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Experimental Methods of Inducing Myocardial Infarction and Its Pathophysiology: A Review

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ABSTRACT

Myocardial Infarction (MI) is one of the major health problem worldwide. There is a need to study MI in order to enrich one's knowledge about its pathophysiology and find better therapeutic options. In order to better understand MI, appropriate animal models need to be developed. Animal models have been used extensively in scientific research. This was intended to help researchers to understand the underlying pathophysiology of MI, compensatory mechanisms involved and efficacy of treatment. Although, it is difficult to create necrosis similar to those found in human heart, various large and small animals were used to create experimental model of MI. Each of these animals and method of selection offers various advantages and disadvantages in relation to clinical studies. The main objective of the present review is to discuss pathophysiology of MI and various methods used to induce MI in the animals. These methods may be beneficial for academicians and researchers concerned with the treatment of MI.

INTRODUCTION

Cardiovascular disease (CVD) is a major cause of global premature morbidity and mortality, with ischemic heart disease and stroke accounted for 24% of all deaths. In India, although data quality challenges exist, the number of years of life lost due to deaths from coronary heart disease before age 60 is projected to rise from 7.1 million in 2004 to 17.9 million in 2030.[1] This is much higher than expected for any other field in Asia and beyond. In India, CVD incidence in southern India is much higher than in northern India. Various cardiovascular risk factors such as dyslipidemia, hypertension, diabetes, obesity, food habits, smoking, and physical inactivity, etc., are contributing 50% to the development of CVDs.[2] World Health Organization estimates that cardiovascular disease kills 17 million people annually. It is well known that CVD is directly or indirectly associated with oxidative damage sharing a common molecular and cellular mechanism. Cardiovascular disease is a group of disorders of the heart and the vasculature includes high blood pressure, coronary heart disease, congestive heart failure, myocardial infarction, stroke and congenital heart defects.[3]

Myocardial infarction (MI) is one of the world's most life-threatening diseases. MI increases the influence of mortality and morbidity statistics in developing countries because of their lifestyle changes most commonly in urban regions.[4]

There is a need to study MI in order to enrich one's knowledge about its pathophysiology and find better therapeutic options. In order to better understand MI, appropriate animal models need to be developed.[5]Two nearly opposing aims serve experimental models of myocardial ischemia, both worth investigating. The first goal is to provide more mechanistic knowledge, from a clinical condition that cannot be accessed. The second objective is to provide mechanistic insight for translation into the clinical situation from an experimental study, and for this purpose experimental models must replicate the clinical setting as closely as possible.[6]

In animal species [7], various methods were tested using either chemical[8], open[9] or closed-chest catheter system [10].The main objective of the present review is to discuss pathophysiology of MI and various methods used to induce MI in the animals. Knowledge of such methods would be beneficial in specifying treatments in experiments related to the MI for researchers.

Myocardial infarction:

Myocardial infarction is an acute CVD that causes heart attack due to blood vessel narrowing that supplies blood to the heart. Therefore, it contributes to damage to the heart muscle. This is the myocardial disease caused by an imbalance between coronary blood supply and demand for myocardium. Generating toxic species of reactive oxygen produces radicals of superoxide, peroxide of hydrogen and radicals of hydroxyl. The primary symptom is pain in the chest and discomfort that can migrate into the shoulder, arm, back, neck and jaw. Often a severe pain left more than a few minutes in the middle tiny. Such an annoyance sounds like heartburn. Secondary signs include shortness of breath, nausea, exhaustion, cold, sweat, and fatigue.[11]

Pathophysiology of MI(Fig.1)

Myocardial infarction ("heart attack") is the irreversible injury of myocardium due to prolonged ischemia and hypoxia. MI is clinically characterized by the cardinal symptoms of varying degree of chest pain, sweating, lethargy, difficulty in breathing and sometimes the individual may become unconscious and may even die. CTnI and/or ECG defects and cardiac cell necrosis are the clinical hallmarks of MI. The acute myocardial infarction pathophysiology is very complex. Atherosclerosis is the prominent cause of MI. Coronary atherosclerosis is caused by subsequent breakdown of atherosclerotic plaque accompanied by coronary artery occlusion by thrombus formation. This leads to the sudden induction of ischemia throughout the occluded artery anatomic area. Under ischemic conditions, mammalian cardiomyocytes are unable to generate sufficient energy through oxidative phosphorylation in the form of ATP to stop the aerobic metabolism. As a result, myocardial ATP has produced reduced and anoxic oxidation products such as lactic acid in seconds.[12] These events lead to reactive oxygen species (ROS) production, reactive nitrogen species (RNS), calcium imbalance, and cell metabolism disturbance. ROS and RNS induce oxidative stress that transmogrifies cellular proteins and membrane permeability. [13] Ischemia decreases heart rate and arterial pressure results in baroreceptor stimulation followed by neurohumor compensatory mechanisms activation. The myocardial infarction-related pain and anxiety further aggravates the condition by further stimulating the sympathetic nervous system (SNS). The activation of the sympathetic nervous system contributes to systemic vasoconstriction and cardiac stimulation leads to increased demand for myocardial oxygen leading to higher myocardial hypoxia, expanding the infarcted area, precipitating

arrhythmias, and further impairing cardiac function. The effects of these insults are ultra-structural cardiomyocyte changes, sarcolemma destruction, DNA damage, necrosis of coagulation and ultimately death of cells. Cardio preventive approaches are not yet available but are one of current MI research priorities.

