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A Review on Exploring Myocardial Ischemia and Reperfusion Consequences through IN VITRO and IN VIVO Animal Models for Targeted Screening of Anti-Anginal Drugs

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The pathophysiological process called myocardial ischemia, which is characterized by an insufficient blood supply to the myocardium with subsequent reperfusion, is crucial in the development of many cardiovascular diseases. With a focus on evaluating the potential of new antianginal drugs, this review aims to fully assess the effects of myocardial ischemia and reperfusion using in vitro and in vivo animal models. An accurate mechanistic view of cellular responses is possible through in vitro models using isolated cardiac cells and tissues to recreate ischemia and reperfusion situations under controlled conditions. These models are important for understanding cell remodeling, oxidative stress processes, and the molecular pathways involved in cardiac damage. A more comprehensive understanding of the systemic consequences of ischemia-reperfusion injury on cardiac function, hemodynamics and overall cardiovascular

homeostasis is provided by in vivo animal models such as rats and pigs. We aim to use a mix of these models to better understand the complex molecular processes underlying ischemia-reperfusion injury and to assess the efficacy of new antianginal drugs. We can assess the effectiveness of a drug treatment in terms of its ability to reduce myocardial damage, improve cardiac function, and improve overall recovery after ischemia by simulating clinical conditions and using state-of-the-art imaging tools. The results of this study have important implications for the development of targeted therapies for ischemic heart disease. Our review helps identify potential antianginal drugs for further clinical trials, thereby bridging the gap between in vitro molecular knowledge and in vivo physiological relevance. The ultimate goal of these studies is to improve patient outcomes by demonstrating state-of-the-art treatments for myocardial ischemia and reperfusion injury.

Keywords: Myocardial ischemia, reperfusion injury, anti-anginal drugs, in vitro models, in vivo models, cardiovascular diseases.

1. Introduction

In vitro models

1. Langendorff Heart Preparation

Langendorff is a highly reproducible preparation which can be studied quickly in large number of at relatively high cost. It allows management of broad spectrum off of biochemical, physiological and morphological indices. Measurements are made in absence of cofounding effects of the organs. Both global and regional ischemia can be studied using this model. It allows experiments to be continued in face of events (myocardial infarction, arrhythmias) which would normally jeopardize the survival of an in vivo experiment. However, it is a deteriorating repression do capable of studying for several hours. The basic principle involved is that heart is perfused in a retrograde direction from the aorta either at constant pressure or constant flow with oxygenated saline solutions. Retrograde perfusion closed the opened right atrium. (Fig.1) Parameters usually measured are contractile force, coronary flow and cardiac rhythm.

Guinea Pigs of either sex weighing 300 to 500g are used for the study. They are sacrificed by stunning. Diaphragm is assessed by Trans abdominal incision and cut carefully to expose the thoracic cavity. Thorax is open by bilateral incision along the lower margins of the last of first trips. Thoracic cage is reflected over the animal's head exposing the heart. The heart is cradled between fingers and lifted before incision the aorta, vena cava and pulmonary veins. Immediately after excision, heart is tipped in cold perfusion solution 4 degrees Celsius to limit ischemic injury during the period between accession and restoration of vascular perfusion. The aorta is located and cut below the point of division. A cannula Inserted into the aorta and tied and the heart is perfused with oxygenated ringer solution. The heart is transferred to a double wall Plexiglas perfusion apparatus maintained at 37 degrees Celsius. [2] Oxygenated ringer solution is perfused at constant pressure of 40 MmHg at a temperature of 37 degrees Celsius from a reservoir. A small steel hook with a string is attached to the epics of the heart.

Contractile force is measured isometrically by a force transducer and recorded on a polygraph. Drugs are injected into the perfusion medium. [3] The antianginal effect of the test drug is indicated by an increase in coronary blood flow. The incidence and duration of ventricular fibrillation, coronary flow, inotropic state and K^+ levels after treatment with drug are compared with control.

This method is very useful for testing coronary vasodilator drugs. It has wide applications in the field of pharmacology and Physiology. It is useful to study positive inotropic effects, negative inotropic effects, coronary vasodilator effect, calcium antagonism, effect on potassium outflow induced by a glycosides and determination of hypoxic damage. Metabolic studies, arrhythmogenic, and the arithmetic, and anti fibrillatory effects can also be assessed using London draw method. Recently this model has also been used to study EDRF release from the coronary rescuer bed and electrophysiological evaluation of cardiovascular agents.

Fig.1 Langendorff Heart Preparation (Heart mounted in Langendroff Apparatus both global and regional ischemia can be studied using this model).

