Past and Future of Neurotrophic Growth Factors Therapies in ALS: From Single Neurotrophic Growth Factor to Stem Cells and Human Platelet Lysates

Flore Gouel1†, Anne-Sophie Rolland1†, Jean-Christophe Devedjian1, Thierry Burnouf2,3,4 and David Devos1,5*

1 Department of Medical Pharmacology, Lille University, INSERM U1171, University Hospital Center, LICEND COEN Center, Lille, France, 2 Graduate Institute of Biomedical Materials and Tissue Engineering, College of Biomedical Engineering, Taipei Medical University, Taipei, Taiwan, 3 International PhD Program in Biomedical Engineering, College of Biomedical Engineering, Taipei Medical University, Taipei, Taiwan, 4 International PhD Program in Cell Therapy and Regeneration Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan, 5 Department of Neurology, Lille University, INSERM U1171, University Hospital Center, LICEND COEN Center, Lille, France

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that typically results in death within 3–5 years after diagnosis. To date, there is no curative treatment and therefore an urgent unmet need of neuroprotective and/or neurorestorative treatments. Due to their spectrum of capacities in the central nervous system—e.g., development, plasticity, maintenance, neurogenesis—neurotrophic growth factors (NTF) have been exploited for therapeutic strategies in ALS for decades. In this review we present the initial strategy of using single NTF by different routes of administration to the use of stem cells transplantation to express a multiple NTFs-rich secretome to finally focus on a new biotherapy based on the human platelet lysates, the natural healing system containing a mix of pleitropic NTF and having immunomodulatory function. This review highlights that this latter treatment may be crucial to power the neuroprotection and/or neurorestoration therapy requested in this devastating disease.

Keywords: Amyotrophic lateral sclerosis, growth factors, therapeutic, stem cell, human platelet lysate

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease affecting the upper and lower motor neurons in the cerebral cortex, brainstem and spinal cord that lead to a progressive, irreversible muscle paralysis, and swallowing and respiratory dysfunctions. Death eventually occurs 3–5 years after diagnosis (1). The majority of ALS cases (90%) are sporadic with unknown cause (2). To date, there is no curative treatment in ALS. Therefore, the development of new and effective treatment is highly urgent. Among the different approaches, the delivery of neurotrophic factors (NTFs) is explored since the 90's because NTFs are necessary to regulate several physiological processes such as neuronal differentiation and survival, axonal outgrowth and synapses maintenance (3–5), proliferation and differentiation of stem cells in the nervous system (6–9). Therefore, these trophic factors represent a promising therapeutic strategy to treat neurodegenerative diseases (10) such as ALS.
PRECLINICAL EVIDENCE OF NEUROTROPHICS GROWTH FACTORS ABILITIES TO TREAT AMYOTROPHIC LATERAL SCLEROSIS (TABLE 1)

Recombinant NTFs Delivery by Injection
Some trophic factors have been demonstrated to promote cell survival and be protective in both in vitro and in vivo models of neuronal degeneration: Ciliary Neurotrophic Factor (CNTF), Brain-derived Neurotrophic Factor (BDNF), Glial-Derived Neurotrophic Factor (GDNF), Insulin-like Growth Factor 1 (IGF-1), Vascular Endothelial Growth Factor (VEGF), and Granulocyte-Colony Stimulating Factor (G-CSF). In vivo experiments performed in ALS models using single recombinant growth factors are described in this section.

CNTF, one of the first NTF studied in ALS models, injected intraperitoneally in pmn/pmn mice, mouse model for human spinal motor neuron disease (11) or subcutaneously in wobbler mice (12) improved motor function and survival, and decreased neuronal degeneration and muscle atrophy (13). In addition, Mitsumoto et al. demonstrated a synergic effect of CNTF and BDNF, respectively, to arrest disease progression for 1 month (14).