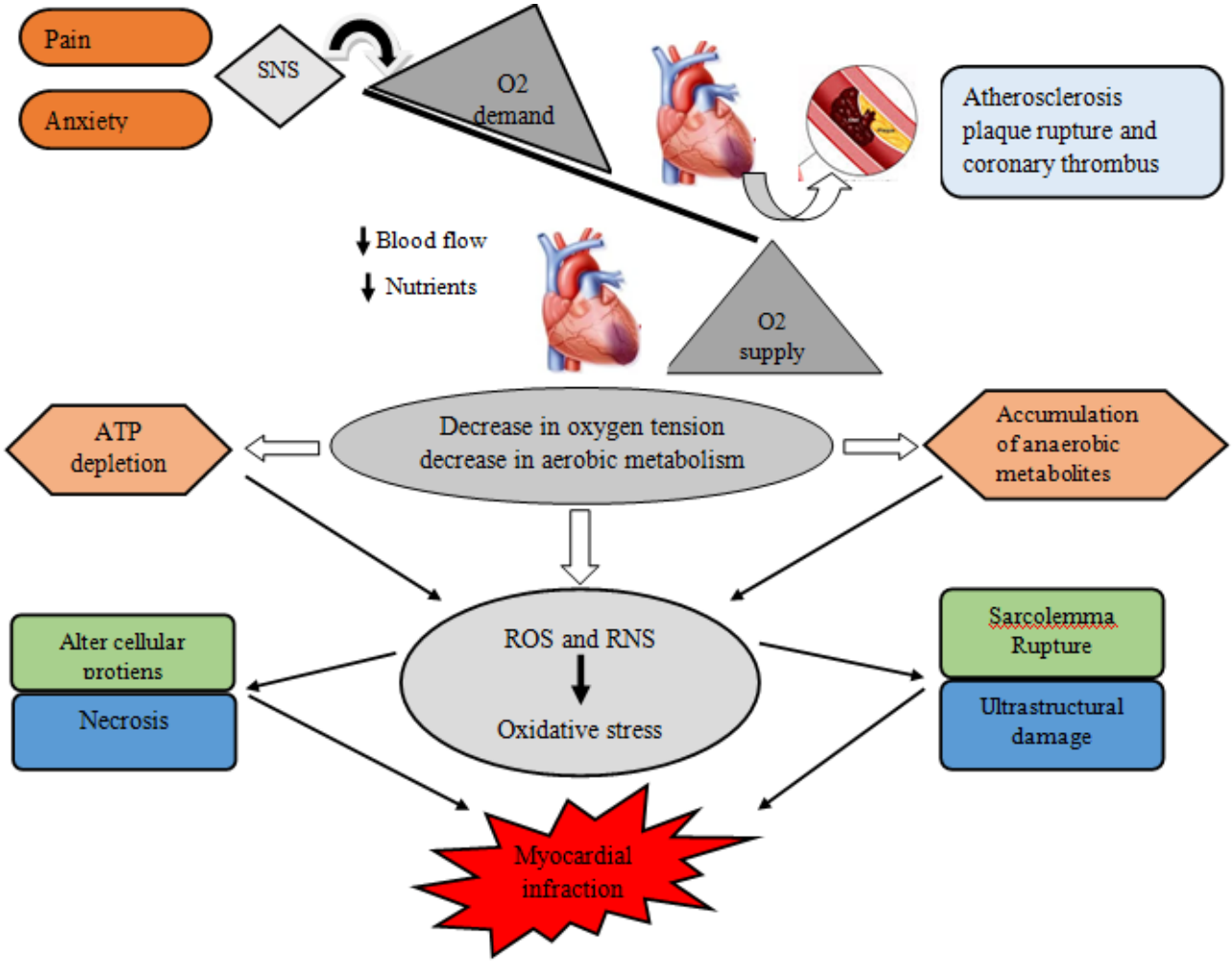

Figure No. 1:

Structural Changes Occurring During MI

Following ischaemia, the remaining blood vessel becomes dilated and collateral vessel develops. The least collateral blood flow, the larger is the infarct size.[14, 15] According to Snell, coronary arteries are functional end arteries and they are not able to provide adequate blood supply to the heart muscle if the large vessel is occluded.[16] However, according to other researchers, in one-third of patients with atherosclerotic lesions, sufficient coronary collateral circulations were functionally available to prevent the patients from myocardial ischemia during brief vascular occlusions.[14]

Structural Differences between the Human and Animal Heart

Even though the macroscopic features of the animal heart resembles that of humans, there are few differences in the gross anatomy of the structures. Due to these dissimilarities, experimental result in animal study may be challenging to be extrapolated, without the human clinical trial.[17] In addition, these differences make it difficult to design good animal model to mimic the pathophysiology found in human MI.[18]

The unique feature of the cardiac muscle cell in human and most animals is its poor capacity of regeneration. However, zebrafish, teleost fish and urodele amphibians are capable of heart regeneration after cardiomyocyte death. It was reported that the scar tissue formation following cryoinjury induced-MI in the zebrafish heart was replaced with new myocardium.[19]

The closer the size of the animals to humans, the more is the similarity of the heart. The body weight bears an inverse relationship with the heart rate.[17, 20] In larger animals like the whale, the heart rate is 10 beats/min[21] while in a small animal like mouse, the heart rate can reach up to 800 beats/min.[20]

Various patterns of collateral coronary network are observed in the animal species. These include guinea pig, cat and dog. They have good sizeable collateral coronary blood flow.[17] Hence, infarction does not occur after hours of coronary occlusion in the guinea pig.[15] In contrast, in animals such as rat, rabbit, pigs and non-human primates, there is sparse collateral coronary circulation. It was estimated that about 50% of ischemic cardiomyocyte became necrotic after 34 minutes of coronary artery occlusion as reported in the rabbits.[22] The pig is also vulnerable to ischemia as the infarction develops 20-35 minutes after ischaemia and it takes 60 minutes for the anterior left ventricular wall to become completely infarcted.[23] Even though primates such as humans, monkeys and baboons have sparse collateral coronary blood flow, the development of infarction was gradually slow when compared to rats, rabbits and pigs.[24, 25]

