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HYPOTHESIS

Intrathecal autologous thrombin-activated condensed platelet cytokines in chronic neurodegenerative disease: A hypothesis for anti-inflammatory and regenerative response.

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Abstract

Choroid plexus insufficiency or glymphatic stasis are often classified as prequels to harmful accretion of toxic proteins in neurodegenerative disease. Cognitive decline and disordered neuronal signaling subsequently become cardinal features of Alzheimer's disease (AD), typically progressing with amyloid-ß and tau protein accumulation. For Parkinson's disease (PD), α-synuclein deposits and dopamine depletion are linked to impaired movement, resting tremor, and rigidity. Importantly, both diagnoses feature hyperinflammation and intrathecal cytokine changes. Thus far, numerous clinical trials have produced nothing effective for AD or PD, yet the anti-inflammatory and regenerative potential of autologous platelet-rich plasma (PRP) remains largely unexamined in this context. Our report explores a proposed Phase I study on intrathecal condensed plasma growth factors processed from thrombin-activated PRP as monotherapy for AD or PD. The concept gains support from related work where cytokines of platelet origin successfully lowered inflammation, corrected background fibrosis, deactivated abnormal cells, and recovered local tissue function—all desirable outcomes in AD and PD. While PRP-mediated effects on membrane potentials, cellular signaling, electrolyte balance, and water clearance are less well characterized, experimental data suggest these pathways could likewise influence glymphatic drainage to ameliorate proteinopathies. As a well-tolerated 'orthobiologic' with no hypersensitivity risk, intrathecal PRP and its derivatives bring advantages over synthetic pharmaceuticals. If age-associated neuroinflammation in AD and PD is an upstream event inciting or contributing to neural disruption, then dampening

local oxidative stress by a patient's own platelet cytokines (successful in other contexts) could offer therapeutic relevance to these neurodegenerative conditions as well.

INTRODUCTION

The need to provide a cure for chronic neurodegenerative diseases has never been more acute. Despite decades of uninterrupted scientific efforts, clinical trials for Alzheimer's disease (AD) have scored the lowest yield of almost any medical research endeavor, while forecasts for breakthroughs in Parkinson's disease (PD) are similarly bleak (Mari & Mestri, 2022; Moutinho, 2022). And as global incidence of these conditions is expected to increase three-fold by 2050 (Dorsey *et al.* 2018; Triaca *et al.* 2022) the spectre of this public health concern is outpacing the research response intended to counter it.

While the poor returns on AD and PD research have been covered elsewhere (Cummings *et al.* 2018; Mari & Mestri, 2022), against this busy background one path to cure remains largely untraveled: Intrathecal platelet-rich plasma (PRP). Given the technology behind novel recombinants designed to squelch production of specified brain proteins, autologous PRP risks being discounted as simplistic by comparison. However, in addition to platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF) and other better-known PRP constituents, activated platelet (PLT) releasate also includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT3), plus other potent neurotrophins (Swift *et al.* 2006; Shen *et al.* 2009). Notwithstanding animal data on inflammatory myelin damage in multiple sclerosis showing recovery after intrathecal PRP (Farid *et al.* 2022) a literature search intersecting 'intrathecal' and 'platelet-rich plasma' returns only seven publications—and none specifically address AD or PD. While the paucity of published data on intrathecal PRP for neurodegenerative conditions is notable, more worrisome is the state of registered clinical trials on the topic, where no investigations are currently underway or planned (U.S. National Library of Medicine, 2023). This means that not only is scant information available on this point today, the research pipeline is unlikely to supply anything relevant on the matter going forward. Accordingly, this account invites fresh scrutiny on intrathecal PRP as an alternative disease-modifying option in AD and PD, with an emphasis on possible anti-inflammatory and regenerative roles.

