Salisbury
UNIVERSITY

Honor College at Salisbury University

Honors Thesis

An Honors Thesis Titled

Optimizing tPA therapy for Stroke victims

Submitted in partial fulfillment of the requirements for the Honors Designation to the

Honors College

of

Salisbury University

in the Major Department of

Chemistry

by

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Date and Place of Oral Presentation: Jan 17. (Posters on the Bay/OC)

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Optimizing tPA therapy for stroke victims

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Abstract

Tissue Plasminogen Activator (tPA) remains one of the most effective thrombolytic agents given to patients that present with acute ischemic strokes. However, increased chances of brain hemorrhaging are observed if administered outside of the accepted time window of 4.5 hours. Patients who already have prior trauma comorbidities are at the highest risk and are therefore not eligible for reperfusion therapy using tPA. In this manuscript, the author aims to assess the feasibility of counteracting tPA mediated blood brain barrier (BBB) degradation and proposes novel diagnostic techniques to quantify the efficacy of potential adjuvant therapies reported in the literature.

Introduction

Acute stroke care holds as one of the most controversial and elusive management anomalies in modern day medicine. As time plays the most important factor with respect to irreversible brain damage post-onset, dissensions between health care professionals as to what is considered the "ideal" combination of therapeutic modalities used in treatment highlights the desperate need for uniform standard care. However, Tissue Plasminogen Activator (tPA) remains the "gold standard " treatment option for stroke victims at this time. Having gained international recognition after a double blind clinical trial by the NINDS revealed impressive thrombolytic capabilities in salvaging healthy brain tissue (N. Engl. J Med 1995), tPA has been accepted as the only FDA approved drug for acute infarctions. However, only 3% of stroke victims meet the eligibility criteria for this approach (Knecht 2017); like many double-edged emergency interventions, the use of tPA becomes deleterious or even fatal if administered beyond 6 hours. As cerebrovascular accidents make up the 4th leading cause of death nationwide and the 2nd

leading cause of death globally, the limitations of tPA poses an impetus on the overall quality of life for many potential candidates. Two decades later, the unintentional tPA-mediated neurological side effects persist despite modern day advances in medical approaches. The lack of irrefutable evidence for tPA's true efficacy raises concerns about primary stroke care, turning health care professionals toward alternative invasive procedures and other clot-busting agents in the hopes of one day replacing this protease.

In response to these limitations, recent research efforts have pushed their focus towards adjuvant therapy as a means to combat the negative molecular and cellular pathogenesis of tPA itself. Bolstered by the evidence of case studies, *in vivo* trials, and theoretical models, the possibility of complementing IV-tPA with antagonists specific to biological targets could potentially extend the stringent selection criteria for patients with more debilitating comorbidities. Nonetheless, integrating additional therapies to counteract tPA mediated hemorrhagic transformations into protocol management with respect to improving stroke therapy needs to be addressed. Given the narrow timeframe, the practicality of adjuvant therapy has yet to be elucidated. In order to determine if it is, indeed, worth expending funds on improving adjuvant therapy as opposed to researching novel approaches, it is important to take a look at the history behind tPA and its origins.

Mechanism of Action

Our own bodies already contain varying amounts of tPA naturally. It is synthesized in endothelial cells and can be secreted into our bloodstream when prompted by increases in fibrin agglomeration. Its concentration in a healthy person's body can range anywhere from 1ug/L-5ug/L, depending on the analytical method utilized. It is

important to note, however, that tPA itself does not work to lyse fibrin clots but instead is an activator of plasminogen. Through tPA mediation, plasminogen is converted into the enzyme plasmin to directly catalyze fibrinolysis. Despite its invaluable role in helping to remove occlusions, tPA is a rather inefficient activator without a strong presence of fibrin, where tPA's substrate binding sites are located. However, binding is reported to decrease the activation energy of fibrinolysis by 1000 times (Diapharma 2018). The half life of free tPA sits at around 4 minutes but is decreased at almost double the rate during thrombosis. Although its known cellular mechanism seems to underly its proven functionality, its relatively poor half life and affinity for fibrin means that the human body cannot catalyze the degradation of cerebral occlusions or embolisms fast enough to salvage healthy oxygenated tissue.