Methods used to Induce MI in Animal Model

Chemical agent

Isoproterenol (ISO): Isoproterenol-induced MI is a simple, fast and less complicated method which is used to induce MI in the animal model. This technique offers indirect approach to

produce MI as open surgery or thoracotomy is not required. Isoproterenol is a synthetic sympathomimetic catecholamine which produces 'infarct-like' myocardial necrosis resembling human myocardial infarction.[26]

Isoproterenol acts on β1 and β2 receptors in a non-selective way. β1 receptors primarily present in the heart, resulting in positive chronotropic, dromotropic and inotropic effects.[27] Due to its depressive effect on circulation, ischemia occurs when isoproterenol is given due to the imbalance between cardiac stimulation and decreased coronary blood flow. Isoproterenol oxidation products are also believed to be involved in the myocardial damage.[26]

Isoproterenol exhibits cardiotoxic lesions even at low dose 8 far below the level of the lethal dose.[26] The lesions produced are dose-dependent.[8] Low dose of Isoproterenol (32µg/kg) shows abnormal histopathological changes as early as three hours post-injection.[8] Moderate dose of Isoproterenol (85mg/kg) shows significant alteration in biochemical parameters and moderate necrosis in the heart.[28] The myocardial injury becomes more extensive with higher dose of isoproterenol.[29]

Isoproterenol produces infarct-like lesion similar to human MI.[30] Isoproterenol causes changes in the hemodynamic, biochemical, histopathological and oxidative stress markers.[31] Histopathological changes can be seen in subendocardial layer, myocardium of the apex, left ventricle, papillary muscle and interventricular septum.[8,32]Fig.2 summarizes the physiological effects brought about by isoproterenol in the myocardium.

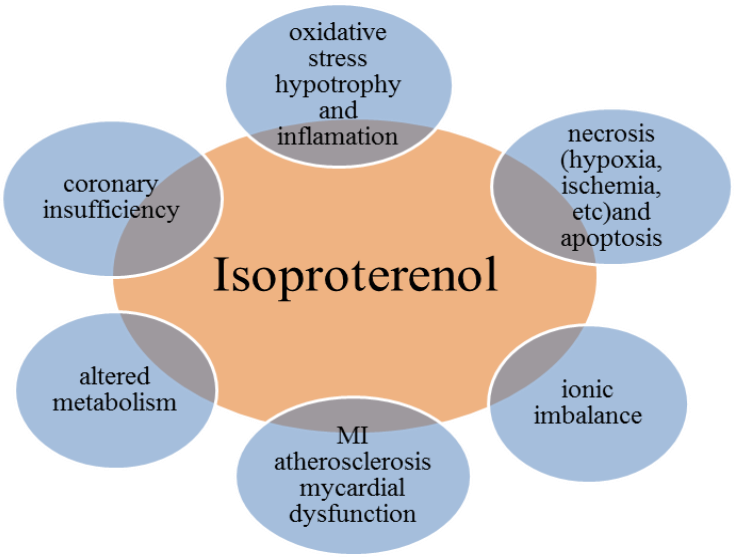

Figure No. 2: Physiological effects of isoproterenol in the myocardium

Isoproterenol-induced MI is simple to execute. It can be administered subcutaneously[8], intraperitoneally [32] or intravenously [33]. However, these different methods of administration affect drug metabolism and its conversion into inactive metabolites. According to previous researchers, the serum level of cardiac troponin was higher when isoproterenol was given subcutaneously compared to animal injected through intraperitoneal method. This suggests that the route of administration affects the metabolism of the drug.[34]

Isoproterenol-induced MI method has been used widely in the research on the protective effect of the natural product that can prevent myocardial necrosis damage [35] in the animals like mice [36], rats [31], and rabbits [32]. In order to evaluate cardioprotective effect of natural product, isoproterenol is given 85 mg/kg on two consecutive days in mice and rats to produce MI [37]. According to earlier research, the rabbits were induced with 3 mg/kg of isoproterenol injected intraperitoneally once prior to sacrifice [32]. Single dose of isoproterenol was enough to produce infarct-like necrosis in the heart but with repeated doses, the myocardial injury became more extensive.[36]

Coronary Artery Ligation

Coronary artery ligation provides precise timing, location and extent of the coronary event due to direct visualisation and observation of the procedure and targeted area of infarct [7, 8]. The procedure is initiated with administration of an anaesthetic agent to the animal, followed by securing the airway with the mechanical ventilator. The skin on the left side of the chest is incised, the muscles reflected and left thoracotomy is performed. The heart is rapidly exteriorized and specific site for ligation identified. The artery is then ligated with suture.[38]

There are various approaches which have been introduced to ligate the coronary artery. If Left Coronary Artery (LCA) or proximal part of left anterior descending branch of the Left Coronary Artery (LAD) near its origin, which emerged from conus arteriosus and left auricle are ligated, large MI is produced with 100% mortality rate, with more than 65% of infarction occurring at the left ventricle[39]. Ligation of the segment of LAD at 2 mm lower than the tip of the left auricle is the most preferred site to induce MI in animals[9]. This artery is also a predominant site for occurrence of atherosclerosis[40]. Permanent occlusion of LAD produces infarction in the antero-apical and lateral wall of the left ventricle and anterior interventricular septum and also global impairment of left ventricular function[41]. Immediately

after ligation of the artery, the ischaemic myocardium become pale in colour and abnormal ECG changes could be detected.[7]

