ZHITTYA
REGENERATIVE MEDICINE INC.

Stroke, Dementia and Alzheimer's Disease: Vascular Disorders of the Brain

Clinical Description and Possible Breakthrough Therapies

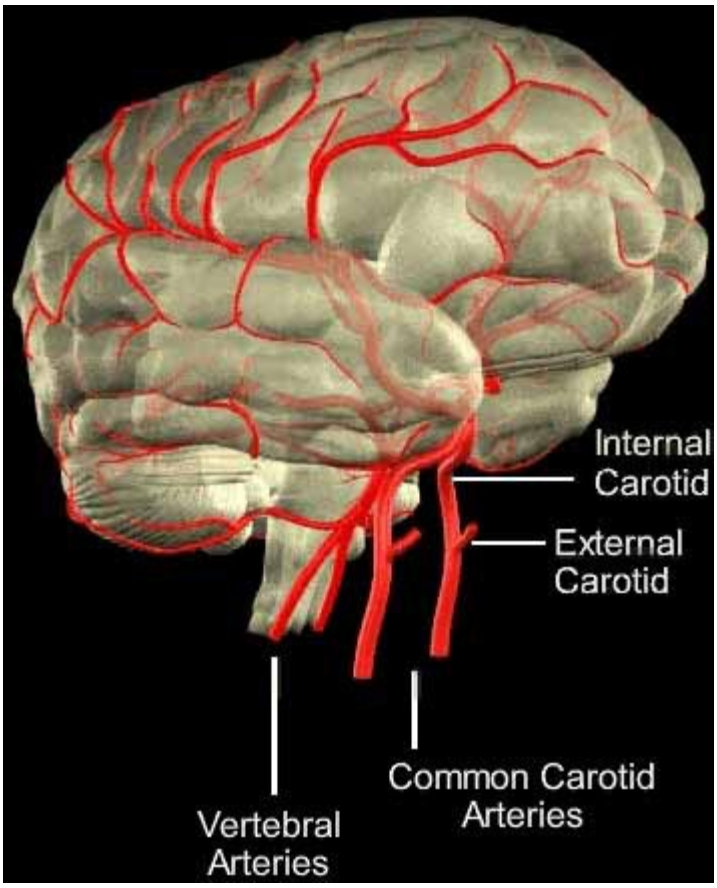
January 6, 2017

I. Introduction

A. The Brain's Vascular System

Although the brain is only about 2% of the total body weight in humans, it receives 15-20% of the body's blood supply. Because brain cells will die if the supply of blood which carries oxygen is stopped, the brain is the body's top priority for blood perfusion. Even if other organs need blood, the body first attempts to supply the brain with a constant flow of blood. Two major groups of arteries supply the brain with blood, including the left and right carotid arteries and the middle, anterior and posterior cerebral arteries (Figure 1 below). As will be discussed in greater detail below, blockage of these arteries leads to acute stroke resulting in neuronal cell death, functional impairment and death in many cases.

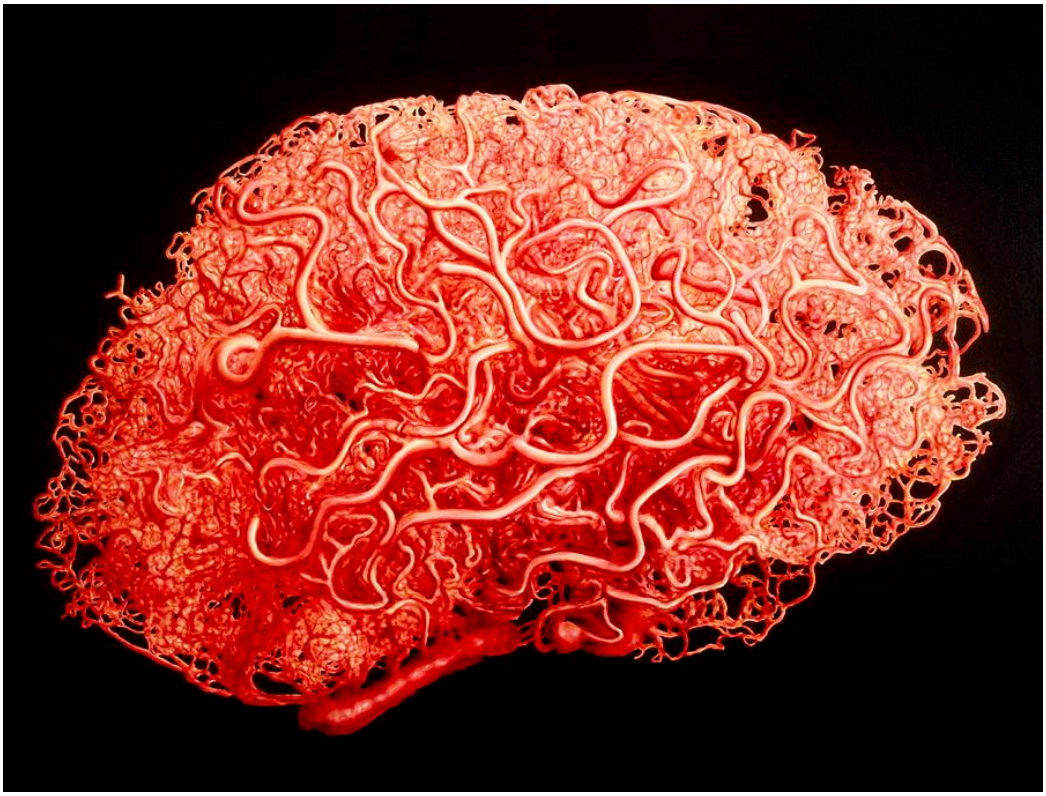
Figure 1: *The Major Arteries Supplying Blood to the Brain, Including the Carotid and Vertebral Arteries*



These major arteries break down into a vast array of smaller arteries, arterioles and capillaries in the brain supplying glucose and other necessary nutrients to the billions of neurons comprising the

brain and removing waste products that these neurons generate as part of their intense metabolic functioning. Figure 2 is a representation of this vast array of blood vessels in the brain. As will be described below, blockage or rupture of these smaller vessels can lead to mini-strokes, which over time can give rise to a condition known as multi-infarct dementia. In addition, recent evidence has pointed to vascular dysfunction in the smaller vessels of the brain as the very earliest step in the progression of events that ultimately leads to Alzheimer's disease.

Figure 2: *The Brain's Vasculature: The supply of blood to the brain is carried out by a vast network of arteries, smaller arterioles and capillaries*



This extensive vascularization of the brain is crucial to bring the brain its primary fuel source, glucose. Glucose is virtually the sole fuel for the human brain, except during prolonged starvation. The brain lacks fuel stores and hence requires a continuous supply of glucose, which in turn is dependent on an intact and functional vascular architecture.

