Undergraduates Explore a New Health Benefit of Green Tea

By Amita Risbud

Barsegh Barseghian and Avi Albert Elhiani research the effects of green tea.

Green Tea as Stroke Prevention

Green tea has long been a subject of study in healthcare for its anti-oxidative properties. More recently, USC undergraduate seniors Albert Avi Elhiani and Barsegh Barseghian have been studying green tea's possible ability to help patients survive, prevent and overcome strokes under the guidance of Dr. Rayudu Gopalakrishna in the Department of Cell and Neurobiology at USC's Keck School of Medicine . Elhiani explains that their project investigates "the role of green tea, specifically it's polyphenols (antioxidants), to precondition brain cells to be able to survive oxygen/glucose free conditions as is exhibited in a stroke."

Researchers' Backgrounds

A kinesiology major from Beverly Hills, Elhiani was initially attracted to this project because he was interested in cancer-related research, and more personally is also a regular consumer of green tea, and so he got in touch with Dr. Gopalakrishna and interviewed for the lab assistant position. Dr. Gopalakrishna's "brilliance and expertise in neurobiology" made the research position a worthwhile experience. Elhiani describes the environment of the lab, "Dr. Gopalakrishna was the brain and we became the hands, by

which we carried out the delicate and intricate research." His partner, Barseghian, is a Health Promotion and Disease Prevention major and also pursuing a Master's in Global Medicine. Born and raised in Los Angeles, Barseghian has always had an interest in the different practices of medicine around the world and has had the chance to travel to India, Armenia, Israel and Peru during his time at USC. He, too, enjoys the lab's cooperative team, as well as the unique data collection methods involved with working with green tea. Elhiani has been working under Dr. Gopalakrishna for two years now, and Barseghian joined the team last January.

What is Ischemic Stroke?

Because stroke is notoriously one of the leading causes of death in the United States, it has warranted copious amounts of research projects to develop drugs to fight it. Sometimes, occlusions occur in blood vessels in the brain, resulting in cell death in the region of the brain containing the occluded vessel; this event is called a cerebral infact, more commonly known as an ischemic stroke. Many current drugs use innovative methods with side effects, but Elhiani and Barseghian's lab employs an "ethnobotanic method" to protect the brain from ischemic stroke.

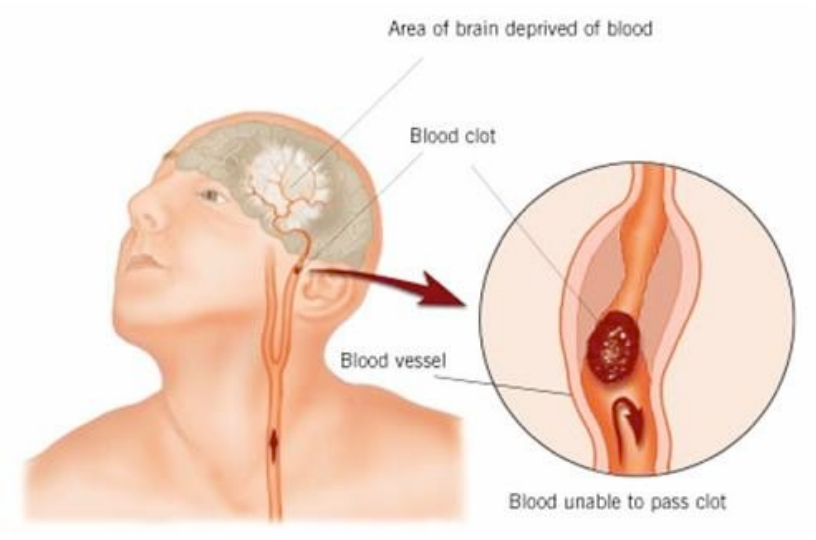

Figure 1. Pathophysiology of ischemic stroke. (http://epilepsy-brain.blogspot.com/2011/05/ischemic-stroke-pathophysiology.html)

Methods—the "Ethnobotanic Method"

A polyphenol is an organic compound that contains more than one phenolic hydroxyl group. Antioxidants with polyphenols, like green tea, are thought to prevent cardiovascular and neurodegenerative conditions. Hence, the research is primarily focused on epigallocatechin-3-gallate (EGCG), a type of green tea

polyphenol (GTTP) that is thought to have neuroprotective properties. The ethnobotanic method involved diluting and adding these polyphenols from a green tea supplement to neuronal cells in a Petri dish.

Dr. Gopalakrishna's lab used an *in vitro* oxygen-glucose deprivation (OGD)/re-oxygenation model on pheochromocytoma (PC-12) cells of rats in order to determine EGCG's neuroprotective effects. The PC-12 cells are grown on polylysine-coated flasks or plates in a minimal essential medium supplemented with 10% heat-inactivated horse serum, 5% fetal calf serum, and antibiotics. The cells are cultured until they ceased to divide. Additionally, they are preconditioned to 3-4 cups daily of GTTP/EGCG for 48 hours before OGD/re-oxygenation. Then, a lactate dehydrogenase (LDH) cell viability assay is used to measure the polyphenol's efficacy. The LSH assay has demonstrated that the preconditioned PC-12 cells were better protected against cell injury and death in the oxygen- and glucose-deprived conditions. Barseghian explains that the most important finding, however, has been that the ε isoenzyme, a protein kinase C (PKC), is activated by GTPP treatments: with an inhibitor peptide, "PKCε membrane translocation is necessary to confer GTPP-induced preconditioning" (Gopalakrishna, et. al 2012). PKC is known, among many other functions, to be an enzyme that helps cells survive stroke.