2. Isolated Rabbit Aorta Preparation

Aortic rings are used to evaluate the smooth muscle relaxant contractile activity in this method (Fig.1). Adding potassium chloride or norepinephrine to the organ bath containing slightly modified Krebs's bicarbonate buffer induces contraction of aorta rings. Using an overdose of pentobarbitone sodium, rabbits of either sex weighing 3 to 4 kg are sacrificed. Thorax is opened by bilateral incision. The descending thoracic aorta is rapidly removed and placed in crabs bicarbonate buffer maintained at 37 degrees Celsius. Tissue is cleaned, fat and connective tissue is carefully removed. Eight rings of 4-5 min width are obtained and each is mounted in 20ml tissue bath containing Krebs solution. A stabilization period of 2 hrs is allowed where in the Kreb's solution is frequently changed followed by stabilization period of one hour. A tension of 1g is maintained during these times. A sustained contraction is generated by addition of KCL. Twenty five minutes after addition of agonist, the test drug is added. The percent relaxation reading is taken every 30 minute after addition of test drug. There is a 30 minute time interval between additions of different test drugs. [4]

An active tension is calculated for the tissue at the time the point just prior to the addition of test compound and also at the point 30 minute after the addition of each concentration of the compound. ID_{50} and free percentage relaxation caused by test drug from the precontracted level is calculated.[5] Test drug with calcium channel blocking activity have a relaxing effect and can be evaluated using this method.

3. Calcium Antagonism in Pitched Rat

This model can differentiate calcium entry blockers from other agents that do not directly block entry of calcium.

Sprague Dawley rats (250-350g) are anesthetized intraperitoneally with methohexitone sodium (50 mg/kg). The trachea is cannulated. Thereafter the rats are pitched through one orbit and immediately maintained on artificial respiration. The pithing rod is used as a stimulating electrode and continuous electrical stimulation of the thoracic spinal cord with square wave pulses at supra maximal voltage (frequency 0.5 Hz and duration 0.5 ms) produces a cardio accelerator response. Only rats with a resulting tachycardia (100 beats/min) are included for the study. The jugular vein is calculated for administration of drugs and blood pressure is recorded via carotid artery using a pressure transducer. In the femoral region, an indifferent electrode is inserted subcutaneously.

When cardio accelerator response is established for 3-5 minute, calcium channel blockers and beta blockers are administered. These test compounds dose dependently block tachycardia [6]. The level of tachycardia immediately prior to drug administration is taken as 100% and response to drug is expressed as a percent of predose tachycardia. ID_{50} is calculated and compared.

4. Relaxation of Bovine Coronary Artery

The relaxation caused by test compounds can be assayed using spiral strips from bovine coronary artery. The tunas of coronary arteries can be regulated by eicosanoids. Prostacylin induces relaxation whereas thromboxane A_2 causes contraction.

Beef hearts are obtained immediately after slaughtering. They are immersed in cold oxygenated Kreb's solution and immediately transported to the laboratory. The left descending coronary artery is cut into spiral strips and suspended in a four ML organ path under an initial tension of 2G and immersed in oxygenated Kreb's bicarbonate solution at 37 degrees Celsius. The Kreb's solution contains a mixture of antagonists to inhibit actions from endogenous acetylcholine, 5-hydroxy-trptamine, histamine or catecholimines. The strips are super fused with the solution of test compound with oxygenated Kreb's solution. Isometric contractions are recorded with force displacement transducers on a grass polygraph. The strips are super fused with Kreb's solution 3 hrs prior to the experiment. Standard compounds are 100ng/ml PGE_2 inducing contraction and 100ng/ml PGI_2 inducing pronounced relaxation [7-8].

The maximal response to 100ng/ml PGE_2 OR 100ng/ml PGI_2 is calculated and the relaxation caused by the test compound is expressed as its percentage.

5. Coronary Artery Ligation in isolation Rat Heart

Langendorff Technique can also be used to produce regional ischemia by clamping the left coronary artery close to its origin. (Fig.2) after removal of the clip, change in the reperfusion

period can be observed. Prevention of these symptoms is an indicator of the efficacy of the coronary drugs.

Wistar rats of either sex weighing 280-300 grams are sacrificed by decapitation. The hearts are removed and dissected free from the epicardium and surrounding connective tissue. [4] A cannula is introduced into the aorta from where the coronary vessels are perfused with the non-circulated perfusion medium according to the Langendorff technique. In the left ventricle a balloon closely fitting the ventricular cavity is placed and connected to an artificial systemic circulation. The balloon is made of silicone material using a Teflon form. The dimensions of the Teflon form are basically derived from CSDS off left ventricle off potassium arrested CSTS by injection of dental cement. During each heartbeat the fluid volume pressed from the balloon corresponding to the stroke volume of the heart, can be recorded groove and an integrator connected in series . The preload and afterload are adjusted separately and the perfusate flow is recorded separately. For coronary artery Occlusion experiment, the isolated working hearts are perfumes for 20 minute with Krebs buffer at 65 mmHG. Acute myocardial ischemia is produced by clamping the left coronary artery close to its origin for 13 minutes. The clip is then opened and the change during reperfusion is monitored for 30 minutes. Hemodynamic parameters like left ventricular pressure, heart rate, cardiac output and coronary flow are measured. [9] From the coronary effluent, samples are taken for Lactate dehydrogenase (LDH), creatine kinase (CK), glycogen, ATP and lactate determinations. The test drug is given on to the perfusion medium either before occlusion oh 5 minute before reperfusion. The incidence and duration of ventricular fibrillation after treatment with test drugs is compared with controls.The incidence and duration of ventricular fibrillation after treatment with test drugs is compared with controls. Left ventricular pressure, left ventricular dP/dt max, coronary flow and myocardial LDH, CPK, glycogen, ATP and lactate measured.