In humans, the brain consumes ~20% of glucose-derived energy making it the main consumer of glucose. Tight regulation of glucose metabolism is critical for brain physiology and disturbed glucose metabolism in the brain underlies several diseases affecting both the brain itself as well as the entire organism. For example, if there isn't enough glucose in the brain, neurotransmitters, the brain's chemical messengers, are not produced and communication between neurons breaks down.

As will be discussed later in this paper, long-term diabetes—either type 1 or type 2—has many consequences for the brain and for its neurons, including that high blood glucose levels can lead to small-vessel disease in the brain, which restricts blood flow, and can lead to cognitive difficulties and, if severe enough, spur the development of vascular dementia.

B. Vascular Dysfunction in the Brain

A healthy and intact vascular system in the brain is critical for normal human functioning, both motor and cognitive. Any disruption in the brain's blood perfusion system can lead to dire consequences, as the brain neurons are deprived of glucose and oxygen and begin to quickly atrophy and die. A number of medical conditions are characterized by a disruption of blood flow to the brain and these will be reviewed below. Some conditions, such as stroke, have long been known to be directly tied to a breakdown in the brain's vasculature system. With others, such as Alzheimer's disease, it has only recently been appreciated that a critical early step in the progression of this disease is a disruption in the functioning of the small arteries supplying blood to the brain.

II. Acute Stroke

Stroke (Figure 4) is the 5th leading cause of death in the US, with one person dying every 4 minutes as a result. Approximately 800,000 people have a stroke each year; about one every 40

Figure 4: *The Onset of Stroke with Destruction of Brain Tissue*

seconds. Only heart disease, cancer, chronic lower respiratory diseases and accidents are more deadly. In the US, approximately 40% of stroke deaths are in males, with 60% in females. According to the American Heart Association (AHA), compared to white people, black people have nearly twice the risk of a first-ever stroke and a much higher death rate from stroke.

Strokes are a direct result of a disruption in the brain's vascular system: either the blood supply is blocked or a blood vessel within the brain ruptures, causing brain tissue to die.

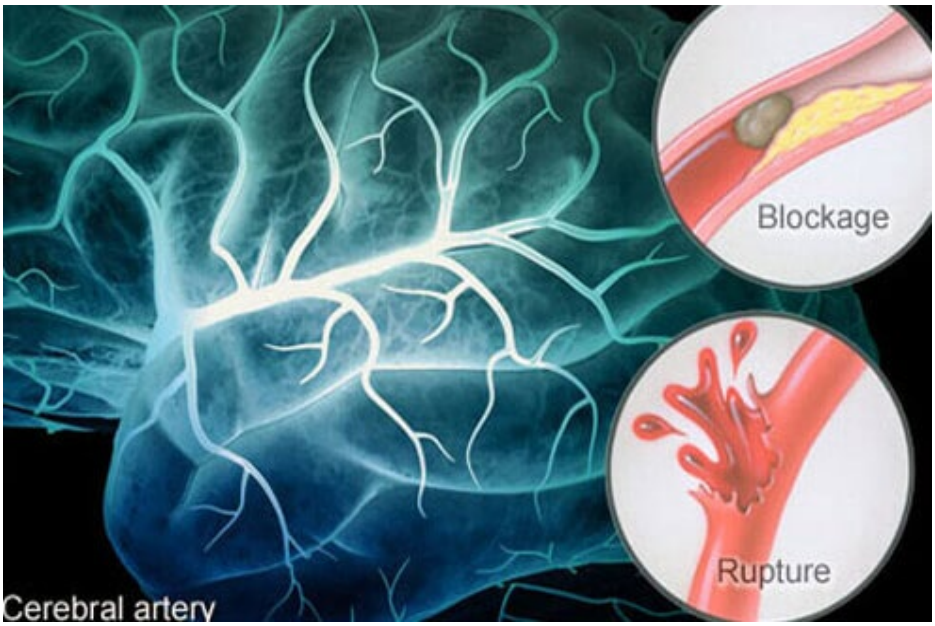
There are three main kinds of stroke:

1. Ischemic strokes
2. Hemorrhagic strokes
3. Transient ischemic attacks (TIAs), also referred to as mini-strokes

1. Ischemic stroke

Ischemic stroke is the most common form of stroke, accounting for around 85% of strokes. This type of stroke is caused by blockages or narrowing of the arteries that provide blood to the brain, resulting in ischemia from severely reduced blood flow. These blockages are often caused by blood clots, which can form either in the arteries connecting to the brain, or in other blood vessels before being swept through the bloodstream and into narrower arteries within the brain. Clots can be caused by fatty deposits within the arteries called plaque as shown in the top of Figure 3 below.

Figure 3: *A depiction of the two most common forms of stroke: ischemic stroke caused by blockages in major arteries perfusing the brain (in this case, the cerebral artery) and hemorrhagic stroke, which results from a rupture of the artery.*



2. Hemorrhagic stroke

Hemorrhagic stroke is caused by arteries in the brain either leaking blood or bursting open, as depicted in the bottom of Figure 3 above. The leaked blood puts pressure on brain cells and damages them. Blood vessels can burst or spill blood in the middle of the brain or near the surface of the brain, sending blood into the space between the brain and the skull. The ruptures can be caused by conditions such as hypertension, trauma, and aneurysms (weaknesses in blood vessel walls).

Intracerebral hemorrhage is the most common type of hemorrhagic stroke and occurs when brain tissue is flooded with blood after an artery in the brain bursts. Subarachnoid hemorrhage is the second type of hemorrhagic stroke and is less common. In this type of stroke, bleeding occurs in the subarachnoid space - the area between the brain and the thin tissues that cover it.

3. Transient ischemic attack (TIA)

TIAs are different from ischemic or hemorrhagic stroke because the flow of blood to the brain is only briefly interrupted. TIAs are similar to ischemic strokes in that they are often caused by blood clots or other debris. They serve as warning signs for future strokes and indicate that there is a partially blocked artery in the brain or a clot source coming from the heart, for example from atrial fibrillation. According to the Centers for Disease Control and Prevention (CDC), over a third of people who experience a TIA go on to have a major stroke within a year if they have not received any treatment. Between 10-15% will have a major stroke within 3 months of a TIA.