CEREBROSPINAL FLUID AND NEURODEGENERATIVE PATHOLOGY

Substantial neuronal signal interference occurs in both AD and PD. In each condition, diagnosis depends on clinical tests where detection of early-stage disease is far from ideal. Sadly, at initial presentation the stage of neurodegeneration may already be quite advanced (Domínguez-Fernández *et al.* 2023). A recent study of noradrenaline, 3-methoxy-4-hydroxyphenylglycol, and noradrenaline transport availability found that PD shows markedly lower levels of noradrenaline and 3-methoxy-4-hydroxyphenylglycol in cerebrospinal fluid (CSF), as well as poor hypothalamic noradrenaline transport availability (Lancini *et al.* 2023). In AD, profoundly reduced amyloid-β clearance is observed compared to healthy controls yet production quantity is unchanged (Mawuenyega *et al.* 2010). While there is disagreement on how much time must pass before such changes can be registered, the relation highlights the role of disordered brain clearance systems (Jessen *et al.* 2015; Tadayon *et al.* 2020) where resident microglia and peripheral mononuclear phagocytes somehow exhaust their governance over cerebral deposition of amyloid-β (Sochocka *et al.* 2017; Taipa *et al.* 2019). Cellular water handling is also relevant to CSF dynamics, and the aquaporin (AQP) family has been extensively studied with at least 12 different AQPs operating in specific cell types.

While function of transmembrane channels is subject to resting voltage potentials (Bulling *et al.* 1999), how PLT cytokines influence cell portal operation is only now being investigated. Loss of membrane integrity from inflammation can be reversed, and ongoing work should extend recent discoveries concerning how gap junctions are targets for selected PLT cytokines (Sassoli *et al.* 2022). If this also applies to the main aquaporin in neural tissue (AQP4), then PLT cytokines might actuate this transmembrane gate to mediate amyloid-β clearance (Ozawa *et al.* 2019) and manage extracellular volume during synaptic action (Haj-Yasein *et al.* 2012). Moreover, our proposal incorporates PLT activation by thrombin (Xu *et al.* 2021) rather than calcium chloride or calcium gluconate (Sills *et al.* 2018) to minimize risk of electrolyte upset.

When CSF cytokines in AD patients were referenced against healthy controls, eotaxin, IL-1ra, IL-4, IL-7, IL-8, IL-9, IL-10, IL-15, GCSF, monocyte chemotactic protein 1, PDGF, and TNF-alpha were all elevated (Taipa *et al.* 2019). Of note, a negative correlation was observed between disease progression and IL-1β, IL-4, IL-6, IL-9, IL-17A, FGF, GCSF, GMCSF, interferon gamma, and macrophage inflammatory protein-1β (Taipa *et al.* 2019). By contrast, CSF features in PD include α-synuclein (αSyn) levels which are often much lower than expected in normal health (Kang *et al.* 2013). This diminished αSyn could be explained by passage of this protein from CSF into adjacent tissues (Mollenhauer *et al.* 2011; Hall *et al.* 2015), precipitates worsened by slowed circulation secondary to local inflammation. Oxidative stress appears central in both cause and course of PD pathology, where neurodegeneration is typified by mitochondrial dysfunction, excitotoxicity, or loss of dopaminergic action (Verma *et al.*

