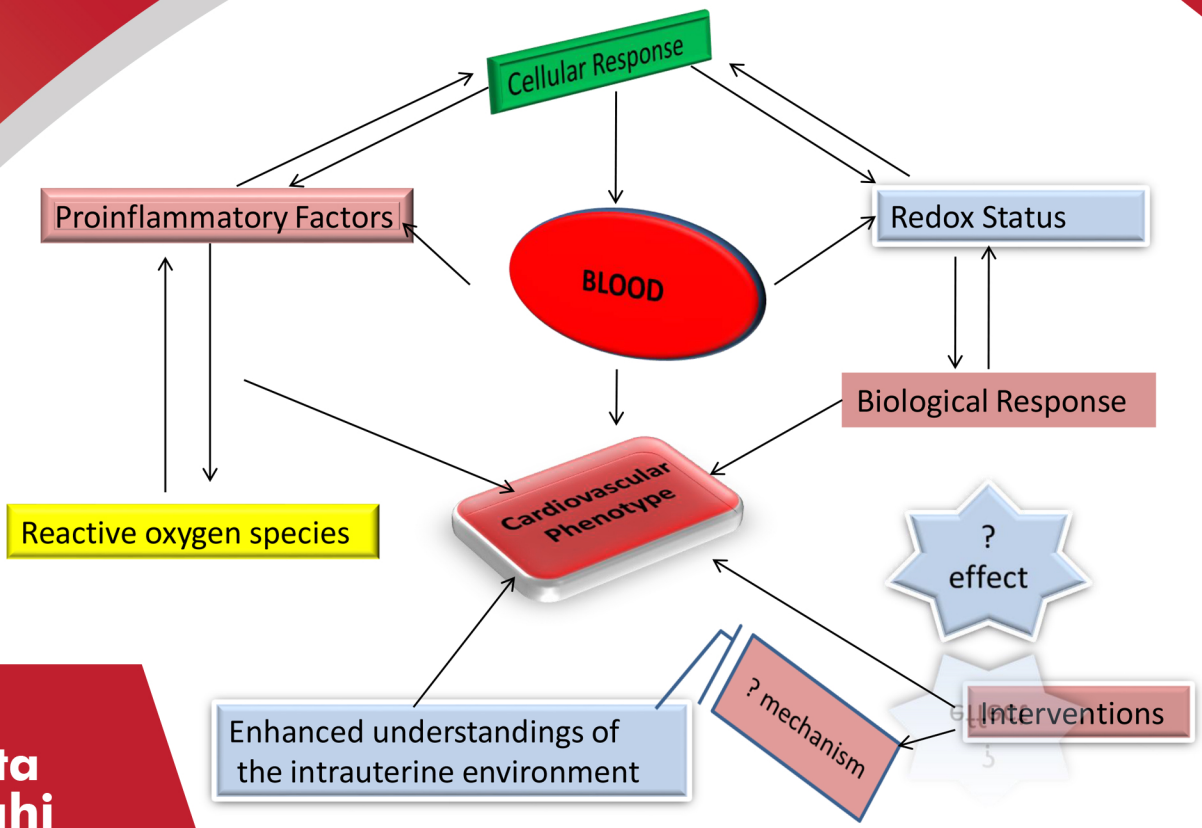
BLOOD OXIDANT TIES:
THE EVOLVING CONCEPTS IN MYOCARDIAL INJURY AND CARDIOVASCULAR DISEASE



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Blood Oxidant Ties: The Evolving Concepts in Myocardial Injury and Cardiovascular Disease

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FOREWORD

Oxidative stress is a major contributor to the pathology and pathophysiology of cardiovascular disease and other related human conditions; however, our understanding of the processes and mechanisms is still limited and warrants further studies and dissemination. Drs Elahi, Day-Walsh and Matata have undertaken a momentous task of assembling and organising topical and interesting topics for this book which starts with expositions in the basic science of Redox signaling, oxidative stress in cardiovascular disease (Bashir Matata & Maqsood Elahi), followed by oxidative stress and leukocytes activation (Bashir Matata & Maqsood Elahi). Dr. Priscilla Day-Walsh discusses the role of lipids and oxidation in cardiovascular disease which are hotly debated in the scientific community. Dr. Sferruzzi-Perri and colleagues elucidate the role of maternal factors in programming and progression of cardiovascular disease. The role of microbiomes in cardiovascular diseases (Day-Walsh & Shehata) has become a very important topic of research and medical practice which is a salient chapter of this book. The role of antioxidants in the modulation of inflammation and cardiovascular disease is explained in detail by Dr. Matata and Dr. Elahi. The last two chapters focus on therapeutic approaches focusing on oxidants, nitric oxide, role of nanotechnology (Gajardo *et al.* and Kogan *et al.* respectively) which advances our knowledge of their usefulness in clinical practice.

The authors of different chapters in this book are experts in their field at clinical and research levels and have crafted their chapters in a way that has appeal and usability at many levels for colleagues in clinical research, medical practice, and education (doctors, nurses and other health care practitioners). This book will serve as a treatise as an excellent update on the role of oxidation, oxidative stress, and antioxidants in cardiovascular disease and its prevention. Authors and Editors are congratulated to bring this important task and book to fruition.

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PREFACE

I would like to thank the authors for their wonderful contributions to this book and I believe this will significantly improve our understanding of the role of oxidative stress in the pathology and pathophysiology of cardiovascular disease and other related human conditions.

Oxidative stress is described as an intracellular and extracellular imbalance between the production of free radicals and the availability of appropriate antioxidant species and the effectiveness of these molecules to counter these species. Evidence from the literature suggests that oxidative stress is a major contributor to the pathogenesis and pathophysiology of cardiovascular disease.

A major contributor to oxidative stress is a family of reactive oxygen species (ROS) that are unstable molecules and contain one or more unpaired electrons in atomic or molecular orbitals that readily react with other molecules. The widespread production of ROS damages the plasma membrane and stimulates the release of various proinflammatory agents. Several proteins become denatured; for example, receptors, ionic channels, transporters, or components of transduction pathways through oxidation by ROS. Altered protein structure inhibits their functions leading to the disruption of vital cellular processes. Not only can ROS contribute to oxidative stress in general, but individual species of ROS also have their own distinct properties and may activate diverse signalling pathways. Activation of these signalling pathways leads to distinct pathological changes associated with cardiovascular disease cascades. For example, the onset of reperfusion injury is further exacerbated by the activation and infiltration of the infarcted area by polymorphonuclear leukocytes (PMNs). Several studies have identified the release of different leukocyte intracellular factors during PMN activation, such as selectins and b2-integrins to be related to the magnitude of tissue damage.

Oxidative stress is a major contributor to ischaemia reperfusion injury-mediated myocardial infarction. The onset of coronary ischemia deprives the heart muscles of nutrients and oxygen in the areas distal to the site of occlusion, rendering cardiomyocytes unable to undergo aerobic metabolism to support their energy requirements. Homeostatic intracellular signalling systems, such as the hypoxia-inducible factor (HIF) transcription factor cascade sense the low oxygen environment. This in turn stimulates the upregulation of numerous compensatory mechanisms which are ultimately involved in elevating anaerobic glycolysis and promoting angiogenesis and vascularization. The increased anaerobic metabolism increases the production of lactic acid hence metabolic acidosis. This leads to myocyte death and the expansion of the size of the original area of the infarct. Under normal aerobic conditions, the myocardium generally metabolises relatively high levels of adenosine triphosphates (ATP). In contrast, during ischemia, the shift in energy production to glycolysis results in the inefficient production of ATP and constitutes a pathological feature, and if not reversed, early may lead to complications such as heart failure and ischemia-induced atrial or ventricular fibrillation. Despite the widespread use of fibrinolytic agents and new types of angioplasty procedures for the treatment of myocardial infarction, often new sets of complications persist. These include the occurrence of extensive tissue injury caused by myocardial reperfusion through the reintroduction of oxygen to previously ischemic tissues because of the excessive generation of reactive oxygen species (ROSs) and depletion of antioxidants.

Interestingly, more recent evidence has shown that the activation of proinflammatory cells in particular PMN, is closely linked to the activation of other cells involved in the inflammatory response. For example, during myocardial ischemia-reperfusion injury, it has been shown that

the activity of neutrophils is also modulated by lymphocytes and macrophages.