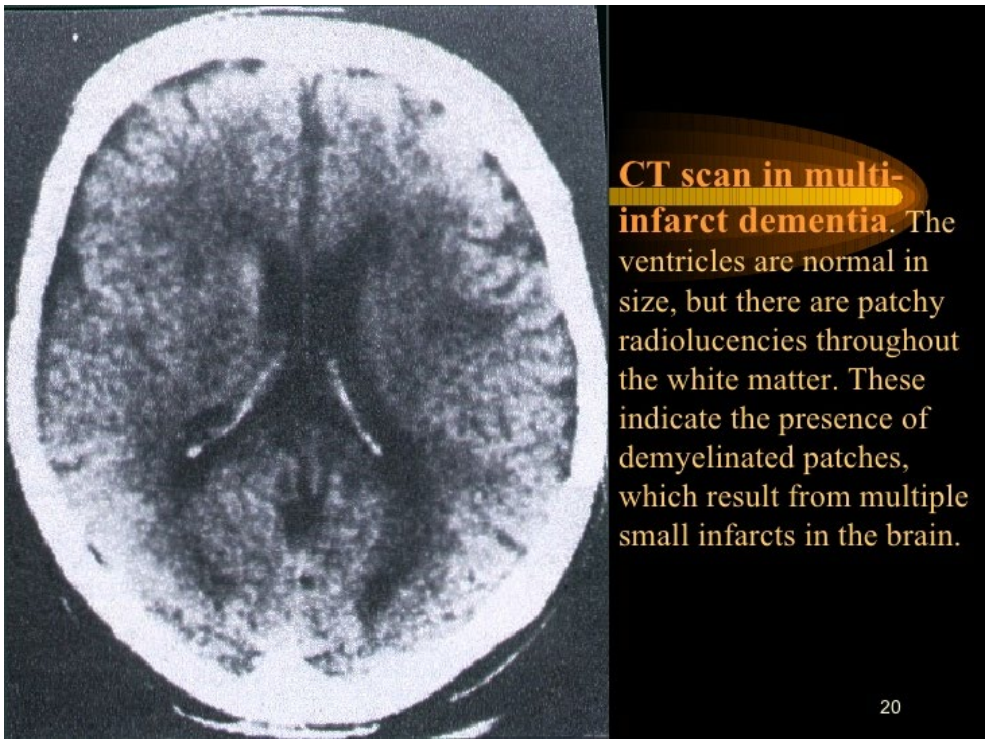
However, for the roughly two thirds of patients with TIAs that do not experience a subsequent major stroke, a large percentage of these individuals will go on and experience similar mini-stokes throughout their lives. Although these mini-strokes are silent and are not detected by imaging studies in the early stages of this disorder, over years the cumulative effect of these brain insults can lead to a significant percentage of these patients developing a condition known as multi-infarct dementia.

4. Multi-infarct dementia

Multi-infarct is the second most common cause of dementia in older people and it is sometimes difficult to distinguish from Alzheimer's disease, which is the most common cause of dementia in older persons. It is possible for a person to have both multi-infarct dementia and Alzheimer's disease, making it hard for the physician to diagnose either.

Multi-infarct dementia usually affects people between the ages of 60 and 75 and can be diagnosed with CT scans of the brain as shown in Figure 4 below, where a diffuse pattern of brain damage can be visualized. Men are slightly more likely than women to have this disease.

Figure 4: *A CT Scan of a patient with multi-infract dementia showing a multitude of small infracts throughout the brain, which are visualized as small white circles on the CT scan*



The most important risk factor for multi-infarct dementia is high blood pressure and it is rare for a person without high blood pressure to develop multi-infarct dementia.

III. Treatments for acute stroke

As the two main kinds of stroke, ischemic and hemorrhagic, are caused by different factors, both require different forms of treatment. It is important that the type of stroke be diagnosed quickly using brain imaging techniques, as a treatment suitable for one kind of stroke can be harmful to someone who has had a different kind.

1. Ischemic stroke treatment

Treatment can begin with drugs to break down clots and prevent further ones from forming. Aspirin can be given, as can an injection of tissue plasminogen activator (TPA). TPA is very effective at dissolving clots but needs to be injected within 4.5 hours of stroke symptoms manifesting themselves. In addition, a specialized catheter can be inserted directly into the affected artery in the brain to physically remove the clot from its obstructive position.

Many neurologists are reluctant to administer TPA, as the drug carries a significant risk to cause further bleeding in the brain, which can be fatal. TPA is generally only available at highly specialized trauma centers that are experienced in the diagnosis and treatment of stroke.

There are other procedures that can be carried out to decrease the risk of future strokes or TIAs. A carotid endarterectomy involves a surgeon opening the carotid artery and removing any plaque that might be blocking it. Alternatively, angioplasty can be done, which involves a surgeon inflating a small balloon in a narrowed artery via a catheter procedure and then inserting a stent (a mesh tube) into the opening in order to prevent the artery from narrowing again.

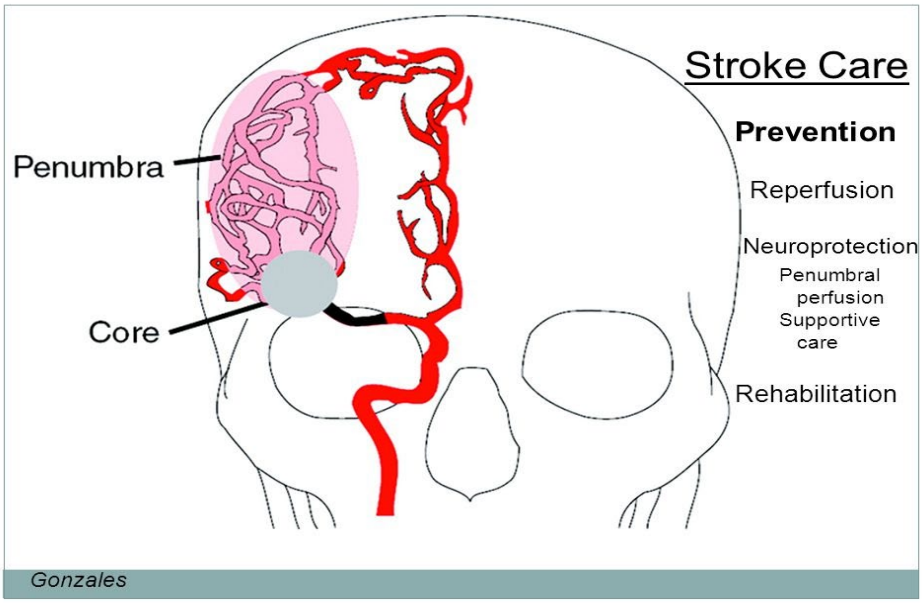
2. Hemorrhagic stroke treatment

Hemorrhagic strokes are caused by bleeding into the brain and so treatment focuses on controlling the bleeding and reducing the pressure on the brain that it is causing. Treatment can begin with drugs that can reduce the pressure in the brain and overall blood pressure. If the patient is taking anti-coagulant or anti-platelet medications such as Warfarin or Clopidogrel, they can be given drugs or blood transfusions to counter the medication's effects. Surgery can be used to place small clamps at the base of aneurysms to prevent rupture and to remove small arteriovenous malformations (AVMs) which are tangled connections between arteries and veins
that are weaker and burst more easily than other normal blood vessels.

3. Neurotrophic Growth Factors for Treatment of Acute Stroke

Recent investigations have led to the promise that neurotrophic factors, a group of naturally

Figure 5: *Depiction of the Penumbra, an area of ischemic neuronal tissue adjacent to the core of the stroke, which is still viable and amenable to treatment with neurotrophic growth factors.*



occurring proteins that the body produces in response to neuronal injury, can be of benefit in the treatment of acute stroke.