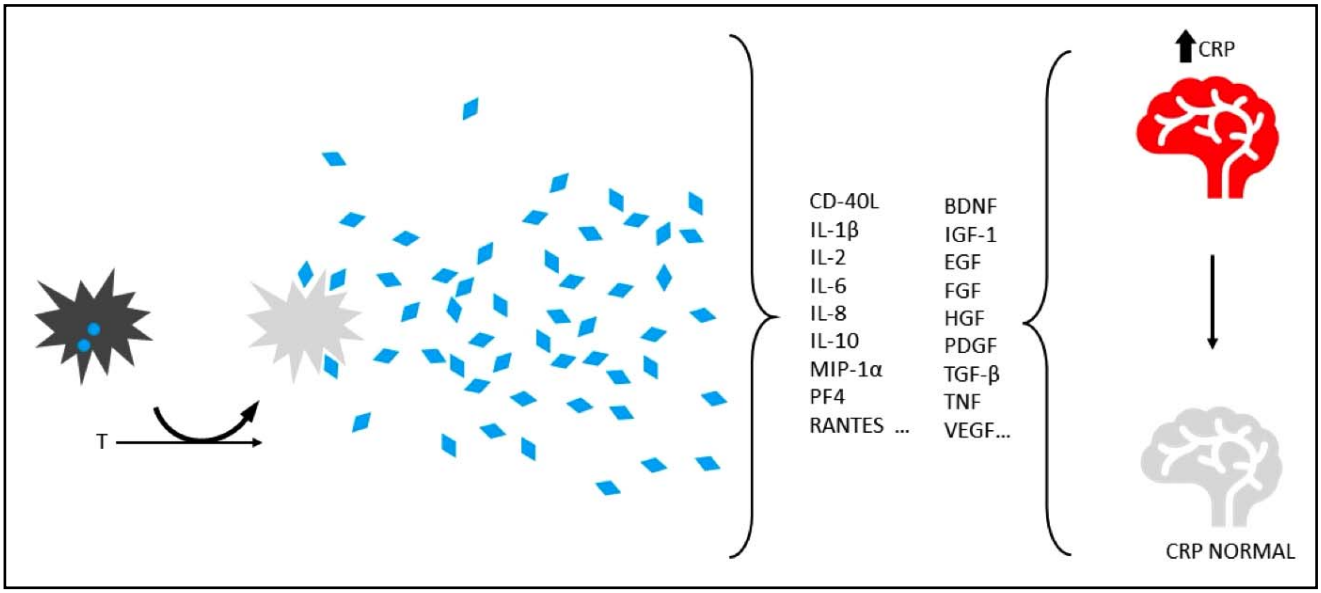

Fig. 1. Anti-inflammatory sequence for processed platelet (black) rich plasma and its soluble mediators (blue), discharged after thrombin (T) activation. After subtraction of depleted PLTs (grey), relevant cytokines include: Brain-derived neurotrophic factor (BDNF), supports neuron survival and induces differentiation of *de novo* neurons and synapses; Epidermal growth factor (EGF), a central element in cellular proliferation, differentiation, and survival; Fibroblast growth factor (bFGF), a mediator with broad mitogenic and survival actions; Hepatocyte growth factor (HGF), stimulates mitogenesis, cell motility, and matrix invasion with a key role in angiogenesis and tissue regeneration; Insulin like growth factors (IGFs) are required for cell stimulation and response to local microclimate; Interleukin-1β (IL-1β), a central inflammatory mediator involved in cell proliferation, differentiation, and apoptosis; Interleukin-2 (IL-2), an inducer of T-helper 1 (Th1) and Th2 cell differentiation and antagonist to inflammatory Th17 cells; Interleukin-6 (IL-6), a metabolic regulator and promoter of hypothalamic PGE2; Interleukin-8 (IL-8, or 'neutrophil chemotactic factor') coordinates local angiogenesis; Interleukin-10 (IL-10) suppresses Th1 cytokines and MHC class II antigens and regulates the JAK-STAT signaling network; Ligand of CD40 (CD-40L), an inflammatory organizer of PLTs, leukocytes, and dendrites; Macrophage inflammatory protein 1-alpha (MIP-1α), a conditional trigger for cell migration, survival, and proliferation; Platelet derived growth factor (PDGF), modulates mitogenesis and mesenchymal proliferation; Platelet factor 4 (PF4), a chemotactic protein involved in PLT aggregation; Regulated after Activation of Normal T-cell Expressed and Secreted (RANTES), a monocyte attractant; Transforming growth factor beta (TGF-β), an activator of multiple secondary substrates involved in tissue repair; Tumor necrosis factor (TNF), highly diverse actions with multiple cross-talk nodes balancing cell proliferation and apoptosis; and Vascular endothelial growth factor (VEGF), a promoter of vascular development for improved perfusion. In addition to recorded changes in selected CSF markers, post-program reduction in serum C-reactive protein (CRP) can be measured to confirm anti-inflammatory effect.

2022; Huang *et al.* 2023). A key meta-analysis of CSF cytokine data from AD and PD patients reported that TGF-β, MCP-1, and YKL-40 levels in CSF were all significantly elevated in AD vs. healthy controls, while PD was characterized by higher CSF levels of TGF-β1, IL-6, and IL-1β (Chen *et al.* 2018). Since CSF biomarkers have discriminatory value for AD and PD (Lancini *et al.* 2023) this becomes a plausible pathway to cytokine normalization, via abatement of their common ramifying term—inflammation.