The fusion protein BDNF with the c fragment of the tetanus toxin (BDNF-TTC) exhibited enhanced neuroprotective effect in SOD1G93A ALS mice model, but no synergic effect was observed compared to TTC alone (55). Recently, motor function improvement and less neuronal loss were observed in SOD1G93A mice treated with the flavonoid 7,8-dihydroxyflavone, a small-molecule mimicking the effect of BDNF (56). Two receptors binding the BDNF, p75NTR and TrkB.T1, were highlighted in SOD1G93A: a decreased of p75NTR expression correlated with a delay of mortality and motor impairment (57); a deletion of the TrkB.T1 increased survival and delayed motor deficit (58).

Treatment with encapsuled GDNF-secreting cells in pmn/pmn mice did not impact motor neuron degeneration and lifespan (15). The authors suggest a combined treatment for GDNF with others NTFs. Recently, astrocytic GDNF triggered by the tumor necrosis factor α (TNFα) was highlighted in the SOD1G93A mice, and found to limit motor neuron degeneration and disease progression (59).

Intraperitoneal (16) or intracerebroventricular (17) injection of VEGF at doses of 1 g/kg/d and 0.2 µg/kg/d in SOD1G93A mice and rats, respectively, increased lifespan and improved motor performance. Similar data were observed in a sporadic model of ALS rats induced by excitotoxic administration of AMPA (60, 61).

Finally, protective properties of G-CSF were observed in SOD1G93A mice when delivered continuously at dose of 30 µg/kg/d (18). Indeed, disease progression was reduced and...
**TABLE 1** | Different routes of NTFs delivery and therapies in pre-clinical models.