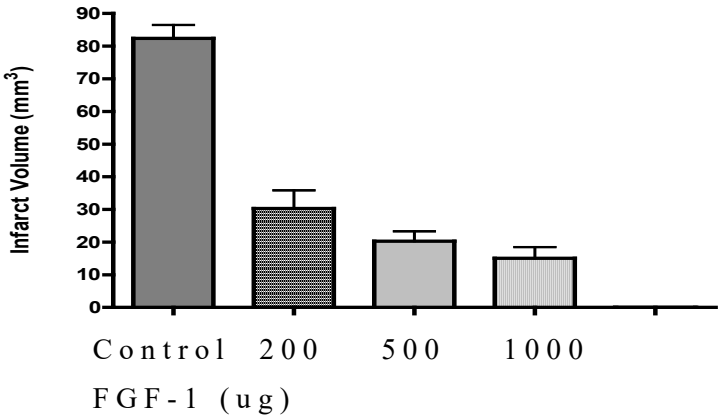
Zhittya Regenerative Medicine, Inc is developing a human neurotrophic factor, human fibroblast growth factor-1 (FGF-1) for acute stroke. These growth factors can stabilize and permit the survival of the neurons at risk in the penumbra area (see Figure 5 above), thereby salvaging brain tissue which can be critical for the preservation of motor and cognitive functions. Research with FGF-1 in animal models of stroke, as summarized below, point to the promise of this molecule as a breakthrough therapy for acute stroke.

4. FGF-1 for the Treatment of Acute Stroke

Previous work in animal neuroprotection models of stroke has demonstrated that the rapid intervention by delivery of FGF-1 can diminish the extent of damage in the penumbra area by protecting the jeopardized cells from cellular death. The mechanism by which this transient intervention works in animal models is not entirely clear but the efficacy of FGF-1 could be related to the prevention of apoptosis (programmed cellular death) of stressed neurons. It should also be noted, that unlike most proteins, FGF-1 has been shown to cross the blood-brain barrier and may be one reason FGF-1 has shown efficacy in stroke models when delivered intravenously.

As reported at the International Association of Regenerative Medicine's 3nd Annual Conference entitled "The New Frontier", FGF-1 was tested in a focal animal model of stroke where a stroke is experimentally induced by temporarily occluding the cerebral artery. As shown in Figure 6 below FGF-1 had excellent activity in this model, reducing stroke infarct volumes significantly.

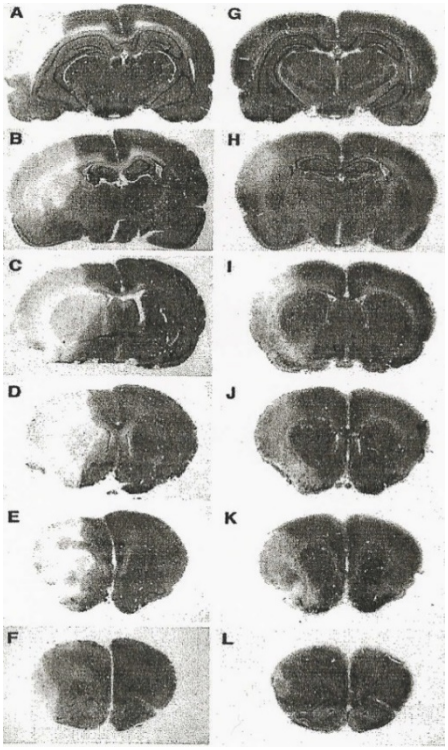
Figure 6: *Effect of FGF-1 on stroke infarct volume in a mouse model of focal brain ischemia. Increasing doses of FGF-1 had a significant effect on reducing the size of the stroke volume.*



Sectioning through the brain followed by subsequent staining of the brain tissue allowed a visualization of the stroke infarct as shown in Figure 7 below, and the dramatic reduction in the volume of the infarct when the animals receive FGF-1 can be seen.

Figure 7: *Effect of FGF-1 treatment in a focal animal model of acute stroke. Sectioning through the brain followed by subsequent staining of the brain tissue allowed a visualization of the stroke infarct. The infarct area appears as a white area in the slices below and the effect of FGF-1 in reducing infarct volume is readily apparent.*

Placebo-treated *FGF-1 treated*



Zhittya is planning on repeating and extending these promising animal studies by conducting animal toxicity studies with FGF-1 administered by both IV and intracranial dosing regimens to support an Investigational New Drug Application (IND) to the US FDA to study the effects of FGF-1 in patients with acute stroke.

IV. Stroke Recovery

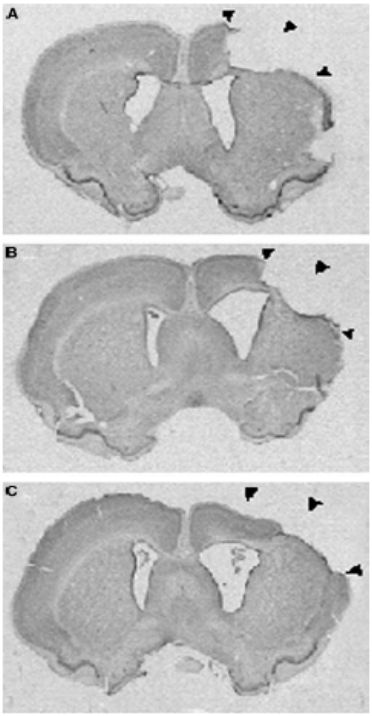
1. Normal Neurogenesis

The production of new neurons was once thought to be restricted to early development. However, neural precursor cells, consisting of stem cells and neural progenitor cells, from which new neuronal and glial cells continuously arise, are now recognized to reside in the normal adult mammalian brain. FGF-1 can stimulate the proliferation of neural stem cells. Although normal neurogenesis declines with age, in humans new neurons are made in the hippocampus at least as late as the 7th or 8th decade of life[31]. Neurogenesis also decreases with age in rodents. However, neural progenitors from older animals appear to be fully responsive to neurogenic stimulation, implying that the environment within the aged brain provides support for new neurons. In vivo, a sustained infusion of FGF for 3 days directly into the brain, increases neurogenesis by 2.5 and 1.4-fold in the brains of old rats.

2. Stroke Recovery with Neurotrophic Factors

Neurotrophic factors such as FGFs, which can inhibit neuronal cell deaths, would be anticipated to promote the survival of newly generated neurons. In response to stroke, neurons sprout new axons, presumably to help establish new connections to compensate for neural damage. In vivo,

Figure 8: *Stimulation by FGF of the growth of new brain tissue over 4 weeks in an animal model of stroke recovery. Arrowheads point to an area of the brain that has healed in the FGF-treated animal (Panel C). Panel A is placebo treated and Panel B is a low dose of FGF.*



biweekly injections of FGF-2 (a member of the FGF family that has similar biological activities as FGF-1) beginning 24 hours after an induced focal stroke and continuing for 4 weeks, resulted in increased axonal sprouting and subsequent enhanced recovery of motor function, including limb movement and coordination in mice. A representative sectioning through the brain at week 4 of an animal receiving FGF-2 for stroke recovery is shown below in Figure 8.