Myocardial reperfusion injury (MIR) accounts for about half of the final damage caused by cardiac ischemia-reperfusion. Considering the importance of clinical models involving cardiac ischemia-reperfusion, such as acute myocardial infarction (AMI) and cardiac surgery (CS), many therapies have been developed to prevent MIR. However, despite encouraging results in experimental studies in this innovation journey, no therapy has shown substantial clinical benefits. The view of the therapeutic role of antioxidants as one of the potential treatments to prevent MIR in these settings arises from the known burst of reactive oxygen species occurring from the onset of myocardial reperfusion. Although antioxidants have shown some clinical benefit in the prevention of reperfusion arrhythmias in CS, no antioxidant alone has successfully reduced the final infarct size or caused improvement in other long-term clinical outcomes. Therefore, recently novel strategies have been developed that combine nanotechnology as a mode of delivery of antioxidant therapies that have shown to result in better clinical benefits throughout the innovation journey.

This book provides an in-depth analysis of the relationship between oxidative stress and cardiovascular as follows:

1. The current knowledge of the biological concept of oxidative stress and its role in the pathogenesis of the cardiovascular disease.

2. The interaction between oxidative stress, activation of different leukocytes, and the release of factors involved in the generation of myocardial ischaemia reperfusion (MIR) injury.

3. The interaction between oxidative stress and lipid metabolism *i.e.* metabolomics as primary drivers for myocardial injury and cardiovascular disease.

4. The interaction between oxidative stress and *in-utero* programming of the cardiovascular disease.

5. The role of antioxidants in the prevention of cardiovascular disease and acute myocardial infarction.

6. An update on the stage of development of new nanotechnologies-based antioxidants therapies for the prevention of myocardial injury.

The authors are collectively known for their extensive contributions in the field of oxidative stress and cardiovascular disease research and would like to dedicate this book to the support of different funding organisations that have contributed to their work over the years. In addition, the authors acknowledge and wish to thank work colleagues, friends, and families collectively for their support especially when balancing daily responsibilities and finding sufficient time to write their contributions to this magnificent book in the middle of the covid-19 pandemic.

We welcome reader's suggestions and comments for future improvements.

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CHAPTER 1

Redox Signaling, Oxidative Stress in Cardiovascular Disease –basic Science and Clinical Aspects

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Abstract: The generation of certain species of biomolecules described as reactive oxidant species (ROS *e.g.*, superoxide, $O_2^{\bullet-}$; hydrogen peroxide, H_2O_2; hydroxyl radicals ($OH^{\bullet}$)) and reactive nitrogen species (RNS *e.g.*, peroxynitrite, $OONO^-$; nitric oxide, $^{\bullet}NO$) is a critical step in health and disease . These species play critical roles in cell defences in both animals, and plants. They also perform an important function in the regulation of key cellular signalling pathways such as cell differentiation, proliferation, migration, and apoptosis (commonly described as redox signalling pathways). The imbalance between the levels of ROS and RNS generated to that of antioxidant species may lead to oxidative stress and biomolecular damage, especially in situations where the latter are depleted. Redox biology and oxidative stress are particularly important in ischaemia-reperfusion associated diseases in particular the pathogenesis of cardiovascular disease (CVD). CVD is a major cause of mortality on a global scale, although the exact mechanisms underlying the pathological process are not fully understood. It is believed that ROS play a pivotal role in the progression of CVD. In particular, recent evidence suggests that the development of atherosclerosis is modulated by ROS and influenced by other factors such as inflammatory responses, disturbed blood flow, and arterial wall remodelling. This chapter provides an overview of the pathways of oxidative stress and redox-regulated signalling underlying the genesis and progression of cardiovascular disease.

Keywords: Cardiovascular disease, Oxidative stress, Redox signalling pathways, Reactive nitrogen species, Reactive oxygen species.

INTRODUCTION

Oxidative stress is a biomolecular characteristic associated with the disruption of the balance between the process of production of reactive oxidant species (ROS)/reactive nitrogen species (RNS) and the effectiveness of the antioxidant

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defence systems in favour of the former [1 - 3]. There is evidence to suggest that many drug-induced complications and diseases are associated with an adverse increase in the levels of ROS and RNS, at the same time, antioxidant defences are ineffective [4, 5].

Reactive Oxygen Species and Reactive Nitrogen Species

There are different types of ROS that at low-level production, maintain redox regulation of physiological signalling broadly divided into oxygen radicals (*e.g.*, superoxide, $O_2^{-\cdot}$), hydroxyl radical ($^{\cdot}OH$), and peroxynitrite ($ONOO^-$) or non-radicals (*e.g.*, hydrogen peroxide (H_2O_2)). Commonly, superoxide is formed by the one-electron donation to molecular oxygen (Equation 1) in a reaction catalysed by Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH) oxidase (Equation 2), with electrons supplied by NADPH [6]:

$$O_2 + e^- \longrightarrow O_2^{-\cdot} \quad \text{(superoxide)} \qquad \textbf{(1)}$$

$$2O_2 + NADPH \longrightarrow 2O_2^{-\cdot} + NADP^+ + H^+ \qquad \textbf{(2)}$$

Superoxide is a short-lived molecule that acts locally and at low pH, it spontaneously dismutases to hydrogen peroxide (equation 3):

$$2O_2^{-\cdot} + 2H \longrightarrow H_2O_2 + O_2 \qquad \textbf{(3)}$$

A number of endogenous free radical scavengers keep levels of hydrogen peroxide in check. Hydrogen peroxide is more stable than superoxide and can diffuse widely and accounts for the majority of distinct effects on redox regulation and underlying established specificity of ROS signalling [6]. However, higher levels of hydrogen peroxide generate hydroxyl radicals in the presence of metal ions *via* the Fenton or Haber-Weiss reactions [6]. Hydroxyl radicals are extremely reactive [6] and would react with the first molecule they contact with and also have a very short half-life.

Nitric Oxide Synthases and the Generation of Reactive Nitrogen Species

Reactive nitrogen species (RNS) also play an important role in redox biology and pathophysiology of diseases with nitric oxide (NO), laying a central role [7, 8]. NO is a vasodilator and inhibitor of platelet aggregation, leukocyte adhesion, and smooth muscle cell proliferation [7, 8]. Endothelial NO modulates vascular tone

and blood pressure by cyclic guanosine monophosphate (cGMP)–stimulated smooth muscle relaxation, inhibition of platelet aggregation and adhesion to the endothelium, and prevention of smooth muscle proliferation (prevents vascular wall thickening) [9].

Three distinct mammalian isoforms of NO synthase (NOS) enzymes responsible for NO synthesis have been identified *i.e.*, neuronal (nNOS), endothelial (eNOS), and inducible (iNOS) [10]. The isoforms are products of different genes and have different localization and regulation properties, with distinct differences in the rate of NO production by these enzymes and the inhibition of NO production by different inhibitors of these enzymes [10, 11].

All three NOS enzymes can catalyse the 5-electron oxidation of L-arginine to L-citrulline [11, 12] in a process that involves the oxidation of Nicotinamide adenine dinucleotide phosphate (NADPH) to the reduced form $NADP^+$. Molecular oxygen acts as a co-substrate for the reaction and tetrahydrobiopterin (BH4), flavin adenine dinucleotide, Flavin mononucleotide and haem are the cofactors involved in the catalytic process [11, 12].

The production of NO occurs twice as fast for nNOS as it does through eNOS although the output by eNOS is significantly higher compared with nNOS [13, 14]. Both eNOS and nNOS are constitutive enzymes (cNOS) with NO production by eNOS and nNOS being calcium-dependent where a calcium/calmodulin complex is needed for NOS activation [13]. iNOS, which is calcium-independent, is a very high output but a slow rate of enzyme activity. NO is produced by cNOS in a pulsatile manner, whereas the production of NO by iNOS is continuous [15]. Unlike nNOS and iNOS, eNOS adjusts in response to a change in the environment or status and is often targeted to the plasmalemma terminal caveolae [15]. The interaction of eNOS with some domains of caveolin-I causes the eNOS to become inactive [13]. However, interaction with the calcium/calmodulin complex with eNOS permits electron transfer through the enzyme and the oxidation of L-arginine [13].