| NTF Delivery route   | Model                                      | Outcomes                          | References |
|----------------------|--------------------------------------------|-----------------------------------|------------|
| **RECOMBINANT NEUROTROPHIC GROWTH FACTORS** |                                           |                                   |            |
| CNTF                 | I.P                                        | pmn/pmnn mice (20–21 d)           | MP+, S+    | (11)       |
|                      | S.C                                        | Wobbler mice                      | MP+, S+/+  | (12–14)    |
| BDNF                 | S.C                                        | Wobbler mice                      | MP+        | (14)       |
| GDNF                 | S.C                                        | pmn/pmnn mice (15–18 d)           | No effect  | (15)       |
| VEGF                 | I.P                                        | SOD1G93A mice (74 d)              | MP+, DDO+, S+11 d | (16) |
|                      | I.C.V                                      | SOD1G93A/LMIF rats (60 d)         | MP+, DDO+, S+10 d | (17) |
|                      | I.S.P                                      | Excitotoxic model in rats         | MP+, DDO+, S+10.5 d, +5 d | (18, 19) |
| **Viral vector based gene therapy** |                                           |                                   |            |
| AAV-NTF              | I.M                                        | SOD1G93A mice (90 d)              | MP+, S+22 d | (20)       |
|                      | I.S.P                                      | SOD1G93A mice (60 d)              | MP+, S+12.3 d, S+ | (21) |
|                      | In D.C.N                                   | SOD1G93A mice (88–90 d)           | MP+, S+14 d | (22)       |
|                      | I.M                                        | SOD1G93A mice (60 and 90 d)       | MP+, S+29 d and +15 d S+, +24 d and +14 d S | (23) |
|                      | I.V                                        | SOD1G93A mice (90 d)              | MP+, S+10 d | (24)       |
|                      | I.C.V                                      | SOD1G93A mice (80–90 d)           | DDO+, S+12 d | (25) |
|                      | GDNF                                       | SOD1G93A mice (90 d)              | DDO+, S+9 d S+, +20 d S | (26) |
|                      | I.T                                        | SOD1G93A mice (90 d)              | DDO+, S+12 d | (27)       |
|                      | GDNF                                       | SOD1G93A mice (90 d)              | MP+, DDO+, S+16.6 d | (28) |
|                      | G-CSF                                      | SOD1G93A mice (70 d)              | MP+, DDO+, S+ | (29)       |
| **Stem cell based therapy** |                                           |                                   |            |
| AAV-NTF              | hSC-NSC                                     | I.S.P                             | SOD1G93A rats (56–62 d) | MP+, DDO+, S+11 d | (30, 31) |
|                      | gm hNSC line (VEGF)                        | I.T                               | SOD1G93A mice (70 d) | DDO+, S+12 d | (32) |
|                      | hSC-NPC (GDNF)                             | I.S.P                             | SOD1G93A mice (40 d) | MP+, S+5  | (33)       |
|                      | gm hNPC (GDNF)                             | I.S.P                             | SOD1G93A rats (~80 d) rats (~80 d) | MP+, S– | (34, 35) |
|                      | iPb                                         | Cortex                             | SOD1G93A rats (~80 d) macaques | DDO+, S+14 d | (36) |
|                      | hBM-MSC                                    | I.S.P                             | SOD1G93Aad mice (28 w) | MP+ | (37) |
|                      | I.V                                         | SOD1G93A mice                     | MP+        | (38)       |
|                      | mBM-MSC                                    | I.V                               | SOD1G93A mice | MP+, S+17.3 d | (39) |
|                      | gm hBM-MSC (GDNF, VEGF, GDNF/IGF-1, BDNF)  | I.M                               | SOD1G93A rats (80 d) | MP+, S+28 d and +18 d for GDNF, +13 d for VEGF, +28 d for GDNF/VEGF | (40, 41) |
|                      | mBM                                        | I.S.P and I.M                      | mdf/ocd mice (6 weeks) | MP+ | (42, 43) |
|                      | mASC                                        | I.V                               | SOD1G93A mice (76–77 d) | MP+, S– | (44) |
|                      | hASC                                        | I.V and I.C.V                      | SOD1G93A mice (70 d) | MP+, DDO+, S+ | (45) |
|                      | hUCBC                                       | I.V                               | SOD1G93A mice (66 d, 68 d) | DDO+, S+21 d, +38.5 d, +23.8 d | (46–48) |
|                      | I.T                                         | SOD1G93A mice (90 d)              | DDO+, S+10 d | (49)       |
|                      | I.S.P                                      | SOD1G93A mice (40 and 90 d)       | No effect  | (50)       |
|                      | I.C.V                                      | SOD1G93A (70 d)                   | MP+, S+6 d for 40 d mice | (51) |
|                      | I.V                                         | SOD1G93A ad mice                  | MP+, S+18 d | (52)       |
|                      | gm hUCBC (VEGF, GDNF, and/or NCAM)          | I.V                               | Wobbler mice (28 d) | MP+ | (53, 54) |

I.P. Intrapitoneal; I.M. Intramuscular; I.V. Intravenous; I.C.V. Intracerebrovascular; I.S.P. Intraspinal; I.T. Intrathecal; S.C. Subcutaneous; DCN, deep cerebellar nuclei; gm, genetically modified for expression of NTFs in brackets; hSC-NSC, human spinal cord neural stem cell; m/hBM-MSC, murine/human bone marrow-mesenchymal stem cell; m/hASC, murine/human adipose derived MSC; hUCBC, human umbilical cord blood cells. Main results are summarized as follow: MP, motor performance; DDO, delay of disease onset; S, survival. The age of the model at the treatment is noted in brackets (d, days old; w, weeks). +, improvement; –, deterioration. ♂, male; ♀, female.
survival increased by rescuing motoneurons. Similar results were obtained with subcutaneous injection of pegfilgrastim, a more stable analog of G-CSF (19).

As protein infusion has known drawbacks (invasive method of delivery, protein stability over time, short half-life) others strategies, such as viral vector-based gene therapy and stem cell-based therapy have been developed to express NTFs of interest and avoid chronic injection.