3. Implications for the Treatment of Stroke Recovery and Multi-infarct Dementia

Given these results, one can speculate that in addition to inhibition of acute damage by FGF-1 administration within the first few hours after stroke, additional benefits on neurogenesis and neurite outgrowth might occur with subsequent continuous or intermittent delivery of FGF-1. This hypothesis could be tested in a primate model of stroke to determine the best dose of FGF-1 to use and the best mode of delivery (IV versus direct injection into the brain). Given the devastating effects of stroke on a patient's quality of life (Figure 9), designing and carrying out a clinical trial is this area should receive widespread support, as the patients have very little to lose.

Figure 9: *A wheel-chair bound stroke patient*

In addition, a potent stimulator of new neuron growth in the brain, such as FGF-1, would be an attractive candidate to study as a new treatment for multi-infarct dementia. Again, as this disease has no known treatment, and is progressive and potentially fatal, testing of potential new therapies would be expected to be highly welcomed in this field.

V. Small Vessel Vascular Disease in the Brain

Stroke is basically an acute event where a blood vessel blockage or rupture leads to permanent brain damage. Even with multi-infarct dementia, the underlying cause of this condition is a series of acute mini-strokes that accumulate over time. A second type of vascular dysfunction in the brain, whose mechanism is very different from stroke, comes about from diseases that can cause damage to the small vessels of the brain over an extended period of time. A well-known example of this is diabetes, a chronic disease that can lead to vascular disease in the brain. In addition, recent evidence also points to a vascular origin of Alzheimer's disease, where it has been shown that damage to the microvasculature of the brain is the very first pathology that can be detected in patients with this disease, long before cognitive impairment or the characteristic beta amyloid plaques can be detected.

1. Vascular Dysfunction in the Brain Caused by Diabetes

The effects of glucose and other forms of sugar on the brain may be the most profound in diabetes, a group of diseases in which high blood glucose levels persist over a prolonged period of time. Type 1 diabetes is a disease in which the immune system destroys the cells in the pancreas that produce insulin, a hormone used by the body to keep blood glucose levels in check. Type 2 diabetes, caused by dietary and other environmental factors, is a condition in which cells become overwhelmed by insulin and fail to properly respond; they become resistant to insulin.

Long-term diabetes—either type 1 or type 2—has many consequences for the brain and for neurons in the brain. High blood glucose levels can affect the brain's functional connectivity, which links brain regions that share functional properties, and brain matter. It can cause the brain to atrophy or shrink. And it can lead to small-vessel disease, which restricts blood flow in the brain, causing cognitive difficulties and, if severe enough, spurring the development of vascular dementia.

Researchers have been studying ways to prevent these effects in people with type 2 diabetes. One of these ways involves an intranasal containing insulin (INI). When used, INI enters the brain and binds to receptors in its memory networks, including the hippocampus, hypothalamus, and insular cortex. As signaling within these memory networks become more efficient, the cognitive functions associated with these areas, such as learning and visual perceptions of spatial relationships, improve. The results of the trial are especially relevant because of the high prevalence of dementia and significant cognitive decline among older adults with diabetes.

In fact, some researchers have begun to call Alzheimer's disease "type 3 diabetes." When a group of researchers reviewed the collections of studies available on Alzheimer's disease and brain function, they noted that a common finding in Alzheimer's disease was the deterioration of the brain's ability to use and metabolize glucose. They compared that decline with cognitive ability, and noted that the decline in glucose processing coincided with memory impairment, word-finding difficulty and behavior changes. Furthermore, scientists determined that as insulin functioning in

the brain worsens, not only does the brain's cognitive ability decline, the size and structure of the brain also deteriorate - all things that occur as Alzheimer's disease progresses.

The notion that perhaps Alzheimer's is a brain-specific type of diabetes termed "type 3 diabetes" is controversial, but there is certainly compelling evidence linking a decline in insulin levels and insulin responsiveness to cognitive impairment. While research on the question, “does type 2 diabetes cause Alzheimer’s disease?” is still ongoing, several studies suggest that while diabetes likely exacerbates and contributes to the development of Alzheimer's disease, it is likely not the sole cause of it.

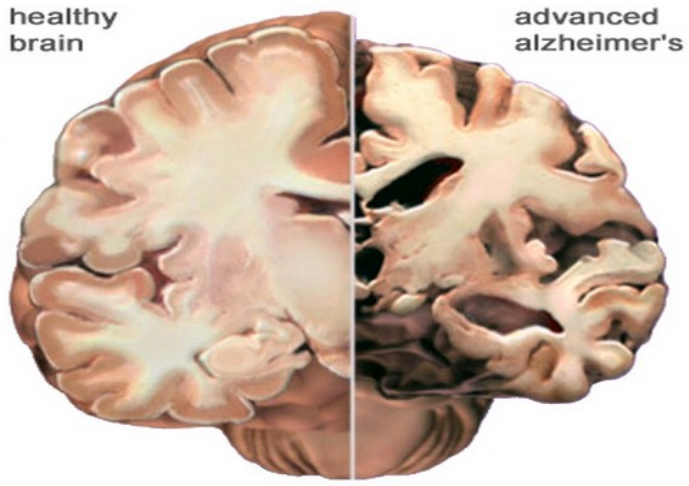
In one study, scientists fed mice a high fat diet which induced the development of type 2 diabetes. They then studied the mice and found that a higher amount of Tau protein was present in their brains, and the mice also developed a resistance to brain insulin, both known characteristics of Alzheimer’s disease. Additionally, the brain structure of these mice also deteriorated somewhat on the high fat diet; however, the cognitive functioning of these mice did not decline significantly to the level they would expected if Alzheimer's disease was present.

In a second, very eloquent elegant study, researchers created “knock-out” mice where they selectively removed the gene for the brain’s insulin receptor, thereby removing all insulin sensitivity and responsiveness in the brains of these animals. Similar to the above study, these animals showed a marked increase in level of Tau protein in their brains, a hallmark of neurodegenerative diseases. However, the animals exhibit no alteration in neuronal proliferation/survival, memory, or basal brain glucose metabolism. Thus, these researchers concluded that a lack of insulin signaling in the brain may lead to changes that are also seen in Alzheimer’s disease, but other mechanisms must come in to play for Alzheimer's disease to develop.