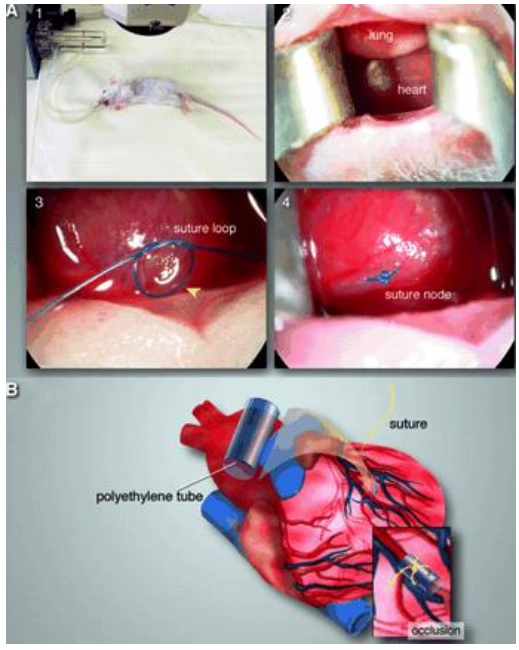


Fig.2 Left Coronary Artery Ligation in isolation Rat Anesthesia, ventilation, and a heating

pad are all applied to a mouse in (A) (1). The heart and lung are visible because to a left-sided thoracotomy that retracts the ribs (2). The left coronary artery is sutured with prolene (suture is shown by an arrowhead in (3) and (4)). The myocardial region downstream of the obstruction appears pale right away after suturing. (B) The suture is wrapped around the LCA and a short piece of polypropylene tubing, enabling it to be cut and removed without harming the myocardial, permitting removal of the suture and hence reperfusion of the ischemic heart. [30]

6. Isolated Heart-Lung Preparation

Isolated heart lung preparation off the dog has been used to study various physiological and pharmacological processes. Now this model has also been established in rights.

Wistar rats (300 to 350 g) are anesthetized intraperitoneally with phenobarbitone sodium 50 mg/kg. The trechea is cannulated and the animal is maintained on artificial respiration. The chest cavity is opened and ice cold saline saline is injected to arrest the heart. The aorta, superior and inferior vena cava are cannulated. The heart-lung preparation is perfused with Kreb's-Ringer buffer containing rat RBC (hematocrit 25%). The perfusate is pump from the iota and is passed through the pneumatic resistance and collected in a reservoir maintained at 37 0 Celsius. It is then returned to the inferior vena cava thus perfusing only the heart and the lung. Best drug is administered into perfusate 5 minute after start of experiment. Cardiac output is recorded with an electromagnetic blood flow meter and mean arterial pressure from the pneumatic resistance. With the help of bioelectrical amplifier heart rate is recorded. Hemodynamic data and recovery time of the test drug group and control group without any treatment is compared using ANOVA and Kruskal-Wallis test respectively [10].

7. Plastic Casts Technique in Dogs

Coronary drug when administered for prolonged duration leads to increase in the number and sites of interarterial collaterals especially in pigs and dogs. Acute or gradual occlusion one of the major coronary branches may also stimulate development of collaterals. In order to quantify the collaterals, the arterial coronary bed is filled with plastic. This provides with the possibility to make the collaterals visible.

Dogs (10-15 kg) are intravenously anesthetized with pentobarbital sodium (30mg/kg). They are maintained on artificial respiration, Chest cavity is open and the heart is exposed. The pericardium is removed and Ameriod cuffs are placed around the major coronary branch. The plastic materials gradually swells and occlude the lumen within 3 to 4 weeks. The animals are administered test drugs or placebo for 6 weeks and then sacrificed. After a recovery of 1 week, dear hearts are removed and coronary bed flushed with saline. Care is taken to maintain the uniformity of the filling pressure, the filling time and viscosity of the filling material. After polymerization is complete, the tissue is digested with 35% KOH. Plastic casts from the drug treated animals are compared with costs from the sham group (dogs subjected to the same procedure without drug treatment). The ability of the test drug to increase the number and size of collateral's is evaluated.

2. IN VIVO MODELS

1. Occlusion of coronary Artery

Compounds that reduce infant size are study using this model. Infarct size is studied after proximal occlusion of the left anterior descending coronary artery in open chess dogs. Nitro blue tetrazolium chloride steam is myocardial sections are used to visualize infarct size in coronary arteriograms made after injection of $BaSO_4$ gelatin mass into the left coronary ostium.