Under some pathological conditions, the vascular endothelium becomes dysfunctional and generates a much greater amount of O_2^- than the normal endothelium. These conditions favour the production of high concentrations of superoxide that reacts with NO to form peroxynitrite, which is directly cytotoxic and in turn reduces NO bioavailability [6]:

$$NO^{\cdot} + {}^{\cdot}O_2^- \longrightarrow OONO^- \text{ (peroxynitrite)} \qquad \textbf{(4)}$$

CHAPTER 2

Oxidative Stress and Leukocytes Activation - The Two Keystones of Ischemia/Reperfusion Injury during Myocardial Infarction, Valve Disease, and Atrial Fibrillation

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Abstract: Oxidative stress is a major contributor to ischaemia reperfusion injury-mediated myocardial infarction. Coronary ischemia deprives the heart muscles of nutrients and oxygen in the areas away from the site of arterial blockage, rendering cardiomyocytes unable to utilise aerobic metabolism to support their energy requirements. Homeostatic intracellular signalling systems, such as the hypoxia-inducible factor (HIF) transcription factor cascade, sense the low oxygen environment. This in turn stimulates the upregulation of numerous compensatory mechanisms which are ultimately involved in elevating anaerobic glycolysis and promoting angiogenesis and vascularization. The increased anaerobic metabolism increases the production of lactic acid hence metabolic acidosis. This leads to myocyte death and the expansion of the size of the original area of the infarct. Under normal aerobic conditions, the myocardium generally metabolises relatively high levels of adenosine triphosphates (ATP). In contrast, during ischemia, the shift in energy production to glycolysis results in the inefficient production of ATP and constitutes a pathological feature, and if not reversed early, it may lead to complications such as heart failure and ischemia-induced atrial or ventricular fibrillation. Despite the widespread use of fibrinolytic agents and new types of angioplasty procedures for the treatment of myocardial infarction, often new sets of complications persist. These include the occurrence of extensive tissue injury caused by myocardial reperfusion through the reintroduction of oxygen to the previous ischemic tissues because of the excessive generation of reactive oxygen species (ROSs) and depletion of antioxidants. Widespread production of ROS damages the plasma membrane and stimulates the release of various proinflammatory agents. Several proteins become denatured for example receptors, ionic channels, transporters, or components of transduction pathways through oxidation by ROS. Altered protein structure inhibits their functions leading to the disruption of vital cellular processes. The onset of reperfusion injury is further exacerbated by the activation and infiltration of the infarcted area by polymorphonuclear leukocytes (PMNs). Several studies have

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identified the release of different leukocyte intracellular factors during PMN activation such as selectins and b2-integrins to be related to the magnitude of tissue damage. Some studies have shown that antagonists for leukocytes intracellular factors such as selectins abrogate PMN activation and reduce the infarct size.

More recent publications have shown that PMN activation is closely linked to the activation of other cells involved in the inflammatory response. For example, during myocardial ischemia–reperfusion injury, it has been shown that the activity of neutrophils is also modulated by lymphocytes and macrophages. This chapter summarises the interaction between oxidative stress, activation of different leukocytes and the release of factors involved in the generation of reperfusion injury.

Keywords: Cardiovascular disease, Ischaemia reperfusion injury, Leukocyte activation, Myocardial infarction, Oxidative stress.

INTRODUCTION

Globally, cardiovascular disease (CVD), specifically coronary artery disease (CAD) is a leading cause of death and disability [1, 2]. CAD develops over decades and is worsened by risk factors such as abnormal lipid profile (dyslipidaemia), smoking, hypertension, diabetes, abdominal obesity, alcohol consumption, psychosocial factors, and lack of consumption of fruits and vegetables and sedentary lifestyle which are common factors worldwide in both sexes and all ages [3]. In addition, dyslipidaemia, which is associated with elevated serum concentrations of low-density lipoprotein (LDL) particles, is a major risk factor for CAD through the progressive formation of arterial plaque. Indeed, the evidence suggests that oxidative stress and inflammation are associated with the instability of atherosclerotic plaque and the development of acute coronary syndrome (ACS) [4].

The blood flowing through the vascular endothelium causes frictional forces termed as shear stresses [5]. The properties of the vascular endothelium are influenced by the characteristics of the type of blood flow through it. Characteristically, high blood flow leads to an inert and anti-inflammatory phenotype [5]. In contrast, the vascular endothelium in areas of turbulent blood flow exhibits properties termed as “activated phenotype” [5]. The “activated phenotype” is characterized by high endothelial expression of proinflammatory factors that attract the recruitment and translocation of stimulated monocytes [5]. The presence of activated monocytes facilitates their transition into monocyte-derived macrophages that fervently engulf endothelial deposits and particles that could potentially trigger further local inflammatory processes. The local inflammatory process in the vessel wall stimulates the recruitment of smooth muscle cells, the formation of a fibrous cap that prevents the rupture of low-density lipoprotein-rich plaque ultimately causing thrombogenesis [5].

In addition, evidence [5] suggests that the formation of atherosclerotic plaques through the progressive formation of more cholesterol deposits at the sites of low or/and turbulent blood flow) *i.e.*, vessel curvatures, branch points, and bifurcations) as illustrated in Fig. (**2.1**), is further triggered by the presence of oxidised low-density lipoproteins (LDL) [5]. The formation of lipid-laden dysfunctional macrophages contributes to the destabilisation and rupture of the atherosclerotic plaques and the formation of circulating thrombi [5].

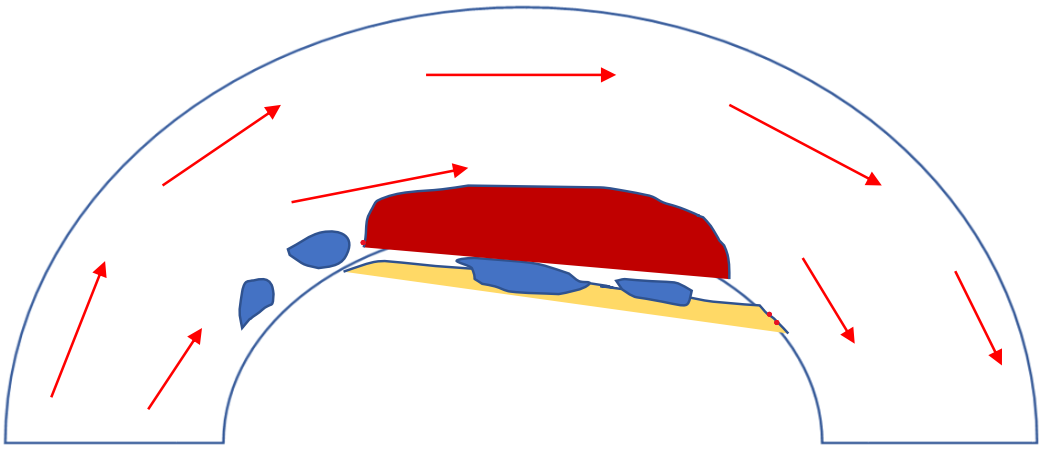

Fig. (2.1). This is a schematic representation of atherosclerotic-plaque formation at the sites of sluggish or turbulent blood flow.

Oxidative Stress as a Putative Mechanism for Atherogenesis and Myocardial Injury

Many intracellular processes including mitochondrial electron transport chain (ETC), nicotinamide adenine dinucleotide phosphate oxidase (NADPH), nitric oxide synthase, and xanthine oxidase are putative mechanisms for the excessive generation of reactive oxidant species (ROS) [6]. The accumulation of ROS in tissues overwhelms local and circulating antioxidant factors leading to oxidative stress [6 - 9]. Antioxidant mechanisms also exist within the cell mitochondrial compartment and in the cytosol that actively prevents the accumulation of reactive oxygen species through a neutralisation process [10, 11].

