ORIGINAL ARTICLE

FLUORESCENT METHOD FOR REAL-TIME DETECTION OF MYOCARDIAL ISCHEMIA IN AN ANIMAL MODEL

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ABSTRACT

Objective: Evaluate the feasibility and sensitivity of Fluorescein to determine and delineate an ischemic area in an experimental model of acute coronary occlusion.

Materials and Methods: The studies were performed at the center for experimental Surgery at Hospital de Clínicas "Jose de San Martin". All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at University of Buenos Aires. All animals were maintained in a pathogen-free environment throughout the experiments. We used 10 New Zeeland rabbits. They served as their own control model.

All the experiments were performed under general anesthesia with tracheostomy. Median sternotomy was performed and the second diagonal artery was ligated. The infracted area was evaluated under xenon and UV (530nm) light after the administration of 0.01mg/kg Fluorescein 10% intravenous (peripherical vein) Electrocardiogram (EKG), pulse oximetry, heart rate (HR), Troponin, CPK, CPK-MB and LDH were determined postoperatively. All the animals were euthanized at the end of the experiment and the heart was harvested for histopathologic examination. Biochemical (enzymatic) and electrocardiography analysis were performed at baseline and at ninety minutes after complete occlusion of the second diagonal artery: Baseline (BL) and post-ischemic (PI) measurements were performed for LDH, CPK, CPK -MB and troponin.

Results: ST segment elevation of 1,8 +\- 0,65 mm was detected in every case after coronary artery occlusion. Oxygen saturation and heart rate were 97 +/- 2 % and 145 ± 5 respectively. Enzymes results: LDH (BL) 159,7 ±112,2 (U/L) vs LDH (PI) 1012 ±359,9 (U/L) (p<0,001). CPK (BL) 1,072 ± 121,7 (U/L) vs. CPK (PI) vs. 359,5 ±95,7 (U/L) (p<0,001), CPK-MB (BL) 0,89 ±0,42 (ng/ml) vs CPK-MB (PI) vs. 3,89 ±1,9 (ng/ml) (p<0,001), Troponin (BL) 0,06 ±0.06 (ng/ml) vs. Troponin (PI) 19,6 ±5,9 (ng/ml) (p<0,001). The Xenon light failed to demonstrate any changes in the ischemic area. However, when evaluated under the UV (530nm wave length) light, a clearly demarcated area lacking fluorescence can be appreciated. The area represented 0,7225 ±0,39cm2 in the anterior aspect of the myocardium distal to the ligated vessel. This was correlated and confirmed by microscopic evaluation.

Conclusion: This study serves as a proof of principle that Fluorescein-detection of myocardial ischemia in an experimental model of acute coronary occlusion is feasible, sensitive and reproducible. However, further clinical studies are required to understand if the findings of our study could be extrapolated into human studies.

KEY WORDS: myocardial ischemia, animal model, fluorescein.

INTRODUCTION

The properties of sodium fluorescein have been known and used in medicine since the end of the 19th century. However, it has never been used for the evaluation and correlation of a myocardial ischemic area in real time. Its ease of obtaining, its fast distribution in tissues highly supplied with blood such as the cardiac one, together with its low rate of adverse effects make it the ideal substance to carry out such experimental work[1].

The purpose was to evaluate the real-time direct visualization of a myocardial ischemic area through the intravenous use of sodium fluorescein, under direct stimulation by UV light (530 nm) in an experimental model of myocardial ischemia.

The surgical significance of the proper recognition of the ischemic area, not clearly visualized under direct vision, lies in:

1. the provision of more information about the anatomy and pathophysiology of the coronary disease in vivo,
2. the improved visualization of the reperfused myocardium after a myocardial revascularization surgery.

Several authors have carried out various experimental models of myocardial infarction in animals, but none of them evaluated the area with a fluorescent method[2].

MATERIALS AND METHODS

All the procedures were carried out at the Center for Experimental Surgery of the Hospital de Clínicas "José de San Martín", Universidad de Buenos Aires, during 2013 with the endorsement of the Ethics Committee of Universidad de Buenos Aires.