Dogs of either sex (30 Kg) are use in this model. The animals are anesthetized with Pentobarbitone sodium (35mg/kg), intraperitoneally which is followed by its continuous infusion at 4 mg/kg/h. Trechea is cannulated and the animal is maintained on artificial compound. ECG is recorded continuously. Femoral vein is calculated and connected to a pressure transducer for measuring peripheral systolic and diastolic pressure. Left ventricular end diastolic pressure, left ventricular pressure and heart rate are also measured using a Miller micro tip catheter PC 350 inserted why are the left coronary artery. Heart is exposed through a left thoracotomy between 4^{th} and 5^{th} intercostal space. The pericardium is opened and the left anterior descending coronary artery is exposed and then ligated for 360 minutes. Test substance or vehicle is administered by intravenous bolus injection. Hemodynamic parameters are monitored and at the end of the experiment, animals are sacrificed with an overdose of pentobarbital sodium. Area at risk of infection is measured using coronary arteriograms. The left ventricle please cut into transverse sections. From each slice angiograms are made with X-ray tube at 40kv to assess the area at risk of infarction by defect opacity: reduction $BaSO_4$ field vessels in infarct tissue. The slices are then incubated in p-nitro-blue-tetrazolium (0.25 g/l) in order to visualize the informal tissue (blue Violet stained healthy tissue, unstained necrotic tissue). The slices are photographed for determination of infarct area. Mortality, hemodynamic parameters and in fact size are determined. Changes in parameters in drug treated animals are compared to vehicle controls.

2. Microspheres-induced Acute Ischemia

This model can be useful in evaluating the effect of test drugs on myocardial performance during acute ischemic left ventricular failure. Microspheres (50μm) when injected repeatedly into the left main coronary artery may induce left ventricular failure in anesthetized dogs. Hemodynamic parameters can be recorded and drugs tested on the basis of the improvement of cardiac performance.

Dogs 30 kg are anesthetized with pentobarbital on sodium intravenously and additionally administered a supplementary dose of 4 mg/kg/h. The trachea is cannulated and the animal maintained on artificial respiration. The brachial vein is cannulated for administration of analgesic and saphenous vein for administration of test compounds. ECG is recorded continuously. The femoral artery is also cannulated and connected to up pressure transducer for the measurement of systolic and diastolic pressure. A Miller micro tip catheter is inserted via the left ventricular end diastolic pressure (LVEDP) is measured on a high sensitivity scale. From the pressure curve, dP/dt and heart rate are calculated. Mean pulmonary capillary pressure, mean pulmonary artery pressure (PAP) and cardiac output are measured using Cardiac Index Computer and a balloon tip triple lumen catheter with the thermistor position in

the pulmonary artery via the jugular vein. Through a left thoracotomy, the heart is exposed. Microspheres are injected through the angiogram catheter into the left ostium initially as 10 ml and later 5 ml boluses about 5 min apart. Embolization is terminated when the LVE DP increases to 16 to 18 mmHg and PAP to 20mmHg and heart rate 200 beats per minute. Test compound is then administered by intravenous route and the above mentioned parameters recorded. In addition to the directly measured hemodynamic parameters, stroke volume, tension index, coronary vascular resistance, total peripheral resistance, pulmonary artery resistance can also be measured. Changes of parameters in drug treated animals are compared to vehicle controls. Also mean embolization time, doses of microspheres and numbers of microspheres are evaluated [13].

3. Isoproterenol-induced Myocardial Necrosis

Synthetic catecholamines like Isoproterenol when injected at high dose produce cardiac necrosis. Rona et al have studied the infarct like lesions in the red myocardium. [14] Several drugs such as sympatholytics all calcium antagonists can totally or partially prevent these lesions.

Wistar rats (150-200g) are pretreated with test drug or standard drug orally or subcutaneously for at least a week. These rats are then injected with 85MG per KG isoproterenol subcutaneously on two consecutive days. Mortality as well as symptoms are recorded in each group and compared to group injected with isoproterenol only. After 48 hours of first dose for histological evaluation or processed for estimation of radius biochemical parameters. Before sacrificing, the animals hemodynamic parameters such as systolic diastolic blood pressures and heart rate can be recorded buy can you relating the carotid artery and connected it To appreciate transducer. By inserting a cannula in the left ventricle, parameters such as left ventricular end diastolic pressure (LVEDP) and DVD can be measured. The degree of histopathological change can be created as follows:

- Grade 0: No change
- Grade 1: Focal areas of necrosis
- Grade 2: Focal areas of necrosis and muscle
- Grade 3: Confluent areas of necrosis, edema and inflammation and muscle fiber fragmentation
- Grade 4: Massive areas of necrosis, edema and inflammation and mural thrombi. Change of parameters histological, biochemical and hemodynamic of drug treated animals are compared to isoproterenol controls.

4. Stenosis-induced Coronary Thrombosis Model

Thrombosis can be induced by stenosis in dogs. This model is characterized by alterations in coronary blood flow with transient platelet aggregation at the site of coronary constriction.

Dogs (15-30 kg) are anesthetized with pentobarbitone sodium (30-40 mg/kg, intraperitoneally) and then maintained on artificial respiration through a tracheal tube using a positive pressure respirator. Through a left thoracotomy the heart is exposed add the 4^{th} and 5^{th} intercostal space and the pericardium is removed. An electromagnetic flow probe is placed on the proximal part

of the left coronary artery to major coronary blood flow. Distal to the flow meter, the vessel is clamped for 5 second. Small plastic constrictor is placed around the artery at the site of damage. The constrictor is exchanged several times until the required narrowing of the coronary artery is achieved. In case the artery is occluded, the coronary artery is lifted to induce reflow. Dogs with regular repeated cyclic flow variations of same intensity within a pretreatment phase of 60 minute are used for experimental purpose. Hemodynamic parameters are recorded. Test compound is administered intravenously and the cyclic flow variations are registered for 2 to 5 h and compared to pretreatment values.