Examples of antioxidant factors include uncoupling proteins, thioredoxins, glutathione, peroxidase, and superoxide dismutase [10, 11]. Although physiological concentrations of ROS have been shown to exert beneficial effects [11, 12], increased quantities lead to oxidative stress if there is an imbalance between the production and clearance of ROS [12, 13]. Under conditions that favour excessive production of ROS, ultimately cell damage and death may be the

CHAPTER 3

Lipids, Oxidation, and Cardiovascular Disease

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Abstract: Cardiovascular disease (CVD) remains one of the leading causes of morbidity and mortality worldwide with altered lipid metabolism as an important risk factor. In the current chapter we discuss processes involved in lipid metabolism, the past and emerging roles of various lipoprotein cholesterol molecules in this process, free fatty-acid metabolism and the various mechanisms of lipid oxidation and their impact on vascular physiology in health and disease. We further describe the role of reverse cholesterol transport (RCT) in the elimination of lipids as bile acids, and finally discuss current clinical interventions based on emerging technologies against dyslipidemia, hypertriglyceridemia, and CVD.

Keywords: Apolipoprotein, Lipid oxidation, Remnant cholesterol, Statins, Triglycerides.

INTRODUCTION

Poor diet, smoking and sedentary lifestyle as well as genetics correlate with CVD risk and other metabolic diseases (MD) such as diabetes and stroke. Traditionally, increased cholesterol with high levels of low-density lipoprotein cholesterol (LDL-C) and low levels of high-density lipoprotein cholesterol (HDL-C) has been used as a marker for dyslipidaemia and CVD. Accordingly, current therapeutic interventions have focused on lowering LDL-C and increasing HDL, although it is becoming clear that the association between high HDL and the reduced risk of CVD is much more complex and poorly understood. Mechanistically, oxidised LDL (OxLDL) plays a key role in the pathogenesis of CVD where a variety of lipid peroxidation products interact with proteins and DNA to elicit both anti-atherogenic and pro-atherogenic signalling pathways. Oxidation products can be formed from free polyunsaturated fatty acids (PUFAs) or from PUFAs present on phospholipids and cholesterol esters *via* enzymatic and non-enzymatic pathways.

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On the other hand, the mechanism such as reverse cholesterol transport (RCT) can mediate the elimination of lipids to prevent lipid accumulation and atherosclerosis. In this chapter, we will discuss the oxidation of various lipids and their effects on various signalling pathways associated with the pathogenesis of CVD. We will further discuss current clinical interventions and their contribution to our current understanding of CVD risk and possible future drug targets for CVD.

PATHWAYS INVOLVED IN CHOLESTEROL METABOLISM AND TRANSPORT

Lipids are critical to human physiology, contributing to mammalian cell membrane integrity and function, energy metabolism through mitochondrial beta-oxidation, and as co-factors, electron carriers, and signalling molecules. The main types of lipids include free fatty acids (FFA), sterols, triglycerides composed of three fatty acids attached to a glycerol molecule, and phospholipids. Phospholipids contain a hydrophilic phosphate group and two molecules of fatty acids which in some cases may be attached to molecules such as choline, serine, and ethanolamine forming phosphatidylcholine (PC)(lecithin), phosphatidylserine (PS) and phosphatidylethanolamine (PE) (cephalin), respectively. PC can also be further hydrolysed to form lysophosphatidylcholine (lysoPC). Sterols include cholesterol and cholesterol esters, bile acids and steroid hormones. With the exception of free fatty acids which are carried through the circulation bound to albumin, cholesterol, triglycerides, and phospholipids are transported through the body *via* lipoproteins of various sizes and densities. After fat consumption, lipids such as triglycerides produced by oral and intestinal lipases are emulsified by bile salts and transferred into intestinal enterocytes [1]. Cholesterol is transported into the enterocytes by the Niemann-Pick C1-Like 1 (NPC1L1), although this can be counteracted by the ATP-binding cassette (ABC) transporters G5 and G8 (ABCG5/ABCG8) which form an obligate heterodimer and eliminate cholesterol from enterocytes [2, 3]. In enterocytes, lipids are packaged into lipoproteins and transported into circulation. Seven classes of lipoprotein particles have been described as forming the exogenous and endogenous system for lipid transport and metabolism [1] (Fig. **3.1**).

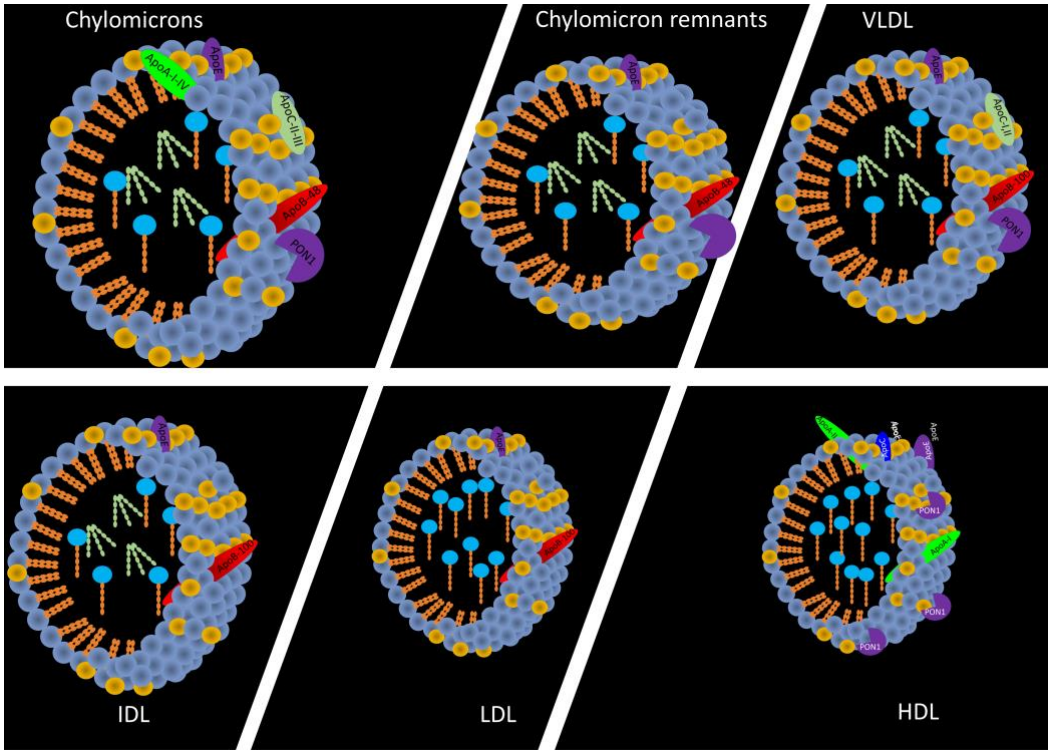

Fig. (3.1). Type of lipoproteins. VLDL (very low-density lipoproteins), IDL (Intermediate density lipoproteins), LDL (low-density lipoproteins) and HDL (high-density lipoproteins).

Chylomicrons

The exogenous system includes chylomicrons and chylomicron remnants. Chylomicrons (75-1200nm diameter) are formed in the intestines from dietary lipids and are composed of mainly APo-48 as the core structural protein as well as Apo-E, Apo-C-(I, II, III, IV) and Apo-A-(I, II, IV, V). Owing to their role in the transport of dietary lipids from the gut to the liver and peripheral organs, the size of chylomicrons is determined by dietary lipid content. Once in the circulation, HDL plays a role in the exogenous system by donating apoproteins such as apolipoprotein C-II (APOC-II) and apolipoprotein E (APOE) to chylomicrons to form mature chylomicrons.

Chylomicron Remnants

Mature chylomicrons are further processed by endothelial lipoprotein lipases which remove some TGs from chylomicrons leaving behind, pro-atherogenic

CHAPTER 4

Maternal Factors and the Placenta: A Programming Environment for Cardiovascular Disease

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Abstract: The risk of chronic diseases such as cardiovascular diseases (CVD) during postnatal life is not only determined by environmental factors in adulthood but also by *intra-uterine* and early life environment according to the Developmental Origins of Health and Disease (DOHaD) concept. Environmental insults including poor nutrition, oxygen availability, maternal stress, alcohol, smoking and drugs, can compromise the maternal uterine and lactational environment leading to short- and long-term adaptations in offspring physiology or programming. While short-term predictive adaptive responses may offer immediate survival value, they can lead to irreversible changes in embryonic/fetal tissues and organs mediated through changes in cellular signalling and metabolic pathways, as well as endocrine axes governing whole-body function. The capacity for developmental adaptation may also be determined by both genetic susceptibility and epigenetic mechanisms, as well as environmentally induced changes in maternal microbiome structure and composition. Basic mechanisms involved in the development of CVD have been described in previous chapters. Here we will focus on how mechanisms involved in developmental programming may contribute to CVD in adulthood.