History of tPA and its Competitors

The origins of tPA as a treatment stem back to the first large scale clinical trial (GISSI-1), that proved the use of streptokinase for thrombolytic therapy to be an effective agent for lowering mortality rates when dealing with Acute Myocardial Infarctions (AMI). However, low fibrin specificity and antigen binding specificities limited the practical applications of this avenue, which prompted further research efforts to determine more optimal thrombolytic compounds. It was during the 1980's, that Dr. Desirée Collen and Dr. D.C. Rijken were able to isolate a pure sample of human extrinsic plasminogen activator (HEPA), which later became known as tPA. With the help of Genentech, an external biotechnology company that collaborated with Dr. Collen, tPA was mass produced through cloning and gene expression using hamster ovary cell lines

(Maroo et al. 2004). Nonetheless, the effectiveness of tPA as a reperfusion agent wasn't promulgated until the first statistically significant randomized controlled trial coined GUSTO-1 (Global Utilization of Streptokinase and tPA for Occluded Coronary Arteries) was completed during the early 1990's. The trial results were promising, as the use of tPA significantly increased blood vessel dilation (over streptokinase), resulting in an impressive 15% decrease in mortality rates (N Engl J Med 1993). However, increased risk of hemorrhaging complications through unknown mechanisms was already evident in patients with AMI (Califf 1988), prompting researchers to shift their focus into elucidating the underlying mechanisms responsible for this additional comorbidity.

Although we would have expected that tPA use for ischemic strokes would have emanated from its first role in fighting acute myocardial infarctions, neurologists were similarly administering streptokinase and urokinase to small cohorts of patients with cerebral infarctions before the 1970's (New Engl J Med 1996). Unfortunately, the tradeoff between desired therapeutic resolutions and hemorrhagic complications remained unfavorable (Fletcher et al 1976). As was globally accepted before the 1990s, the use of tPA for ischemic stroke was deemed a failure unlike its co-use for heart attacks. The reason for the lack of progress made toward treating brain injuries (notably for the treatment of stroke) before that time remains unknown. But taking into consideration the golden age of the cardiology community in the 1980's, new-founded treatment for vascular occlusions may have smothered any noise made for the advocation of thrombolytic therapy for any other distal parts of the body, including the brain. In addition, the lack of statistically significant tPA-centered clinical studies during that time may have also contributed to its limited popularity.

It was not until the 1990s that substantial improvements were made in stroke care with tPA. Researchers at the University of Cincinnati treated a relatively large number of patients (74) with varying concentrations of tPA. The administration of less than or equal to 0.85mg/kg of tPA within 90 minutes post stroke onset prompted no hemorrhagic transformations in 58 patients while 22 patients showed neurological improvement after 2 hours and in 34 patients after 24 hours. Although this study showed promising results, there remains no correlation between improving neurological outcomes and increasing levels of tPA dose (Brott 1992).

In comparison, researchers at the University of Virginia gave patients expressing symptoms of acute ischemic strokes varying doses of tPA and reported 4 fatalities; 2 who received a dose of 0.85mg/kg and 2 who received a dose of 0.9mg/kg. Interestingly, 3 patients did greatly improve neurologically after 24 hours (Haley 1992), sparking the impression that tPA may, in fact, have potential therapeutic advantages for certain patients if administered within a certain time window and under controlled doses.

These two primary case studies became the stepping stones for further investigation of tPA as a novel avenue for stroke treatment at the National Institutes of Health (NIH). Being at the pinnacle of medical research, the National Institute for Neurological Diseases and Stroke (a branch of the NIH) published the largest randomized tPA clinical trial to date. The experiment had two distinct parts; the first part assessed tPA for clinical activity while the second part utilized statistical parameters to determine clinical outcomes after three months. In part 1, a total of 144 patients were administered tPA vs 147 patients in the placebo group. Results showed that there was no significant neurological amelioration in the experimental group compared to the control group.