Increase simple clamping off the coronary artery does not produce cyclic flow variations, additionally adrenaline (0.2 µg/kg) is infused into the peripheral vein, 30 minutes before and 30 minutes following Drug Administration. Also platelet activating factor (PAF 0.2 nmol/kg/min) when infused for a similar duration as adrenaline into the cannulated lateral branch of the coronary artery may produce cyclic flow variations. Cyclic flow variations are registered and compared to the drug treated group [16].

5. Electrical Stimulation-induced Coronary Thrombosis

Electrical stimulation can induce thrombosis in the coronary artery in pigs. An alteration in coronary blood flow with transient platelet aggregation at the site of coronary construction is assessed using this model.

German landrace Pigs (20-40 kg) are anesthetized with ketamine (2mg/kg, intramuscularly) additionally metomidate (10mg/kg intraperitoneally) and then maintain on artificial respiration through a tracheal tube using a positive pressure respirator. Through a left thoracotomy the heart is exposed at the 4^{th} and 5^{th} intercostal space and the pericardium is removed. A electromagnetic flow meter is placed on the proximal part of the left coronary artery to measure the coronary blood flow. A vanadium steel electrode is placed in the vessel with the intimal lining and connected with the Teflon coated wire of 9 Volt battery, a potentiometer and an amperometer [17]. Complete the electric circuit, a disk electrode is placed on the thoracic muscle layer. Intima is stimulated with 115 µA for 6 hours during which time in occluding thrombosis occurs. The test drug is administered either subcutaneously with the electrical stimulation or 30 minute following the electrical stimulation. Hemodynamic parameters systolic, diastolic, mean blood pressure and heart rate are measured by cannulating the femoral artery and connecting it to up pressure transducer. Left ventricular pressure, left ventricular and diastolic pressure, dP/dt Are measured by inserting a micro tip catheter why are the carotid artery retrogradely. ECG is also recorded using lead II. The time interval until the thrombotic occlusion of the vessel occurs and thrombocytes are determined. At the end of the experiment the animals are sacrificed with an overdose of anesthesia. Percent change in mean values for Occlusion time and thrombus size in drug treated groups is compared to the control group. Also change in hemodynamic parameters, cyclic number and cycle area after drug treatment is compared to pre-treatment values.

6. Myocardial Ischemia Preconditioning Model

Myocardial preconditioning brief duration of ischemia and reperfusion can reduce the damage produced by prolonged ischemia and reperfusion. Preliminary pre conditioning of the myocardium reduces infarct size, reduces leakage of cellular protein indicative of myocyte

death, induces post-ischemia ventricular function, as well as attenuates cardiac arrhythmia associated with frequent ischemia reperfusion. Fig. 3

Rabbits (New Zealand ring 3-4 kg) are anesthetized with ketamine (50 mg/ml) /xylazine (10mg/ml) at a dose of 0.6 ml/kg. The trachea is cannulated End animal maintained on artificial respiration (30 inflations per min). The right femoral artery and vein are catharized for measurement of arterial pressure and administration of drugs respectively. Hemodynamic parameters like systolic, diastolic, mean blood pressure, heart rate, left ventricular pressure; left ventricular and diastolic pressure and dP/dt are measured. A 4-0 suture is looped loosely around the marginal branch of left coronary artery to facilitate coronary occlusion during the experiment. Ischemic preconditioning is induced by tightening the loosening around the coronary artery for five minute and then losing to refuse the myocardium for 10 minute prior to our subsequent 30 minute occlusion. After 30 minutes in ischemia, ligature is released for 120 minute of reperfusion. Prior to 30 minute of occlusion the rabbits are selected to receive ischemic preconditioning, no preconditioning or preconditioning along with the administration of test compound. The animals are sacrificed after the reperfusion duration. Comparisons between systemic hemodynamic data and in fact size studies are analysed by ANOVA using statistical software. [18]

