



CARDIO VASCU-GROW™ AND STROKE

1. Summary

Stroke resulting in brain damage is most often caused by a lack of blood flow to a selected part of the brain. A stroke results in permanent damage to the brain tissue—and in many cases, permanent disability to the patient. Stroke is the third leading cause of death and a leading cause of serious, long-term disability in the United States. The probability of stroke increases as people get older, with those over age 65 at greatest risk. According to the American Heart Association, approximately 700,000 Americans suffer a stroke each year; about 25% of these strokes are fatal. Stroke is responsible for an estimated \$40 billion in health-care costs and lost productivity each year.

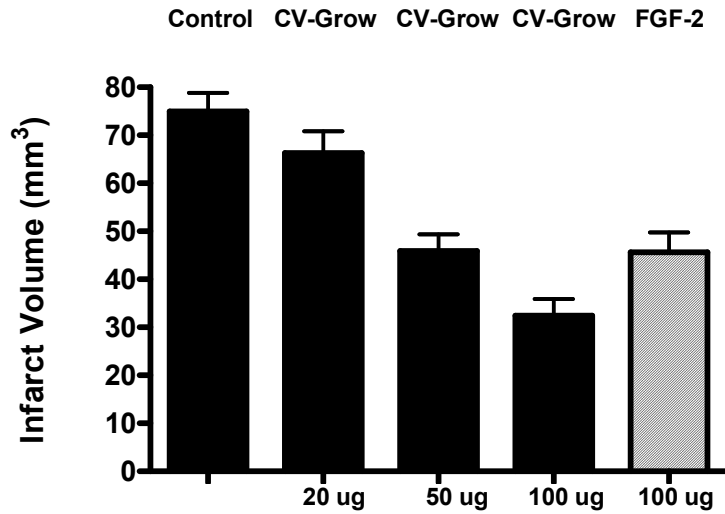
Ischemic or occlusive strokes, which account for approximately 80 percent of all strokes, are caused by an obstruction in an artery, generally one of the neck carotid arteries that carry oxygen-rich blood from the heart to the brain. There is limited treatment available to patients who have suffered a stroke. Thrombolytic therapy using plasminogen activators is sometimes tried in these patients to unblock the arteries supplying blood to the brain, but safety and bleeding issues have prevented this treatment from gaining wide-spread acceptance in the medical community.

Animal studies have shown the potential of growth factor therapy in limiting the severity of brain ischemia after a stroke. A stroke is characterized by an infarcted area of the brain, which cannot recover, surrounded by an underperfused area of risk, which would be the target of growth factor treatment.

2. Results from CVBT's Current Studies in Animal Stroke Models

We have tested **Cardio Vascul-Grow™** in an animal model of stroke. In this model, the mice are given an experimental stroke by blocking the flow of blood into the brain for 1 hour after which either animals were dosed by i.v. infusion with control or Cardio Vascul-Grow™ for 3 hours. The volume of the stroke is measured along with behavioral tests that indicate the degree of neurological deficits in the animals after 24 hours. The first study in mice tested **Cardio Vascul-Grow™** at doses of 20, 50 and 100 ug/kg/hr. The 50 ug/kg/hr dose was protective, and the 100 ug/kg/hr dose was highly protective. A second trial, in which mice received increasing doses of **Cardio Vascul-Grow™** of 200, 500 and 1000 ug/kg/hr, has also been completed. There was a dose-dependent decrease in the volume of the stroke area as more **Cardio Vascul-Grow™** was given to the animals. The 1000 ug/kg/hr dose group displayed stroke volumes that were decreased by over 80% when compared to control animals. Also seen in these studies was a significant improvement in neurological defects as the dose of **Cardio Vascul-Grow™** increased.