**NTFs Delivery by Viral Vector-Based Gene Therapy**

Many studies focused on IGF-1. The intramuscular injection of adeno-associated viral (AAV)-IGF-1 in SOD1G93A mice before or at the time of disease symptoms delayed disease onset and increased lifespan (20). Intraparenchymal spinal cord delivery was also tested, showing higher expression of IGF-1 but partial rescue (21), whereas a stereotaxic injection into the deep cerebellar nuclei significantly extended mice lifespan (22). Recently the injection of self-complementary adeno-associated viral vector 9 (scAAV9), a more efficient transducing agent for IGF-1, extended survival, and motor performance of SOD1G93A mice when injected either intramuscularly (23) or intravenously (24). Also, the intracerebroventricular injection of AAV4-VEGF was studied and gave similar results than AAV4-IGF-1 by slowing disease progression. No combined effect of these 2 constructions was observed in SOD1G93A mice (25). Similarly the intrathecal injection of scAAV9-VEGF showed positive impact on lifespan and motor performance in mice (26). The AAV-GDNF injected intramuscularly in SOD1G93A allowed expression of the protein at the sites of injection, a retrograde transport in anterior horn neurons, and was associated with a delay in the onset and the progression of the disease (27). However, the systemic injection of AAV9-GDNF in SOD1G93A rats showed limited functional improvement and no survival extension (28). Finally the efficacy of intraspinal delivery was showed for AAV-G-CSF in SOD1G93A mice with minimal systemic effects (29).

**NTFs Delivery by Stem Cell-Based Therapy**

Different types of stem cells exist—based on their source, clonogenic capacity, differentiation potential and availability—and exert a paracrine effect, suitable for therapy in neurodegenerative disease such as ALS (62–65). We mainly focus here on stem cells with potential clinical application, engineered or used as such, e.g., a mix of NTFs.

**Neuroprotection With Neural Stem Cells (NSC) and Neural Progenitor Cells (NPC)**

Human NSC graft into lumbar protuberance of SOD1G93A rats was shown to delay the onset and the progression of the disease, with their integration into the spinal cord (30, 31). Similarly, the intraspinal administration of human NPC delayed the progression of the disease in SOD1G93A mice (33).

NSC were also engineered to secrete specific one. Intrathecal transplantation of human NSC overexpressing VEGF in SOD1G93A mice delayed the onset of the disease and increased survival with an integration and differentiation of NSC-VEGF into the spinal cord (32). Human neural progenitor cells NPC (hNPC) were also genetically modified to secrete GDNF. The transplantation of such engineered cells in SOD1 rats were integrated into the spinal cord, limited motoneuron degeneration but failed to improve motor function (34, 35). However, the transplantation of hPNC-GDNF into the cortex extended the survival of SOD1G93A rats and was safe for primates (36).

**Mesenchymal Stromal Cells (MSC)**

Bone marrow (BM) MSC (BM-MSC), when injected intraspinally (37, 38) or intravenously (39) in SOD1G93A mice, allowed decreased motoneurons degeneration, improved survival and motor function, prevented pro-inflammatory factors. Indeed, MSC display immunomodulatory properties by secreting anti-inflammatory cytokines such as TGF-β or IL-10 (66) Since neuroinflammatory markers were detected in neural tissues of ALS patients (67) promising results can be expected with MSC based therapy. Moreover, intramuscular transplantation of human BM-MSC genetically modified to secrete GDNF in SOD1G93A rats, showed a decrease in motoneuron loss and an overall increased lifespan (40). In addition they demonstrated a synergic effect of the combined intramuscular delivery of hMSC-GDNF and hMSC-VEGF with an increased survival, protection of neuromuscular junction and motoneuron degeneration, greater than either growth factor delivered individually (41). Even though human BM-MSC injections have positive effects on the disease progression, it should be noted that the whole BM intraspinally transplanted showed a greater improvement of motor functions than BM-MSC in mdfr/odc mice (42) and increased motoneurons survival when intramuscularly transplanted (43).

Others reported positive results with adipose derived MSC when administrated by systemic (44), or intracerebroventricular administration (45).