2. Alzheimer's Disease as a Vascular Disorder

Alzheimer disease (AD) is an insidious disorder that progressively ravages the brain, destroying its memory, intellect, and dignity in the process. The main stumbling block in the clinical management and in the search for a cure of AD is that the cause of this disorder has remained uncertain. For more than 30 years, AD has been classified and managed as a neurodegenerative disorder, distinct from vascular forms of dementia, such as multi-infarct dementia, based on the characteristic huge loss of brain tissue as seen in Figure 10 below

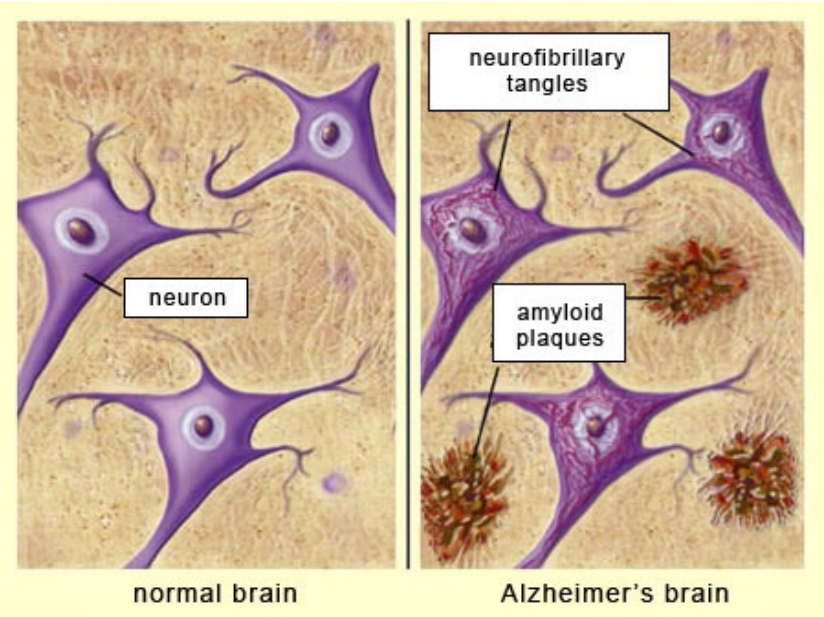
Figure 10: *A healthy brain and the brain from a patient with advanced Alzheimer's disease*



Most investigators in the field have supported the notion that AD and the dementing processes are the result of changes in brain structure, most notably brain atrophy, brought about by the inexorable neurodegenerative process that characterizes AD. Dramatic loss of brain volume, similar to what is seen in Figure 10 above, is readily apparent in virtually all autopsies of patients suffering from late stage Alzheimer's disease.

As shown in Figure 11 below, neuronal death in AD is thought to be caused by the toxic accumulation of a naturally occurring protein, tau, inside the neuron. This protein becomes

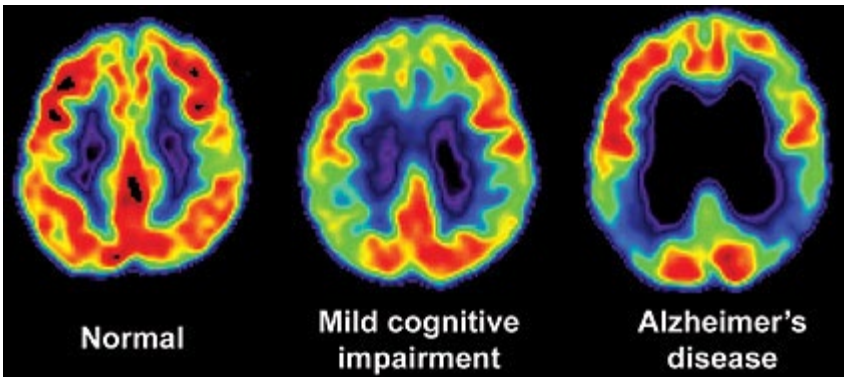
Figure 11: *The characteristic plaques and neuronal tangles seen in AD*



over-phosphorylated and accumulates inside the neuron leading to the development of "neurofibrillary tangles" and cell death. In addition, another hallmark of AD is the accumulation of plaques, composed of the beta-amyloid protein, that occurs outside the neuron and disrupts neuron-neuron communication in the brain. It is the current conventional wisdom that these two process combine to destroy memory and cognition in selected areas of the brain (such as the hippocampus and frontal brain regions involved in reasoning and logic).

Metabolic brain imaging utilizing PET scans that monitor glucose utilization has also been extensively used to diagnose and follow the progression of Alzheimer's disease. As shown below in Figure 12, a significant decline in neuronal glucose utilization can be appreciated in patients with only mild cognitive impairment.

Figure 12: *Metabolic PET imaging of normal brains and brains from patients with AD. Disease progression leads to a significant decrease in neuronal metabolic activity as measured by glucose uptake.*

