Potential Therapies to Promote Tissue Revascularization and Remodeling after Ischemic Stroke "A Systematic Review"

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ABSTRACT

INTRODUCTION: After analysis of current reviews and research into current clinical tries, a novel combination therapy which is phased to tackle the pathophysiological events in the brain over time may be suggested which may provide more positive results in clinical trials, overcome some of the limits that current therapies in trial are facing, and improve ischaemic stroke sufferers long-term recovery and outcome. **METHOD**: Only those articles that provide original research were reviewed, such as primary research articles, cohort studies, and animal model studies and phase 1 clinical trial. Multiple electronic databases using a variety of outlined search terms were used to gather suitable papers. The sources used were Google Scholar, PubMed, Science Direct, Web of Science, Scientific Web Plus etc. **RESULT**: The result of this review shows that the number of pre-clinical animal model studies for potential therapies after stroke outnumber clinical human trials on a ratio 2.6:1 (pre-clinical animal : clinical human). Therapies after TIA to prevent major stroke recurrence appear to be more advanced, with all potential therapies in phase 1 clinical trial for safety and efficacy. **DISCUSSION**: There are many trials which has shown potential for better stroke recovery and results from those studies support the safe and effective use of many chemical derivatives like 2-growth factor therapy initiated 24 to 48 hours after stroke onset, therapy using adenovirus-expressing heparin-binding epidermal growth factor which significantly enhanced recovery after ischaemic stroke, human umbilical mesenchymal stem cells transplantation for ischemic stroke, and monocyte-derived multipotential cells which are potentially effective candidate for use in cell transplantation therapy for cerebral ischaemia.

Key words: Ischaemic stroke, Transient ischaemic attack (TIA), Tissue remodeling, Tissue revascularization, Neuroprotection.

الملخص

مقدمة : بعد تحليل عدة بحوث ودراسات سريرية، يمكن اقتراح مجموعة من العلاجات تنفذ على مراحل لمعالجة التغيرات الفيسيولوجية المرضية في الدماغ والتي قد توفر نتائج أكثر إيجابية في التجارب السريرية ، والتغلب على بعض الصعوبات التي تواجه تجارب العلاجات الحالية, وتحسين شفاء الذين يعانون من السكتة الدماغية على المدى الطويل . **المنهج:** تم استعراض ومراجعة البحوث الأصلية فقط (مثل مقالات البحوث الأساسية ودراسات الزمر و دراسات التجارب علي الحيوان و التجارب السريرية) . تم تجميع الاوراق المناسبة من قواعد بيانات إلكترونية متعددة باستخدام مجموعة متنوعة من مصطلحات البحث الواردة. وكانت المصادر المستخدمة هي (Google Scholar, PubMed, Science Direct, Web of Science, Scientific Web Plus, etc) **النتائج:** اوضح الاستعراض ان دراسة العلاجات المحتملة لما بعد السكتة الدماغية علي حيوانات التجارب فاقت الدراسات السريرية على الإنسان بنسبة ٢,٦ الي ١ , كما ظهر تطور كبير في العلاجات التي تعطي بعد حالات نقص الدم المؤقت للمخ لمنع تكرار السكتة الدماغية الكبرى . **المناقشة:** هناك العديد من التجارب التي أظهرت إمكانات لتحسين الشفاء من السكتة الدماغية ونتائج هذه الدراسات تدعم امكانية الاستخدام الآمن والفعال للعديد من المشتقات الكيميائية مثل عامل النمو ٢ اذا بدأ العلاج به بعد ٢٤ إلى ٤٨ ساعة من الإصابة بالسكتة الدماغية ، والعلاج باستخدام الهيبارين المرتبط بعامل نمو البشرة والذي عزز بشكل كبير التحسن بعد السكتة الدماغية ، و زرع الخلايا الجذعية من الحبل السري البشري للسكتة الدماغية الناتجة من نقص التروية الدموية، و لوحيدات الخلايا الناشئة من الخلايا متعددة الامكانات و التي يحتمل أن تكون فاعلة عند استخدامها في زراعة الخلايا كعلاج للسكتة الناتجة من نقص التروية.

