Scientific Literacy: Exogenous mitochondrial transplantation improves survival and neurological outcomes after resuscitation from cardiac arrest

Jocelyn D. Bonham

Department of Biology, Old Dominion University

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Dr. Christina Steele

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In the United States, there are over 356,000 recorded cardiac arrests outside of hospitals, of all ages, genders, and ethnicities. That is nearly one thousand people each day, in one country alone, that face this health disaster outside of a hospital.

Heavy alcohol use, recent use of cocaine, amphetamines, or marijuana, and ingesting too much caffeine. Physical exertion or stress in sports, meaning that extreme activity may cause cardiac arrest during exercise or sleep. Severe emotional stress may also contribute. Most risk factors are inherited, such as cardiomyopathy: issues with the heart muscle that weakens one's heart with irregular heartbeats or heart failure. Heart problems such as coronary heart disease, for example, coronary artery disease, caused by high cholesterol which clogs the lining of the arteries, ultimately blocking the blood flow in the arteries of the heart. Arrhythmias such as atrial fibrillation are issues with the beat of the heart--too fast, too slow, or overall irregular. Congenital heart defects, an issue that one is met with at birth, can damage the heart. An example is irregular heart structure. If the heart is damaged by infections or other existing medical conditions, it can cause heart inflammation. Medical issues such as respiratory arrest, pneumonia, seizures, and diabetes can contribute to the increased risk of cardiac arrest. Therefore, the main risk factor for younger people surrounds genetic issues.

Ninety percent of all cardiac arrests are fatal, and only ten percent of those who experienced cardiac arrest outside of a hospital survive after EMT care and transportation to a hospital. However, one who survives one cardiac arrest greatly increases the risk of having another. Twenty percent of cardiac arrest survivors will experience another life-threatening arrhythmia within the next year.

Ischemia-reperfusion, also known as I/R, is an injury that occurs within reperfusion. Ischemia of organs results in severe consequences, such as myocardial and cerebral infarction.

Both lead to irreversible tissue damage that worsens the injuries. There are multiple pathological processes involved with ischemia-reperfusion injuries, including cell damage (apoptosis, necrosis, or ferroptosis), inflammatory responses, extracellular matrix remodeling, angiogenesis, fibrosis, and cardiomyocyte hypertrophy. The signaling pathways during an ischemia-reperfusion injury creates an extensive network to regulate the injury. These injuries are a paradoxical process which occurs when the blood flow returns to an organ that has been ischemic (without blood flow), which results in further cellular damage and possible cell death. There is a list of organs that can be affected by these injuries, such as the heart, lungs, kidneys, and brain. Reperfusion is an injury in which the multifactorial process results in extensive tissue or organ destruction.

Mitochondrial dysfunction is common in many diseases, such as metabolic disorders and cancer. It can lead to issues with energy metabolism and an increase in oxidative stress, resulting to issues within homeostasis and contributing to disease pathogenesis. Intercellular mitochondrial transfer is defined as a procedure that facilitates the substitution of exogenous injured mitochondria, aiding in mitochondrial deficiency. This is performed by transferring mitochondria from one cell to another. There are different ways to transport intercellular mitochondria, such as gap junction channels, extracellular vesicles, and tunneling nanotubes. The importance of mitochondrial transfer to maintain its function and prevent cell death within disease has been recognized. The transfer of mitochondrion exchanges components including DNA, RNA, and proteins. It has been shown to reduce oxidative stress in various cell types as well as improve mitochondrial function. Through multiple approaches, there have been successful results revolving around the treatment of multiple diseases,

including Parkinson's disease. Transporting the mitochondria across the neurons aids in cellular mediation and the fitness of bodily systems, as well as homeostasis within the organism.

The test from this scientific research hypothesizes that the transplantation of mitochondria (MTx) can improve the outcome after a cardiac arrest resuscitation. Mainly, it is claimed that there are improvements in multiple vital areas after applying fresh mitochondria, increasing the survival of the test subjects (rats). It compares the acceptance of transplanted mitochondria that were freshly obtained versus the mitochondria that were frozen. The fresh mitochondria seemingly increased the subjects' survival by 72 hours after cardiac arrest, in contrast to the frozen mitochondria that increased the survival only by 24 hours post cardiac arrest.