NEURAL TARGETS, INTRATHECAL ACCESS

Therapeutic results for neurodegenerative conditions, mass lesions, infections, or refractory pain depend on direct drug delivery to bypass the blood-brain barrier (Yi *et al.* 2014), explaining why most systemically administered agents never achieve therapeutic levels in brain tissue (Xie *et al.* 2015). The current design extends prior work where drug delivery was via transdermal adhesive patch placed dorsally on the neck (Lehrer & Rheinstein, 2019), by intranasal insufflation (Lehrer, 2014), or similar techniques (Slavc *et al.* 2018; Muschol *et al.* 2023). Access to cerebrospinal and intraventricular space by intrathecal dosing (Manuel *et al.* 2023) has the benefit of placing PLT cytokines near glymphatic control sites most likely affected by oxidative stress, inflammation, or fibrosis (Prineas *et al.* 2016; Hsu *et al.* 2021; Buccellato *et al.* 2022).

The glymphatic system is a perivascular network for CSF transport connecting distally to the dural/meningeal lymphatic system. As the chief efflux channel for brain tissue (Nedergaard, 2013), the glymphatic network clears metabolic products to prevent pathologic accretion in neural parenchyma and interstitial fluid (Sepehrinezhad *et al.* 2023). While intrathecal dosing was first considered therapeutically relevant only to structures in direct CSF contact, tracer studies have shown this can be a viable route to impact deeper brain tissues (Hladky & Barrand, 2014; Naseri Kouzehgarani *et al.* 2021). While there is broad consensus that CSF originates from choroid plexus in humans, anatomical exit routes beyond arachnoid granulations are less well characterized (Astara *et al.* 2023). Volumes ranging from 0.5 to 5mL can be safely

placed here via intrathecal/lumbar catheter inserted at L3-L5. While experience with intrathecal therapy for neurodegenerative disease is underdeveloped, placement of autologous blood products here is not: Needle access near a lumbar puncture site is a familiar procedure (in obstetric anesthesia) to place a 'blood patch' to remedy spinal headache and promote dural healing (Beckman *et al.* 2023; Epstein & Agulnick, 2023). The priority paper outlining intrathecal dosing of conventional PRP in the management of AD and PD may be attributed to Shen *et al.* (2009). Their insight was predicated not merely on anti-inflammatory action (see Figure 1) but on the observed neurotrophic capacity of PLT growth factors which, individually or in concert, could ameliorate neurodegenerative loss. Interventions to remove biochemical residue to assuage neuropathology gained momentum from animal research, where glymphatic dysfunction was noted after traumatic brain injury or stroke as well as animal models for normal aging and AD (Peng *et al.* 2016; Rasmussen *et al.* 2018; Jiang *et al.* 2022; Butler *et al.* 2023).

These findings helped frame workable ways to rectify physiologic elimination of waste solutes (Benveniste *et al.* 2019; Butler *et al.* 2023). Downshifting tau production would be another logical process to address AD pathology, and favorable results from a first-in-human Phase Ib clinical trial described a tau-targeting antisense oligonucleotide given intrathecally for this purpose (Mummery *et al.* 2023). While devices adapted to alleviate neurodegenerative symptoms await clinical approval (Allievex Corporation, 2023), fresh autologous condensed PLT cytokines used in this context is an application not previously investigated. Indeed, recent laboratory and clinical gains in PLT processing include better plasma cytokine preparations and delivery—including as cell-free substrates following PLT subtraction. But could intrathecal use of an autologous PLT product have efficacy in AD or PD?