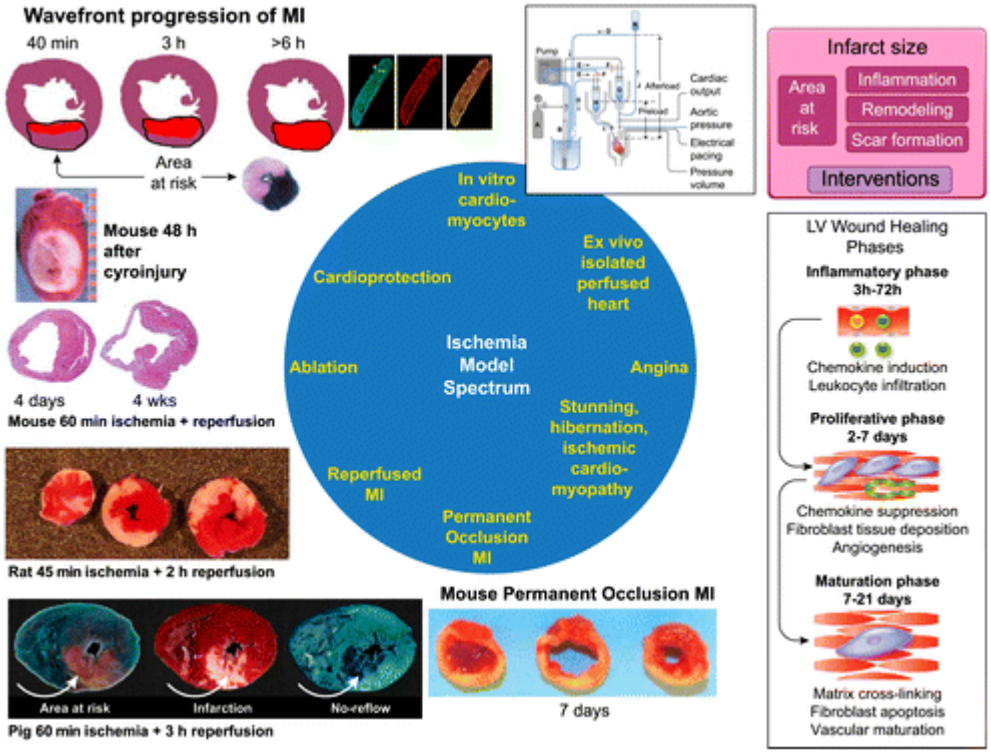


Fig.3 The spectrum of ischemia includes in vitro, ex vivo, and in vivo ischemia models with acute to chronic outcomes and durations ranging from brief to extended. The bottom-left pig portion was changed from Heusch et al [31]. Left ventricular, myocardial infarction, and MI.

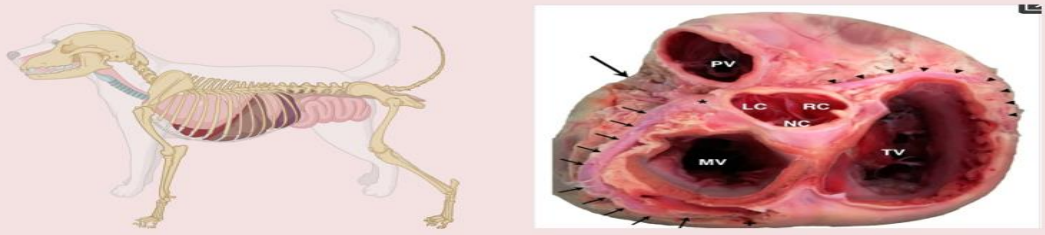
7. Models of Coronary Flow Measurement

These models are based on measurement of coronary outflow in open and closed chest animal preparations. Radius drugs can be screened for the empty anginal potential, on the pieces of the coronary artery dilating properties.

I. Coronary Inflow Measurement in Anesthetized Dogs

Dogs are anesthetized with pentobarbitone sodium (30-40 mg/kg) intraperitoneally and then maintained on artificial respiration using a positive pressure respirator. Through a left thoracotomy, the heart is exposed add the 4^{th} and 5^{th} intercostal space and the pericardium is removed. Through the jugular vein a catheter is inserted two cannulated the coronary sinus. In the in vitro studies cannula is inserted through an opening in the arterial appendage into the coronary sinus and drained to major coronary flow. Fig 4 Hemodynamic parameters systolic, diastolic, mean blood pressure and heart rate are measured by cannulating the femoral artery and connecting it to appreciate transducer. The test drug is administered through the other jugular vein. Change in coronary flow and hemodynamic parameters after test Drug Administration is compared to values before test Drug Administration.

Advantage of this method is that approximately 95% of the total coronary venous flow can be measured. [19] Disadvantage of this method are that only 60% of coronary flow returns through coronary sinus. No constant proportion between coronary winners outflow and coronary sinus flow is present. [20] Another method with slight modification in the above mentioned model could also be used to measure coronary blood inflow. In this method blow from superior vena cava is diverted to pulmonary artery and flow from right ventricle is measured.



- ***Coronary Inflow Measurement in Anesthetized Dogs:-*** **The left coronary artery (*) can be seen arising from the left-adjacent cusp (LC) of the aortic valve and dividing into the paraconal interventricular branch (large arrow) and circumflex branch (multiple small arrows), which wraps around the mitral valve (MV) annulus to descend as the subsinuosal interventricular branch (+). The right coronary artery (arrowheads) can be seen arising from the right-adjacent cusp (RC) of the aortic valve and passing to the right and caudal of the tricuspid valve (TV) annulus. PV = pulmonary valve; NC = non-adjacent cusp of aortic valve.**

Fig.4 The left coronary artery (*) can be seen emerging from the left-adjacent cusp (LC) of the aortic valve. It then splits into the circumflex branch (many small arrows) and paraconal interventricular branch (large arrow). The circumflex branch then descends as the subsinuosal interventricular branch (+) after wrapping around the mitral valve (MV) annulus. It is possible to show the right coronary artery (arrowheads) emerging from the aortic valve's right-adjacent cusp (RC) and traveling to the tricuspid valve's (TV) annulus on the right and caudally.

Pulmonary valve (PV) and non-adjacent aortic valve cusp (NC) [32]

II. Coronary Ouflow Measurement in Anesthetized Dogs

Radius devices have been used to eat for measuring coronary flow, of which in vogue is electromagnetic flow meter. It is used to measure:

A. Phasic flow: An additional probe displaced around the aorta to record changes in coronary flow with changes in aortic pressure.

