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Commentary: The Effects of Different Fluorescent Indicators in Observing the Changes of The Mitochondrial Membrane Potential During Oxidative Stress-Induced Mitochondrial Injury of Cardiac H9c2 Cells

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This article involves some research results entitled “The Effects of Different Fluorescent Indicators in Observing the Changes of the Mitochondrial Membrane Potential during Oxidative Stress-Induced Mitochondrial Injury of Cardiac H9c2 Cells”. Myocardial ischemia/reperfusion (MIR) injury, a main pathological manifestation of coronary artery disease, aggravates heart damage after myocardial ischemia, circulatory arrest, or cardiac surgery. Mitochondrial oxidative stress injury is one of the major types of damage caused by MIR injury, while a decrease in the mitochondrial membrane potential (ΔΨm) is the earliest indicator of oxidative stress-induced mitochondrial injury[1]. Therefore, it is very essential to detect changes in ΔΨm accurately and immediately for cardioprotection. Although a previous study has reported that mass spectrometry can be used to detect ΔΨm in animal models, ΔΨm fluorescent indicators, including tetramethylrhodamine ethyl ester (TMRE), 5,5´,6,6´-tetrachloro-1,1´,3,3´-tetraethylbenzimidazolyl carbocyanine iodide (JC-1), and rhodamine 123 (R123), are the most popular agents for ΔΨm detection in single cells and isolated mitochondria[2]. Meanwhile, many methods have been used to observe the fluorescence intensity of ΔΨm fluorescent indicators in various experimental models, and each has its advantages and disadvantages. In this study, we evaluated the ability of three different fluorescent indicators by various analytical instruments, including a laser scanning confocal microscope (LSCM), fluorescence plate reader, and flow cytometer (FCM), to measure the ΔΨm of cardiac H9c2 cells during oxidative stress-induced mitochondrial injury. Among the instruments assessed in this study, the LSCM was the most suitable to detect dynamic changes in ΔΨm, while all three instruments could be used to detect ΔΨm at the endpoint. Furthermore, R123 was less effective than JC-1 and TMRE in measuring ΔΨm by the LSCM.

Fluorescent Indicators Toxicity Comparison

Although Scaduto RC Jr et al. has reported that the state 3 respiration rates of isolated mitochondria were reduced by TMRE, followed by R123[3], the effects of fluorescence indicators on mitochondria is still unclear. Therefore, we tested whether these probes could affect the mitochondrial function and morphology by

testing the mitochondrial oxygen consumption rate and a transmission electron microscope, respectively. Our data showed that neither the mitochondrial morphology nor basal respiration was significantly changed by TMRE, JC-1 or R123[4]. But the maximal respiration decreased markedly after staining cells with these indicators[4].

Fluorescent Indicators' Effects on ΔΨm Detection

The degree of binding, the sensitivity and the specificity are three indicators of ΔΨm detecting effects evaluation. Each fluorescent probe has its advantages and disadvantages. Although TMRE has less potential-independent binding to cells[5,6], it would induce fluorescence quenching when used at high concentrations[6]. Previous study indicated that JC-1 is a reliable fluorescent probe to assess ΔΨ changes in intact cells comparing to R123[7]. Recent studies demonstrated JC-1 may not suitable for ΔΨm detecting for the proportion of aggregates in the matrix is distributed red fluorescence and green monomer fluorescence in the cytoplasm are invalid and the relatively hydrophilic probe penetrates the plasma membrane very slowly, which means it may be misled[8]. The inconvenience in practice also limits the application of the dual-color dye JC-1[9]. In addition, JC-1 has poor water solubility, so it is necessary to repeatedly calibrate the culture concentration in the experiment[9]. Compared with other dyes, R123 is less photostable[10]. Therefore, evaluating the effects of different fluorescent indicators on ΔΨm detection may be somewhat confusing. It's better to compare results obtained with different fluorescent dyes according to a particular experiment before selecting the most accurate probe. S Salvioli et al. demonstrated that TMRE is more effective than R123 to measure mitochondrial depolarization because it can be taken up by live cells rapidly and reversibly[11]. Whereas JC-1 detects ΔΨm changes in spermatozoa more specifically than other dyes tested, TMRE fluorescence is easily analyzed and these fluorochromes are particularly suitable for multiparametric staining[6]. JC-1 is a reliable probe for analyzing ΔΨm changes with flow cytometry, while R123 shows a lower sensitivity[11]. JC-1 appeared to be a more convenient and simple way to detect a functional P-glycoprotein in clinical acute myeloid leukemia (AML) samples than R123[11]. R123 and JC-1 do not appear to be good probes for the screening of mitochondrial activity in rainbow trout hepatocytes[12].