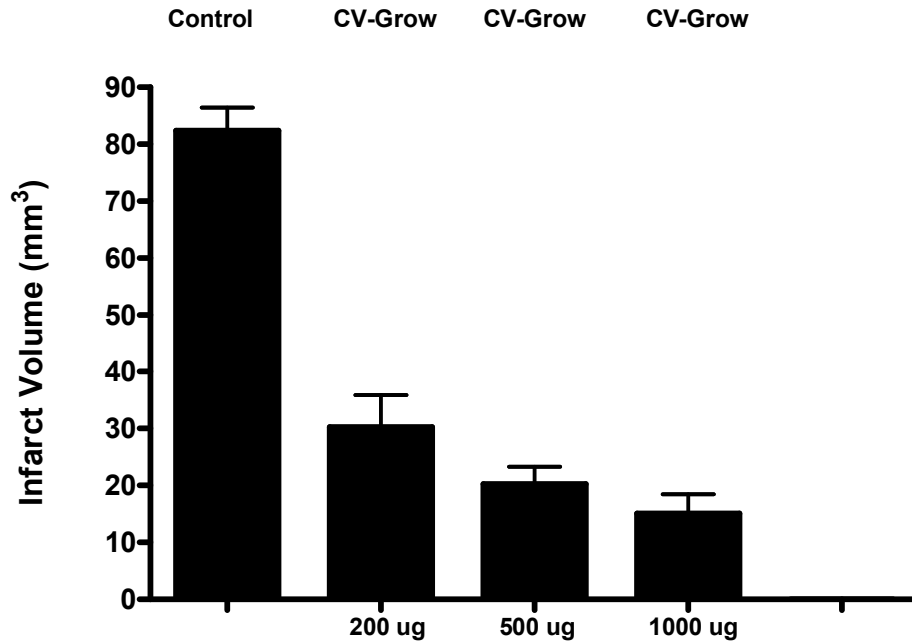
Graphs of the results from these animal studies are shown below.



$p < 0.001$ for 10 ug/kg/hr **Cardio Vascul-Grow™** compared to control.

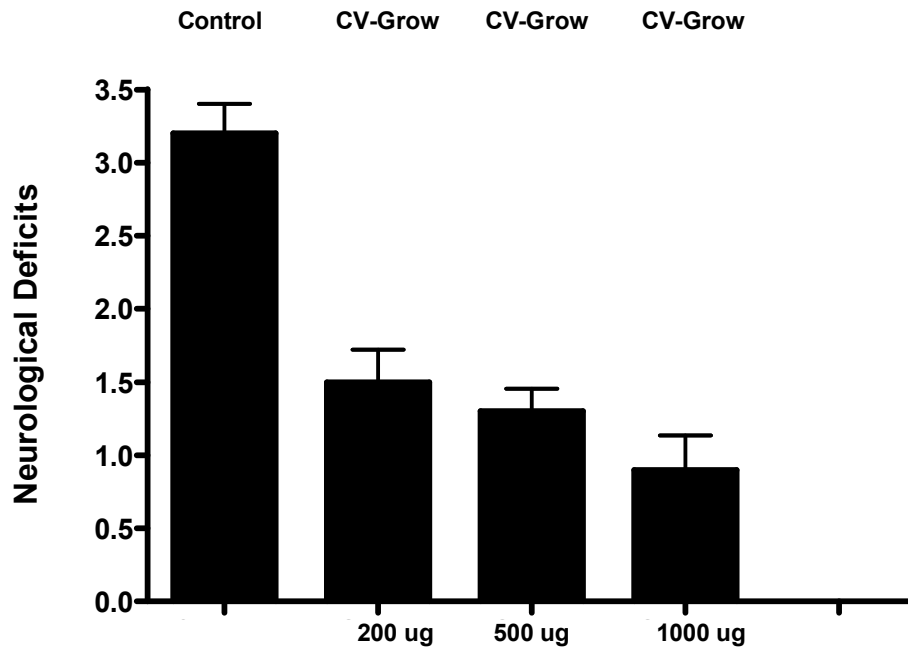
$p < 0.0001$ for 50 and 100 ug/kg/hr of **Cardio Vascul-Grow™** and 100 ug/kg/hr of FGF-2 compared to control.

Figure 1. First study in mice with experimental stroke: effect of Cardio Vascul-Grow™ on infarct volumes in the mouse following transient ischemia. All mice were subjected to 1 hour of cerebral ischemia followed by 24 hours of reperfusion. Animals were infused with vehicle (control), **Cardio Vascul-Grow™** or FGF-2 intravenously at the end of ischemia for 3 hours. Animals were sacrificed on day 2 and processed to determine the infarct volume.