Keywords: Developmental programming, Epigenetics, Foetal growth, Hormones, Hypoxia, Metabolism, Nitric oxide, Placenta.

INTRODUCTION

The risk of chronic diseases including CVD is not only determined by lifestyle and environmental factors in adulthood but also by factors prior to conception, during pregnancy, and in early life according to the Developmental Origins of Health and Disease (DOHaD) concept. These factors include maternal nutrition,

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environments such as altitude, lifestyle as well as maternal health status, and gut microbiota, with their influence on the offspring depending on and being mediated by both genetic and epigenetic mechanisms.

DOHaD is a concept that proposes that exposure to environmental insults during critical windows of development may have later consequences for an individual's long-term health including the risk of developing cardiovascular diseases (CVD) [1]. Developmental windows span conception, organogenesis, fetal growth and early postnatal development. Environmental insults including altered nutrition, oxygen availability, maternal stress, alcohol and drugs, can compromise the maternal uterine and lactational environment with consequences for the developing offspring. In utero, the fetus may respond to the poor environmental condition to increase its chance of immediate survival but also to prime itself for improved viability if a similar environmental disruption reoccurs later in life. This is termed the short-term predictive adaptive response and includes changes in metabolic and endocrine function and the sparing of growth of particular organs or systems over others in the foetus [2]. One example of this is the fetal brain-sparring response that is seen in response to low levels of oxygen/hypoxia during development and is conserved across species, from the reptilian and avian embryo to the mammalian fetus, including in humans [3]. The fetal brain-sparring response refers to the redistribution and prioritisation of blood flow from the peripheral tissues to the brain and is dependent on adaptations in the function of the cardiovascular system. For example, under acute hypoxia, the heart rate is reduced to limit oxygen consumption [4] and more blood is therefore, able to refill the heart so that cardiac output and perfusion pressure are maintained [5]. While short-term predictive adaptive responses may offer immediate survival value, they can lead to irreversible changes in embryonic/fetal tissues and organs by altering gene expression, signalling pathways, as well as cell lineage differentiation, proliferation, and function. These alterations can amplify as the offspring grows and impose a survival disadvantage if the future environment is not compatible with these 'programmed' changes in tissues/organs. Programming effects depend on the nature, exposure period and the duration of environmental insult. They are also influenced by the timing and growth rates of individual tissue/organ systems and hence sensitivity to the insult, in the offspring [6]. Due to programmed changes in organ function, an offspring can be more prone to develop non-communicable diseases including diabetes mellitus, respiratory diseases and CVDs during postnatal life.

The Maternal Environment and Programming of Cardiovascular Disease

In humans, a number of studies have explored the association between maternal nutritional state and cardiovascular health outcomes of their offspring. Children

from obese and overweight mothers have a higher risk of increased birthweight and cardiovascular abnormalities, such as high blood pressure in young adulthood compared to those of mothers with a normal body mass index [7, 8]. Children of overweight mothers also show structural changes including increased left ventricle thickness at birth, as well as diastolic dysfunction and increased stroke volume at one year of age [9]. Other studies also show that maternal obesity and overnutrition are linked to an increased risk of coronary heart disease [10], peripheral artery diseases such as narrowing of blood vessels and adverse cardiovascular events, such as myocardial infarction, stroke and angina in adulthood [11 - 13]. Children of mothers who were hypercholesterolaemic during pregnancy also had an increased risk of atherosclerotic lesions at an early age and aortic fatty streak formation in adult life [14]. Epidemiological work on humans and studies on experimental animals have supported the importance of DoHAD in the development of CVD. These works have provided evidence that the structure and function of the heart and blood vessels can be programmed by the *in-utero* environment. In particular, gestational insults such as maternal nutritional alterations, low oxygen availability and endocrine manipulations during critical phases of somatic development are linked to the elevated risk of CVD in the offspring's later life. As well as confirming the link between poor *in utero* environment and CVD, animal models have been indispensable in providing mechanistic data highlighting changes in gene expression and hormonal signalling in the heart and vessels in the offspring.

In support of human observational data, studies on rodents have shown that maternal obesity induced by an energy-rich diet is associated with programmed structural and phenotypical changes in the cardiovascular system of the offspring. In particular, maternal obesity is associated with increased heart mass and the development of pathological cardiac hypertrophy (increased cardiomyocyte area and size), systolic dysfunction, diastolic dysfunction, cardiac sympathetic dominance, and high blood pressure in the offspring [15 - 22]. Also changes in aortic stiffness, aortic cellular composition (decreases in endothelial layer volume and vascular smooth muscle cell number), atherogenic-like lesions [15, 23], abnormal fatty acids composition in the aorta, programmed changes in the heart of offspring from obese mice are also coupled with the re-expression of cardiac fetal genes in adulthood [18, 20, 24]. Furthermore, hormonal changes such as the reduction of endothelial-derived hyperpolarizing factor by the mesenteric arteries [25] increased noradrenaline contractile response [21] and a decreased endothelium-dependent relaxation in response to acetylcholine [15, 19, 24] have been observed in high fat fed and over-nourished mothers.

Insufficient nutrient provision to the fetus during gestation is also linked to CVD risk. In humans, initial studies by Barker and Osmond highlighted an association

CHAPTER 5

The Emerging Role of Microbiome in Cardiovascular Diseases

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Abstract: Cardiovascular disease (CVD) has become one of the leading causes of poor lifelong health and well-being. Meanwhile, the microbiome has emerged as one of the key determinants of human cardiometabolic homeostasis and the risk of CVD. While the clustering of the microbiome into phylum ratios or enterotypes has been correlated to specific disease phenotypes and population characteristics, the composition of a typical 'healthy human microbiome' is yet to be defined. Several population-based studies have shown an association between certain microbial species with CVD, although the inconsistencies have made the interpretation of such associations very difficult as it is not possible to pinpoint microbial populations associated with CVD. However, here we discuss current evidence on the role of the microbiome and its metabolites on the risk of CVD. We further explore current clinical studies investigating prebiotics and probiotics as potential therapeutic targets to modulate the microbiome for the benefit of the host to prevent cardiometabolic diseases. We highlight that further work to understand the role of specific species/sub-species, strains and polymorphisms within those strains, as well as microbial gene expression profiles and their respective metabolites is required. Coupled with high-resolution metagenomics and metabolomics as well as a unified approach in characterising common gut microbial communities based on global population observations, this would provide better indicators of disease phenotype and a better framework for a divergence to dysbiosis. The challenges that will need to be overcome in order to define a healthy microbiome and advance the clinical use of prebiotics and probiotics as well as faecal microbiota transplantation will also be discussed.

Keywords: Bile acids, *Bacteroidetes*, Cholesterol, Enterotypes, Faecal microbiota transplantation, *Firmicutes*, Microbiota, Prebiotics, Probiotics, Short chain fatty acids, TMAO.

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INTRODUCTION

Cardiovascular disease (CVD) has become a global epidemic and is one of the leading causes of compromised lifelong health and wellbeing. Susceptibility to CVD is determined by social-economic factors, nutrition, lifestyle, environment, and genetics. The recent COVID-19 pandemic has highlighted the role of the host-microbiome interaction in human physiology in health and disease with emphasis on metabolic syndrome and inflammation. The human body contains trillions of microbiomes/microbiotas consisting of bacteria, archaea, viruses, and microeukaryotes. Some of these are deleterious to human health (pathobionts) while others are commensals (symbionts) that contribute to the maintenance of human physiological homeostasis as well as provide colonisation resistance. Preventing the colonisation of opportunistic pathogens, commensal bacteria are responsible for the production of essential substrates such as vitamins and amino acids. Certain microbes can produce beneficial metabolites such as short chain fatty acids (SCFA) but also sequester essential dietary precursors such as amino acids, L-carnitine, and choline to produce toxic/proatherogenic metabolites including uremic toxins and trimethylamines (TMA) that are associated with increased CVD risk. Thus, the microbiome is central to regulatory pathways involved in human physiology and the pathogenesis of CVD, although the multi-dimensionality of microbial signatures renders associations and causality complex.