B. Mean flow: Average flow through coronary arteries plus cardiac cycle. [18]

a. Electromagnetic Flow meter

Dogs are anesthetized with pentobarbital sodium (30-40 mg/kg) IP. Their trachea is cannulated and animals are maintained on artificial respiration using a positive pressure respirator. Through the left thoracotomy, hot is exposed at the 4^{th} and 5^{th} intercostal space and the pericardium is removed. Two poles of electromagnet are placed in opposite sides of coronary vessel. Distal to the Electro magnets, true chromium vanadium electrodes are placed adhering to the coronary artery. A magnetic field perpendicular two blood flow generates voltage in the conductor blood stream. It is picked up by electrodes, amplified and recorded. This method mostly records phase flow. Mean flow is recorded by electrical damping. Jugular vein is cannulated for the administration of test compound and carotid artery for measurement of blood pressure. Change in coronary outflow and hemodynamic parameters before and after test drug administration n are compared.

To avoid polarization add pick up electrodes, magnetic current is reversed by oscillator ether of square wave or sine wave type. Initially probes were big but with advanced technology, nowadays, small size probes are available. They are used mainly in chronic and anesthetized whole animal experiments by running lead wire to the skin. [21-22]

III. Other Models to Measure Coronary Flow

The following methods can be employed to major coronary flow:

1. Inert gas technique

Mainly helium is used as nitrous oxide. A mixture of room air an inert gas is inhaled (known quantity). A series of blood samples are withdrawn simultaneously from a peripheral artery (using needle) and coronary sinus/cardiac vein (using catheter). A-V difference is calculated. AV difference is the difference between the integrals of arterial and coronary sinus. [20] Blood flow through the organ/time is calculated as:

Amount of substance taken up in unit time/A-V difference

It can measure only mean flow but not regional flow. [23] It takes around 10 minutes for one determination.

2. Radioactive technique

The radio isotopes mainly used are ^{121}I, ^{3}H and rubidium. Isotopes are inhaled injected and change in rate over chest wall is measured using giega counter. By appropriate calculations a major of coronary flow can be determined. It has a close correlation with the above described

method. It is a fast and simple technique. [24]

3. Radioactive Microsoft sphere technique

This method determines original blood flow including distribution of coronary flow across the ventricular wall. A batch of radioactive microsphere (9-15 µDM) is suspended in a saline detergent solution and injected into the left atrium. Microspheres launch in only a few capillaries, so no damage/effect on flow is observed.

The number of spheres trapped/unit of myocardial tissue is directly proportional to myocardial blood flow. [25]

4. Thermo dilution technique

A catheter having an end hole is passed to the beginning of coronary sinus. A temperature sensor (thermometer) is placed further down the coronary sinus. Cold saline of known temperature is injected continuously through catheter diluted by coronary sinus blood flow. Modified temperatures are measured by thermometer. The temperature difference obtained is proportional to the blood flow. [26-27]

5. Coronary arteriography

Radio-opaque solutions are injected buy a catheter into a coronary artery at the root of aorta. Fig.5 With high speed cinematography clear visualization of coronary circulation before and after drug administration is done. This technique is the most direct, reliable and advanced method. [28] Angina is extremely variable pain syndrome with new anatomical pathophysiological entity. There is no direct relation between degree of pain and coronary insufficiency. Small areas of ischemia can produce discomfort equal to that of large area ischemia. [29] Also, all antianginal drugs must not be assumed to be coronary vasodilators nor all coronary vasodilators always relieve and angina. So, any compound screen positively for angina has to be tested cautiously in a well-planned and controlled clinical trial.

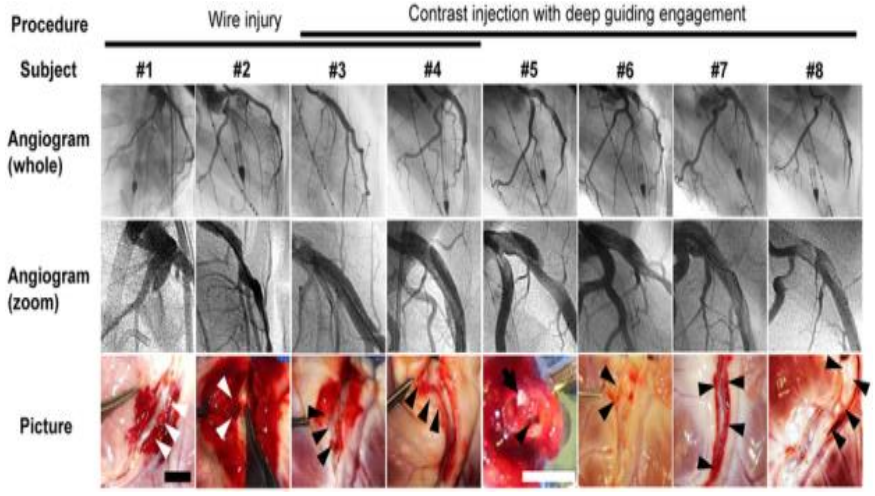


Fig.5 The top indicates the procedure for creating a coronary dissection. Following the development of coronary dissection of the left anterior descending artery (LAD), left coronary angiograms were obtained. The first angiogram was obtained using a 30° left

