reduce post-stroke disability has wide-ranging implications

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ABSTRACT

Developing effective drug treatments for neurodegenerative disorders has always been hamstrung by the accepted inability of large molecules (roughly those with a molecular weight greater than 600 Daltons) to cross the blood-brain barrier (BBB) in therapeutic quantities when administered systemically. The dogma has been that a simple, noninvasive way to accomplish this goal is not possible with many agents, including biologicals, because they are too large. Various novel technologies to breach the BBB have been attempted, but with little success. A randomized double-blind, placebo-controlled clinical trial (RCT) administering a widely used anti-tumor necrosis factor (TNF) biological, etanercept, given via perispinal injection, which bypasses the BBB, turns this dogma on its head. This new trial holds much promise for stroke survivors, as well as having implications for developing treatments based on other large molecules for this and other brain disorders.

ARTICLE HISTORY
Received 13 January 2020
Accepted 5 February 2020

KEYWORDS
Blood-brain barrier; etanercept; double-blind controlled trial; perispinal route of administration; post-stroke therapy; TNF

1. Relevance of TNF

The polypeptide tumor necrosis factor (TNF), first described in the mid 1970s, has proved to be an extremely pleiotropic cytokine that has a central role in physiology, pathology, and the innate immune system in organisms ranging from corals to humans. At physiological levels, it is an important and widespread signaling molecule. Once TNF had been appreciated to be generated and act in the brain as well as elsewhere, it proved to be a multifunction gliotransmitter that caused trouble if generated excessively.

2. The novel perispinal route of administration

The Key Paper discussed here [1] employs perispinal delivery of etanercept, a biological agent widely used to treat chronic systemic inflammatory disease, to address post-stroke syndromes. The outcome is discussed below. Etanercept acts through potently and specifically neutralizing TNF. Edward Tobinick, whose extensive collection of published observational studies over a decade this trial formally tests, published an extended review on perispinal etanercept delivery to the brain in Expert Review of Neurotherapeutics in 2010 [2] and an update elsewhere six years later [3]. Parenthetically, it should be noted that the term perispinal had sometimes been used in the 1970s as a regional anatomical term [4], which is quite different to its precise usage here [3]. With much attention being drawn to this cytokine's roles in chronic degenerative disease in the central nervous system, as well as its central involvement in disease pathogenesis generally (see [5,6] and [7] for reviews), any excess generation of it is an obvious therapeutic target. The challenge is how to get enough of these large TNF-neutralizing molecules through or past the BBB into the brain, where studies employing intracerebroventricular injections in mice over the years had demonstrated activity. Over 15 years ago Tobinick farsightedly addressed this challenge. Equipped with intimate knowledge of the anatomy and physiology of a long-forgotten venous system, he reasoned that it plausibly constituted a direct vascular route for drug delivery to the brain [8]. In this publication he used the term 'cerebrospinal venous system' (CSVS) to describe these vessels. In the same year (2006) Tobinick and colleagues reported the effects of perispinally injected etanercept followed by Trendelenburg positioning in a six-month open trial in Alzheimer's disease [9]. The results were very promising, but by 2008 both of the Big Pharmas who had earlier acquired the etanercept patent inexplicably refused to discuss furthering the perispinal approach or funding the trials needed to achieve regulatory approval.

Years earlier, during aviation medicine research into the effects of negative gravity in rabbits, Wen and coworkers had demonstrated that head-down positioning for a short period made the blood-cerebrospinal fluid (CSF) barrier permeable to plasma albumin [10]. Mindful of this, in 2009 Tobinick and colleagues from Stanford demonstrated, in a rat model, that perispinal injection of radiolabelled etanercept, followed by head-down (Trendelenburg positioning), enabled it to rapidly reach the choroid plexus and the CSF within the cerebral ventricles [11]. This was consistent with Wen's report with albumin, despite etanercept being a larger molecule (150,000 vs. 66,000 Daltons). Delivery of a labeled anti-TNF molecule via perispinal injection to the choroid plexus plus head-down positioning has recently been confirmed in an additional rat model [12].

In addition, collections of observational studies using this perispinal method of delivering etanercept to the brain, beginning in 2010, have reported impressive outcomes in treating post-stroke neurological dysfunction in many patients [13–17]. To summarize a recent text [12] that illustrates and quotes additional anatomical detail, perispinal injection followed by a short

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Article highlights

- The blood-brain barrier has effectively excluded the brain from much of the biotech revolution. Much research has attempted to clear this roadblock, but without success to date.
- Perispinally injected etanercept, which involves injecting this anti-TNF biological into the cerebrospinal venous system before a short period of head-down tilt, has been commonly used by the originator of the technique since 2011 to treat post-stroke syndromes. Without witnessing the treatment, the Big Pharma owners of the patent for etanercept and the American Academy of Neurology have actively discouraged a trial.
- Funding from the Australia public has made possible the first formal controlled trial of perispinal etanercept on post-stroke patients. Within the goals set, the outcome was statistically significant, often markedly so.
- If confirmed in larger trials, this technique will likely have widespread usefulness in getting larger pharmaceuticals, particularly biologicals, into the brain in many different brain disease states, including cancer.

period of head-down positioning [10] may therefore be expected to enable etanercept to be delivered to the brain through the choroid plexus, the cerebral venous system, and the cerebrospinal fluid, thus bypassing the BBB. Such a route is consistent with the reported presence of labeled etanercept within the brain in experimental studies [11,12].