In the small animals like mice, identification and ligation of LAD is difficult to be conducted as the coronary arteries lay within the myocardium, not on the epicardial surface. Furthermore, the same red color of coronary arteries and myocardium make them difficult to be distinguished. Application of soft pressure at the apex was required to induce slight paleness of the myocardium.[42]

Modification in the method of coronary artery ligation has been made to allow the study of reperfusion in the preclinical research. In human MI, myocardial reperfusion is performed to restore the balance between oxygen supply and myocardial demand, either by fibrinolytic therapy or Percutaneous Coronary Intervention (PCI). Timely reperfusion of the coronary artery after myocardial infarction is important to salvage the viable myocardium, limit infarct size, preserve LV systolic function and prevent the onset of heart failure.[43] However, with increasing duration and severity of ischemia, reperfusion of an ischemic area results in myocardial cell necrosis. Thus, reperfusion injury is a term referring to the occurrence of tissue damage when blood supply return after the period of ischaemia.[44]

To produce a reperfusion injury model, temporary occlusion of LAD is performed. It can be done by tying slipknot over the LAD[41]. After a certain period of time, the slipknot is released to allow the blood flow through the previously occluded artery. Hanging weight system is another alternative for an immediate release of coronary occlusion or reperfusion to avoid tissue trauma which can be obtained during manipulation of the knot.[45]

Even though open-chest surgical ligation of coronary artery offers direct access to the heart, there are many disadvantages related to surgical operation such as complicated surgical procedure, anaesthetic complications, long operative times and low success rate [38]. Furthermore, the surgical procedure itself affects the whole balance of bodily function and modifies local and systemic immunological and inflammatory responses.[7]

Closed-Chest Method to Induce Myocardial Infarction

In order to avoid complications related to the invasive procedure of open thoracotomy in the surgical open chest, percutaneous catheter method is introduced. This method is more suitable for large animals.[10, 46]

Coronary Artery Embolization

Percutaneous intracoronary embolization technique is a method to induce myocardial ischemia by catheterization of the coronary artery with the insertion of material into the coronary artery to form a thrombus. This model closely resembles human course of atherosclerotic disease superimposed by thrombus formation during MI event.[10, 46] It offers minimal invasive method as no procedure of thoracotomy and pericardium incision is involved. However, this method requires anticoagulant therapy to prevent blood clot formation during instrumentation[47] and an anti-arrhythmic protocol to prevent arrhythmia and ventricular fibrillation.[48]

Various materials have been inserted into the coronary artery for embolization such as sponge foam[48], various type of coils[8,10,49], polystyrene microspheres[47], alcohol injection[10] balloon catheter[49]. Sponge foam is easily available and inexpensive. The sponge foam is cut into small pieces of 5-7 mm of diameter. The fragmented sponge foam is placed over the wire and pushed into the targeted artery with the assistance of partially inflated balloon catheter. The mechanical obstruction is confirmed via angiography. Then the wire and balloon catheter are removed[48]. Delay ST-segment elevation of ECG may be observed 15 minutes after embolization[8].

The challenge in the percutaneous technique is the difficulty to control the exact location, length and duration of the coronary artery occlusion, and the overall volume of myocardial necrosis[8]. Occlusion of the proximal part of LAD resulted in severe heart failure, ventricular fibrillation with massive infarct, while embolization of distal part of LAD causes the disturbance of atrioventricular conduction system. Researchers suggested that the reliable area for inducing infarction with 25% decrease in LV ejection fraction is in the middle of the right coronary artery and circumflex coronary artery. Another challenge in percutaneous technique is that it requires advanced technical skills and highly trained personnel to manipulate the catheter for deployment of the material for embolization.[10]

Balloon Inflation Model: Due to its depressive effect on circulation, ischemia occurs when isoproterenol is given due to the imbalance between cardiac stimulation and decreased coronary blood flow**.** This simulates the effects of ischemia-reperfusion of percutaneous transluminal coronary angioplasty (PTCA) in patients with occlusion of coronary artery. (50) In swine, pigs or dogs, respectively, a diagnosis of ischemia accompanied by reperfusion is

possible by balloon inflation and deflation. Throughout clinical settings, Ischemia-reperfusion injury is known to be the major event during percutaneous therapy. The generation of free radicals originating from oxygen, such as superoxide and hydroxyl radicals, was thought to be a potential ischemia-reperfusion injury mechanism. The process involves several steps, anesthetized by ketamine, xylazine and pentobarbital in short animals. Following cannulation of the right carotid artery and jugular vein, a balloon dilation catheter is inserted in the proximal/distal portion of the LAD coronary artery under fluoroscopic or x-ray guidance. [51, 52] Occlusion (ischemic myocardium) caused by balloon swelling, while balloon deflation induces reperfusion (normal myocardium). Following inflation and before balloon deflation, the angiogram is performed to check the occlusion and the right balloon positioning. This approach has many advantages over surgical procedures, such as minimal surgical techniques and time specifications, strict control over the location and time of occlusion, and suitability for MRI studies.[53]

Ameroid Constrictor:

Litvak and colleagues first introduced this model in 1957. In this model mechanically chronic ischemia in porcine was produced by ameroid constrictor. The ameroid constrictor consist of casein (a hygroscopic material) enclosed within steel jacket.[54] The constrictor implanted around coronary artery under general anesthesia, casein by absorbing water swells and constricts the artery and produce ischemia over a period of weeks to a month.[55] LCx is the smallest coronary vessels in swine accounts for 20% of the blood supply to the left side of the myocardium and most commonly used coronary artery for occlusion.[56] Variables such as size, shape, and stiffness of material encasing the ameroid as well as the temperature of the surrounding fluid alter the constriction rate of ameroid. Because of the metal outer ring, the constructor will always be visible on x-rays of the chest. Ameroid constrictor is the most extensively used porcine MI models[56, 57] employed for preclinical testing of various cell therapies[58], gene therapy[59], pro-angiogenic therapies (recombinant vascular endothelial growth factor and basic fibroblast growth factor) and studying collateral circulation physiology.[54]

