INTRODUCTION

Percutaneous coronary intervention (PCI) is an established mainstay in the treatment of coronary artery disease (CAD), especially acute ST-elevation myocardial infarction (STEMI). However, successful reopening of epicardial coronary artery does not always mean optimal myocardial reperfusion in a sizeable portion of patients, mostly because of no-reflow phenomenon. Myocardial no-reflow is associated with worse contractile dysfunction and higher incidence of complications [1] and is an independent predictor of death and myocardial infarction after PCI. [2]

In the setting of PCI, no reflow is best defined as inadequate myocardial perfusion in the target vessel territory without evidence of mechanical epicardial vessel obstruction. Angiographic no-reflow - defined as less than Thrombolysis in Myocardial Infarction (TIMI) 3 flow - occurs in 2% of all PCIs. [3]

The no reflow phenomenon is considered a dynamic process characterized by multiple pathogenetic components: (1) distal atherothrombotic embolization; (2) ischemic injury; (3) reperfusion injury; and (4) susceptibility of coronary microcirculation to injury. Each of these mechanisms is variably involved in the pathogenesis of no-reflow in the individual patient. [4]

Over the past years, many targets of therapy have been identified. In this context, prevention of distal embolization of thrombotic/plaque material has been the focus of trials employing thrombus aspiration. [5] Furthermore, several studies have assessed the beneficial effect on microcirculation of drugs given by either the systemic or intracoronary route, targeting different cellular types as platelets [6], or acting as vasodilators. [7]

Use of a vasodilator to treat no-reflow was first described by Wilson et al in 1989 who reported a favourable response to papaverine in a single patient. [8] Since this time the effect of several different vasodilators on no-reflow has been investigated including nitrates, verapamil, papaverine, adenosine, nicardipine and sodium nitroprusside, but interestingly a vasoconstrictor like epinephrine may also have a role through acting on beta-receptors to produce coronary vasodilatation. [9, 10]

Huang D et al compared the effect of 3 different vasodilators (diltiazem, verapamil, nitroglycerine) injected through selective microcatheter on coronary no-reflow & found that intracoronary infusion of diltiazem or verapamil can reverse no-reflow more effectively than nitroglycerin during primary PCI for acute myocardial infarction. The efficacy of diltiazem and verapamil is similar, and diltiazem seems safer.[11] However, no study has been conducted to compare the route administration of different vasodilators whether through a microcatheter or through the guiding catheter.

AIM OF THE WORK

To study the efficacy and safety of distal intracoronary drug delivery in treatment of no-reflow phenomenon in comparison to conventional intracoronary drug delivery through the guiding catheter.

Chapter One

CORONARY MICROCIRCULATION

I] Anatomy of coronary microcirculation

Coronary vasculature can be divided into two anatomically & functionally different segments. The extramural coronary vasculature consists of large conduit coronary arteries & veins running on the epicardial surface of the heart, whereas the intramural coronary vasculature consists of smaller coronary arteries, arterioles and capillaries along with their accompanying vein within the myocardium itself. [12]

The intramural coronary circulation represents a complex vascular tree with a large number of branching orders and arborizations supplying the various vascular compartments of the myocardium. Ventricular branches with a perpendicular take off run vertically from the epicardium to the endocardium. Some of these branches reach the endocardium while others bifurcate at variable depths within the myocardium. In contrast, atrial branches ramify on the external surface without penetrating walls of the rather thin myocardium. [13]

An important components of the intramural system are the arterial-arterial anastomoses, also termed coronary artery collateral vessels. These vessels link intramural branches originating either from the same coronary artery or from different

coronary arteries. Also communications can be observed between intramural vessels and cardiac chambers. [13]

Small coronary arteries (150-400 μm) run intramyocardially and are, similarly to epicardial vessels, characterized by a monolayer of endothelial cells embedded with the tunica intima, internal elastic lamina, and several layers of tunica media, external elastic lamina and tunica adventitia. [14]

Coronary arterioles (< 150 μm) display a single layer of endothelial cells, two to three circular and concentric layers of smooth muscle cells and an outer adventitia. [14]

Myocardial capillaries (5-10 μm) are thin-walled structures containing a single layer of endothelial cells frequently associated with pericytes. Capillaries run parallel to myocardial fibers; usually two capillaries can be observed surrounding a single myocardial fiber. This symmetrical pattern is similar to the capillary networks observed in the striated muscles. [14]

II] Physiology of coronary microcirculation

Flow across the myocardium depends on the *pressure gradient* between the aortic root and the right atrium and the *resistance* of the coronary vasculature. Under normal conditions, the driving pressure is fully maintained along the epicardial conduit vessels with little if any pressure loss in the

distal epicardial arteries. However, intra-coronary pressures decline along the microvasculature (with most of the pressure dissipating in the 300-100 μm diameter vessels) until reaching a pressure of 20-30 mmHg, still adequate to ascertain a gradient across the capillaries. Major determinants of the resistance to flow include the intra-vascular pressure, the velocity of flow, the length of the vessel and, importantly, its diameter. Applying the Hagen-Poisseuil equation, resistance to flow depends on the fourth power of the vessel diameter. [15]

$$R \propto \frac{\eta \cdot L}{r^4}$$

Resistance to flow (R) is directly proportional to the length (L) of the vessel and the viscosity (η) of the blood, and inversely proportional to the radius to the fourth power (r^4).