INTRODUCTION

Cerebrovascular accident (CVA) or stroke is a neurological disorder caused by altered cerebral circulation, defined as an 'acute neurological dysfunction of vascular origin with sudden or at least rapid occurrence of symptoms and signs corresponding to the involvement of focal areas in the brain [1]. Those over the age of 65 are most at risk, but 25% of strokes occur in people under the age

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of 65. Certain nationalities, for example south Asian, African or Caribbean are pre-disposed to conditions such as diabetes and heart disease, which are two major risk factors for stroke.2 Other risk factors include high blood pressure, high cholesterol and diabetes, all of which could be due to unbalanced diet and a lack of exercise, but also age, gender and family history. Smoking can double the risk of having a stroke as it narrows arteries and makes the blood more likely to clot [2].

There are two major mechanisms causing brain damage in stroke; ischemia and hemorrhage. Ischemic stroke represents about 80% of all strokes, the blood supply to the brain is interrupted, and the brain cells are deprived of the glucose and oxygen they need to function sufficiently. The effects of ischemia can be fairly rapid as the brain does not store glucose, the main energy substrate, and is incapable anaerobic metabolism [3]. Ischemic stroke is a complete entity with multiple etiologies and variable clinical manifestations. Hickey [4] approximated that 45% of ischemic strokes are caused by small or large artery thrombus, 20% are embolic in origin, and others have unknown cause.

Normal cerebral blood flow is approximately 50 - 60ml/100g/min and varies in different parts of the brain. Cerebral auto-regulatory mechanisms compensate for reduced CBF in response to ischemia by local vasodilation, opening collaterals, and increasing oxygen and glucose extraction from blood. When CBF is reduced below 20ml/100g/min, electrical silence ensues and synaptic activity is greatly diminished in an attempt to preserve energy stores. CBF less than 10ml/100g/min is irreversible neuronal injury [5].

Understanding the ischemic cascade has led to the concept of a therapeutic time window, or timed approach for treatment possibilities. There is a core zone of severe ischemia with dead cells and blood flow below 10 – 25%. This is surrounded by an area of hypoperfused tissue, known as the penumbra, in which the cells may remain viable for several hours. This is because the penumbral zone is supplied with blood by collateral arteries anastomosing with branches of occluded vascular system. If reperfusion is not established during the early hours, the cells here will also die since collateral circulation cannot maintain the neuronal demand for oxygen and nutrients [5].

Krupinski *et al*. [6] demonstrated an increase in capillary density, active angiogenesis around infarcts, more developed in the penumbra in post-mortem brains of patients who had survived acute ischemic stroke for up to several weeks. These recent developments in the understanding of pathophysiological events following acute ischemic stroke suggest an important role for angiogenesis, which, through new blood vessel formation, results in collateral circulation and may influence positively on the medium to long-term recovery of patients. Future treatment regimens focus on optimization of this process in the penumbra.

Currently a thrombolytic drug called alteplase is used to dissolve the clot, but has proven to be effective only within the first hour after stroke onset. Tissue plasminogen activator (tPA) was approved in 1996 by the U.S. Food and Drug Administration (FDA) as a thrombolytic drug with a 3 hour window for those with severe stroke (NIHSS score > 22) [7]. However, it remains a limited option due to its narrow therapeutic time window and the related risks of intracranial hemorrhage. Aspirin will be given regularly as an anti-platelet medication to prevent further clotting. Anticoagulants such as heparin and warfarin are given to prevent further clotting7. Medication to control blood pressure and cholesterol may also help.

The level of brain damage after a stroke can vary greatly depending on what vessels are affected, but can be widespread and long-lasting. Although many people recover their former independence after extensive rehabilitation with teams of specialists including physiotherapists, psychologists, occupational and speech therapists and specialist nurses and doctors, approximately 90% of stroke survivors are left with some residual deficit [8].