The study used a combination of in vitro and in vivo methods. The researchers first investigated how the exogenous mitochondria migrate in vitro. 24-hour co-cultures were ran with neural cells from the brains and muscles of the test rats, as well as endogenous mitochondria from the brain. The mitochondrial transfer was observed by microscopy. The effects were compared between the fresh and frozen-thawed mitochondria and its cell survival by measuring the adenosine triphosphate (ATP) levels contained. The in vivo experiment used the test rats subjected to 10 minutes of asphyxial cardiac arrest, followed by resuscitation by a dose of epinephrine. After the rats were successfully resuscitated, they were assigned to receive one of the three intravenous treatments: vehicle, frozen-thawed mitochondria, or fresh mitochondria. Throughout a 72-hour period, the survival rate of each subject was monitored in order to assess the efficacy of each treatment, as well as the endurance of the donor mitochondria and its effects in the key organs was observed 24 hours after the cardiac arrest episodes with the use of microscopy.

After isolating the tissue from the brain and muscle in the rats, the donor mitochondria were stained red and the endogenous mitochondria were stained green. The transferred mitochondria shared the area with the preexisting mitochondria inside the cells, when observing the images merged together, the stain was yellow. The exogenous muscle-derived mitochondrial transfer was also observed to be localized in a shared space, which suggests that the donated brain and muscle mitochondria can be efficiently transferred into neural cells. When studying the neurological recovery of the post-cardiac arrest subjects in the three groups, the ATP contents was measured, and a flow cytometry analysis was conducted to compare how the fresh versus the frozen mitochondria fared post-cardiac arrest. The ATP levels in the fresh mitochondria was four times above the level that the frozen/thawed mitochondria displayed. The flow cytometry also confirmed that the mitochondrial membrane potential ($\Delta\Psi m$) was higher in the fresh mitochondria compared to the frozen mitochondria. These results suggest that both the frozen and fresh mitochondria are sufficiently functional, but the frozen can disrupt the membrane potential.

The 72-hour survival rate was equal in the controls: the vehicle and the frozen/thawed had a 54% survival rate (6 out of 11). However, the rats that received the intravenous therapy containing fresh mitochondria had survival rates of 90.9% (10 out of 11). The value for vehicle was $p=0.048$; while the value for frozen was $p=0.038$. The freshly transported mitochondria therefore had a significantly higher rate of survival than the others. The health factors that were measured during the experiment include the neurological functional scores, in which there was a significant difference of $p=0.0469$ between the fresh and the frozen combined with the vehicle; the fresh mitochondria scored significantly higher. The blood lactate levels were measured in time after resuscitation, where the freshly transplanted mitochondria levels were

significantly lower than the controls, with a value of p=0.0171. The cardiac arrest-induced lung edema was measured and concluded that the 72-hour post resuscitation swelling was reduced only by the fresh mitochondria. Additionally, the glucose levels of each rat were measured and resulted in mixed effects. The fresh mitochondria were the first to return to the baseline that was recorded before cardiac arrest, while the vehicle and frozen took the almost the same amount, a little longer than the fresh.

The arterial pH was measured before and after the cardiac arrest, and the freshly transplanted mitochondria stayed the closest to its baseline throughout the two hours post-cardiac arrest, from 7.45 to about 7.42; compared to the frozen that dropped from 7.45 to 7.2, and only returned to 7.35 after the two hours. The CO2 levels within the rats were also recorded, and every variables were all given two hours to measure how close they would return to baseline. The frozen mitochondria rats began at 40mmHg, peaking at 50mmHg and returning to around 41mmHg. The vehicle variable was slightly lower, from 40mmHg to 45mmHg, back to 40mmHg. The fresh mitochondria displayed the lowest peak, however, starting below 40mmHg, peaking at around 41mmHg, and lowering to below 40mmHg. The CO2 levels in the animals are especially important in this study, because the effects of too much CO2 on the body, due to cardiac arrest or otherwise, can leave detrimental long-term effects. Finally, the body weight of the rats was measured: a baseline, and then the increments of 24 hours, until the 72 hours ended. All three variables began at 450g or higher, and all three variables' weights dropped, however; only the fresh mitochondria variable returned significantly close to its baseline, from 500g to about 480g. Using confocal fluorescence imaging, the donated mitochondria were labeled with red staining and observed in the brain, kidney, and spleen. The fresh mitochondria persisted within the organs between 1 and 24 hours after the cardiac arrest.

Conversely, the vehicle variable did not view any donated mitochondria in any of the three major organs after 24 hours.

To conclude the findings, the mitochondrial transplantation used to mitigate the injuries' damages due to the asphyxial cardiac arrest were introduced and the findings were recorded. The primary finding states that the freshly transplanted mitochondria improved the survival of the rats after 72 hours of the cardiac arrest by 91%, while the 2 control variables only survived 54%. Therefore, if the therapy is pursued or continued elsewhere, the use of frozen mitochondria is not recommended, since it yielded near identical results to the variable that did not introduce donated mitochondria, and the fresh mitochondria yielded much more desirable results.

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