Auto-regulatory mechanisms coordinate the interaction between intra-coronary driving pressure and microvascular resistance in order to maintain adequate flow across the capillaries for substrate delivery and removal. Through this mechanism (also defined as ‘‘coronary autoregulation’’), decreases in driving pressure are compensated for by decreases in resistance and conversely, increases in driving pressure by increases in resistance so that flow remains constant for a given cardiac workload. This regulatory mechanism operates within the range of physiologic arterial pressures but fails during hypotension when flows become strongly dependent on the driving pressure. The coronary autoregulation operates between 40 to 130 mmHg. [16]

Changes in myocardial work and energy demand, are accompanied by proportionate changes in coronary and myocardial blood flow. Work-related flow increases are initiated by a ***metabolically-mediated*** decrease in microvascular resistance, possibly involving adenosine as a metabolite of adenosine monophosphate and causing vascular smooth muscle relaxation. The resulting flow increase is augmented by ***endothelium-dependent factors***; higher flow velocities exert greater shear-stress upon the endothelium with stimulation of the endothelial nitric oxide synthase (eNOS) and release of the smooth muscle relaxing nitric oxide (NO). In this scenario, endothelial cells closely interact with vascular smooth muscle cells in order to adjust the vessel diameter to changes in flow velocities (''*flow-mediated dilation*''), both at the level of the microvessels and the epicardial conduit vessels. [17]

Both, endothelial and vascular smooth muscle cells express adreno-receptors and thus respond to regional and systemic sympathetic stimuli. For example, local release of norepinephrine from adrenergic nerve terminals in the coronary arteries and release of catecholamines from the adrenal glands into the circulation during sympathetic stress or physical exercise lead to an ***α-adreno-receptor-mediated*** vascular smooth muscle constriction which under normal conditions is opposed by an ***adrenergically-mediated release of vasodilator substances*** (primarily NO) from the endothelial cell. This finely tuned interaction between vasoconstrictive (mostly vascular

smooth muscle dependent) and vasodilator forces (mostly endothelium related) recalibrates the vessel diameter against the flow velocity in order to maximally reduce resistance to flow. [15]

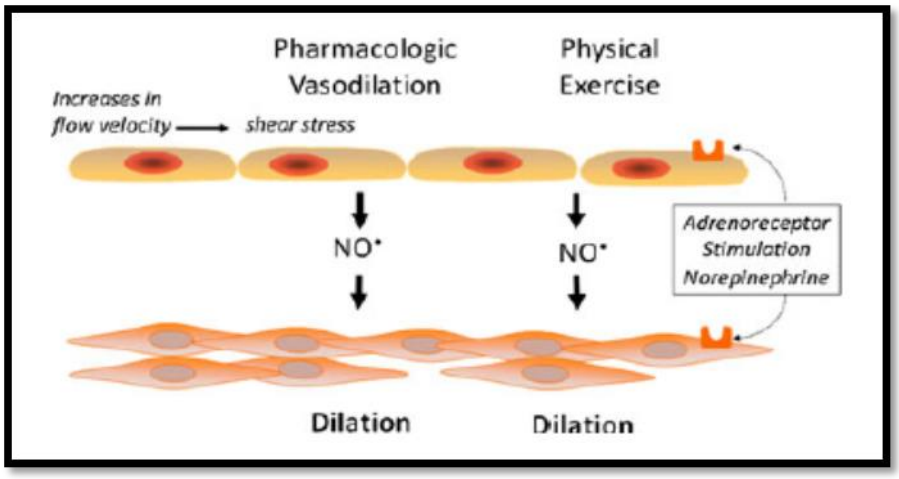

Figure (1): Schematic representation of the factors implicated in the interaction between endothelial and vascular smooth muscle cells during vasodilatation during physical stress [15]

Chapter Two

NO-REFLOW PHENOMENON

I] Historical background

The term no-reflow was first used by Majno and colleagues in the setting of cerebral ischemia in 1967. Brains of rabbits that suffered a brief 2 ½ minutes of ischemia had normal blood flow when the ischemia was relieved. When the rabbits were exposed to longer ischemic periods, normal flow to brain tissues was not restored, even after relief of the vessel obstruction. Prolonged ischemia resulted in significant changes in the microvasculature that interfered with normal flow to the brain cells. [18]