Due to the complexity of physiological regulation of blood vessel formation, involving numerous critical growth factors expressed differentially in time, space and concentration, ongoing therapeutic efforts using single agents and aimed at treatment of ischemic vascular disease are of limited potential. Optimization of therapeutic treatments might involve a complex series of interventions beginning with administration

of a cocktail of drugs within the first few hours of illness to reduce inflammation, but at the same time maintaining neuronal viability with neurotrophins and stimulating growth factor-induced angiogenesis. Perfusion pressure in the penumbra region could be increased using thrombolytic therapy, and susceptible neurons could be protected from apoptosis by viral transfer of genes such as Bcl-2 [9]. With angiogenic therapies there is a tendency for inflammation and vascular permeability, so therapies to prevent this, perhaps by inducing blood vessel maturation, will be analyzed.

After analysis of current reviews and research into current clinical tries, a novel combination therapy which is phased to tackle the pathophysiological events in the brain over time may be suggested which may provide more positive results in clinical trials, overcome some of the limits that current therapies in trial are facing, and improve ischemic stroke sufferers long-term recovery and outcome.

METHODS

Male and female adults were included, and they must have a mean age of 60 or older after ischaemic stroke onset or following a transient ischaemic attack (TIA). Animal models, such as mice and monkeys, will also be included as many potential therapies have not been through stage 1 clinical trials yet. The intervention to ischemic stroke will be therapies that focus on inducing and sustaining angiogenesis, revascularisation and vascular remodelling, also to induce neuroprotection, inflammation. The use of novel combination therapies will be reviewed. Medications to prevent inflammation and vascular permeability following angiogenic therapies will be analyzed. Therapies to prevent major stroke recurrence after transient ischemic stroke will also be analyzed. The aim is to review current potential therapies to be able to suggest a novel combination therapy which is phased to tackle the pathophysiological events in the brain over time may be suggested which may provide more positive results in clinical trials, overcome some of the limits that current therapies in trial are facing, and improve ischemic stroke sufferers long-term recovery and outcome.

The interventions to ischemic stroke will be compared to other potential therapies. As they are not in clinical use it would not be appropriate to compare interventions to currently used thrombolytic therapy for ischemic stroke, tissue plasminogen activator (tPA) and alteplase. Only articles that provide original research are used, such as primary research articles, cohort studies, and animal model studies and phase 1 clinical trials.

Inclusion Criteria

For inclusion, trials need to satisfy the following criteria:

- Articles published since the year 2005
- Adult participants (>18 years) males or females with a mean age of 60 years or older or animal models
- All nationalities are included
- Articles are freely available
- Trials must be primary and randomized
- Pre-clinical and any phase of clinical trial included

Exclusion Criteria

- Peer reviews, surveys, questionnaires
- Any previous haemorrhagic event (including haemorrhagic stroke)
- Previous cardiovascular conditions
- Any known intracerebral pathology other than stroke
- Any other serious medical condition which is likely to affect outcome assessments

Sources of Information

Google Scholar, PubMed, Science Direct, Web of Science, Scientific Web Plus

Search Strategy

Make use of multiple electronic databases using a variety of outlined search terms to gather suitable papers. Review papers related to the topic of cerebral angiogenesis and current and future therapeutic targets for ischemic stroke were used to identify relevant primary studies.

DISCUSSION

The 90 day risk of stroke after a transient ischemic attack (TIA) has been reported as being as high as 17% [7], the stroke recurrence rate has been calculated at 15% within 2 years, and as high as 30% within 5 years [28]. Anti-platelet therapies are still the most

successful method to prevent secondary stroke. Neuroprotection is a key target for stroke treatment and the therapies with current potential are outlined in table 1. As well as for protection against secondary stroke, chronic statin treatment has also been suggested for decreasing stroke severity and improve outcome.