However, tPA did overall improve outcome measures for the experimental group at the 3 month period, with almost a 4 point decrease on the NIHSS stroke scale. (Note the NIHSS scale is used to measure the severity of impairment due to stroke and is scored out from 0-4, 0 indicating normal function while 4 indicating complete impairment). Part 2 results confirmed tPA's favorable outcomes, as patients who were in the experimental group were 30% more likely to experience little to no comorbidities associated with post stroke recovery (N. Engl. Med 1995). But like many investigational medical approaches, there is a caveat to tPA's thrombolytic success. Improvements were only observed in patients who were given tPA alteplase within 90 to 180 minutes of stroke onset, which was a unique parameter of the study. However, it was also reported that increases in intracerebral hemorrhaging occurred in 6.4% of patients in the experimental group compared to 0.6% in the control group. Despite the known tPA neurological deficits, the NIH coined the trial an enormous success and a giant leap in stroke management.

Soon after these novel findings were published, the FDA approved tPA as a legal stroke intervention agent. More recently, however, the European Cooperative Acute Stroke Study III ran a META analysis in the publicly accessible NINDS patient database used in their 1995 publication. The purpose of the META was to combine patient data from the multiple centers that were a part of the study to derive a conclusion (backed up by statistical evidence) about the efficacy of tPA past the accepted time window. As the efficacy of tPA was not proven to be significant after 3 hours of stroke onset, the authors of the ECASS III study set out to investigate the certainty of this time window using multivariate analysis. The selection criteria were subsequently changed, including only patients with no prior history of diabetes, stroke, or administration of anticoagulants. The

results came back conclusive for a wider time window of 4.5 hours. It turns out that 52% of patients who were administered tPA for varying stroke intensities had a positive response and improved quality of life after a 3 month baseline (Hacke et al. 2008). As the study showed promising results, standard tPA protocol guidelines have increased the time window to 4.5 hours.

Although the ECASS III study included a relatively large cohort (499 patients) in their statistical analysis, 225 patients in the original NINDS manuscript were excluded. Furthermore, the study was conducted 18 years later, raising concern about the evolution of stroke therapy and the progress that has been made over the past two decades. Even considering an extended time window, only 8% of patients who have received tPA alone for severe strokes are reported to have improved clinical outcomes (Lutsep 2018). Such a low percentage in part accounts for all patients who do not meet the tPA administration criteria or where alternative approaches are favored. But as tPA remains the "gold standard" of stroke therapy, one would expect more optimistic results. Unfortunately, tPA remains a solid option only for patients who meet the criteria for administration and where the occlusion can be reached via intravenous injection.

Fortunately, the 1990's also gave rise to endovascular therapy in order to make up for tPA's anatomical limitations. Also known as mechanical thrombectomy, the approach utilizes a catheter that is threaded through an artery in the groin and is brought up the neck until it reaches the occlusion. A built-in stent then latches onto the occlusion while the catheter is being retracted, thus removing the clot and restoring normal blood flow. The need to stray away from intravenous approaches was first brought to light by Gregory del Zoppo, a hematologist who observed that administering IV-tPA could

recanalize distal middle cerebral arteries but had trouble doing the same for much larger, proximal arteries. Soon after, Zoppo et al created the Prolyse in Acute Cerebral Thromboembolism trials (PROACT) to assess the efficacy of another thrombolytic agent known as recombinant prourokinase (r-pro UK) through intraarterial exploration. The PROACT trials showed increased rates of successful recanalization outcomes for patients with occlusions in the middle cerebral artery. However, the trials were approved solely for the purpose of proving the effectiveness of r pro UK and did not allow for any operational manipulations of the occlusion itself. Surprisingly the FDA did not approve r pro UK, pinning the blame on the lack of statistical evidence and their fairly small patient cohort. Nonetheless, the PROACT trials sparked the interest of many researchers in building mechanical devices for thrombus removal that could be inserted intraarterially. By 2004, the first Mechanical Embolus Removal in Cerebral Ischemia (MERCI) device was approved by the FDA, sparking further disagreements between the agency and the medical community. This time, neurologists weren't at all happy with the swift integration of the MERCI device in stroke care, as the PROACT II trials showed supporting evidence for the results reached in PROACT I. Additionally, the committee refused to give credibility to the PROACT II trials as the data was not available for analysis at the time being (the study was published later that year). Further investigations were conducted, and a second multi centered trial was published in 2008. The initiative sought to improve the MERCI design, ultimately achieving higher recanalization rates (57.3% vs 48% in 2004) but indicated hemorrhagic rates in 10% of patients (Smith 2008).