**Human Umbilical Cord Blood (hUCB)**

The first study performed on SOD1G93A mice irradiated and transplanted intravenously with hUCB mononuclear cells (MNC), showed a delay in the onset of symptoms and increased the survival (46, 47). Transplanted cells integrated regions of motoneuron degeneration and expressed neural markers (48). Recently, the efficiency of chronic intravenous injections of UCB MNC in symptomatic SOD1G93A mice was demonstrated, with increased lifespan and reduced inflammatory effectors (49). Similarly, the intraspinal as the intracerebroventricular injection of hUCB in pre-symptomatic SOD1G93A or wobbler mice increased survival and motor performance (51, 52). However, intrathecal administration of hUCB did not affect the lifespan of motor function of ALS mice (50).

Some authors engineered hUCB MNC to secrete some NTFs or to enhance homing at the site of degeneration (68, 69). Recently, transplanted hUCB transduced with AAV encoding VEGF, GDNF and/or neural cell adhesion molecule (NCAM), led to a high rate of SOD1G93A mice survival and improved motor function. Moreover, transplanted cells were detected 1 month after grafting into the lumbar spinal cord (53, 54).
CLINICAL TRIALS WITH GROWTH FACTORS: EVIDENCE AND HYPOTHESIS FOR THE FAILURE

Regarding the promising effects obtained in ALS animal models, clinical trials were conducted to examine the neuroprotective effects of these growth factors therapies in ALS patients (Table 2).

Trials Involving NTFs Protein Systemic Injections

**CNTF**
In 90's the ALS CNTF Treatment study group published results obtained in phase I (70) and phase II/III (72) clinical trials where enrolled patients received subcutaneous administration of recombinant human CNTF (rHCNTF) at different doses, 15 or 30 µg/kg, three times a week for 9 months. The phase II/III randomized, placebo-controlled evaluated the safety, tolerability, and efficacy. No statistically difference between rHCNTF-treated patients and placebo-treated patients were observed and side effects were sufficiently severe to limit dosing in many patients. A second trial, same year, did not show any positive effect either (71).

One year later, Penn et al. published results of a phase I clinical trial with intrathecal pump delivery (73). The disease progression was not modified either but no systemic side effects were observed. Thus, intrathecal administration may be the preferred route of administration. To our knowledge, no further clinical study are under investigation.

**BDNF**
Due to a promising phase I/II clinical trial showing the safety and efficacy of subcutaneous administration of BDNF in 1995, a phase III was designed (74). Results failed to demonstrate an effect on survival but post-hoc analyses showed that those ALS patients with early respiratory impairment showed benefit (75). One year later a phase I trial showed the feasibility of intrathecal method of delivery (76) but two other trials conducted in 2003 and 2005 felt to detect any efficacy (77, 78).

**IGF-1**
In the late 90’s, two clinical trials used IGF-1 at a dose of 0.1 mg/kg/d by subcutaneous delivery and found contradictory and opposite results (79, 80). In 2008, a phase III showed no benefit of this route of delivery in 2 years of trials (82). In a pilot study conducted in 2005, intrathecal administration had beneficial effect using high doses of IGF-1 (3 µg/kg every 2 weeks) but it was not placebo-controlled (81).

**G-CSF**
Ten years ago, two pilot clinical trials with subcutaneous G-CSF administration at a dose of 5 µg/kg/d reported a trend for slowing down the disease progression (84) and a delay in motor decline (83). A Phase II clinical trial is under investigation but results are not yet available.

**VEGF**
Three clinical trials assessed the safety, tolerability, and the possible motor function improvement as well as survival time of the intracerebroventricular administration of 4 µg/d VEGF. To our knowledge, no results are published.