The effects of intravenous versus intra peritoneal administrations of rosuvastatin in a pre-clinical mouse model of focal cerebral ischemia at different dosages were studied [10]. The results of this study showed that i.v rosuvastatin at relative pharmacologically relevant concentrations up to 4 hours after an event shows significant signs of neuroprotection; in this study a dosage of as low as 0.02mg kg^{-1} achieved neuroprotection. The protective effects extended to 5 days after ischemia and were accompanied by improved functionality based on a few simple functionality tests, namely the wire hanging test, pole test, and Bederson score. Intraperitoneal administration only appeared effective within 1 hour of ischemia, and at a high dose of 2mg kg-1, which is a restrictive time window. It has been demonstrated that the protective effects may be mediated by up-regulation of endothelial-derived nitric oxide synthase (NOS) in the vascular wall and direct activation of endothelial nitric oxide synthase (eNOS) via protein-kinase Akt. The increased availability of eNOS seems to improve endothelium function, increase cerebral blood flow, inhibit platelet aggregation and exert anti-inflammatory effects, all of which enhance neuroprotection [10]. A safe, efficacious formulation of intravenous statins should be developed in order to move statin treatment in to the phases of human clinical trials.

An epidemiological inverse association has been found between high-density lipoprotein (HDL) cholesterol levels and cerebrovascular events [29]. A study in 2010 tested timing and dosage of intravenous HDL administration compared with saline control. There were positive pre-clinical results with HDL showing neuroprotection up to 5 hours after stroke onset at higher dosages in a rat model of embolic stroke. This model of embolic stroke is suggested to be the closest to human pathophysiology, as there are majorities of ischemic strokes, which involve an embolic mechanism. The results may possibly be attributed to the ability of HDL to protect the blood-brain barrier and limit neutrophil recruitment as shown by immunostaining [18].

Table 1: Novel therapies to induce neuroprotection and combat apoptosis following ischemic stroke

Study	Result
Prinz et al [10], 2008	Intravenous rosuvastatin given at pharmocologically relevant concentrations up to 4 hours after an event can be neuroprotective of focal brain ischemia in an animal model. Development of intravenous statin formulation is warranted for phase 1 acute stroke trials with statins in humans.
Dávalos et al [11], 2012	Citicoline appears not to be efficacious in the treatment of moderate-to-severe acute ischaemic stroke.
Ehrenreich et al [12], 2009	Erythropoietin showed no favourable effects on functional outcomes of ischemic stroke.
Zhao et al [13], 2007	Functional restoration in chronic brain ischemia can be attained using haematopoietic growth factor.
Yang et al [14], 2012	VEGF reduced ischemic neuronal danger with a therapeutic time window within the first 3 h of transient MCAO and may be useful in the treatment of acute ischaemic stroke in humans.
Zhao et al [15], 2011	Postischemic treatment with intravenous Tf-VEGF-PLs promoted neuroprotection and angiogenesis and vascular remodelling.
Emerich et al [16], 2010	Intracerebral implantation of an alginate provides therapeutic sustained delivery of VEGF in a rodent model of stroke provides anatomical and neurological protection.
Pathipati et al [17], 2009	Delayed, chronic central treatment of unilateral stroke with rGH in adult rat was associated with slightly more rapid recovery of motor function and spatial memory.
Lapergue et al [18], 2010	Administration of HDL is neuroprotective when performed up to 5 hours after stroke onset.

Breakdown of the blood-brain barrier (BBB) is a key step associated with ischemic stroke and its increased permeability causes extravasation of plasma proteins and circulating leukocytes, including polymorphonuclear neutrophils, which may use their associated matric metalloproteinases to contribute further to BBB breakdown and basal lamina degradation. In addition to reverse transport of excess cholesterol from tissues back to the liver, HDL particles exert anti-inflammatory, antioxidant, anti-elastase, and anti-thrombotic effects that may protect endothelial cells from acute injury [30].

Lapergue *et al* [18] demonstrated by immunostaining that HDL was taken up by endothelial cells and glial cells, but not neurons, which suggests a beneficial role on astrocyte and cerebral endothelial cell function, and prevent ischemia induced BBB disruption. Many further studies are required to confirm whether HDL based therapies are applicable, efficacious, and effective in human ischemic stroke.