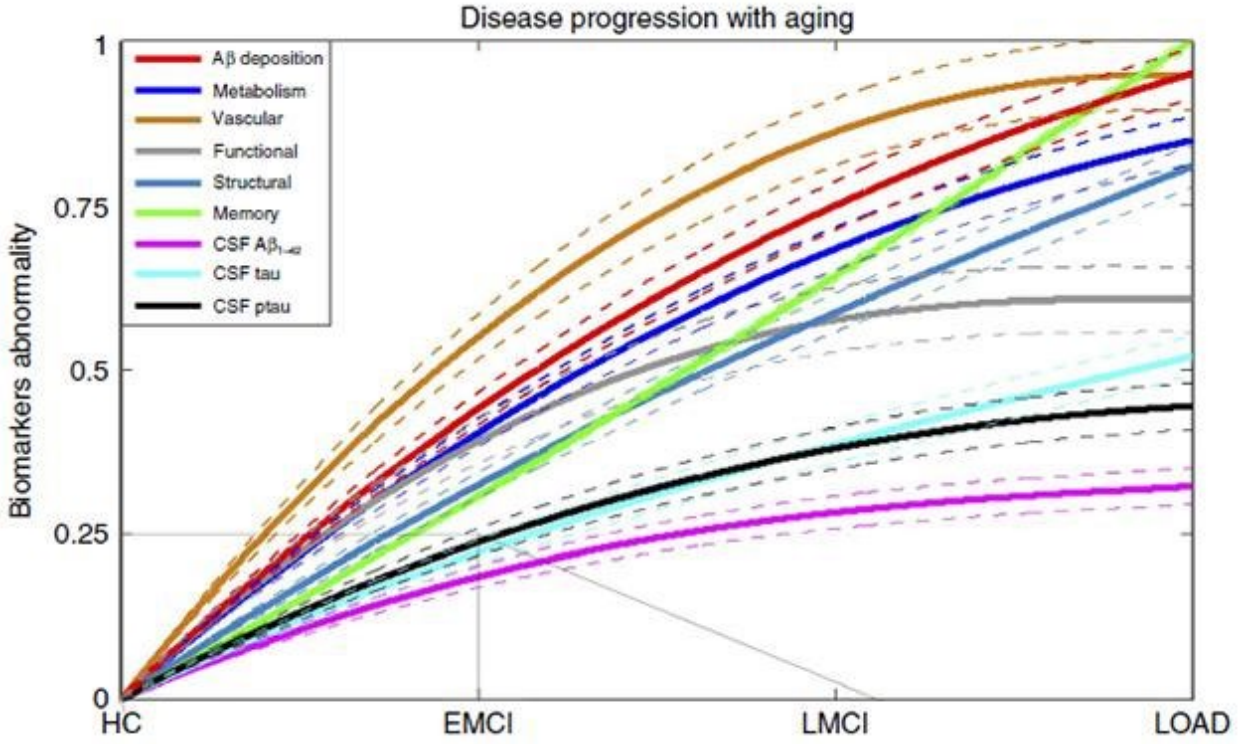


More recently, however, a substantial and ever-growing amount of evidence indicates that AD is initiated by vascular factors that precede the neurodegenerative process. The question of whether AD is first provoked by a neurodegenerative process, as the prevailing paradigm maintains, or by vascular-related events, which then propel neurodegenerative changes is of crucial importance. Establishing the correct pathogenesis for this dementia could help to unravel the exact mechanisms responsible for the cognitive failure and, in so doing, target specific therapy to overcome or treat this disorder more effectively. If AD is a vascular disorder that initiates its pathology through cerebral microvascular abnormalities, then its origin, clinical signs, diagnosis, and potential treatment should revolve around a vascular-centered view.

In probably the largest study of its kind, researchers from McGill University in Montreal recently completed a study reported in the journal *Nature* in June 2016, that studied the disease progression in 1171 patients with AD. The researchers analyzed close to 8000 brain imaging studies and examined a number of biomarkers for AD, including brain hypo-perfusion, brain atrophy, cognitive decline, glucose under-utilization and the accumulation of beta amyloid protein and tau protein. They then developed an algorithm to look at the 30-year trajectory of each of these markers in AD and as seen in Figure 12 below they showed that the very first changes seen in AD was a deterioration of the microvasculature leading to under-perfusion of brain neurons.

These vasculature changes occurred before any of the symptomatic changes in AD occurred, including structural changes in the brain, accumulation of beta amyloid plaques or cognitive decline.

Figure 12: *Disease progression in AD patients as analyzed by brain imaging and biomarkers for AD. Vascular deterioration (the brown line in the figure) preceded all other abnormalities, including plaque formation and cognitive decline.*



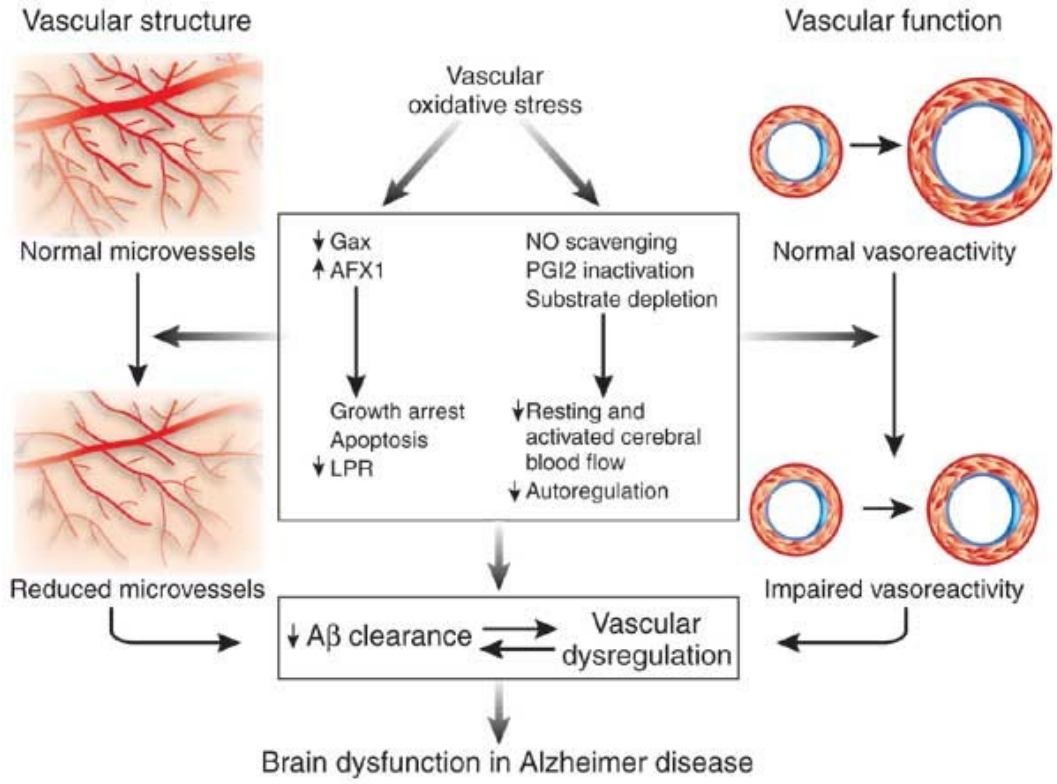
Cerebral capillary degeneration has been shown to be present in practically all AD brains examined postmortem and in cortical biopsy material from pathologically confirmed AD. These cerebral micro-vessel aberrations have been consistently observed in AD brain tissue by a large number of investigators using a variety of histological techniques.

The degenerate capillaries appear more prevalent in the hippocampus, a region that is linked to memory and learning and is an initial target for neurofibrillary tangle formation in AD. Micro-vessel changes in AD brains show no correlation to the stage of the disease, a finding that suggests that such capillary anomalies are not a consequence of AD pathology. In addition, brain capillary distortions do not appear to be significantly targeted by amyloid deposition.

It has been proposed that both a reduction in the number of brain micro-vessels and a decrease in their vascular functions leads to a decline in cerebral blood flow, apoptosis or cell death of neurons and the reduced clearance of beta amyloid deposits. This is depicted in Figure 13 below.

Figure 13: *Stress on the brain vasculature system (in the figure below oxidative stress is shown, but this stress could also come from other environmental, metabolic or genetic factors). This stress*

leads to both a reduction in the number of micro-vessels in the brain, as well as a reduction in the function of these micro vessels. Brain dysfunction and Alzheimer disease symptoms appear as toxic beta amyloid aggregates can not be cleared from the brain by the damaged vascular system and accumulate in the brain, resulting in neuronal death.



VI. Alzheimer's Disease as a Vascular Disorder: New Therapeutic Options

If Alzheimer's disease begins as primarily a vascular disease, then new therapeutic options warrant consideration. As a significant finding in these studies is the reduction in the number of small vessels in the brain, then an agent that could stimulate new vessel growth would be an attractive candidate for clinical testing. Growth factors that stimulate new blood vessel growth or "angiogenesis" would appear to be prime targets for such clinical testing.