Although complications associated with invasive IA approaches remained fairly common, the use of mechanical stents showed consistent favorable clinical outcomes. Of

course, this warranted additional studies to compare IA and IV-tPA as two different treatment modalities and settle the debate as to which method truly has a bigger impact on patient quality of life. The Interventional Management of Stroke III, SYNTHESIS (Intra-arterial Versus Systemic Thrombolysis for Acute Ischemic Stroke Expansion, and MR-RESCUE (Mechanical Retrieval and Recanalization of Stroke Clots using Embolectomy) trials were simultaneously developed to test out the true efficacy of IA vs IV-tPA approaches to stroke treatment, none of which produced any statistically significant results. Luckily, a final trial coined MR CLEAN conducted in the Netherlands enrolled 500 patients with acute ischemic strokes to either undergo intraarterial treatment with IV-tPA administration (233 patients) or IV-tPA alone (267 patients). The study reported better functional recoveries in patients with intracranial arterial occlusions if IA treatment with standard care was given within 6 hours (Berkhemmer 2015). Although these findings have led to the communal acceptance of IA treatment as a viable treatment option, the study fails to report any clinical outcomes for patients in the control group. In addition, the centers utilized either urokinase or tPA for standard care but did not report any statistical difference between the two; therefore, we cannot fully accept the study to be irrefutable evidence for tPA's administration alone to be less effective than if paired with IA treatment.

Regardless, 11 years after del Zoppo's original work, the MR CLEAN trial finally credited IA mechanical thrombectomy as a "successful" treatment option that could seem to replace tPA for patients where the infarct is difficult to access intravenously (ie. proximal occlusions). However, there still remains to this day no concrete evidence that this approach is better than tPA. In fact, current literature indicates that physicians are not

trying to consider if IA treatment is better than tPA but instead are assessing which method may be better suited for each individual patient and the type of occlusion they are experiencing. Coming back to the original idea of extending the use of tPA for patients with previous medical comorbidities with respect to adjuvant therapy, understanding the rationale behind the choices medical professionals have to make when choosing the appropriate approach for stroke care may provide a more realistic view.

Interview and Subsequent Analysis

Over the summer of 2018, neuro-intensivists Dr. Case and Dr. Cava from the University of Colorado Hospital (a level 1 trauma center) were interviewed on their daily exposure with tPA. Both physicians verbally consented to speak over the phone and were informed that their feedback would be used to complement this manuscript without the need of IRB approval. The following questions were asked of them and then summarized:

- What does the "ideal" tPA patient look like?
- How do you go about making a preliminary assessment of someone who is admitted with signs of stroke?
- Why are so few patients eligible for tPA administration?
- Are there any known adjuvant therapies that are currently used in primary treatment in combination with tPA?
- Is it possible to tell through radiological screening or bloodwork the susceptibility of a patient to endure tPA mediated deleterious side effects?
- What should the scientific community be focusing on as a next step to increasing the time window and/or selectivity criteria?

Dr. Case's view on tPA protocol management:

To be considered "fit" enough for tPA administration, the treating physician must first determine when the patient was last known to be normal. Ideally, the patient will have had no prior history of traumatic brain injury or hemorrhaging and would have been brought to the hospital within one hour of stroke onset. Following the ECASS III 4.5 hour time window, it is left up to the discretion of the treating physician in determining tPA as a viable care option. Primary assessment of the patient includes trauma level and blood platelet count. Patients excluded from the selection criteria include onsets reported after 4.5 hours and patients with prior trauma comorbidities. Depending on the type of stroke they're having (ie. embolism or large vessel occlusion), tPA may not be the emergency intervention of choice. Actually, it turns out 25% of all strokes are large vessel occlusions where intra-arterial administration of tPA is not proven effective. If this is the case, the physician may choose to order an angiography to visualize the arteries and veins using real-time imaging. A thrombectomy may then be performed to remove the clot. As far as Dr. Case is concerned, tPA is not paired with any adjuvant medications to counteract its disruption of the blood brain barrier. In addition, there haven't been any reported cases of matching patient bloodwork to inflammatory or other immune system responses caused by tPA administration. Dr. Case also believes the scientific community should turn their focus on getting the "info" out to the general public about the importance of time in stroke management, urging people to make informed decisions about seeking immediate medical attention if symptoms arise.