There have been some recent studies which showed no positive results for therapies to induce neuroprotection. Ehrenreich *et al* [12] used recombinant human erythropoietin (EPO) in a small early phase clinical trial on 522 human patients, which suggests pre-clinical studies showed more positive results. Wang *et al* [31] demonstrated that EPO enhanced functional recovery in rats, possibly due to increased levels of vascular endothelial growth factor (VEGF) and brain-derived neurotrophic factor (BDNF). This is a clear example to prove that animal models do not always relate well to human therapies. EPO is a hematopoietic cytokine that promotes proliferation and differentiation of erythroid progenitors and the survival of maturing erythroid cells [31] in theory it represents and ideal compound for neuroprotection in brain disease acting as anti-apoptotic, antioxidant and anti-inflammatory amongst other things [12]. Recombinant human EPO or a placebo control was given intravenously over 30 minutes within 6 hours of stroke, and repeated 24, then 48 hours thereafter. The use of one of the only recommended treatments for stroke, namely rtPA, was allowed and stratified for in this study. Although the EPO group showed a slight reduction in infarct lesion size compared with the placebo group, neither primary outcome BARTHEL index on day 90 or any other outcome showed any favorable protective effects of stroke outcome in patients. In fact mortality rates were increased in the EPO group at 16.4% compared with 9.0% in the placebo group. Due to the unsuccessfulness of this clinical trial, strict adherence to comprehensive safety protocols would be necessary for any further EPO trials, as well as more balance in the stroke severity of those enrolled on to the study and an exclusion of those in receipt of rtPA treatment.

It has been recently suggested that therapies with the potential to enhance endogenous brain plasticity and repair could reduce brain damage and improve functional recovery in animal models of stroke, even when administered several hours after the onset of the ischemic event [32]. Citicoline is a naturally occurring endogenous compound and an essential precursor in the synthesis of phosphatidylcholine, a key cell membrane phospholipid. It is increasingly recognized as a neuroprotectant that may be able to act through the stages of ischemic damage inhibiting different stages of the ischemic cascade. This is most probably due to its anti-apoptotic effects, its ability to synthesize and repair membranes, inhibit free fatty acid release, decrease free radical formation, and favor the synthesis of nucleic acids, proteins and neurotransmitters [32]. Citicoline is approved in some countries for the treatment of ischemic stroke, the drug required more testing for efficacy in pooled analysis in an international, randomized, multicenter, placebo-controlled study. A very recent phase 3 clinical trial of citicoline compared with a placebo after having received 2000mg of citicoline daily for 6 weeks in 2298 patients has shown not to be efficacious in the treatment of ischemic stroke. This confirms extensive studies previous to this, which report similar safety profiles compared with placebo, and previous failures to prove efficacy. The International Citicoline Trial on acute Stroke (ICTUS) trial deems citicoline safe but not efficacious in the treatment of ischemic stroke [11]. A paper yet to be published online from 'Nature' seems to be set to draw attention to the fact that rodent models, which have seen positive efficacious results following citicoline treatment after ischemic stroke models, metabolize citicoline differently when it is administered in different ways (intravenously or orally) from human models. This therefore suggests that perhaps if a clinically relevant method of administration of citicoline to humans following stroke, for example in a liposomal formulation, it may still provide the

potential neuroprotective effects seen in pre-clinical models, and improve functional outcome in humans following ischemic stroke [33].

Growth factors make up one of the most studied therapies for stroke treatment. It has previously observed that stem cell factor (SCF) and granulocyte-colony stimulating factor (G-CSF), the growth factors that regulate hematopoiesis, have neuroprotective and functional effects on acute cerebral ischemia [13]. In this study the combined administration of both factors at 14 weeks (3.5 months), which is entering the chronic phase after ischemic stroke onset, resulted in improved functional outcomes and reduced infarction size in pre-clinical rat models.