Different Instruments on ΔΨm Detection

Compared with fluorescence indicators, choosing a suitable instrument is also a key factor in testing ΔΨm. In this study, we evaluated the ability of various analytical instruments, including an LSCM, FCM, in measuring the ΔΨm of cardiac H9c2 cells during oxidative stress-induced mitochondrial injury[4]. LSCM provides a visual approach to measure ΔΨm by time-dependent scans. Compared with an LSCM, more samples can be observed at one time by a fluorescence plate reader, and all adherent cells can be analyzed simultaneously. FCM can observe multiple cells at one time. Among the instruments assessed in this study, the LSCM was the most suitable to detect dynamic changes in ΔΨm, while all three instruments could be used to detect ΔΨm at the endpoint[4]. It is necessary to test the damage effect of laser illumination for high-intensity laser illumination may cause photodamage and photobleaching. We tested the effect of laser excitation of an LSCM on cell oxidative stress in our experiments and found the lasers could not increase oxidative damage of the cells in our experimental conditions. Our data implied that TMRE is a suitable fluorescent probe that can be used to detect changes in ΔΨm caused by oxidative stress in live cells by the three instruments, especially the LSCM and fluorescence plate reader. Furthermore, the sensitivity of R123 was less than JC-1 and TMRE to measure ΔΨm by the LSCM.

Limitation

Besides the ΔΨm losing, oxidative stress may exacerbate the myocardial damage through inducing reactive oxygen species (ROS) generation, calcium overload, endoplasmic reticulum stress and so on[13]. If we can observe these indicators by staining cells with multiparametric probes simultaneously, it would contribute to many related experiments. Thanks to recently developed instruments and additional probes for cell surface and intracellular markers, detecting ΔΨm along with other biological parameters is used by many researchers[14]. Previous studies have shown that ΔΨm decreases during the process of cell apoptosis[15]. For example, Thomas Zuliani et al. proposed reliable and efficient staining, with JC-1 and a fluorescent nuclear acid stain TOTO-3 (1,1'-[1,3-propanediylbis[(dimethyliminio)-3,1-propanediyl]]bis[4-[3-(3-methyl-2(3H)-benzothiazolylidene)-1-propen-1-yl]-,iodide) to discriminate three functional cellular states: intact, apoptotic, and necrotic/late apoptotic cells by flow cytometry[9]. The three dyes did not significantly change the mitochondrial morphology and basic respiration, but caused a significant decrease in the maximum respiration value. Studies have shown (2-(4-(dimethylamino)styryl)-N-ethylpyridinium iodide) DASPEI is also a fluorescent probe for detecting changes in mitochondrial membrane potential, but its limitation is that it can only be applied to the detection of live cell mitochondria[15]. When choosing an accurate fluorescent probe, it should be selected according to the advantages and disadvantages of the specific experiment combined with the probe. However, the affection among different fluorescent probes including the ROS indicators and calcium indicators has not been discussed in this paper, which is a limitation.

Conclusions and Closing Thoughts

This article aims to explore the role of different fluorescent indicators in observing changes in mitochondrial membrane potential during oxidative stress mitochondrial damage in cardiac H9c2 cells, and to provide references for the selection of fluorescent probes in related experiments in the future. We should also focus on the relationship between ROS indicator and calcium indicator and fluorescent probe. Therefore, the interaction between fluorescent indicators needs further study.

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