anterior oblique view. Angiograms #2–#8 came from a 90° anterior oblique view of the right. In LAD, zoomed angiograms reveal coronary dissections. The postmortem photos at the bottom show the LAD sliced open either cross-sectionally (#5) or longitudinally (#1–#4 and #6–#8). A dissected lumen is shown by black and white arrowheads. Relatively small dissections with periarterial hematoma are shown by white arrowheads. Dissections with smooth interior surfaces and hematomas around them are indicated by black arrowheads. None of the animals shown any signs of re-entering. A 24-gauge cannula with a white tip is inserted into the real lumen (black arrow) in Picture #5. The image scale bars are 10 mm for black (#1–#4) and 6–#8 and 5 mm for white (#5). Left coronary artery, or LCA [33]

3. Conclusion

In the study of cardiovascular biology, both in healthy and disease conditions, there is a vast spectrum of measurable indices of function and injury. This is particularly so in the case of myocardial ischemia, which contributes to the majority of death in both the developing and developed countries. Each experimental model, each species and each end point has its own inherent advantage and disadvantage and appreciating these will help the investigator select the most appropriate study system for the particular investigation under question.

The key feature of the in vitro isolated animal hearts is there global our original ischemia and reperfusion can be imposed at will and the contractile, biochemical, physiological and morphological consequences can be easily assessed. Furthermore, are various degrees of ischemia from zero flow to low flow can be induced and the rate and nature of reperfusion can be manipulated. On the negative side, in vitro preparations have limited laboratories lifespan that's rarely exceeds a few hours, they deteriorate progressively with time and cannot be used for chronic studies. Furthermore, they are deprived of their normal central neural connections; they are isolated from the systemic circulation and are no longer exposed to the host of peripheral neurohormonal factors. The in vivo, preparations allow measurement of hemodynamic functions such as ECG, ventricular wall motion, an ejection fraction that are of major diagnostic importance. They also provide scope for biochemical, pharmacological, morphological and physiological study. Markers of cardiovascular injury such as lactate cytokines, catecholamine, creatinine kinase are troponin-T from the peripheral circulation can be analyzed.

Nowadays, techniques such as Nuclear Magnetic Resonance (NMR) and Positron Emission Tomography (PET) have increased our ability to study in a non-invasive manner, some aspects of cardiac metabolism, function and coronary flow. However, invasive catheterization procedures will allow the collection of cardiac biopsies, measurement of arteriovenous difference and more sophisticated electrophysiological recording.

4. Limitations

The review titled "Unveiling the Abyss: Exploring Myocardial Ischemia and Reperfusion Consequences through IN VITRO and IN VIVO Animal Models for Targeted Screening of Anti-Anginal Drugs" presents valuable insights into the consequences of myocardial ischemia and reperfusion, utilizing both in vitro and in vivo animal models. However, several limitations

should be considered when interpreting the findings. Firstly, the specificity of animal models may not fully replicate the complexity of human responses, raising concerns about the direct translation of results to clinical settings. Ethical considerations surrounding animal experiments and potential variations in anatomy, physiology, and drug metabolism between species also impact the study's applicability to human scenarios. Additionally, the focus on specific pathways and potential oversimplification of in vitro models may limit the comprehensive understanding of drug interactions within a living organism. Variability in ischemia-reperfusion protocols, challenges in translating findings to human clinical trials, and potential publication bias further underscore the need for cautious interpretation. Despite these limitations, the study offers a foundation for understanding anti-anginal drug screening, emphasizing the importance of further research and validation in human clinical contexts.

Declarations

Ethical approval and consent to participate

Not needed.

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Conflict of Interest

There are no conflicts of interest in said manuscript. It is stated that there are no potential funding sources. Further the material collected for research has been taken form research articles that have obtained ethical approval from the country they belong.

The manuscript is in the accordance with the nature editorial policies (https://www.nature.com/nature-portfolio/editorial-policies) have included a statement in submitted manuscript confirming the study is reported in accordance with ARRIVE guidelines (https://arriveguidelines.org). Moreover it is review article so everything produces have proper referencing and citation (in text and figures also). The references inserted in text are approved by their local ethics committee of their country.

Availability of data and materials

Availability of data and materials statements: All authors declare that there are no raw stand supplementary files to be shared in the said manuscript.

We are able to confirm in this review article that ethically approved research review articles, references has been taken wile referring and citing form these research/review articles from the country they belong, for all the text and figures from where they are taken. I feel that it is relevant to our study (Properly ethically approved protocols, figures and citations).

Abbreviations

IPC Ischemic conditioning

CPCSEA Committee for the Purpose of Control and Supervision of Experiments on Animals

MI Myocardial Infarction

LAD left anterior descending artery

PV Pulmonary valve

ECG Electro cardio gram

Author Contribution

JT- Writing original draft

AKS- Review and supervision

RKS- providing information of various sources

SS- Provided figures

MB- Writing assistance

KG- Writing assistance

MZ- Correction of Grammatical mistakes

RPS- Supervision

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