According to Zhao *et al* [13] this was the first evidence that pharmaceutical intervention as late as 3.5 months after stroke can improve neurological deficits and reduce penumbra and focal infarct size. Pathipati [17]used an infusion of rat growth hormone on rats 4 days after induction of ischemic stroke for 6 weeks, followed by 6 weeks to allow for restorative processes to occur. The results showed a slightly more rapid recovery of motor function and spatial memory compared with the buffer treated group. However, the most recent work on growth factor treatment to provide neuroprotection following ischemic stroke is on vascular endothelial growth factor (VEGF).

VEGF is a potent pro-angiogenic peptide, its administration has been considered as a potential neuroprotective strategy following cerebral stroke [16]. There have been previous studies, which confirm the ability of VEGF to promote neuroprotection, promote neurogenesis and cerebral angiogenesis [34], and therefore improve functional outcome and recovery following ischemic stroke through multiple mechanisms.

Previous studies are all pre-clinical animal models, the majority with rat models. Due to the poor permeability of the BBB, in previous studies, in which the majority are pre-clinical rat models, the models have been subjected to intra cerebral injections or craniotomies to administer the VEGF, both very invasive procedures.

Emerich *et al* [16] demonstrated a successful method of prolonged VEGF delivery to the brain by injection of the drug directly to the striatum of a rat model using hydrogels and alginate scaffold. The results showed VEGF was still viable and detectable around 2 weeks after the hydrogel delivery. This is promising to provide chronic VEGF treatment, therefore increase the scope of anatomical and neurological recovery and result in an improved outcome following ischemic stroke.

However, the invasive nature of delivery poses obvious ethical, safety and methodological issues for the relation of VEGF treatment for neuroprotection from pre-clinical models to a safe, efficacious human treatment of ischemic stroke. A study by Zhao *et al* [15] aimed to achieve enhanced delivery of VEGF to the ischemically disordered parts of the brain; the penumbral region and the infarct cortex. The results indicate that brain-specific expression of exogenous genes, such as VEGF, is possible with the combined use of a cationic gene therapy system such as stabilized liposomes, and a brain targeting ligand. In this case transferrin-targeted coupled liposomes (Tf-PLs) are used, as transferrin, the iron-transporting protein, has free access across the intact BBB as it is a key carrier of essential nutrients to the brain. The intravenous administration of VEGF-loaded Tf-PLs also achieved high VEGF expression in the ischemic cerebral tissues, and seemed to induce neovascularization. Although the full mechanisms for this delivery method are still under investigation, Zhao *et al* [15] believe that Tf-VEGF-PLs hold promise for efficient, non-invasive, and specific brain-targeting gene delivery, and advanced developments in ischemic stroke treatment.

The most recent study into VEGF focuses on the dose response and time window, the results of which may help to bring about safe, efficacious human clinical trials of VEGF for the treatment of stroke targeted to neuroprotection. A dose-response relationship was established indicating the mid-dose level was the most efficacious, which was set at 2.5ng/µl relative to rabbits weighing 2.5–3.0kg. A therapeutic time window for the neuroprotective effects of VEGF in a rabbit model was defined as being within 3 hours of the MCAO ischemic stroke model onset [14]. The future of VEGF therapy to treat ischemic stroke should focus on the multi-potential aspects of VEGF; it's promotion of angiogenesis and brain plasticity leading to improved neurological function. There

is a need to define how these properties can come together for the development of new therapies, it is also important to consider any unfavorable side effects [35].

Inflammation is a key contributor in the pathogenesis and progression of stroke. Inflammatory biomarkers such as high-sensitivity C-reactive protein have been identified as predictors of initial stroke and for prognosis predictions after stroke [36]. Some of the most important studies on therapies to target the inflammation stage if the ischemic cascade are outlined in table 3. Statins have proven to be therapeutic in preventing secondary stroke following TIA, and they have also been suggested to play a role in treatment due to their anti-inflammatory properties. In a small phase 1 clinical trial in a small group of 55 patients received 20mg of lovastatin daily for 90 days or no treatment, however after a 90 day follow-up using the NIHSS and the BARTHEL index, no significant improvement was shown in the lovastatin group [19].