Fluorescein is a hydrosoluble organic coloring substance used in the angiography of eye vessels and in certain dental techniques. It was discovered by Prof. Johann Friedrich Wilhelm Adolf von Baeyer (1835-1917), winner of the 1905 Nobel Prize in Chemistry. It is a water-soluble yellow substance of the xanthine group that produces a deep green fluorescent color in alkaline solutions (with PH above 7). When exposed to light, fluorescein absorbs certain wavelengths and emits fluorescent light of long wavelength, in this case close to 530 nm *(Figure 1)*.

Ten New Zealand rabbits (n = 10) with an average weight of 3.1 ± 0.6 kg were used.

Each individual was its own control. General anesthesia was used with 35 mg/kg ketamine and 5 mg/kg intramuscular xylazine as anesthetic induction. Ear and sternum shaving was done and a 20 G catheter was inserted into a vein of the left pinna. A lateral tracheostomy was performed securing the airway with a endotracheal tube No. 3. The level of anesthesia was maintained with the continuous dripping of propofol. After placing the individual in dorsal decubitus position with its legs stretched, the surgical technique consisted of:

1. sternotomy,
2. pericardiotomy,
3. cardiac luxation,
4. ligation of the second diagonal artery with 6.0 polypropylene suture using 3.5 x loupes.

The ischemic area was visualized directly with xenon light.

Then, peripherally, 0.01 mg/kg of 10% sodium fluorescein was injected intravenously. Visualization with xenon light alternated with visualization with 530 nm UV light.

Blood samples were taken for the determination of cardiac enzymes (CPK, CPK-mb, LDH and troponin) before and after myocardial ischemia (90 minutes). Animals were monitored continuously, recording respiratory rates, heart rates, oxygen saturation levels and electrocardiographic values.

Subsequently, measurements were taken of the non-fluorescent area related to the ischemic area.

Euthanasia was practiced in all animals, the heart was removed in block and was sent to anatomic pathology in 10% formaldehyde.

RESULTS

A total of 10 New Zealand rabbits (n = 10) with a weight of 3.1 ± 0.6 kg underwent the experimental model of myocardial ischemia. No perioperative mortality was observed and euthanasia was practiced in 100% of cases two hours after the anesthetic induction began. The ECG, heart rate, breathing rate and pulse oximetry of all individuals initially did not evidence alterations and were taken as baseline values. Heart rate levels showed values of

145 ± 5 beats per minute, with no significant variations upon acute myocardial infarction, as well as the respiratory rate, which was 28 ± 5 breaths per minute. No significant changes in oxygen saturation levels (97 +/- 2%) were observed. The ST segment elevation after the second diagonal artery ligation was immediate, showing an average rise of 1.8 ± 0.65 mm.

Baseline biochemical parameters (considered in this model as normal) and post coronary ligation biochemical parameters were compared, obtaining statistically significant p-values < 0.05 (using Student's T) *(Table 1)*.

In exposing the infarcted area to xenon light and 530 nm UV light, it was not possible to visualize clearly either the area or the limits of the ischemic sector *(Figure 2)*.

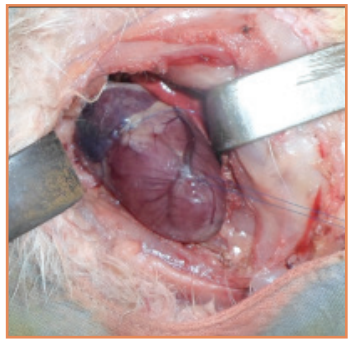


Figure 2. Ligation of the second diagonal artery.

NZW rabbits	**n=10**	**P**
Weight (kg)	3.10 ± 0.6	n/a
ECG		
ECG abnormalities	Immediate	n/a
ST elevation (mm)	1.8 ± 0.65	n/a
Blood biochemistry		
Baseline troponin (ng/ml)	0.06 ± 0.06	n/a
Post-ischemia troponin (ng/ml)	19.6 ± 5.9	<0.0001
Baseline CPK (U/L)	1.072 ± 121.7	n/a
Post-ischemia CPK (U/L)	359.5 ± 95.7	<0,0001
Baseline CPK-mb (ng/ml)	0.89 ± 0.42	n/a
Post-ischemia CPK-mb (ng/ml)	3.89 ± 1.9	0,0003
Baseline LDH (U/L)	159.7 ± 112.2	n/a
Post-ischemia LDH (U/L)	1.012 ± 359.9	<0,0001
Anatomic pathology		
Infarct size (cm^2)	0.722 ± 0.39	n/a

Table 1. Experimental results.