3. Unusual delay in an RTC testing perispinal etanercept

Unfortunately, a clinical trial of these promising observational studies continued to be delayed for over a decade. In the course of much favorable off-label treatment of post-stroke patients, many independent observers, from 2011 to the present, including non-neurological medical practitioners, nurses, speech pathologists, and neuroscientists, have witnessed this negligibly invasive treatment technique and its outcome in post-stroke patients. When faced with a striking mix of rapid onset, effectiveness, and persistence of outcome in an important circumstance where the usefulness of present treatments is very low, a common conclusion by these observers has been that this novel approach warrants an independent RCT. Nevertheless, the American Academy of Neurology (AAN), despite no member of its governing board having witnessed the treatment, or having addressed the science behind it, continues to display an on-line Clinical Advisory that explicitly discourages its members, and indeed any neurologist who reads it, from any association with this approach. In effect, the AAN fell in line behind the Big Pharma patent owners. Their position continues unchanged, despite the validity of the AAN's actions being questioned in an editorial some years ago in Expert Review of Neurotherapeutics [18] and the publication of additional supportive evidence [19,20]. Thus almost all neurologists, following the AAN's advice, have ignored invitations to observe or engage in this work, thereby establishing, for years, a quite unjustified barrier to clinical translation of the perispinal method, with its potential for wide application in disease and research.

4. Validation of perispinal etanercept technique in a randomized controlled trial

This bottleneck has now been overcome by a clinical trial outside the US funded by the community-based Stroke Recovery Trial Fund (https://strokerecoverytrialfund.org), a national health promotion charity formed by Dr Coralie Graham in 2015 in Queensland, Australia, and funded by individual donations from the public, to compensate for AAN and Pharma intransigence. The first publication arising from the funding of this organization is a modestly-sized university-conducted randomized, placebo-controlled, double-blind trial of perispinal etanercept for chronic intractable central post-stroke pain [1]. This condition is notoriously difficult to treat and its unmet medical need is substantial. The trial subjects were selected for having had, among their symptoms, unrelenting central post-stroke pain for an average of more than 4 years. Approval of the study was obtained from the Griffith University Human Research Ethics committee (MSC/10/14/HREC).

Results were consistent with the previously published observational studies, in that shoulder flexion and pain attenuation demonstrated statistically significant improvements in study participants receiving perispinal etanercept compared to the placebo control. Indeed, in an appreciable percentage of those receiving perispinal etanercept, despite their history of years of daily intractable pain, there was rapid (within 30 minutes) and often nearly complete pain abatement, whereas no change occurred in the saline control group with the same pain. This outcome is remarkable, and quite unmatched by any present therapeutic approach for post-stroke pain. From the limited trial duration it was possible to fund, this relief lasted for at least 30 days. In addition, 90% of the etanercept group, but none of the placebo group, showed highly significant rapid enhancements in both active and passive shoulder flexion range of movement, indicating less spasticity of arm muscles. The effect was clear cut ($p = 0.003$) after the first treatment and more so ($p = 0.001$) after the second, 14 days later. A dose response such as this, Bradford-Hill's 'biological gradient', is one of the standard causation indicators.

Clearly, the larger trials necessary for regulatory approval are a pressing need. The rapidity and unprecedented nature of outcomes in patients achieved by perispinal delivery of etanercept in this initial trial is especially notable. This indicates a direct effect of etanercept on the brain following its perispinal injection, and is consistent with the location of labeled etanercept within the brain in animal models after perispinal delivery [11,12].

5. Wider ramifications of this RCT

Moreover, since this trial was the first RCT testing of perispinal administration of any agent, other therapeutics aspiring to access the brain might well benefit from its further validation. An example is the novel experimental anti-TNF therapeutic, XPro1595, an engineered dominant negative inhibitor of TNF [21]. Unfortunately, as with etanercept, its size greatly retards brain entry, with about one-thousandth of the concentration attained in the plasma after peripheral injection being detected in the cerebrospinal fluid [22]. Given the outcome of the present RCT, XPro1595 may be most effective in human

brain disease if also administered perispinally to bypass the BBB. Once proven safe and effective in humans, its unique characteristics [23] may give XPro1595 an advantage over etanercept, when frequent administration is required, of allowing the TNF-dependent innate immune system to keep latent *Mycobacterium tuberculosis* suppressed. Even so, regular testing for evidence of this organism has allowed regular subcutaneous etanercept to thrive as a treatment of rheumatoid arthritis, where the dose is much higher that was used in the RCT under discussion here. Much off-label experience indicates that only one or two doses of perispinal etanercept, and therefore predictably its biosimilars, are required to treat a number of acquired brain injury states, including stroke.

6. Five-year view

The tantalizing prospect now emerges of perispinal delivery revolutionizing the treatment of a range of brain disorders, including the neurodegenerative states, by enabling effective brain delivery of not only etanercept, but also other large molecules. This includes other biologicals, but the principle is open ended. Regulatory approval of perispinal etanercept will, through widely utilizing the perispinal route in science, broaden the research base of chronic neurodegenerative states, and other cerebral conditions, such as brain cancer.

Funding

This paper was not funded.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. Comments were received from three further referees

Reviewer disclosures

A reviewer on this manuscript has disclosed a direct financial conflict of interest with respect to the subject of this article. The three further peer reviewers who provided comments on this manuscript have no other relevant financial relationships or otherwise to disclose.

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