Microbiome Characteristics in Health and Disease

Taxonomically the human gut bacterial microbiome is ordered into phylum, class, order, family, genus, and species, with *Firmicutes*, and *Bacteroidetes* phyla contributing to approximately 90% of all the human gut microbiota, the rest being, *Actinobacteria, Proteobacteria, Fusobacteria* and *Verrucomicrobia* and many other more (Fig. **5.1**) [1]. The distinction between a “healthy” and a “diseased” microbiome has not yet been established owing to intra- and inter-individual variations in the microbiome. Everyone has core microbial populations, which are critical for the symbiotic survival of microbial communities and remain stable for a considerable length of time from three years old and throughout adulthood although it starts to destabilise with ageing [2]. In humans, the microbiome is determined by early life events such as maternal environmental and nutritional factors, mode of delivery, gestation age at delivery and whether the infant is breast or formula fed [3, 4]. Nevertheless, the abundance of each microbe may fluctuate over time because of perturbations induced by antibiotic use, diet, lifestyle, age, cultural practices, and geography [2]. As a result, the ability for each microbial population to remain stable, in response to perturbations, will determine the individual’s propensity to dysbiosis and disease [5]. In general, a

large microbial diversity as well as functional similarity between microbial populations are considered more beneficial to the host [4, 6]. This is mainly due to the ability for such species to compensate for each other and the other group of species fluctuate in response to perturbations.

Earlier studies defined a healthy microbiome based on *Firmicutes/Bacteroidetes* ratio with a higher ratio being associated with the disease phenotype [7]. However, contrasting observations have been reported by others who show no differences in *Firmicutes/Bacteroidetes* ratio and attribute the differences to sample size and methodological approaches [8, 9]. The association of *Firmicutes* with disease is also contrary to expectations since *Firmicutes* are associated with higher butyrate production than *Bacteroidetes*, and butyrate is known as a beneficial SCFA [10, 11]. As such classification of diseases phenotype based on *Firmicutes* to *Bacteroidetes* is still a matter of debate, particularly as several genera in the *Firmicutes* phylum such as *lactobacillus* may be beneficial for host physiology while some of the genera in the *Bacteroidetes* phylum may be deleterious to host physiology [7, 12]. This is mainly collaborated by further efforts that have characterised microbiome structures into three clusters of microbial communities termed enterotypes [13]. Enterotype I predominantly consists of the genus *Bacteroides* with functional capacity to metabolise western diet food constituencies such as animal fat, carbohydrates, and proteins [14]. In contrast, enterotype II is predominated by *Prevotella* with functional capacity to metabolise simple sugars and carbohydrates mainly found in plant-rich diets consumed by non-westernised agricultural communities and western communities on Mediterranean diet (Agrian). Enterotype III which predominated by *Ruminococcus* although in some cases such as in infants, other genera including *Enterobacteriaceae, and Bifidobacterium* as well as *Proteobacteria* also exist. *Enterotype III* favours diets that are less variable but mainly consisting of fibre-rich grains and resistant starch. Enterotype III is dominated by *Firmicutes* species, while the former two belong to the *Bacteroidetes* phylum. Consequently, when the function potential to metabolise protein, lipids and carbohydrates was assessed, *Bacteroides* exhibited high proteolytic, lipolytic and saccharolytic potential than the other enterotypes, while *Prevotella* enterotype had a high saccharolytic potential than *Ruminococcus* although enterotype *Ruminococcus* had higher lipolytic potential than *Prevotella* enterotype [14, 15]. Additionally, *Bacteroides* were shown to have low functional redundancy and were less resilient to environmental insults with predicted manifestation of low-grade inflammation and diseased phenotype. However, as will be discussed in the next sections, correlation studies do not seem to support the associations of *Bacteroides* with the disease phenotype. Additionally, diet which is determined by urbanisation seems to be a major predictor of individuals' enterotypes [16].

CHAPTER 6

Oxidants and Antioxidants Interplay in the Modulation of Inflammation and Cardiovascular Disease

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Abstract: Oxidative stress and inflammation are parallel self-perpetuating mechanisms that when triggered, appear to be strongly linked with several complications of cardiovascular disease (CVD). Unchecked production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are largely the responsible factors that operate *via* the activation of several transcriptional messengers and a series of inflammatory pathways. Such messengers include Nuclear Factor-KappaB, known to contribute to a plethora of pathological complications such as endothelial dysfunction, the initiation and progression of atherosclerosis, irreversible ischemic reperfusion injury, and arrhythmias, particularly atrial fibrillation. Although much is known about the link between oxidative stress and CVD, the development of direct therapeutic interventions has remained elusive. In experimental animal models, the use of antioxidants in the form of dietary supplements has been shown to quench ROS/RNS or catalyse the break-up of free radical chains and has resulted in some measure of success. However, these findings have not been able to be replicated in human clinical trials for several different well-known agents, such as vitamin E and beta-carotene. Many potent naturally occurring antioxidants have been exploited by nature such as the oxygenated carotenoids (xanthophylls) and researchers have tested several of them in their natural form in clinical trials but sadly many of them have not translated into useful therapeutic tools. Questions, therefore, remain as to whether the reasons may be solely the inability to find the "right" compound(s) or delivery strategy, or the exact mechanisms of action of existing compounds have unknown targets or whether correct dosages are used. This chapter reviews existing evidence on the thesis that antioxidant/anti-inflammatory compounds may present an opportunity for the development of future therapeutic agents for both cardiovascular oxidative stress and inflammation.

Keywords: Antioxidants, Cardiovascular disease, Ischaemia reperfusion injury, Leukocyte activation, Myocardial infarction, Oxidative stress.

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INTRODUCTION

It has been estimated that cardiovascular diseases (CVD) account for approximately 30% of annual deaths and disability on a global scale [1 - 3]. In 2010, there were an estimated 16.7 million deaths globally attributed to CVD with a statistical projection that approximates about 23.3 million deaths by 2030. CVD mortality rates are considered equivalent to the combined number of deaths due to nutritional deficiencies, infectious diseases, and maternal and perinatal conditions [4]. The predicted increasing trend in the growth of CVD for the next 10 years is largely related to the increasing incidence in low-and middle-income countries during the last decade [5].

For the most part, the greatest contributing factors include an ageing population and the spread of the Western diet and sedentary lifestyle [5, 6]. The consequences of the increasing burden of CVD are rising costs of medical care and economic impact particularly on economies for low-and middle-income countries [5, 6]. There is therefore a need for a better understanding of the mechanisms underlying CVD and its clinical consequences.

Oxidative Stress as a Putative Mechanism for CVD

While reactive oxygen species (ROS) at physiological levels function as signalling molecules that regulate a wide range of processes and contribute to the maintenance of cardiovascular homeostasis [7 - 9], oxidative stress brought about by the generation of excessive and/or sustained increase in ROS production plays a pivotal role in the initiation, progression, and resultant clinical consequences of CVD [10 - 16].

Oxidative stress occurs when there is an imbalance between the production of free radicals and the availability of fully functioning antioxidant species [10 - 16]. The pathogenesis of a number of cardiovascular diseases include atherosclerosis [17], hypertension [18 - 20], heart failure [21, 22], cardiac ischemic reperfusion injury [22, 23], postoperative arrhythmias [24 - 26], oxidative heart failure [27], chemotherapy-induced cardiotoxicity [28], and ischemia-reperfusion (I/R) injury of the myocardium [29].

A number of studies have explored the effectiveness of antioxidant therapies in the prevention and treatment of cardiovascular diseases [29]. Small trials on small molecules, such as astaxanthin and omega 3, have shown potential beneficial roles in cardiovascular diseases [30]. However, while some clinical trials have shown positive results, findings from others have remained controversial [30]. Nevertheless, there is new optimism that the new types of antioxidants being

developed can be turned into useful therapeutic interventions specifically because of the better understanding of the most effective delivery approaches.