Cryoinjury Induced Myocardial Infarction

Cryoinjury is another method used to induce MI. This method has been used in various animals such as zebrafish [19], mouse[60], rat[61] and pig[62]. According to Van Den Bos EJ et al.,

this is an ideal model used for the assessment of therapeutic intervention to restore cardiac function or cardiac regeneration after a case of MI.[60]

Left chest incision is made following administration of anaesthetic agents. After left thoracotomy is performed, cryoprobe which has been precooled with liquid nitrogen is applied to the anterior left ventricular wall for few seconds. Therefore, anterior myocardial infarct occurs with modest adverse remodeling.[60] Van DenBos EJ et al., compared the outcome of the myocardial injury produced by cryoinjury with the coronary artery ligation method.[60] He concluded that both showed the similar loss of contractility and diastolic dysfunction, however, more modest LV remodeling observed in the cryoinjury model with no overt heart failure. No overt heart failure is seen due to a small necrotic lesion of disc-shaped, induced by the cryoprobe. The lesion produced has similar cellular patterns of coagulation necrosis of myocardial infarction. Thus, it is a suitable model used to demonstrate myocardial repair.[61]

The pathophysiology of MI in the cryoinjury method is different from other methods because acute cell death occurs following the cryoinjury without concomitant ischemia. It is due to mechanical forces induced by formation of ice crystals in the intra and extracellular space and in the vasculature.[60] This method has been used for research on intracardiac cell transplantation for myocardial repair.[61] It is because the transplanted cell is easily injected at its well-defined locations and presence of vascular reperfusion is beneficial for the cellular repair.[60]

Surgical and Interventional Methods used to Induce MI in Different Systems of the Body:

Surgical Method

A method used to induce myocardial infarction also recapitulated in the research related to an intestinal ischemic injury. Intestinal ischemic injury model is produced to mimic a clinical condition in which there is deprivation of intestinal blood flow occurring during acute mesenteric ischemia, necrotizing enterocolitis or volvulus. This model is used to study the pathophysiology of ischemia. In this model, Superior Mesenteric Artery is either ligated[63] or embolized.[64]

Interventional Method:

Ameroid Constrictor Method

Ameroid constrictor was used in a dog with a single portosystemic shunt for surgical management. Ameroid constrictor is favored because the single extrahepatic vessel is slowly occluded Prior to portal hypertension, it is important to allow the production of collateral shunting vessels.65 According to Murphy, the surgical occlusion of the single extrahepatic portosystemic shunt with the ameroid constrictor is as effective as the method of ligation with the benefit of shortening the operating time and the risk associated with shunting vessel ligation.[65]

Hydraulic Occluder Model:

It is a type of chronic ischemia that is close to the model of fixed stenosis. This model of persistent ischemia was developed by Bolukoglu and his co-worker, in which an adjustable hydraulic occluder was mounted around an epicardial coronary artery either proximal or distal to a myocardial doppler flow stenosis study.[66] Hydraulic occluder is a mechanical device made up of an expandable silicone membrane inside a stretch-resistant polyester collar. The diameter of the coronary artery is decreased by percutaneous, guided fluid injections into a device's subcutaneous injection tube, connected to a balloon. The hydraulic occluder can be successfully implanted for 32 months and can be adjusted for up to 12 months. It has several benefits such as allowing single implantation surgery in order to achieve incremental and complete vascular occlusion and the ease of reversing occlusion. With recent advances, there are few improvements in the Bolukogluand coworkers66 model, such as putting a hydraulic occlude on the proximal LCx and applying an ultra-sonic flow probe to calculate myocardial blood flow, providing accurate stenosis control and minimal coronary artery harm.[67]

Table No. 1: Summary of advantages and disadvantages of various methods used for inducing MI in experimental animals

Method	Advantages	Disadvantages
Isoproterenol	Simple technique [36] Non-surgical method, non-invasive[36] High success rate[36] Low mortality rate[36]	Indirect method - visualisation of the effect on the targeted area is impossible during the procedure[36]
Coronary artery ligation	Precise control of site of occlusion via direct observation on the ligated artery[7] Able to control exact location and extent of the infarct area Suitable for small and large animals [41,62]	High mortality rate[9] Require artificial ventilator[9] Surgical and anaesthetic related complication[9]
Intracoronary embolization	Minimally invasive technique [10] Non-surgical method [10] Suitable for large animal[10,46]	Difficult to control exact location, length and duration of coronary ligation[10] Require artificial ventilator, require advanced method, expensive equipment and highly trained personnel to manipulate the catheter[10] Require anti-arrhythmic protocol[47]
Ameroid contrictor	Gradual occlusion of the coronary artery[62] Study on development of collateral artery[62]	May require open thoracotomy[62]
Cryoinjury	Suitable for research on study of cell transplantation for myocardial repair, heart regeneration and cellular remodeling [19]	Require open thoracotomy [62]