$p < 0.0001$ for **Cardio Vascul-GrowTM** (all doses) compared to control.

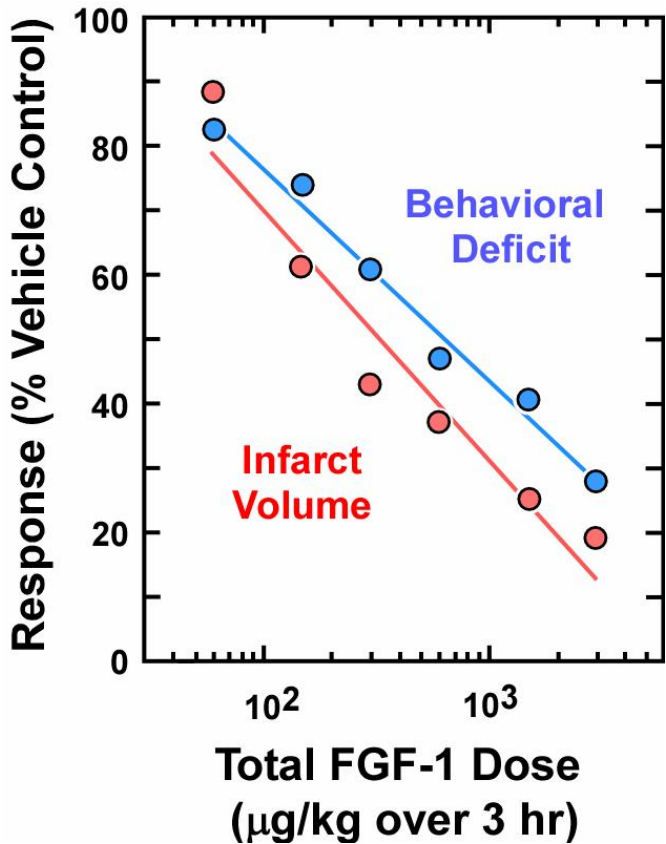
Figure 2. Second study in mice with experimental stroke: effect of Cardio Vascul-GrowTM on infarct volumes in the mouse following transient ischemia. All mice were subjected to 1 hour of cerebral ischemia followed by 24 hours of reperfusion. Animals were infused with vehicle (control), **Cardio Vascul-GrowTM** or FGF-2 intravenously at the end of ischemia for 3 hours. Animals were sacrificed on day 2 and processed to determine the infarct volume.



P < 0.0001 for each treated group compared to control.

Figure 3. Effect of Cardio Vasco-Grow™ on neurological deficits in the mouse following transient ischemia. All mice were subjected to 1 hour of cerebral ischemia followed by 24 hours of reperfusion. Animals were examined for neurological deficits at 22 hours after ischemia.

Figure 4: Plot of Behavioral Deficit versus Infarct Volume. In the figure below it can be observed that as the volume of the infarct, or area of the stroke damage, is decreased after treatment with increasing doses of Cardio Vascul-Grow™, the behavioral deficits decrease in a similar manner.



3. Summary and Conclusion

These studies demonstrate the potential of **Cardio Vascul-Grow™** to dramatically decrease the area of a stroke in experimental animal models. In a dose-dependent fashion, **Cardio Vascul-Grow™** decrease the volume of a stroke to a statistically significant extent over control animals. At the highest dose of **Cardio Vascul-Grow™**, over an 80% decrease in the volume of the stroke area was noted with a significant increase in the neurological functioning of these animals as assessed by behavioral studies.

There is currently no treatment in the marketplace that has widespread acceptance for the treatment of stroke. The debilitating effects that patients suffer following a stroke, as well as the expensive medical treatment needed to care for stroke patients is a primary motivator for new drug development in this area. Given the very promising pre-clinical results CVBT has obtained with **Cardio Vascul-Grow™** in animal models of stroke, the company has decided to aggressively expand its efforts in this area including the initiation of clinical testing. In addition, the company will also begin exploring testing the efficacy of **Cardio Vascul-Grow™** in chronic stroke animal models. Over 5 million Americans

have suffered a stroke and a product that could possibly restore any function to these patients would be a true breakthrough therapy.