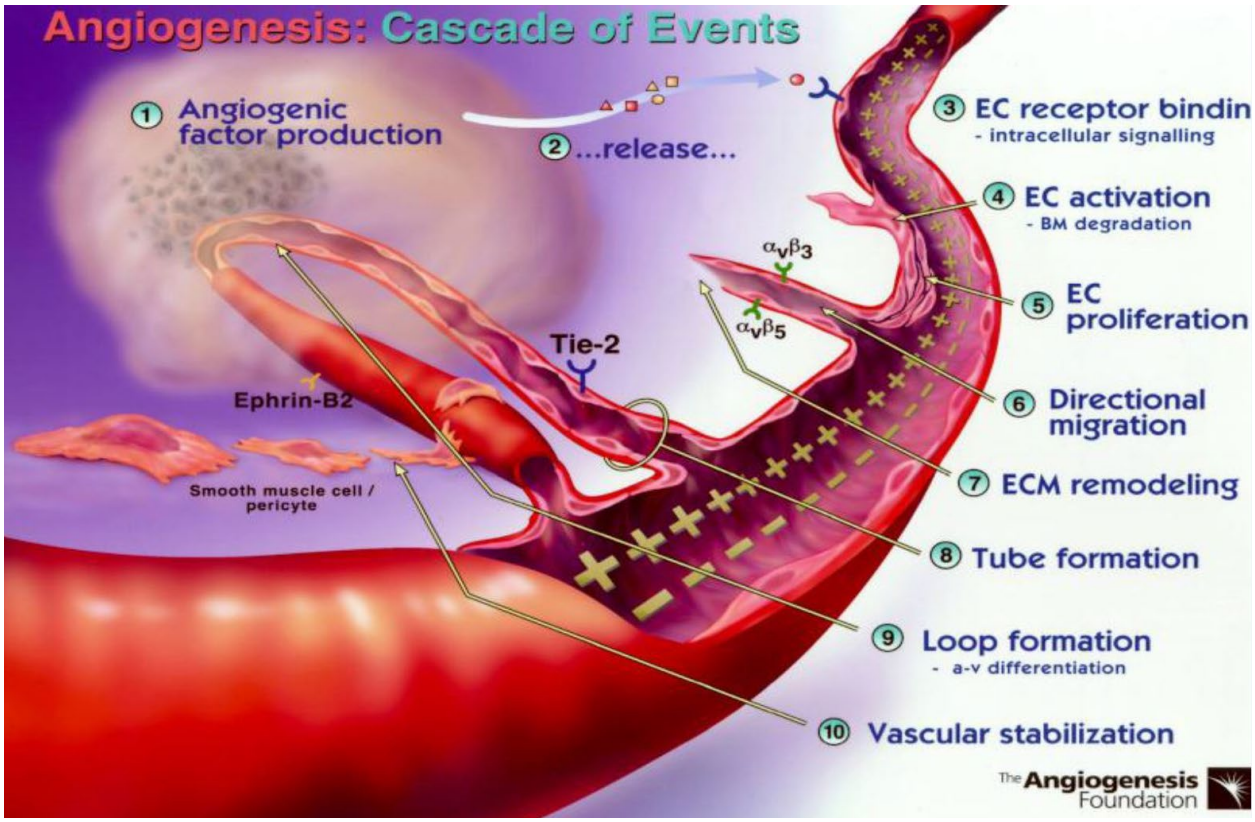
1. Angiogenesis: A Solution to Restore Brain Perfusion and Treat Alzheimer's?

Can agents that stimulate new blood vessel growth or angiogenesis be used as a potential treatment for Alzheimer's disease? Angiogenesis is an important natural process used for healing. The body induces angiogenesis in damaged tissues by releasing a barrage of growth factors that stimulate new vessel growth. Insufficient blood perfusion is now recognized as a "common denominator" underlying many deadly and debilitating conditions, including diabetic ulcers, cardiovascular disease and peripheral artery disease.

Therapeutic angiogenesis stimulates angiogenesis where it is required but is lacking. This technique is used to replenish the blood supply to chronic wounds to speed healing, and it prevents unnecessary amputations. New research suggests this approach can also be used to save limbs afflicted with poor circulation, and even oxygen-starved hearts. There is no reason therapeutic angiogenesis could not be applied to Alzheimer's disease to reverse and reestablish the growth of small vessels in the brain, thereby restoring an adequate amount of blood perfusion to allow the brain's neurons to remain healthy.

Figure 3 below gives a schematic view of how angiogenesis occurs in our body. A key concept in this process is the fact that new vessels branch off of pre-existing blood vessels as shown in the figure. Important cellular components in this process are the endothelial cells which line the

Figure 3: *Schematic Representation of Angiogenesis and New Blood Vessel Formation: Formation of a new vessel occurs as endothelial cells bud off of an existing blood vessel to form hollowtubes which are furthered modeled into capillaries.*



interior of all blood vessels. These cells migrate into the adjacent tissues and form hollow tubes, which are the precursors of new capillaries. Fibroblasts also help direct and guide the formation

of these new vessels. Finally, a coating of smooth muscle cells gives strength and elasticity to these newly formed blood vessels, and these vessels are now ready to perfuse the adjacent tissue.

2. Fibroblast growth factor 1 (FGF-1), a Potent Stimulator of Angiogenesis

Several growth factors have been shown to stimulate angiogenesis, including VegF (vascular endothelial cell growth factor) and EGF (epidermal growth factor), but one growth factor in particular, FGF-1, stands out due to its potency and its ability to stimulate the production of not only capillaries, but larger arterioles, which are critical in bringing more blood into the injured tissue.

FGF-1 is a natural protein contained within our bodies. It is released upon tissue injury and is the body's natural response to tissue repair. FGF-1 is mitogenic for endothelial cells and smooth muscle cells, the two principal cell types necessary for the formation of new capillaries and arterioles. It has also been shown to be a neurotrophic factor, as discussed above, and it is this characteristic that is being exploited for its development as a drug to treat acute stroke and stroke recovery.

Human FGF-1 binds strongly to heparin and this is one reason FGF-1 is such a potent angiogenesis agent as it can stay resident in an organ or tissue for days bound to heparin moieties found in abundance on basement membranes of damaged tissues. The simplicity of the FGF-1 structure also makes it a very stable molecule and in the presence of heparin, FGF-1 is stable for 18 months at 4° C, a desirable quality for a pharmaceutical. Although the growth factor is only present in minute quantities in our bodies, the protein has been bioengineered and unlimited quantities of this growth factor can now be made in biological manufacturing facilities.

Finally, FGF-1, unlike most other proteins, has been shown to cross the blood brain barrier. This characteristic means that long term administration via the IV route, perhaps employing a pump, could be attempted in brain-specific diseases, such as Alzheimer's disease. Given the dearth of current treatment options for Alzheimer's disease and its 100% mortality rate, FGF-1 becomes an attractive candidate to consider for pilot testing in this patient population.

VII. Summary and conclusions:

In this White Paper, we have discussed in detail various medical conditions that result from vascular dysfunctions in the brain, including stroke, vascular dementia and Alzheimer's disease. Currently there are no treatments which can significantly alter the medical outcomes of these conditions. We hope that we have been able to convey to the reader the future promise of neurotrophic and angiogenic agents to treat these vascular disorders of the brain. Such therapies, if successful, will undoubtedly significantly reduced the suffering and loss of life world-wide.

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