DISCUSSION

AD and PD are characterized by dysregulated, excessive inflammation causing disordered neural signaling and eventually, functional collapse. How specific cell populations respond to this stress is difficult to predict, although changes observed in AD include neurofibrillary tangles/tau protein variants as signature features. These findings were originally considered to be the defect responsible for the AD phenotype, although further research has reexamined the disease sequence such that the observed tangles may be but one part of a compensatory response to local inflammation, kinase activation, and subsequent tau phosphorylation (Bonda *et al.* 2011). Neurofibrillary changes can confer protection against redox damage in neurons (Lee *et al.* 2005), resolving the apparent paradox of tangles occurring absent disease where such neurons function satisfactorily for many years (Lee *et al.* 2005; Bonda *et al.* 2011). This question received close attention in a recent Mayo Clinic study, where some cytokines were negatively associated with CSF nanoplaque levels yet CSF cytokines showed similarity between amyloid-positive vs. amyloid-negative patients (Aksnes *et al.* 2021). While there is no consensus on how these related processes lead to AD, there is closer agreement on inflammation being the inciting stress which culminates in neurodegenerative pathology.

PLTs enter the therapeutic roster perhaps best known for contributions to hemostasis, but this designation has since broadened to include other roles. Specifically, upon activation PLTs expel a suite of cytokines and soluble growth factors which communicate with varied targets to modulate inflammation, normalize cellular metabolism, and organize tissue healing (Scherlinger *et al.* 2023). Thus, to reestablish quiescence in a hyperinflammatory background, intrathecal PLT-derived cytokines would be a well-suited 'orthobiologic' (Abdul Ameer *et al.* 2018; Kumar *et al.* 2022; Rodeo, 2023; Wongjarupong *et al.* 2023). Indeed, diffuse neural tissue inflammation (Tarkowski *et al.* 2003) can be attenuated by hepatocyte growth factor (HGF) transforming growth factor-β (TGF-β), insulin-like growth factor 1 (IGF-1), interleukin (IL)-1 receptor antagonist, IL-4, IL-6, IL-10, IL-11, or IL-13 (Zhang *et al.* 2013; Abdul Ameer *et al.* 2018; Southworth *et al.* 2019; Ziegler *et al.* 2019; Opal *et al.* 2000), all of which are components of the activated PLT releasate.

Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT3) are also discharged from activated PLTs and control neuron survival, development, and function via Trk-p75 NTR receptors, including suspension of programmed cell death (Mitsiadis *et al.* 2016; Boukhatem *et al.* 2021; Burk, 2023). First isolated in brain tissue, BDNF is now known to be stored in PLTs at concentrations 100 to 1000-fold greater than neurons and plays a major role in synaptic plasticity and neuron development (Boukhatem *et al.* 2021). Importantly for our study design, these signals also promote progenitor cell differentiation to induce formation of replacement neurons—most notably involving the hippocampal dentate gyrus and subventricular zone (Park & Poo, 2013; Triaca *et al.* 2022). While additional PLT releasate constituents (i.e., EGF, IGF-1, and TGF-β) exhibit neurotrophic potential, widefield effects of these signals have only been partially mapped (see Figure 2). Chen *et al.* (2018) were the first to describe intrathecal PRP placed into rat spinal cords where microglia and astrocyte activation was observed with PDGF-B and ICAM-1 expression. Their intrathecal PRP protocol for spinal cord injury (SCI) successfully recovered locomotor function with white matter sparing, and interestingly, ongoing treatment at reduced levels yielded therapeutic effects superior to single-dose PRP