CONCLUSION

Many animal models were designed to mimic clinical condition related to cardiovascular disease. A good animal model must be reproducible, able to provide consistent injury and can be translated to the human clinical condition. As per authors' review of literature, no statistical data has shown the best method to study all aspect of MI nor the most commonly used method to induce MI. Each method offers a different approach to induce ischaemia. For instance, occlusion of LAD, isoprenaline and cryoinjury are methods suitable to mimic acute MI, while ameroid constrictor and hydraulic occluder are more appropriate to study the effect of MI. To conduct MI in small animal, occlusion of LAD, isoprenaline, cryoinjury is the option of choice, whereas, for the large animal, occlusion of LAD, coronary artery embolization, balloon catheterization and ameroid constrictor are the list of selection. However, to study the effect of drug or natural product on MI, isoprenaline is the most commonly used method to induce MI. It is easy to execute. As isoprenaline acts non-selectively on β1 and β2 receptor, isoprenaline might as well affects other systems such as

central nervous system, gastrointestinal, endocrine and metabolic system. Future improvement of isoprenaline is required to ensure it acts solely on target organ. Coronary ligation of LAD is also widely used in the experimental animals. However, the technique for thoracotomy, severity of occlusion as well as the agent of anaesthesia and technique for ventilation should be improvised to minimise the complication related to surgical procedure and anaesthesia. Perhaps in future, improvement is needed in the methods to induce MI as it would help researchers immensely in the cardiovascular-related field.

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REFERENCES

1. Peiris D, Praveen D, Mogulluru K, Ameer MA, Raghu A, Li Q, Heritier S, MacMahon S, Prabhakaran D, Clifford GD, Joshi R. SMARThealth India: A stepped-wedge, cluster randomised controlled trial of a community health worker managed mobile health intervention for people assessed at high cardiovascular disease risk in rural India. PloS one. 2019 Mar 26;14(3):e0213708.
2. Rajasekhar D, Srinivasa RP, Latheef SA, Saibaba KS, Subramanyam G. Association of serum antioxidants and risk of coronary heart disease in South Indian population.
3. Mudagal MP, Goli D. Preventive effect of Rhododendron arboreum on cardiac markers, lipid peroxides and antioxidants in normal and isoproterenol-induced myocardial necrosis in rats. African Journal of Pharmacy and Pharmacology. 2011 Jun 30;5(6):755-63.
4. Mudagal MP, Swathi K. Role of quercetin and catechin in attenuation of isoproterenol induced bioflavonoids, myocardial infarction in rats. Asian Journal of Pharmacy and Pharmacology. 2018;4(5):662-7.
5. HALIM A, SYED SA, GHAFAR NA, JUBRI Z, DAS S. Induction of Myocardial Infarction in Experimental Animals: A Review. Journal of Clinical & Diagnostic Research. 2018 Nov 1;12(11).
6. Heusch G. Critical issues for the translation of cardioprotection. Circulation research. 2017 Apr 28;120(9):1477-86.
7. Katsanos K, Mitsos S, Koletsis E, Bravou V, Karnabatidis D, Kolonitsiou F, Diamantopoulos A, Dougenis D, Siablis D. Transauricular embolization of the rabbit coronary artery for experimental myocardial infarction: comparison of a minimally invasive closed-chest model with open-chest surgery. Journal of cardiothoracic surgery. 2012 Dec;7(1):16.
8. Zhang J, Knapton A, Lipshultz SE, Weaver JL, Herman EH. Isoproterenol-induced cardiotoxicity in sprague-dawley rats: correlation of reversible and irreversible myocardial injury with release of cardiac troponin T and roles of iNOS in myocardial injury. Toxicologic pathology. 2008 Feb;36(2):277-8.
9. Xu Z, Alloush J, Beck E, Weisleder N. A murine model of myocardial ischemia-reperfusion injury through ligation of the left anterior descending artery. JoVE (Journal of Visualized Experiments). 2014 Apr 10(86):e51329.
10. Isorni MA, Casanova A, Piquet J, Bellamy V, Pignon C, Puymirat E, Menasche P. Comparative analysis of methods to induce myocardial infarction in a closed-chest rabbit model. BioMed research international. 2015;2015.
11. Diwakar M, Subramaniam S, Subramaniam S. Curative role of modified Arjunarishta on isoproterenol-induced myocardial infarction in rats. International Journal of Research in Pharmaceutical Sciences. 2019 Jan 5;10(1):179-85.