Table 2: To show novel anti-inflammatory therapies to target the pathophysiological stage of inflammation following ischemic stroke.

Study	Result
Zare *et al* [19], 2012	Lovastatin therapy shows no role in improved stroke recovery over 90 days.
Jung *et al* [20], 2010	High dose nitrite therapy within 24hrs after ischemia reduced inflammatory cytokine levels and caspase activity in sub-acute period.
Herz *et al* [21], 2012	Anti-inflammatory effects, neural plasticity, promoting effect of VEGF accompanied by immunosuppressive effects were observed.

A pre-clinical trial on rats on nitrite therapy showed more successful results. NO is known to mediate, proliferation and differentiation of brain cells and VEGF-induced angiogenesis, all of which improve functional recovery following ischemic stroke. This trial was especially interested in measuring the anti-inflammatory effects of NO therapy, and found that inflammatory cytokine levels were reduced as was caspase activity [20]. RT-PCR was performed to measure the mRNA of cytokine-related genes, IL-1β IL-6, TNF-a, and Fas-L, all of which are usually raised in the ischemic brain, were reduced in nitrite-treated rats. Western blotting measured reduced expression of PARP, a family of proteins involved in DNA repair and programmed cell death and caspase-3 which is involved in cell apoptosis.

VEGF has also shown to have ant-inflammatory properties; in a study by Herz *et al* [21] VEGF mechanisms providing neurological recovery were accompanied by a profound immunosuppressive effect and anti-inflammatory mechanisms. However, there were negative side effects associated with administration of VEGF in the acute phase such as hemorrhage and vascular permeability [21]. These may be associated with the angiogenic and neovascularization properties of VEGF, as vessels that are developed rapidly can often be described as 'leaky', or permeable, the new vessels haven't had a chance to mature.

Table 3 concentrates on therapies to induce angiogenesis, tissue revascularisation and remodelling. An early study on neurotrophic factors, which have been shown to promote neurogenesis after cerebral ischemia, used heparin-binding epidermal growth factor-like growth factor (HB-EGF) injected into the lateral ventricle in the ischemic side 3 days after ischemic onset in rats, and again on the 6th and 7th day [23]. The study shows improved functional recovery by inducing neurogenesis and angiogenesis. However, during the searches, the results of this study didn't seem to lead to any further developed studies which have been published as of yet.

A practical idea demonstrated by Levy *et al* [27] was intracranial stent deployment. Patients with contraindications to rtPA treatment, or those who failed to improve following 1 hour of rtPA treatment were assigned to receive the self-expanding stents (SES) intracranialy within 8 hours of stroke onset. This was a small-scale phase 1 clinical trial on humans to evaluate the safety and efficacy of primary stent deployment for revascularisation following acute ischemic stroke, with encouraging 1-month follow-up results and the conclusion that it may be a valuable addition to stroke treatment. Due its successful potential in this small scale trial, the therapy should advance through the clinical trial phases in bigger

patient groups to assess the best candidates for this type of treatment, as it is more invasive than intravenous therapies.

Following on from the unsuccessful trial of EPO on neuroprotection therapy after ischemic stroke, EPO plus β-human chorionic gonadotropin (β-hCG) was used in an effort to induce neurogenesis. β-hCG is in the same growth factor family as nerve growth factor and is able to cross the BBB. The time window of B-E therapy suggests it acts as a restorative rather than neuroprotective therapy, as the therapy remains effective when administered up to 48 hours after stroke onset and continued for 9 days [22]. This was a small patient sample, therefore not representative of the population as a whole, although the inclusion and exclusion criteria would have maintained some consistency. A placebo-controlled, double-blind, phase IIb trial of B-E therapy had been initiated at the time of publication, the results of which are unpublished as of yet.

Table 3: Novel therapies to induce angiogenesis, tissue revascularization and tissue remodeling.