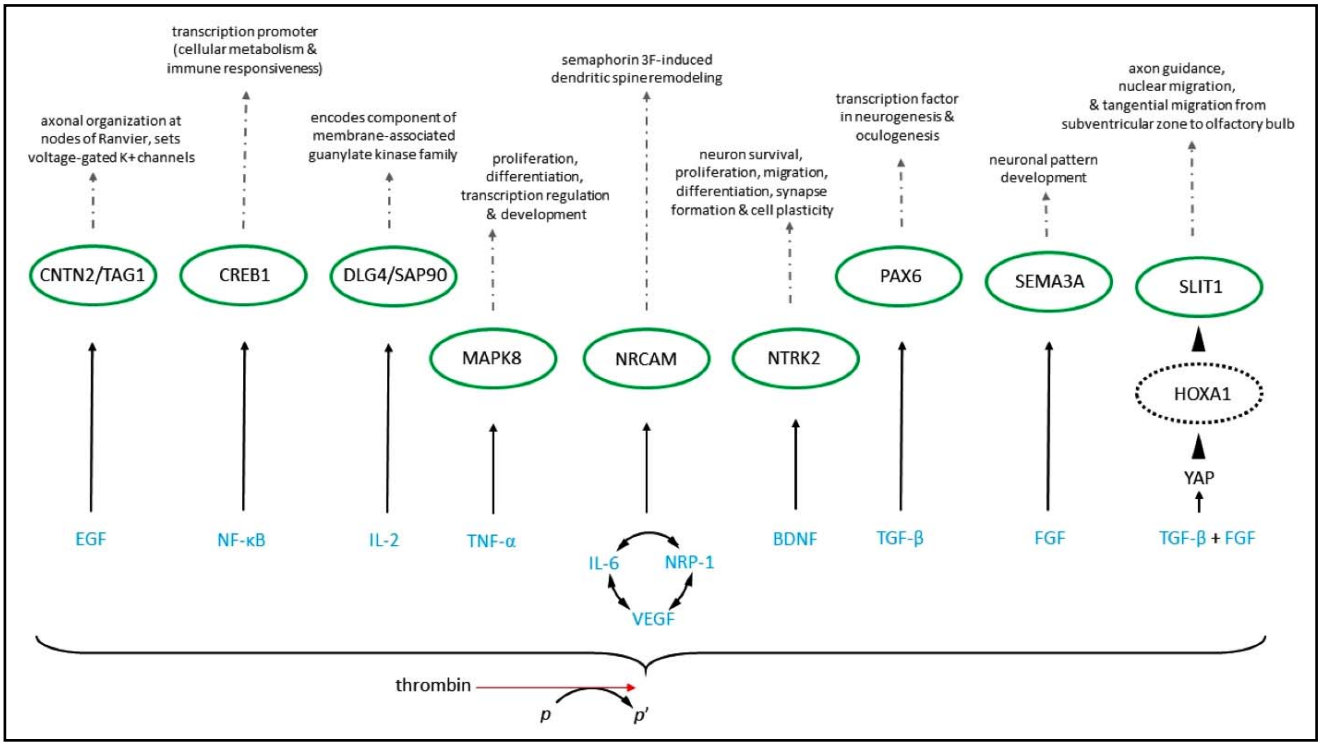

Fig. 2. Gene ontology functional schematic (adapted from Su *et al.* 2017) depicting representative cytokines (blue) discharged after thrombin activation of autologous platelets (*p, p'*) shown with modulated intermediate (dashed) and hub gene (green) targets associated with neuronal differentiation and downstream tissue effects (top). *Where:* CNTN2 = Contactin-2, CREB = cAMP responsive element binding protein-1, DLG4 = Discs large MAGUK scaffold protein-4, HOXA1 = Homeobox A1, MAPK8 = Mitogen-activated protein kinase-8, NRCAM = Neuronal cell adhesion molecule, NTRK2 = Neurotrophic receptor tyrosine kinase-2, PAX6 = Paired box protein-6 (aniridia type II protein), SEMA3A = Semaphorin-3A, SLIT1 = Slit guidance ligand-1, YAP = Yes-associated protein. Condensed plasma cytokines: BDNF = Brain derived neurotrophic factor, EGF = Epidermal growth factor, FGF = Fibroblast growth factor, IL-2 = Interleukin-2, IL-6 = Interleukin-6, NF-kB = Nuclear factor kappa-light-chain-enhancer of activated B cells, NRP-1 = Neuropilin-1, TGF-β = Transforming growth factor-β, TNF-α = Tumor necrosis factor-alpha, VEGF = Vascular endothelial growth factor.

(Chen *et al.* 2018). Remarkably, this axonal regeneration evoked by PLT growth factors persisted even after an extended interval between injury and treatment.