Dr. Cava's view on tPA protocol management:

The physician should be immediately notified of the last time the patient was known to be normal. Platelet count, trauma level, and patient history should all be considered before deciding on the treatment option. Depending on the severity of the symptoms, thromboelastrography tests may be ordered to determine the efficiency of blood coagulation. If the results and location of the blood clot warrant invasive approaches, thrombectomies may usually be preferred. However, tPA is not usually given in conjunction with thrombectomies except for cases that deal with complete occlusions of the carotid artery. Even then, anti-coagulants may be used instead of tPA to decrease the risk of additional hemorrhaging. Similarly to Dr. Case, Dr. Cava is not aware of any other adjuvant modalities given alongside tPA and states the blood is too complex in terms of its varying pathophysiological properties during stress to exactly target specific pathways for inhibition. Generally speaking, the best use of tPA can be observed in cases where the patient does not have a flagged history for head injuries, the location of the occlusion does not require surgical intervention, and subsequent MRI scans confirm no prior comorbidities.

Confirmed by both Dr. Cava and Dr. Case's plan of action, time is the most important factor with regards to stroke treatment. tPA is automatically ruled out for patients who present to the emergency room post 4.5 hours after stroke onset or with previous brain injuries and may not even be considered for patients with proximal IA occlusions. Both physicians also mention ordering additional contrast enhancing scans to determine the location and magnitude of the clot, which proves useful in determining if a mechanical stent would be considered a more suitable option compared to tPA. Given the

chances that tPA may induce hemorrhagic transformation, it also seems that physicians would prefer to opt for a more invasive method in order to minimize the risk of further complications.

Interestingly, Dr. Cava did mention the use of thromboelastography (TEG) to analyze the efficiency of blood coagulation. But as TEG remains a fairly novel analysis technique for stroke patients, the approach can only be conducted at certain centers and is by no means fully understood. Considering its limited accessibility, TEG is subject to the file drawer phenomena, as current interpretation of its results in the literature varies across the medical community. Nonetheless, TEG can provide a rapid estimation regarding the strength and speed of clot formation by probing viscoelastic properties of blood in a small sample.

TEG has recently been used to obtain clot data pre and post tPA administration in patients with acute ischemia. Overall, post tPA bolus results showed reduced levels of fibrin formation but concluded that tPA seemed to affect the strength of the clot and "not the kinetics of the enzymatic portion of the coagulation" (Elliott et al. 2015). The authors also call for greater uniformity of future studies when reporting data to assess the accuracy of TEG. Although the technique could still be labeled as being experimental, TEG may provide a medium through which varying adjuvant therapies may be tested. As was implied in the literature and in both Dr. Case's and Dr. Cava's reports, mechanical thrombectomies are preferred over tPA for specific cases where tPA cannot catalyze thrombolysis. However, mechanical thrombectomies are not meant to replace tPA, which is still extensively used every day. Revisiting the idea of exploring which extrinsic inflammatory pathways are being triggered by tPA may constitute an effective approach

to expanding the treatment window. As was previously mentioned, TEG has shown that tPA affects the formation of the occlusion itself but not necessarily the rate at which it is formed, which may indicate that enzymatic catalysis of coagulation is not affected by tPA. This in turn may prompt further research efforts to look into proteins and other factors responsible for blood vessel formation.