6- Failure Hypothesis
Most of the clinical trials based on direct protein administration gave disappointing outcomes in view of the promising preclinical results. Different hypotheses can be raised to explain those failures (70–84):
- The route of administration: subcutaneous injection seems less efficient than the intrathecal one
- The minimal ability of these growth factors to cross the blood brain barrier
- The dose: highest safe dose in humans can be lower than those determined in animals, as the clinical trial with CNTF demonstrated
- The treatment start time: in animals, treatment start before the onset of the disease whereas in humans the diagnosis is performed at later stage
- The need of synergic association of numerous neurotrophic factors

Trials Involving Adeno-Associated Viral Gene Therapy
To our knowledge, there is no reported clinical trial using adeno-associated viral gene therapy despite promising results obtained with SOD1G93A mice. AAV2 and AAV9 are vectors having the greatest potential, one specific for neuron tissue, one passing the blood brain barrier and exhibiting neuronal tropisms, respectively. One of the drawbacks of genes therapies for ALS can be the safety. Indeed to stop delivery will not be possible if serious adverse events occur during the treatment.

Trials Involving Stem Cell Therapy
Twenty-two trials involving stem cells-based therapy are registered on ClinicalTrials.gov. Most of them use MSC from different origins and few have results available. This section is an overview of all the known clinical trials.

**Neural Stem Cells**
In 2012, two trials sponsored by Neuralstem used NSC by intraspinal injection. The phase I did not show any adverse events (85, 86), but the phase II has an unknown status on the ClinicalTrials.gov website.

Recently, published results of a phase I trial, proposing transplantation of human NSCs into the lumbar spinal cord, demonstrated the safety and reproducibility of this cell therapy. Moreover, because the brain tissue used was from natural miscarriages, ethical concerns may be eliminated (87). An ongoing clinical trial concern neuronal progenitor cells engineered to produce GDNF. This is a phase I/IIA trial, active but not recruiting. No results are available for now.
### TABLE 2 | Clinical trials with growth factors.

| NCT number | NTF | Delivery method | Phase and status of the trial | Cohort size | Outcomes | References | Year |
|------------|-----|----------------|-------------------------------|-------------|----------|------------|------|
| NCT01348451 | NSC | ISP | Phase I | 12 | No major adverse events | (85, 86) | 2012 |
| NCT01730716 | NSC | ISP | Phase I, unknown status | 18 | Not available | | |
| NCT02943850 | NPC | ISP | Phase I/IIa, active, not recruiting | 18 | Not available | | |
| NCT01640067 | NSC | ISP | Phase I, completed | 6 | Safe approach, no increase of disease progression | (87) | 2015 |
| NCT00781872 | MSC | IT, IV | Phase I/II, terminated | 19 | Safe and feasible, ALS-FRS score stable the first 6 months | (88) | 2010 |
| NCT02085706 | PBMC | ISP | Phase NA, completed | 14 | Not available | | |
| NCT01933321 | HSC | IT | Phase I/II/III, completed | 14 | Not available | | |
| NCT01609283 | MSC | IT | Phase I, active, not recruiting | 27 | Not available | | |
| NCT01142856 | MSC | IT | Phase I, completed | 1 | Not available | | |

(Continued)
**Blood Cells**

Two clinical trials, one using autologous peripheral blood mononuclear cell for intraspinal transplantation and one in phase II/III using hematopoietic stem cells for intrathecal injection were conducted and completed but no results were reported to our knowledge. One trial using autologous bone marrow mononuclear cells (90) for intraspinal injection showed the safety of the procedure.

**Mesenchymal Stromal Cells**

Among 14 clinical trials using MSCs from diverse origin such as bone marrow, adipose tissue or engineered to secrete particular NTFs, through diverse types of delivery (intrathecal, intraspinal, intramuscular, intravenous, or intraventricular), 5 have no published results, 4 are ongoing, and 5 are completed with published results. All of them are listed in the Table 2 and the last 5 are detailed below and involved the use of the bone marrow derived MSCs.

In 2012, a phase I/II, using autologous bone marrow MSCs administered by intraspinal delivery, was conducted. No severe adverse event were observed, no acceleration of the disease progression noticed and an increase of the motoneurons in the treated segments compared with the untreated segments for patients who died for unrelated reasons to the procedure. Thus, this trial demonstrates the safety of intraspinal infusion of MSCs and suggests their neurotrophic activity (89). In 2013, a phase I/II confirmed the safety of BM-MSC infusion (91).