Molecular Effects of Oxidative Stress

The production of intra- and extracellular but highly active and unstable ROS takes place under a number of pathological conditions many of which may be linked with the pathogenesis and propagation of cardiovascular disease [30]. Consequently, the defence mechanisms become overwhelmed leading to the modification of proteins, lipids, and the nucleus [30]. In addition, excessive ROS can lead to the alteration of regulation of protein function and signal pathways. Cell membrane lipids, including polyunsaturated fatty acids (PUFAs) and cholesterol are highly susceptible to attack by free radicals leading to oxidative stress [31]. The process of oxidation of lipids occurs in three steps: initiation, propagation, and termination [32]. The initiation of lipid oxidation starts by the formation of free radicals through the loss of a hydrogen atom on the unsaturated lipid molecule [32]. During the propagation process, the formation of peroxyl radicals follows the reaction between lipid radicals and oxygen [32]. The highly reactive peroxyl radicals then attack other lipids in proximity to form more lipid peroxyl radicals. Examples of such peroxyl radicals include 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA), which are products of lipid peroxidation that play an important role in the pathogenesis of many diseases [33] with the levels in blood shown to be predictive of the magnitude of disease progression [34, 35]. ROS and ROS-derived lipid peroxyls also attack nearby protein molecules causing the protein to be modified in a process called carbonylation [36] where nucleophilic amino acids, such as cysteine, histidine, and lysine combine with ROS to form inactive complexes [32]. Protein carbonylation is one the main ways through which enzymes can become inactivated as a result of interacting with ROS, and also proteins are degraded [37-38].

ROS also attack nucleic acid bases at sites with double bonds and the by-product of the reaction includes molecules such as 8-oxo-deoxyguanosine, thymine glycol, 5-hydroxymethyluracil, 6-hydroxy-5, 6-dihydrocytosine, 5-hydroxyuracil and 8-oxo-deoxyguanosine [39]. The attack is known to induce G-T transversions [39], and DNA and histone methylation [40]. Indeed, DNA and histone methylation may cause a change in the stability of the genome to significantly alter the chromatin structure and function [40]. Lipid peroxides also directly modify DNA to such an extent that normal nucleus activities such as excision repair of nucleotides, homologous recombination, or translational protein synthesis become impaired [41].

CHAPTER 7

Prevention of Reperfusion Injury in Acute Myocardial Infarction: A "flashback" Journey of Novel Strategies Based on the Potential Therapeutic Role of Antioxidants

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Abstract: It has been recognized that oxidative stress plays a key role in the development of cardiac alterations derived from events of ischemia followed by reperfusion, such as in the clinical setting of acute myocardial infarction of patients subjected to coronary angioplasty. During ischemia, due to the occlusion of a coronary branch, biochemical events responsible for anaerobic metabolism, ATP availability and impairment of cell ionic homeostasis are the major deleterious effects. Following the onset of reperfusión, a burst of reactive oxygen species occurs, thus accounting for increased tissue damage due to the endovascular intervention. This iatrogenic damage has not been adequately treated to date. Among the many pharmacological attempts, cardioprotection with antioxidants should be mentioned; however, the experimental studies have not been translated into successful clinical trials aimed to prevent this enhancement of cardiac damage, despite some beneficial effects have been reported in the clinical outcome of the patients. This chapter aimed to present the hypothesis that the combination of antioxidant effects should improve the cardioprotection of the patients subjected to coronary angioplasty following acute myocardial infarction. Therefore, we present an update of previous attempts at cardioprotection with an antioxidant alone and give the basis for the expected improved protection by using two or more antioxidant compounds exerting different mechanisms that could enhance the beneficial protective effect.

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Keywords: Acute myocardial infarction, Antioxidant enzymes, Antioxidants, Apoptosis, Combined antioxidant treatment, Ischemia/Reperfusion injury, Lipid peroxidation, Necrosis, Oxidative stress, Reactive oxygen species.

INTRODUCTION

Acute myocardial infarction has remained a leading cause of mortality worldwide [1], and it is associated with high health expenses and diminished life quality in survivors. The best treatment for these patients is to open the occluded artery, which is better achieved by coronary angioplasty [2]. Nevertheless, this procedure causes a reperfusion injury leading to enhanced myocardial mass damage that could account for up to 50 percent of the final infarct size [3]. The extent of myocardial injury relates to the clinical outcome, the appearance of complications, and reduced quality and life expectancy in survivors [4]. At cellular level, various processes have been involved in the mechanism of the paradoxical heart damage occurring after achieving revascularization, including pH changes, intracellular calcium homeostasis impairment, and oxidative stress, among others [5]. The damage mediated by oxidative stress is partly dependent on the increased activity of pro-oxidant enzymes during the phase of ischemia; then, a burst of reactive oxygen species occurs early during the onset of reperfusion due to the increased oxygen delivery [4]. Considerable effort has been devoted to prevent reperfusion myocardial damage, as it is responsible for impairing the clinical outcome of acute myocardial infarction survivors; however, the results so far are unsatisfactory. The development of oxidative stress is derived from the ischemia-reperfusion sequence. Metabolic events of ischemia give rise to increased activity of pro-oxidant enzymes, leading to radical anion superoxide production, but this result is not completely achieved until the recovery of coronary blood flow by the endovascular percutaneous intervention. Experimental *in vivo* studies have demonstrated a burst of reactive oxygen species during the first minutes following the restoration of oxygen delivery to the affected tissue, thereby providing evidence for a new cell death mechanism. Reactive oxygen species can trigger cell death pathways and myocardial structural and functional impairment. Consequently, the effects of increased reactive oxygen and nitrogen species result in deleterious structure and cell function as the antioxidant defense system has been overwhelmed. Therefore, it seems reasonable to argue that administering antioxidants before reperfusión could lead to cardioprotection against damage mediated by oxidative stress [6]. However, this proposal's results have been controversial up to date. The explanation for this inconsistency remains to be determined. Previously, we performed a clinical trial of patients subjected to high doses of ascorbate administration before and during coronary angioplasty [7, 8]. Although the patients showed beneficial effects in ejection fraction and other heart functional parameters, they did not change the infarct size; besides, they

showed diminished reduced/oxidized glutathione ratio, showing signs of an impaired redox state. Considering the characteristics of therapeutic strategies performed in clinical trials, aimed with the objective of cardioprotection against reperfusion damage, it can be said that (i) these studies are lacking robust pharmacological characterization of the effects of the drugs, as controls are not quite appropriate for comparisons, and (ii) two or more drugs acting through different mechanisms should be expected to improve the cardioprotection. As the cause of this disturbance seems to be multifactorial, the group of researchers leading the cardioprotection against ischemia/reperfusion injury has hypothesized that to translate cardioprotection to patients effectively, a multitarget cardioprotective therapy is necessary, thus suggesting that optimal cardioprotection may require the combination of additive or synergistic therapies [9]. Accordingly, in turn, we hypothesize that an association of antioxidant agents, pro-antioxidant and anti-ferroptosis is necessary to achieve an enhanced response against the challenge of oxidative stress.

PATHOPHYSIOLOGY OF MYOCARDIAL ISCHEMIA–REPERFUSION INJURY

Sources of Reactive Oxygen and Nitrogen Species in the Heart

The formation of reactive oxygen species at the myocardium corresponds to 5% of the total reduction of oxygen at the mitochondrial level, producing a series of intermediates in the process (Fig. **7.1**) such as superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$) and singlet oxygen ($^1O_2$). Although all are oxidants, the most reactive radical species are $O_2^{\bullet-}$ and $OH^{\bullet}$, free radicals. Physiologically, $O_2^{\bullet-}$ is converted into H_2O_2, which is less reactive but capable of diffusing to other sites due to its greater lipophilicity and half-life. The problem arises when an overproduction of H_2O_2 occurs, which can be produced by the Fenton or Haber-Weiss reaction, $OH^{\bullet}$, which, although it has a short half-life, is the most reactive oxygen radical species, capable of interacting with other macromolecules, and is able to generate lipoperoxidation and sarcolemmal disruption [10].