These encouraging results agree with those reported by others working in a cat model, where intrathecal PRP given 14d post-SCI produced significant benefits on hindlimb motor function, sometimes as early as d20. On MRI exam, lesion size was also significantly reduced after PRP treatment, while histopathology comparisons showed PRP fostered significant remyelination with improved structure and organization of white matter (Farid *et al.* 2022). Brain atrophy in AD or PD may sometimes overshadow equally important spinal cord involvement. As the spinal cord sends and receives sensorimotor signals involving cortex and periphery, these motor pathways often undergo abnormal rewiring even before cognitive decline (Fu *et al.* 2018). This would explain why fine motor impairment and deteriorating handwriting are common preludes to dementia onset in AD. Degenerative atrophy may thus extend beyond hippocampus and temporal lobes, with AD spinal cord changes akin to spinal atrophy present in multiple sclerosis (Azodi *et al.* 2017; Lorenzi *et al.* 2020). Notably, intrathecal PRP attained functional recovery, remyelination, oligodendrogenesis, and curtailed inflammation in an animal model for multiple sclerosis (Borhani-Haghighi & Mohamadi, 2019). As PLT cytokines also regulate heat shock protein expression by oligodendrocytes in human glial cell cultures and other fibroblasts (D'Souza *et al.* 1994; Hasan *et al.* 2008) when acting near abnormal brain tissue, they may also influence local unfolded protein responses (Tao *et al.* 2017). For example, heat shock proteins under cytokine control drive apoptosis to deactivate abnormal cells, slowing progress of inflammation and associated neurodegeneration (Terrab & Wipf, 2020). Even for mechanical hyperalgesia and induced inflammation, intrathecal PRP experience in a mammal model gave a post-treatment response of higher pain threshold, inhibited astrocyte activation, diminished aerobic glycolysis, and lower PKM2 expression (Wei *et al.* 2020).

As with all early investigations, it is impossible to predict if intrathecal autologous condensed plasma cytokines/PRP can deliver a useful answer for the problems of AD or PD. Given the long history of prior interventions aspiring to halt and reverse these conditions, the odds are not especially favorable. While most PRP applications do tend to support tissue regeneration, reservations have been voiced (Filardo

& Kon, 2012; Hamid *et al.* 2014). Notwithstanding the growing interest in PRP and its derivatives in new domains (Sills & Wood, 2022), nonuniform sample preparations (Andia & Maffulli, 2018; Rickers & Sills, 2022), have made randomized controlled trials difficult to standardize and almost impossible to interpret. While any diffuse hyperinflammation corrected by PLT cytokine use is welcome, numerous CNS signal pathways are also impacted upon PLT activation to facilitate tissue repair, angiogenesis and neurogenesis (Padilla *et al.* 2017). Our proposal therefore expands prior descriptions of PRP treatment for CNS disorders (Shen *et al.* 2009) given its recuperative effects already documented in SCI and peripheral nerve damage (Sánchez *et al.* 2017; Su *et al.* 2017). This approach also builds on earlier PRP use in kidney disease, balancing difficulties of treating an encapsulated solid organ with the need to dose PRP locally (Martín-Solé *et al.* 2016). Because an autologous PRP-based clinical trial entails no FDA new drug clearance to power the study program, the project joins the AD/PD research portfolio as an uncommon outlier. Nevertheless, the central research questions cover common ground: What is the preferred baseline PLT concentration needed for the optimal releaseate after activation? Is there an ideal growth factor profile or cytokine ratio which will dependably deliver desired clinical effects? How will best dosing schedules be determined? Do the observed results help clarify a mechanistic model for disease? Are there any associated features which may predict treatment response? If yes, can these be modified? With institutional review board oversight, repurposing PLT-derived cytokines as outlined here invites comment on a readily available, relatively low cost, low-risk, and underutilized therapeutic option for use in AD/PD.

AUTHORS' CONTRIBUTIONS

ESS developed the protocol as Principal Investigator; ESS, HIC, J-WW, SHW and SLT advised on the project, edited drafts, and reviewed the literature. All authors approved the final manuscript.

CONFLICT OF INTEREST

U.S. Trademark #88505430 has been awarded to ESS for specified process and method using autologous platelet cytokines. HIC, J-WW, SHW and SLT have no disclosures.

AVAILABILITY OF SUPPORTING DATA

Not applicable.

FUNDING

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