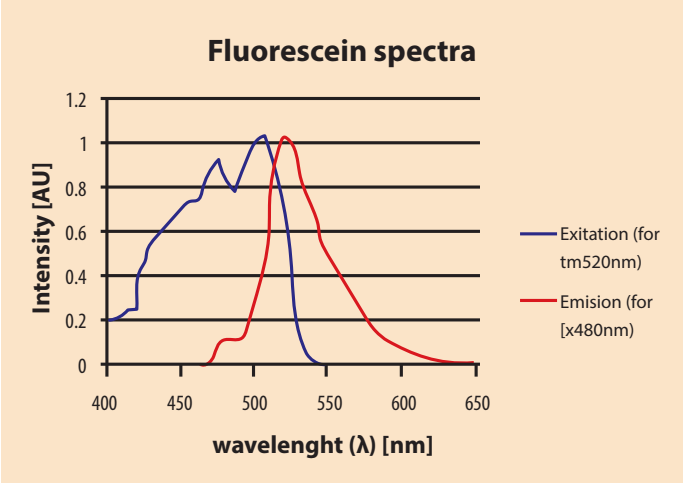


Figure 1. Fluorescein spectra.

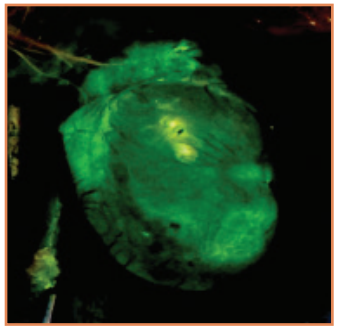


Figure 3. Área no fluorescente (isquémica).

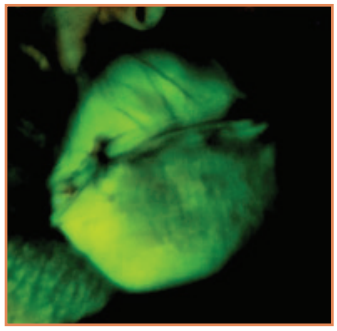


Figure 4. Non-fluorescent area (ischemic).

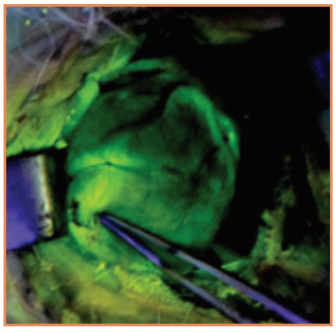


Figure 5. Non-fluorescent area (ischemic).

Sixty seconds after ligating the second diagonal artery, 0.01 ml/kg of 10% sodium fluorescein was injected by periferal catheter. Under the xenon light, fluorescence was not identified clearly. However, when 530 nm UV light was used, it was observed that the vascularized area showed strong fluorescence. Instead, the infarcted area showed no fluorescence and the ischemic area was clearly delimited *(Figures 3, 4, 5 and 6)*. They were measured yielding values of 0.722 ± 0.39 cm^2.

The histopathological analysis of the surgical specimen produced findings consistent with acute myocardial infarction, such as disorganization of muscle fibers with more eosinophilia and inflammatory infiltrate sectors. Vacuolization of cytoplasm of myocytes and undulation of muscle fibers *(Figure 7)*.

DISCUSSION

The anatomy of the rabbit similar to that of the human myocardium, the easy access to the mediastinum and the sufficient size of coronary arteries were the main reasons for the choice of the animal model.

After the ligation of the second diagonal coronary artery, a significant increase of the enzymes LDH, CPK, CPK-mb and troponin and electrocardiographic changes with ST segment elevation were confirmed in all individuals, proving a myocardial injury or ischemia. Kobayashi T. et al describe high sensitivity for the detection of myocardial ischemia in the case of ST segment elevation[3].

Normally, the methods for the determination of the affected ischemic area, such as the electrocardiogram and the echocardiography, are indirect. The ECG also has the advantage of being a non-invasive method and is read through the electrical changes produced by the heart. The echocardiography can determine myocardial akinesia areas and calculate values that provide guidance on the ventricular anatomy and geometry[4].