It is important to note that current investigations into tPA adjuvant therapies are limited to animal models. Only two adjuvants have been proven to be safe for human administration, so notable clinical trials remain sparse in the literature. In addition, this manuscript expands on the feasibility of using current diagnostic methods to pin-point which respective tPA mediated deleterious cascades are being activated in each individual patient. However, as Dr. Cava mentioned, the complexity of blood, the enormous amount of proteins involved in clot formation, and varying genetic conformations of each patient would cause this approach to require exorbitant amounts of time and funding. With respect to patient care, however, developing adjuvant therapies that are proven to work even without the knowledge of how they are hindering tPA mediated pathways may be a faster solution for tPA salvage therapy.

Search Rationale

Considering the numerous amount of adjuvant therapies and proposed tPA mediated deleterious mechanisms that are currently being investigated, studies in this paper were included based on the following criteria: The authors should hail from reputable neurosurgical institutions or the therapy at hand must have been previously explored and shown to be effective in other publications. Articles older than 10 years

were disregarded. The search query was performed under PRISMA (Preferred Reporting Items for Systemic Reviews and Meta-Analyses) guidelines using a combination of Boolean search terms and key words. Although PRISMA is meant to provide a set of items intended to help investigators report statistical studies, the guidelines are geared towards extrapolating targeted information specifically for medical interventions and presents itself as a relevant approach for data collection in this literature review. Databases chosen for this literature review included Web of Science, PubMed, and Academic Search Ultimate. Access was granted using a Salisbury University subscription. The search results are listed below:

Database	Search results for ["Adjuvant Therapy" AND "Tissue Plasminogen Activator"]	Search results for [Tissue Plasminogen Activator' AND "Blood Brain Barrier"]
PubMed	30	355
Web of Science	23	733
Academic Search Ultimate	14	250

Proposed Mechanisms and Adjuvant Therapies

One notable tPA mediated mechanism was proposed by the Department of Neurosurgery at the Barrow Institute (AZ) in conjunction with the Department of Neurological Surgery at the University of Pittsburgh. It involves an induced inflammatory immune response, where tPA acts as a protagonist to C3 cleavage associated with aggravated edema. One of those product proteins, C3a, is shown to be in high concentration in surrounding ischemic endothelium and is also linked to increased BBB permeability and inflammatory cell influx. Complement inhibition using an

antagonist molecule to block exogenous C3a receptors was shown to protect against breaches in the BBB (Zhao et. al 2017). This study was the first to show a substantial upregulation of cascade activation in inflammatory proteins caused by tPA, meriting a switch from *in vitro* models to experimental clinical applications.

Two years earlier, researchers at the University of Michigan Medical School looked at the role of Platelet Derived Growth Factor-CC (PDGF-CC) in increasing membrane permeability. PDGF-CC is a protein component of the neurovascular unit (NVU), which is made up of neurons, astrocytes, microglia, neurons, and endothelium tissue that work synergistically to maintain a healthy BBB. The authors discovered through immunohistochemical staining of wild type and tPA deficient mice that PDGFF-CC was confined in abundance where tPA was expressed in the brain. With this new finding, they then demonstrated that treating mice with Imatinib (a type of kinase inhibitor) to block the formation of PDGF-CC alongside tPA administration reduced cerebrovascular permeability while keeping the thrombolytic effects of tPA (Su et.al 2008). Imatinib is a multi-purpose drug approved by the FDA that is currently used in many cancer fighting regimens and is widely available for sale. Its accessibility and relatively low complication rates makes it an interesting drug for tPA adjuvant therapy. Although these inhibition mechanisms present promising therapeutic avenues, in vitro models and studies using knockout mice will eventually need to be replaced with randomized, controlled clinical trials.

However, antioxidants have recently taken the spotlight as a potential therapeutic amendment for reperfusion injury. Glycyrrhizin, an antioxidant found in liquorice root, was administered intravenously alongside tPA to male rats subjected to ischemia.

Interestingly, the authors reported decreased levels of hemorrhagic transformation, a lower mortality rate, and increased improvements in neurological outcome. More specifically, two discrete proteins known as HMGB1 (high mobility group box 1) and MMP9 (matrix metallopeptidase 9) were "significantly inhibited" post glycyrrhizin + tPA treatment but were originally at high levels prior to the experiment (Jiangang 2016). Similarly, Liang et. al (2015) reported lower levels of MMP9 in the surrounding ischemic endothelium when they exposed rats to 100% O_2, also known as normobaric oxygen (NO), to rats subjected to middle cerebral arterial occlusions. The study also concluded that NO + tPA slowed down BBB deterioration and also improved neurological outcomes.