To measure the efficacy of GTTP/EGCG, Barseghian and Elhiani used cell cultures and various assays that identified the behavior and interactions of various neurons that are affected during a stroke. Western immuno-blotting and a radioactive assay were used to determine PKC levels. A lactate dehydrogenase assay was used to measure the amount of cells that had died under stroke-like conditions, and it was determined that GTTP reduced the number of cell deaths.

Polyphenols and Green Tea--- Conclusions

From the above experimentation, Barseghian and Elhiani have been able to conclude that when brain cells are pretreated with green tea polyphenols(the equivalent of 3-4 cups/day) they are able to survive significantly longer than those who had not been treated with green tea. (This was demonstrated by reduced numbers of cell deaths in the lactate dehydrogenase assay.) They also found that this occurs to some extent if green tea is administered during or after a stroke. The reperfusion injury, or injury caused by lack of nutrients and oxygen, would then be able to heal more rapidly and less cells would die upon initial oxygen/glucose deprived insult. Through this study, the mechanisms behind how green tea offers neuroprotective benefits was elucidated. These findings are elaborated on in the American Journal of Biochemistry, published very recently on February 28.

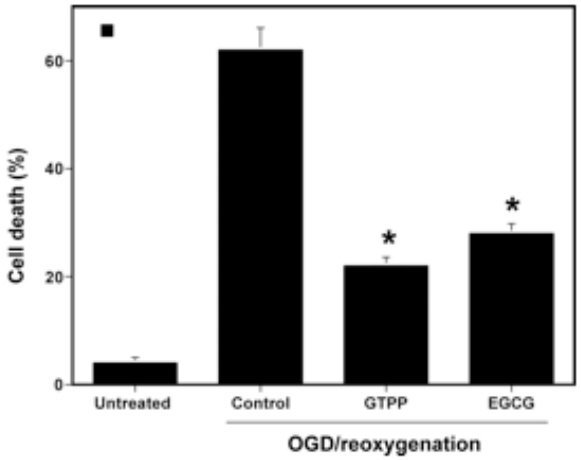

Figure 2: GTPP and EGCG protect PC12 cells from OGD/re-oxygenation induced cell death. PC12 cells were treated with GTPP (0.2 µg/mL) or 2 µM EGCG for 48h. Cell death was determined by assaying LDH (lactate dehydrogenase). The values are expressed as mean and SEM (n=4).
*p<0.05 significantly different from control.

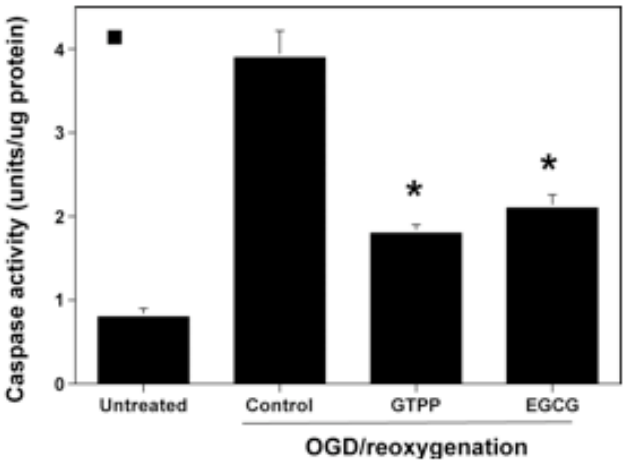

Figure 3: GTPP and EGCG inhibit elevation of caspase-3 activity after OGD/re-oxygenation. PC12 cells were treated for 48h with GTPP or EGCG and then subjected to OGD/re-oxygenation. The caspase activity is expressed as mean and SEM.
*p<0.05 significantly different from control.

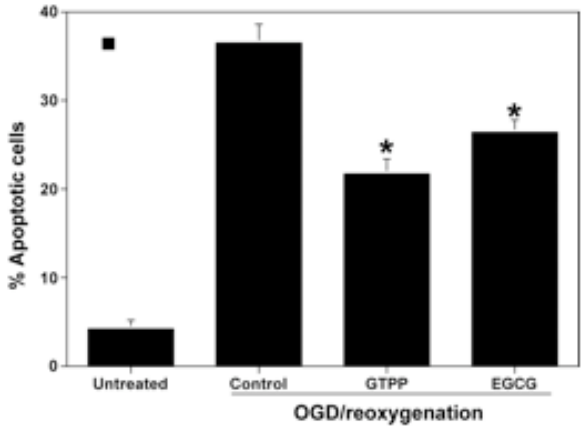

Figure 4: GTPP/EGCG-induced preconditioning protects PC12 cells from apoptosis induced by OGD/re-oxygenation. Cells in culture slides were pretreated with GTPP or EGCG for 48h and then subjected to OGD/re-oxygenation. Treated cells were stained with DAPI. Apoptosis in each sample was analyzed by counting 500 cells and determining the percentage of apoptotic cells.
*p<0.05 significantly different from control.

Future Implications

The research findings ultimately suggest that in the event of an occlusion in the brain, the consumption of green tea helps affected cells heal and withstand too much damage. While the findings in Dr. Gopalakrishna's lab are promising, they are based only on molecular experimentation. *In vitro* testing has shown the efficacy of a natural product in health, and suggests that it may be safe to test GTTP from green tea in humans. Hence, the efficacy of GTTP/EGCG in neurogenic protection would be better determined by preclinical and clinical testing (not on animals in mere cell cultures). Until these clinical studies are conducted, the results from *in vitro* testing cannot be confirmed.

From the *in vitro* testing, however, Barseghian and Elhiani's lab has established the advantages in the natural protect of green tea and in the near future, we can safely test the effects of GTTP in green tea in humans, especially in high-risk populations.