In 2016, two clinical trials in small groups of patients, phase I/II, used bone marrow MSCs engineered to secrete NTFs. Intramuscular transplantation for early ALS patients and intrathecal transplantation for progressive ALS patients were evaluated. They concluded that both route of administration are safe and provide indications of possible clinical benefits that need to be confirmed on a bigger cohort (93).

In 2018, a phase I/II trial was initiated to evaluate the safety and efficacy of these cells through intrathecal delivery. A possible benefit seems to last at least 6 months with apparent safety (92). A phase II is required to evaluate long-term efficacy and safety.

Finally, recent phase I/II trials showed safety and feasibility of intravenous and intrathecal transplantation of autologous bone marrow MSCs (94). Indeed, no adverse events were reported and the ALS-FRS score and the force vital capacity percentage were significantly reduced. Additional trials with bigger cohort are needed.

To conclude, stem cells-based therapy as a future therapy to treat ALS patients is premature due to the lack of results. As for the protein infusion, some questions need to be considered:
- The delivery method
- The timing of intervention
- The number of cells to transplant to obtain a therapeutic efficacy
- The capacity of transplanted cells to migrate to the area of interest and to mature in the hostile environment
- The evaluation of the long-term efficacy

Nevertheless, trophic factors remain essential for neuronal maintenance and survival and remain a promising candidate to treat ALS patients. Another source of those factors can be the natural healing system, namely the platelet lysate, and a continuous infusion into the brain by intracerebroventricular (ICV) injection can be a route of administration, avoiding the potential problem with the blood brain barrier crossing.

**HOW TO IMPROVE GROWTH FACTORS THERAPEUTICS IN ALS: A NEW THERAPEUTIC APPROACH BASED ON THE HUMAN PLATELET LYSTATE**

The lack of clinical efficacy of single NTF infusion, despite a good diffusion, required increasing the dose to a point where they finally induced poor tolerance (i.e., µg). A single NTF was therefore unable to induce the complex set of signaling pathways required to promote efficient neuroprotection. Platelets constitute abundant, natural sources of physiological balanced mixtures of many growth factors [e.g., Platelet Derived Growth Factor (PDGF), VEGF, IGF-1, EGF, or TGFβ] (95) and are used to enhance wound healing and tissue repair (96). In addition, they express adhesion molecules, secret chemokines (97) giving thus neuroinflammatory property to the platelet lysate that could be of an additional interest in ALS therapy. Interestingly, it was demonstrated that ICV injection of human platelet lysates significantly reduced infarct volumes in rats with permanent middle cerebral artery occlusion, improved motor function and promoted endogenous neural stem cells proliferation (98). Similar results were obtained with platelet rich plasma in ischemic rats (99). Moreover, intranasal (IN) administration of platelet lysates was demonstrated to be neuroprotective in Alzheimer and Parkinson’s disease animal models (100, 101). To pursue with the neuroprotective potential of platelets lysate in neurodegenerative diseases, we developed a heated low protein human purified platelet lysate (HPPL) preparation, compatible with ICV and IN intermittent administration, to deplete fibrinogen, avoid thrombogenic, and proteolytic activities. We demonstrated its neuroprotective effect in *in vitro* and *in vivo* model of Parkinson’s disease and its anti-inflammatory properties (102). To extend the concept to ALS, HPPL was tested on a motoneuron-like model and strongly protected from apoptosis and oxidative stress (103). Higher neuroprotection was obtained with HPPL compare to single growth factor or combination of 4 (PDGF, BDNF, BFGF, VEGF) and involved specific signaling pathway such as Akt and MEK (103). These results give a real hope for neuroprotective therapy and need to be confirmed in *in vivo* ALS model with ICV or IN administration of HPPL.

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FG and A-SR wrote the manuscript. J-CD, TB, and DD critically revised the manuscript. All authors read and approved the submitted version.

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