Study	Result
Cramer *et al* [22], 2010	Results support the safety of this sequential, 2-growth factor therapy initiated 24 to 48 hours after stroke onset.
Suqiura *et al* [23], 2005	Gene therapy using Ad-HB-EGF significantly enhanced recovery after ischaemic stroke in rats.
Lin *et al* [24], 2011	There were therapeutic benefits of hUMSC transplantation for ischemic stroke.
Hattori *et al* [25], 2012	MoMCs are a potentially effective candidate for use in cell transplantation therapy for cerebral ischemia.
Yu *et al* [26], 2010	NSCs and collagen type 1 implantation facilitated structural and functional recovery of neural tissue following ischaemic injury.
Levy *et al* [27], 2009	Primary intracranial stenting for acute stroke may be a valuable addition to stroke treatment.

One of the most studied methods of revascularisation and remodelling is by the use of stem cells. The transplantation of pluripotent stem cells hold the greatest potential, however, the use of human embryonic stem cells is controversial and sparks many ethical issues from pro-life groups and others alike. As a result, adult stem cells are the favorable option, although there has been a lack of sufficient adult human tissue and appropriately differentiated cells to provide therapeutic potential.

A stem cell, which appears to show potential, was neural stem cells (NSCs), as, studied by Yu *et al* [26], due to its importance in basic research of neural development as well as their broad multi-potential properties for stem-cell based therapies for neurological diseases such as stroke. In a pre-clinical rat model, NSCs were taken from 14-day old embryos in gestating female rats, and transplanted back into the female rat subjects following middle cerebral artery occlusion to replicate human ischemic stroke and 4 hours of observation. The issue as to whether the transplanted cells which differentiate into neurons and proliferate are capable of acquiring full neuronal functionality and synaptic contact [26]. Yu *et al* [26] suggested that an ideal cerebral remodelling in stroke should be composed of a biodegradable material strong enough to resist damage, be permeable to diffusion of nutrients, enable normal cellular processes and facilitate extracellular matrix formation; collagen was used in this study. The results show that in the group administered with NSCs and collagen type 1, the majority adopted a neuronal fate, and demonstrated significant improvements in structural and functional neurological function following ischemic injury.

The use of neural stem cells has been preceded by more modern sources of stem cells. Human umbilical mesenchymal stem cells (hUMSCs) from the Wharton's jelly of the umbilical cord can be cultured in abundance in vitro. They are less controversial to use and aren't associated with many ethical issues as the umbilical cord isn't required once a new baby is born, the cord would usually be discarded so is often donated by new mothers to harvest the stem cells. Although the hUMSCs are multipotent, it has been shown that they are able to differentiate into neural cells when cultured with neuronal-condition medium (NCM) for 6 days24. In the pre-clinical model by Lin *et al* [24], both treated and untreated hUMSCs were grafted into the infarct cortex of rats in 2 injections

to observe the differences, and compared with a control group, which received only phosphate-buffered saline (PBS). Therapeutic benefits were demonstrated in both the NCM-treated and non-treated hUMSC groups, there appeared to be no major significant difference in revascularisation, motor function or metabolic activity of cortical neurons between the treated untreated groups. HUMSCs are abundant and easily accessible, therefore were said to be a promising and reliable source for the future. Lin (2010) confirmed that additional research was required to define the therapeutic potential for chronic treatment of ischemic stroke.

In the most recent study, Hattori *et al* [25] used monocyte-derived multipotential cells (MoMCs) in a pre-clinical rat model, which have the potential to differentiate in to mesenchymal, neuronal and endothelial lineages. Similar significant improvements in revascularisation as shown by microvessel-like structures in the penumbra, and angiogenesis as demonstrated by increased numbers of angiogenic factors being expressed (Hattori, 2012). These are adult stem cells, so are a safe, abundant and readily available in the adult peripheral blood system; they are without ethical issues, and due to them being transplanted by autologous methods they are without the immunological complications of other stem cell transplant methods. Hattori [25] suggests that further studies should determine optimal therapeutic timing and recommended dosages, as well as an appropriate method of autologous transplantation to maintain therapeutic levels in the penumbra and ischemic cortex.

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