12. Frangogiannis NG. The immune system and cardiac repair. Pharmacological research. 2008 Aug 1;58(2):88-111.
13. Ceconi C, Cargnoni A, Pasini E, Condorelli E, Curello S, Ferrari R. Evaluation of phospholipid peroxidation as malondialdehyde during myocardial ischemia and reperfusion injury. American Journal of Physiology-Heart and Circulatory Physiology. 1991 Apr 1;260(4):H1057-61.
14. Seiler C. The human coronary collateral circulation. Heart. 2003 Nov 1;89(11):1352-7.
15. Heusch G. The coronary circulation as a target of cardioprotection. Circulation research. 2016 May 13;118(10):1643-58.
16. Snell RS. Clinical Anatomy by Regions, 9th ed. Philadeplhia: Lippincott Williams & Wilkins; 2012.
17. Ibanez B, Heusch G, Ovize M, Van de Werf F. Evolving therapies for myocardial ischemia/reperfusion injury. Journal of the American College of Cardiology. 2015 Apr 14;65(14):1454-71.
18. Bracken MB. Why animal studies are often poor predictors of human reactions to exposure. Journal of the Royal Society of Medicine. 2009 Mar 1;102(3):120-2.
19. Chablais F, Veit J, Rainer G, Jaźwińska A. The zebrafish heart regenerates after cryoinjury-induced myocardial infarction. BMC developmental biology. 2011 Dec;11(1):21.
20. Milani-Nejad N, Janssen PM. Small and large animal models in cardiac contraction research: advantages and disadvantages. Pharmacology & therapeutics. 2014 Mar 1;141(3):235-49.
21. Race GJ, Edwards WLJ, Halden ER, Wilson HE, Luibel FJ. A large whale heart.Circulation.1959;19(6):928–32.
22. Miura T, Downey JM, Ooiwa H, Ogawa S, Adachi T, Noto T, Shizukuda Y, Iimura O. Progression of myocardial infarction in a collateral flow deficient species. Jpn Heart J. 1978;30(5):695–708.
23. Pich S, Klein HH, Lindert S, Nebendahl K, Kreuzer H. Cell death in ischemic, reperfused porcine hearts: a histochemical and functional study. Basic Res Cardiol. 1988;83(5):550–59.
24. Hedström E, Engblom H, Frogner F, Åström-Olsson K, Öhlin H, Jovinge S, Arheden H. Infarct evolution in man studied in patients with first-time coronary occlusion in comparison to different species - implications for assessment of myocardial salvage. J Cardiovasc Magn Reson. 2009;11(1):38.
25. Yang XM, Liu Y, Liu Y, Tandon N, Kambayashi J, Downey JM, Cohen MV. Attenuation of infarction in cynomolgus monkeys: preconditioning and postconditioning. Basic Res Cardiol. 2010;105(1):119–28.
26. Rona G. Catecholamine cardiotoxicity. J Mol Cell Cardiol. 1985;17(4):291–306.
27. Shukla AC, Steven JM, Jr FXM. Cardiac Physiology and Pharmacology. In: A Pract. Anaesth. Infants Child, Philadeplhia: Saunder; 2009, Pp. 361–395.
28. Peer PA, Trivedi PC, Nigade PB, Ghaisas MM, Deshpande AD, Goel RK. Cardioprotective effect of Azadirachta indica A. Juss. on isoprenaline induced myocardial. Int J Cardiol. 2008;126(1):123-26.
29. Ribeiro D, Buttros J, Oshima C, Bergamaschi C, Campos R. Ascorbic acid prevents acute myocardial infarction induced by Isoproterenol in rats: role of nitric oxide synthase production. J Mol Histol. 2009;40(2):99-105.
30. Rona G, Kahn DS, Chappel CL. Study on the healing of cardiac necrosis in the rat. Am J Pathol. 1961;39(4):473–89.
31. Shikalgar TS, Naikwade NS. Evaluation of cardioprotective activity of fulvic acid against isoproterenol induced oxidative damage in rat myocardium. Int Res J Pharm. 2018;9(1):71-80.
32. Pinelli A, Trivulzio S, Tomasoni L, Brenna S, Bonacina E, Accinni R. Isoproterenol-induced myocardial infarction in rabbits. Protection by propranolol or labetalol: a proposed non-invasive procedure. Eur J Pharm Sci. 2004;23(3):277–85.
33. Hoffbrand BI, Forsyth RP, Melmon KL. Dose-related effects of isoprenaline on the distribution of cardiac output and myocardial blood flow in conscious monkeys. Cardiovasc Res. 1973;7(5):664–69.
34. Brady S, York M, Scudamore C, Williams T, Griffiths W, Turton J. Cardiac Troponin I in isoproterenol-induced cardiac injury in the hanover Wistar rat: studies on low dose levels and routes of administration. Toxicol Pathol. 2010;38(2):287–91.
35. Upaganlawa A, Gandhi H, Balaraman R. Isoproterenol induced myocardial infarction: protective role of natural products. J Pharmacol Toxicol. 2011;6(1):1–17.
36. Brooks WW, Conrad CH. Isoproterenol-induced myocardial injury and diastolic dysfunction in mice: structural and functional correlates. Comp Med. 2009;59(4):339–43.