Interestingly, a subsequent clinical trial explored the use of NO as a single therapeutic modality. The purpose of the trial was to see if NO could be administered to tPA ineligible patients and extend their time window so that they may become candidates for tPA administration. The results obtained for an 85 patient cohort were inconclusive. No changes in BBB degradation or neurological outcomes post ischemia were observed on MRI scans at varying time intervals from 0 hours to 3 months (Singhal 2016).

The latter study raises an interesting point. If NO works alongside tPA administration but not by itself, there may be a common pathway involved between the two therapies. On the other hand, the use of NO has also previously been reported to salvage healthy sclera (eye) tissue during ischemia (Dana et al 2012). Considering its extensive use in stroke therapy, NO stands as one of the most promising adjunctive therapies under study to this day.

Breathing in high levels of oxygen can often be harmful, as it can actually increase the levels of reactive (radical) oxygen species in the blood (Yu 2017). However, hyperbaric oxygen (HO), which utilizes 100% oxygen at 1 atmosphere of pressure was shown to promote the same therapeutic effects as NO (Chazalviel 2016) and could be complemented with hydrogen gas to counteract the creation of free radicals (Yu 2017).

Valuable information also lies hidden in the shortcomings of adjuvant therapy for reperfusion injury. The Surgical Neurology Branch at the NINDS believed that the intravenous administration of sodium nitrite would help combat aggravated hemorrhaging as the compound is able to move freely across the BBB and was previously shown to shield against reperfusion injury in the heart (Duranski et al 2005). Using albino rats as their test subjects, the authors administered sodium nitrite in conjunction with tPA reperfusion 6 hours after (induced) stroke onset. The results showed that sodium nitrite was in fact not successful in improving blood flow deficits (Schatlo et al 2008).

Although utilizing alternative concoctions non-adjuvantly with tPA is not within the scope of this manuscript, the extent to which the following two drugs were considered as potential thrombolytic agents deserves a mention. Desmetoplase, a chemical agent found in the saliva of vampire bats, reached a phase III clinical trial (coined DIAS-2) to assess the clinical efficacy of its intravenous administration for patients who presented with acute ischemic strokes between 3-9 hours after onset of symptoms. Backed up by prior phase II trials, Desmetoplase initially showed improved clinical outcomes (Furlan 2006). However, the DIAS-2 trial results showed no benefit from the drug, arguing the placebo group had too high of a response rate compared to the patients who received the

treatment. Furthermore, the increased mortality rate (14% or 17/123 patients) was too high compared to the use of tPA (Hacke et al. 2009).

Ancrod, an enzyme found to hold fibrinolytic properties that can be isolated from the pit viper (Sherman 2000), received the attention of the scientific community in the Stroke Treatment with Ancrod Trial (STAT). The multicentered initiative involved a double blind, placebo controlled cohort of 500 patients, with half being administered Ancrod and the other half given regular treatment. The study determined that the patients in the Ancrod group achieved decreased fibrinogen levels and better clinical outcomes. However, further phase III trials under the Ancrod Stroke Program (ASP 1 and 2) were cut short to due increasing safety concerns (Stroke trial).

Discussion

The literature does present some certain trends that may be of interest for future research. First mentioned by Szocs et al. 2004, thrombolysis for stroke therapy seems to disrupt the BBB and further ischemia through an unknown influx of oxygenated free radicals. However, the use of animal models above by no means guarantee this to be irrefutably true. In their defense, though, current research still has not indicated exactly how tPA and thrombectomies can increase the chance of reperfusion injury. Nonetheless, multiple trials seem to indicate some therapeutic advantages to targeting said radicals. Although Jiangang et al. 2016 did not mention why they chose to experiment with glycyrrhizin, antioxidants remove damaging oxidizing agents and can even protect against cellular damage. In fact, the study indicated lower levels of HMGB1, a protein that has been shown to increase levels of free radicals (Klune 2013) and MMP9. NO was