In 1974 Kloner *et al.* sought to find out whether the no-reflow phenomenon would be observed in ischemic canine hearts and whether it was related to microvascular damage. Dogs were subjected to 40 or 90 minutes of proximal coronary artery occlusion. When the coronary occlusion was relieved after 40 minutes of occlusion, the blood flow was restored to the damaged myocardium as assessed by markers of perfusion such as thioflavin S and carbon black. However, after 90 minutes of coronary occlusion, there was only partial restoration of blood flow to the myocardial tissue, despite virtual elimination of the coronary occlusion. Anatomic perfusion defects were prominent in the subendocardial myocardium when thioflavin S or carbon black was injected

into the vasculature after restoration of epicardial coronary flow. Electron microscopic examination of the cardiac microvasculature within the anatomic no-reflow zones revealed significant capillary damage in the form of swollen endothelium and intraluminal endothelial protrusions and, less commonly, intraluminal platelets and fibrin thrombi. These changes, coupled with interstitial and myocardial cellular edema, could compress the capillaries and be responsible for the no-reflow phenomenon. The longer ischemia lasts, the more likely the no-reflow phenomenon is to occur. [19]

The first clinical observation of coronary no-reflow was reported by Schofer *et al* in 1985 in patients with a first anterior myocardial infarction. [20] One year later, Bates *et al* reported the angiographic correlation of no-reflow as abnormally slow antegrade contrast filling in the infarct-related artery. [21]

In 1989, Wilson *et al.* concluded that intense microconstriction was a possible mechanism for the no-reflow induced ischaemia. It was notable that there was a lack of response to nitroglycerin and thrombolytic drugs but a favourable reaction to papaverine in one patient. [8] In 1991, Pomerantz *et al.* reported five more cases of no-reflow successfully treated by intracoronary verapamil. [22] The first clinical case of no-reflow during PTCA for acute myocardial infarction was reported by Feld *et al.* in 1992. [23] Thereafter, Piana *et al.* and Abbo *et al.* presented the results of two large

clinical series, where no-reflow was reported between 0·6% and 2% of all patients. [3, 24]

II] Definition and classification

No-reflow has been variously referred to as slow flow, slow re-flow, no-flow and/or low-flow. As these terms all describe, essentially, the same phenomenon and are all equally indicative of myocardial ischaemia. [25] A common definition that suits well may be adapted from the work of Kloner *et al*., who described the condition as the **inability to adequately perfuse myocardium after temporary occlusion of an epicardial coronary artery without evidence of persistent mechanical obstruction, thus implying ongoing myocardial ischaemia**. [19] Angiographic no-reflow is defined as less than TIMI 3 flow without angiographic evidence of mechanical vessel obstruction. [3, 24]

E. Eeckhout *et al*., proposed a new classification for no-reflow phenomenon. **Experimental no-reflow**, which occurs during experimental conditions, **myocardial infarction reperfusion no-reflow**, which occurs in the setting of pharmacological and/or mechanical revascularization for acute myocardial infarction and **angiographic no-reflow**, which occurs during PCI. [25]

III] Pathophysiology

Understanding the pathophysiology of the no-reflow phenomenon is the key for managing this condition. After prolonged cessation of coronary occlusion and restoration of blood flow to the epicardial coronary arteries, there is sufficient structural damage to the microvasculature to prevent restoration of normal blood flow to the cardiac myocytes. This may lead to inadequate healing of the cardiac scar. In addition, it may prevent the development of future collateral flow. [26] This phenomenon appears to be more pronounced in the subendocardium. It is more pronounced with longer periods of coronary occlusions. [19] No reflow appears to be a process rather than an immediate event that occurs at the moment of reperfusion. Experimental studies showed that the no-reflow area increases with time after reperfusion. Although it is clear that abnormalities at the level of the microvasculature caused the no-reflow phenomenon, the exact mechanism is uncertain; a variety of factors probably contribute to it. [26]

It is important to emphasize the human model, a critical duration (40 min to 90 min) of ischemia is needed in dogs before no-reflow occurs, whereas in humans, no-reflow generally occurs abruptly during coronary interventions following only a brief interruption (less than 60 s) of coronary blood flow. [19]

Also it is important to outline that no-reflow is only one of the four types of cardiac dysfunction (myocardial stunning,

no-reflow, reperfusion arrhythmias, and lethal reperfusion injury) caused by myocardial reperfusion as recently summarized by Yellon and Hausenloy, and it refers to the high impedance of microvascular blood flow encountered during opening of the infarct-related coronary artery. [27]

The multifactorial nature of no-reflow has been summarized recently into four interacting processes (**Fig. 2**): ischaemic injury, reperfusion injury, distal embolization, and susceptibility of microcirculation to injury. [4]