37. Hassan MQ, Akhtar M, Ahmed S, Ahmad A, Najmi AK. Nigella sativa protects against isoproterenol-induced myocardial infarction by alleviating oxidative stress, biochemical alterations and histological damage. Asian Pac J Trop Biomed. 2017;7(4):294–99.
38. Wang J, Bo H, Meng X, Wu Y, Bao Y, Li Y. A simple and fast experimental model of myocardial infarction in the mouse. Texas Hear Inst J. 2006;33(3):290–93.
39. Yeap XY, Dehn S, Adelman J, Lipsitz J, Thorp EB. Quantitation of acute necrosis after experimental myocardial infarction. Methods Mol Biol. 2013;1004:115–33.
40. Wasilewski J, Niedziela J, Osadnik T, Duszańska A, Sraga W, Desperak P, et al. Predominant location of coronary artery atherosclerosis in the left anterior descending artery. The impact of septal perforators and the myocardial bridging effect. Polish J Cardio-Thoracic Surg. 2015;12(4):379–85.
41. Gao E, Lei YH, Shang X, Huang ZM, Zuo L, Boucher M, et al. A novel and efficient model of coronary artery ligation and myocardial infarction in the mouse. Circ Res. 2010;107(12):1445–53.
42. Redel A, Jazbutyte V, Smul TM, Lange M, Eckle T, Eltzschig H, et al. Impact of ischemia and reperfusion times on myocardial infarct size in mice in vivo. Exp Biol Med. 2008;233(1):84–93.
43. Hausenloy DJ, Yellon DM. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. J Clin Invest. 2013;123(1):92–100.
44. Verma S, Fedak PWM, Weisel RD, Butany J, Rao V, Maitland A, et al. Fundamentals of reperfusion injury for the clinical cardiologist. Circulation. 2002;105(20):2332– 36.
45. Eckle T, Grenz A, Köhler D, Redel A, Falk M, Rolauffs B, et al. Systematic evaluation of a novel model for cardiac ischemic preconditioning in mice. Am J Physiol Circ Physiol. 2006;291(5):H2533–40.
46. Lukács E, Magyari B, Tóth L, Petrási Z, Repa I, Koller A, et al. Overview of large animal myocardial infarction models (review). Acta Physiol Hung. 2012;99(4):365– 81.
47. Hanes DW, Wong ML, Jenny Chang CW, Humphrey S, Grayson JK, Boyd ED, et al. Embolization of the first diagonal branch of the left anterior descending coronary artery as a porcine model of chronic trans-mural myocardial infarction. J Transl Med. 2015;13:187.
48. Dariolli R, Takimura CK, Campos CA, Lemos PA, Krieger JE. Development of a closed-artery catheter-based myocardial infarction in pigs using sponge and lidocaine hydrochloride infusion to prevent irreversible ventricular fibrillation. Physiol Rep. 2014;2(8):e12121.
49. Krombach GA, Kinzel S, Mahnken AH, Günther RW, Buecker A. Minimally invasive close-chest method for creating reperfused or occlusive myocardial infarction in swine. Invest Radiol. 2005;40(1):14–18.
50. Liu J and Li X (2012) Novel Porcine Models of Myocardial Ischemia/Infarction–Technical Progress, Modified Electrocardiograms Validating, and Future Application. Advances in Electrocardiograms–Clinical Applications:175-190.
51. Kren L, Meluzin J, Pavlovsky Z, Mayer J, Kala P, Groch L, Hornacek I, Rauser P and Vlasin M (2010) Experimental model of myocardial infarction: Histopathology and reperfusion damage revisited. Pathol. Res. Pract. 206:647-650.
52. Saeed M, Martin AJ, Saloner D, Do L and Wilson M (2010) Noninvasive MR characterization of structural and functional components of reperfused infarct. Acta. Radiol.51:1093-1102.
53. Krombach GA, Kinzel S, Mahnken AH, Günther RW and Buecker A (2005) Minimally invasive close-chest method for creating reperfused or occlusive myocardial infarction in swine. Invest. Radiol. 40:14-18.
54. Hughes GC, Post MJ, Simons M and Annex BH (2003) Translational physiology: porcine models of human coronary artery disease: implications for preclinical trials of therapeutic angiogenesis. J. Appl. Physiol. 94:1689-1701.
55. Elzinga WE (1969) Ameroid constrictor: uniform closure rates and a calibration procedure. J. Appl. Physiol. 27:419-21.
56. Roth D, Maruoka Y, Rogers J, White F, Longhurst J and Bloor C (1987) Development of coronary collateral circulation in left circumflex Ameroid-occluded swine myocardium. Am. J. Physiol. 253:H1279-H1288.
57. O'konski M, White F, Longhurst J, Roth D and Bloor C (1987) Ameroid constriction of the proximal left circumflex coronary artery in swine. A model of limited coronary collateral circulation. Am. J. Cardiovasc. Pathol. 1:69-77.

58. Fuchs S, Baffour R, Zhou YF, Shou M, Pierre A, Tio FO, Weissman NJ, Leon MB, Epstein SE and Kornowski R (2001) Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. J. Am. Coll. Cardiol. 37:1726-1732.
59. Mack CA, Patel SR, Schwarz EA, Zanzonico P, Hahn RT, Ilercil A, Devereux RB, Goldsmith SJ, Christian TF and Sanborn TA (1998) Biologic bypass with the use of adenovirus-mediated gene transfer of the complementary deoxyribonucleic acid for vascular endothelial growth factor 121 improves myocardial perfusion and function in the ischemic porcine heart. J. Thorac. Cardiovasc. Surg. 115:168-177.
60. Van Den Bos EJ, Mees BME, De Waard MC, De Crom R, Duncker DJ. A novel model of cryoinjury-induced myocardial infarction in the mouse: a comparison with coronary artery ligation. Am J Physiol Hear Circ Physiol. 2005;289:1291–300.
61. Murry CE, Wiseman RW, Schwartz SM, Hauschka SD. Skeletal myoblast transplantation for repair of myocardial necrosis. J Clin Invest. 1996;98(11):2512–23.
62. Hirano A, Fujita J, Kanazawa H, Kawaguchi S, Handa N, Yamada Y, et al. Cryoinjury-induced acute myocardial infarction model and ameroid constrictor-induced ischemic heart disease model in adult micro-mini pigs for preclinical studies. Transl Med Commun. 2017;2(1). doi:10.1186/s41231-017-0011-y.
63. Gonzalez LM, Moeser AJ, Blikslager AT. Animal models of ischemia-reperfusion induced intestinal injury: progress and promise for translational research. Am J Physiol Gastrointest Liver Physiol. 2015;308(2): G63-75.
64. Acosta S, Nilsson TK, Malina J, Malina M. L-Lactate after embolization of the superior mesenteric artery. J Surg Res. 2007;143(2):320–28.
65. Edwards R, Yousef Z, Rakhit R, Wright M, Rosenthal E, Redwood S, et al. A model of closed chest regional myocardial infarction in the rabbit: a clinically relevant in vivo assay system of post-infarction remodelling. Basic Res Cardiol. 2002;97(5):374-83.
66. Bolukoglu H, Liedtke AJ, Nellis SH, Eggleston AM, Subramanian R and Renstrom B (1992) An animal model of chronic coronary stenosis resulting in hibernating myocardium. Am. J. Physiol. 263:H20-H29.
67. Dean DA, Jia C-X, Cabreriza SE, D'ALESSANDRO DA, Dickstein ML, Sardo MJ, Chalik N and Spotnitz HM (1996) Validation study of a new transit time ultrasonic flow probe for continuous great vessel measurements. ASAIO J. 42:M671-676.