also shown to decrease levels of MMP9, which could indicate a relationship between metalloproteinase and increased levels of free radicals. Su et. al explored the use of PDGF-CC in tPA adjuvant therapy because they observed that PDGF-CC was activated when tPA was injected into CSF and believed the two components shared a mutual pathway. However, PDGF-CC was also shown to block the release of radical damage by downregulating the activation of neutrophil mediated oxidative influx (Wilson 1987). Even though the research was completed 3 decades ago, the level of scrutiny achieved by publishing in the Proceedings of the National Academy of Sciences of the United States of America cannot be overlooked. In light of the overbearing amount of current literature on adjuvant therapy, further research efforts should be aimed at increasing the amount of pre-clinical trials in the hopes of one day developing novel drugs that target pathways specific to the formation of free radical oxidative species.

But as each respective study explored a discrete pathway that tPA may be mediating , the amount of potential therapeutic agents that could be developed are enormous. Unfortunately, only two of the aforementioned experimental therapeutic agents (Desmetoplase and Ancrod) reached phase III clinical trials, but to no avail. This may be explained, in part, by their extensive role in prior research efforts. Desmetoplase was first found to have fibrinolytic properties back in 1932 and was later discovered to induce fibrinolysis when in the presence of human specific fibrin (Medcalf 2012). Ancrod was already being used in Europe for reperfusion therapy in patients with peripheral vascular diseases and deep vein thrombosis (Sherman, 2000), so the risk of known complications was already known to physicians. Coming back to recently explored adjuvant therapies, clinical trials may not be warranted without prior indication

that the approach shows promise in obtaining better outcomes compared to standard treatment during phase III trials.

There also remains a lack of uniformity amongst reported outcomes across the literature. To account for this, authors should agree to report progression free survival and overall survival rates for their respective cohorts. Authors should also agree to publish under a similar journal and utilize key words that solely relate adjuvant therapy with tPA. Including all relevant publications under one database should also be considered, as this would allow for easier extrapolation of duplicate articles pertaining to one specific approach (ie. All articles that studied NO as adjuvant therapy alongside tPA). Future investigations should also be aimed at performing META analyses on duplicate articles to provide statistical significance for each respective adjuvant therapy, which could in turn initiate the selection process for future clinical trials.

The use of TEG as a comparison method for adjuvant therapies that may potentially reach the stage of human experimentation is also warranted. Current analysis of exploratory therapeutic agents are limited to chemical analyses that do not measure colligative properties in human blood. However, blood plasma contains a wide array of proteins including fibrinogen when separated from blood serum. It could be theorized that adjuvant doses could directly be added into blood plasma and analyzed for coagulation under a laboratory setting. By mimicking similar hemic conditions, adjuvant therapies could be tested alongside tPA under realistic conditions without the need to put human subjects at risk.

Conclusion

As the underlying tPA mediated biological mechanisms responsible for aggravated hemmorhagic transformations remain obscured, the enhancement of tPA in stroke management has been slow to progress. Its current use still revolves around immediate salvaging of healthy brain tissue, meaning only patients with no prior brain trauma or flagged commorbidities can benefit from this infamous clot-busting agent without carrying the risk of further complications. Although many adjuvant therapies have been proposed, some even including outcomes from animal models, the absence of specific reported data points makes it hard to integrate results from multiple studies. In order to draw a specific conclusion for the whole body of a particular adjuvant therapy (and measure its true efficacy with more stringent statistical evidence), authors must agree upon what outcomes are to be reported, including progression free survival and overall survival rates. Additionally, novel instrumental techniques that measure the physical properties of blood (like TEG) are promising therapeutic enhancers and should be incorporated into future experiments to observe real time changes in coagulation respective to each adjuvant therapy.

The struggle in the global medical community to accept tPA as the messiah of stroke treatment is evident. However, tPA cannot be discredited for saving millions of lives since its revelation and merits novel investigative approaches aforementioned in this manuscript to better study its ever so elusive properties. In turn, the opportunity to fix the negative effects of tPA through adjuvants may remold a much more holistic approach for stroke treatment, finally providing a solution for patients who were left in the dark.

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