Measuring Infarct Size in Rat Hearts Following Global Myocardial Ischemia

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Introduction

Cardiac ischemia occurs when heart muscle no longer receives oxygenated blood. If left untreated, this can lead to heart muscle death, known as myocardial infarction. As a result, the overall ability of the heart to effectively pump blood through the circulatory system is greatly diminished. Investigations into medical therapies that aim to increase the heart's resistance to infarction require an objective metric to measure their effect. Tetrazolium-based stains are widely used to measure infarction in animal hearts. In healthy tissue, dehydrogenase enzymes react with the tetrazolium salts to form a formazan pigment, staining it red. Infarcted tissue without active enzymes does not take up the stain and appears white. These areas can then be measured and the percent of infarcted tissue quantified.

Objective

Measure the infarct size in rat hearts undergoing 30 or 35 minutes of global ischemia and 90 minutes of reperfusion, then staining with **triphenyltetrazolium chloride (TTC)** to measure infarct size.

Methods

Hearts from Sprague-Dawley rats (~350 g) that underwent 30 minutes (n=7) or 35 minutes (n=8) of global ischemia were procured then re-perfused with KH buffer on a Langendorff apparatus for 90 minutes. Then the hearts were stored at -20°C in an aluminum foil. The TTC solution was made by dissolving 0.5 g of TTC in 50 mL of sodium phosphate buffer (0.1M Na^2PO^4 in distilled water) and placed in a water bath at 37°C. The frozen hearts were then sectioned longitudinally into four pieces (~2-3 mm in width). These sections were then placed in the TTC solution at 37°C and incubated for 20 minutes followed by submersion in 10% formalin overnight at 4°C. The following day, each set of four heart sections were dried, weighed and scanned to a computer. A computerized planimetry tool (ImageJ, National Institute of Health, USA) was used to calculate the percentage of infarcted area compared to the overall area of myocardium at risk. The percent of infarcted tissue by weight was then calculated and compared between the 30 and 35 minute ischemia groups using a compare means T-test.

Results

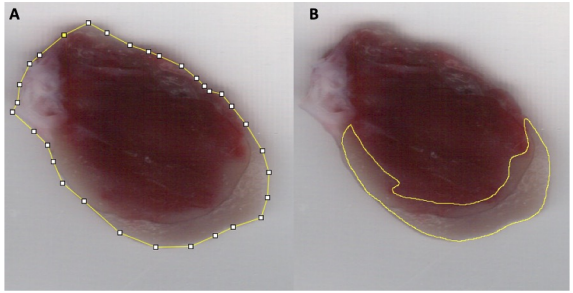
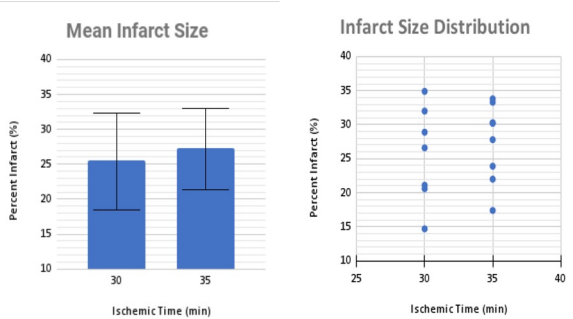


Figure 1: Representative section of rat heart stained with TTC. Healthy tissue appears red while infarcted tissue appears white. Panel A shows the ImageJ tracing of the entire area of myocardium. Panel B shows the Image J tracing of infarcted tissue.



The average percent of infarcted myocardium in the 30-minute (n=7) ischemia group was 25.54% ± 7.11 compared to 27.34% ± 5.78 for the 35-minute (n=8) ischemia group (p=0.7861).

Conclusions

TTC Staining is an effective method to accurately quantify infarct size in rat hearts that have undergone a global ischemic. The 30-minute ischemia group did demonstrate a slightly lower amount of infarcted tissue when compared to the 35-minute group. This did not; however, reach statistical significance ($p<0.05$).

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