Reactive nitrogen species (RNS) are also formed, although the primary function of nitric oxide (NO) produced by NOS is cardioprotection due to its coronary vasodilator effects [11] inhibitors of platelet aggregation, and inhibitors of adhesion of neutrophils and platelets. Faced with prolonged ischemia, an acidotic environment, and the presence of ROS, particularly O_2, will lead together with NO to the formation of peroxynitrite anion ($ONOO^-$); which can give rise to species such as peroxynitrous acid (ONOOH), capable of decomposing into more reactive species such as $OH^{\bullet}$ and $NO_2^{\bullet}$. When mitochondrial oxidative phosphorylation

CHAPTER 8

Improvement of Nitric Oxide Availability in Myocardial Ischemia/reperfusion: Role of Nanotechnology as a Therapeutic Approach

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Abstract: In the search for an effective treatment against myocardial damage caused by oxidative stress, it has become necessary to generate new therapies that overcome the difficulties and failures observed in conventional therapies. Therefore, nanotechnology and nanoparticle development may open new horizons for the control and therapy of oxidative stress and associated myocardial damage. The term nanomaterials describe materials with nanoscale dimensions (< 100 nm). In this chapter, different nanoparticle drug delivery systems, along with their targeting strategies, and how they can help to improve therapeutic failure in oxidative stress using nanoparticles in the control of myocardial infarction and oxidative stress will be discussed. Achieving an inhibition of oxidative stress producers or improving the endogenous antioxidant capacity through drug delivery by nanoparticles increases the drug's aqueous solubility, protects its degradation, allows prolonged release, and improves the bioavailability, determining a targeted delivery, and decreases the toxic side effects. It leads to new therapeutic opportunities for both monotherapies and combined therapies, benefiting from nanoparticles' particularities associated with increased solubility, bioavailability, and specificity.

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Keywords: Combined antioxidant treatment, Drug delivery system, Ischemia/Reperfusion injury, Myocardial infarct, Nanotechnology, Nanoparticles, Oxidative stress.

INTRODUCTION

World health organization in its annual 2020 statistics reported that cardiovascular disease (CVD) caused 17.9 million non-communicable disease related deaths globally [1], keeping it as the world's major killer. Ischemic heart disease, was responsible for 16% of the total deaths, rising by more than 2 million since the year 2000, to 8.9 million deaths in 2019 [2]. In the face of this, there is a constant development of new treatments aimed to slow its progress.

A significant alteration in CVD is the blockage of blood vessels, thus leading to ischemic heart disease. Acute revascularization is a standard treatment in myocardial ischemia, restoring blood flow and oxygen supply. Nevertheless, this procedure results in a secondary condition known as reperfusion injury [3]. Ischemia/reperfusion (I/R) injury increases oxidative stress species that activate the inflammatory response, increasing oxidative stress species generation [4].

Various pharmacological agents have been shown to reduce IR injury in animal models; however, none have been developed as cardioprotective modalities for IR injury in clinical practice [5].

Today nitric oxide (NO) is regarded as one of the most important mediators of biological processes in the heart and blood vessels [6]. NO mediates many of the processes contributing to I/R injury, including attenuating platelet aggregation, neutrophil–endothelium interaction, inflammatory response, and cardiomyocyte apoptosis [6]. However, NO exhibits extremely sensitive and concentration-dependent effects in models of I/R injury [7]. The difficulty of storing gaseous NO, ensuring appropriate delivery, and quantification is a big problem. As an alternative, several small-molecule drugs that store and release NO have been reported [8]. However, conventional pharmacological NO donors have shown significant limitations, including indiscriminate NO release, short half-life, instability during storage, vehicle toxicity, and triggers of NO generation [9, 10]. For this, the nanoparticles appear as an alternative that can increase cellular uptake, bioavailability, and therapeutic efficacy while also reducing systemic toxicity [11].

Nanotechnology corresponds to technological research at a nanometric scale (10^{-9} meters), which has allowed the vanguard development regarding the application of nanoparticles (NPs) and nanostructures ranging from 1 to 100 nm even slightly more extensive for the prevention, diagnosis, and treatment of diseases [12]. The

emerging concept of nanomedicine is defined by the US National Nanotech Initiative as the application of nanotechnology to medicine [13]. NPs have been shown to improve conventional drugs' pharmacological properties, for example, the decrease in degradation and controlled release by associating them with nanocarriers. They also can cross biological barriers such as the blood-brain barrier, being more effective and selective, reducing their toxicity and side effects by avoiding the accumulation of drugs, which results in the use of lower doses [14]. These characteristics allow NPs to be targeted to specific cardiovascular system injury and control the release of therapeutic molecules, which can be encapsulated, entrapped, conjugated, or adsorbed [12]. Some nanoparticles' drug delivery platforms (Fig. **8.1**) include biological substances such as gelatin, albumin, and phospholipids for liposomes, polymeric micelles, protein-based nanoparticles, dendrimers, carbon nanotubes, polymer-drug conjugates, and polymers solid metal-containing NPs [15].

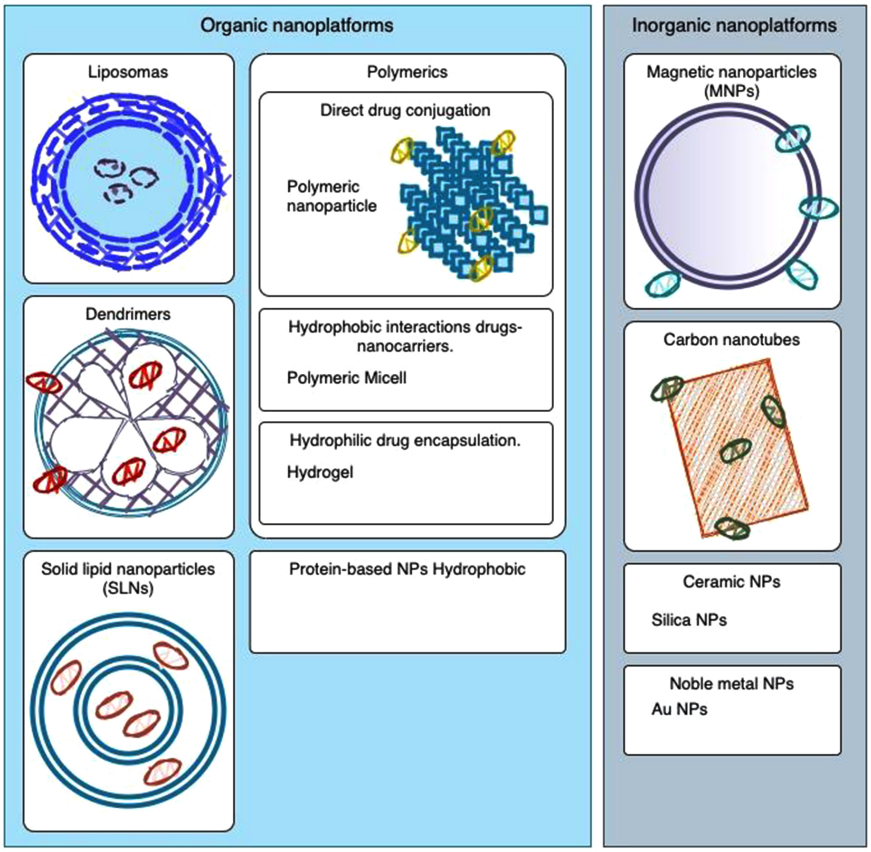

Fig. (8.1). Drug delivery systems based in the use of nanoparticles.

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Bashir Matata

Dr. Bashir Matata, is a healthcare professional with a background in cardiac physiology working within the National Health Service in the UK for more than 25 years. He graduated with a BSc in healthcare science, received a postgraduate certification in echocardiography from Leeds University, UK. He completed Ph.D. in bioengineering from the University of Strathclyde, UK and has a master's in public health from the University of Liverpool, UK. He also holds honorary academic titles at the University of Liverpool, UK, and Edge Hill University, Ormskirk, UK.

Dr. Matata is an empirical researcher who has mastered the art of conducting both clinical and basic molecular science studies. As a researcher, he is firmly grounded in postpositivist epistemology. Majority of his clinical research work has so far been designed to reduce the effect of bias, especially in terms of his role as a researcher. In the studies he used the techniques, such as random sampling, double blinding for both researchers and participants, and statistical analysis of data.

Dr. Matata has skills and techniques to conduct the basic molecular research and has over a period of more than 15 years of studies to produce highly innovative findings in the field of cardiovascular research.