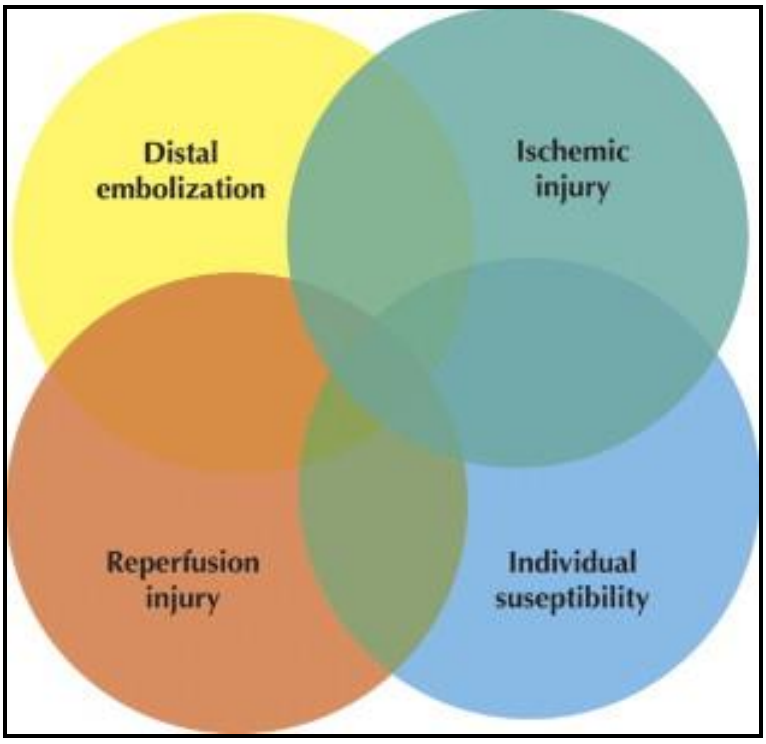

Figure (2): Four interacting mechanisms (distal embolization, ischemia-related injury, reperfusion related injury, and individual susceptibility to microvascular injury) are responsible for no-reflow phenomenon. The individual contribution of these mechanisms to the pathogenesis of no-reflow is likely to vary in different patients. [4]

A. Ischemic injury

The ischemia duration is the key predictor of the occurrence of no-reflow. Ischemia affects endothelial cells, causing the formation of intraluminal blebs, which obliterate vessel lumen, & expression of P-selectins. [28]

During ischemia there is an increase of intracellular content of sodium (Na^+) due to accumulation of hydrogen (H^+) that are exchanged by the Na^+/H^+ exchanger. The subsequent exchange of calcium ion (Ca^{++}) with Na^+ by sarcolemmal Na^+/Ca^{++} exchanger produces a Ca^{++} overload that triggers uncontrolled hypercontraction and stimulates opening of the mitochondrial permeability transition pores (MPTP), which further enhances calcium overload. Furthermore, Na^+ extrusion through Na^+/potassium (K^+) adenosine triphosphate (ATP)-ase is impaired and together with Ca^{++} accumulation leads to myocyte swelling, which contributes to subsequent rupture of the cell membrane when the extracellular osmolality is rapidly normalized by reperfusion.

Of note, cyclosporine, which blocks the MPTP, has been recently shown to reduce infarct size by 20% when administered intravenously in patients undergoing PPCI. [29]

Finally, ischemic pre-conditioning might also reduce infarct size by blockade of MPTP. [30]

B. Reperfusion injury

Reperfusion injury causes further obliteration of vessel lumen by neutrophil-platelet aggregates which in turn produce large amount of vasoconstrictors and inflammatory mediators. At the cardiomyocyte level, reperfusion stimulates the production of radical oxygen species (ROS) by mitochondria. In turn, ROS and rapid normalization of intracellular pH lead to severe opening of MPTP with subsequent cellular and mitochondrial swelling and cell disruption. Both cell swelling and interstitial oedema contribute to microvascular obstruction due to compression. Vasoconstriction also contributes to microvascular obstruction. [28]

Importantly, neutrophils have been shown to have a causative role in reperfusion injury. Indeed, neutrophils are a major source of oxidants in hearts reperfused in vivo after prolonged ischaemia. [4] Accordingly, a reduction in radical generation by R15.7, a monoclonal antibody against neutrophil CD18 adhesion molecule, was associated with a significant reduction in infarct size and no-reflow. [31] Adenosine also has been shown to inhibit neutrophil function and, in particular, neutrophil-mediated injury to endothelial cells. [32] Of note, it has been demonstrated in experimental models that exogenous or endogenous adenosine can inhibit neutrophil adhesion and injury to myocytes by an A2-mediated mechanism on cells activated with TNF-α. [33] Finally, the beneficial effects of abciximab in man may in part be mediated by neutrophil