Also, the coronary angiography is a diagnostic and therapeutic method that allows to infer which the affected coronary artery is but does not determine the area actually infarcted[5].

The literature has not described yet any techniques for visualization during an open myocardial revascularization procedure, for delimiting the ischemic area during surgery and the behavior of the myocardial tissue after being revascularized[7-9].

This experimental work reveals the possibility of real-time visualization of the affected area with the use of sodium fluorescence and UV light. Fluorescence is the property of a substance that glows after having absorbed light or other form of electromagnetic energy[1,6].

The fluorescent substances used in medicine include indocyanine green, with a 780 nm excitation spectrum and an 830 nm emission spectrum. This drug was used to test liver functionality in the past, and today its fluorescent properties are used in the visualization of the biliary pathway in order to avoid surgical injuries during laparoscopic cholecystectomies[6,10].

Methylene blue is widely used in medicine for the evaluation of anastomosis or for the treatment of methemoglobinemia[10,13]. Its fluorescent properties began to be used recently for ureteral visualization during pelvic surgery[11].

In our case, the substance used was sodium fluorescein and the excitation source was ultraviolet light with a wavelength of 530 nm. In comparison with the other drugs, it has a lower excitation spectrum and a larger quantum of energy emitted upon excitation, and therefore the light emitted is even stronger[1].

Its molecular weight is 332.306 g/Mol, which would prevent its use in the study of lymphatic and sentinel ganglia due to its tendency to spread into small capillaries[14]. It is such property that we considered for its use in injury and infarction areas.

To differentiate the possibility of visualizing the infarcted area with the naked eye, we used white light. We did not detect areas of distal hypoperfusion into the ligated artery, as usual in cases of injury.

However, after the administration of fluorescein and with the use of 530 nm light, it was possible to visualize, in 100% of cases, the distribution of fluorescein in areas well vascularized and a filling defect (black color) in areas not vascularized. In our case, the use of filters for the observation of light, as described by Ishizawa et al, was not needed when using indocyanine green, since the emission of fluorescein is visible to the naked eye[15].

Remarkably, the fluorescence area was maintained even after the animals were euthanized, which we interpreted as a delay in the washout of the fluorescent material.

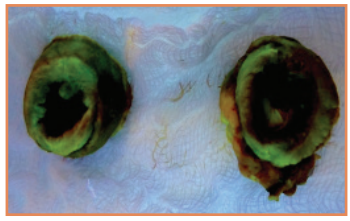
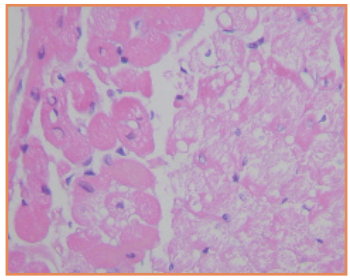


Figure 6. Non-fluorescent area (ischemic) in cross-section of the heart.



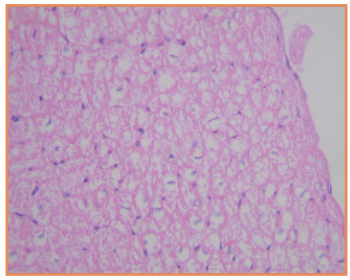


Figure 7. Microscopy. Disorganization of muscle fibers with more eosinophilia and inflammatory infiltrate sectors. Vacuolization of cytoplasm of myocytes and undulation of muscle fibers.

It was not possible to access the use objective methods for the measurement of fluorescence, such as the software used by Diana et al, which by digital subtraction determines values for the degree of fluorescence[16].

In our case, the determination of the absence or presence of light in the tissue was performed by the interpretation of the authors and was compared with anatomic pathology results, which were consistent as regards the ischemic area and the area not illuminated with fluorescein.

This initial work will be useful for the design of future study options in the evaluation of the consequences of myocardial revascularization.

CONCLUSIONS

The intravenous administration of sodium fluorescein allowed to visualize clearly the ischemic area in 100% of the individuals of this series after a heart attack, in consistency with ECG, enzymatic and anatomic pathology results.

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