

DRUG INDUCED ENDOTHELIAL DYSFUNCTION: FUNCTIONAL ROLE OF OXIDATIVE STRESS

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ABSTRACT

Reactive oxygen species (ROS) are increasingly recognised as a major cause for altering normal endothelial cell functions. Several studies have revealed that pharmacological agents in the treatment of various diseases can increase ROS load in the body and result in endothelial dysfunction. Anti cancer drugs, immunosuppressive drugs, anti-retroviral drugs, aldosterone and aldosterone antagonists, diethyldithiocarbamate, nanoparticle drugs and drug carriers have been found to cause endothelial dysfunction through oxidative stress. ROS mediated endothelial dysfunction can adversely affect bioavailability of nitric oxide, endothelium-dependent vasodilatation, cell permeability, endothelial cell growth and survival. Whether anti oxidant therapies would really be beneficial to prevent the endothelial oxidative stress associated drugs is unclear. Redox biology of drug induced endothelial dysfunction involves highly complex pathways. Understanding mechanisms of regulated generation of ROS in endothelial cells and downstream effects are necessary to design appropriate therapeutic measures. The functional role of ROS in drug induced endothelial dysfunction and currently known mechanisms are reviewed in this article.

Keywords: Endothelial dysfunction; reactive oxygen species; oxidative stress; anti oxidants; nitric oxide; drug toxicity

[I] INTRODUCTION

Endothelium is a massive organ with diversified functions [1]. The endothelial cell surface in an adult human is composed of approximately 1 to 6×10^{13} cells and the total area of this organ is close to several thousand square meters. Its total mass is equal to the weight of several hearts. For these reasons, endothelium is considered as the biggest gland of the human body [2, 3].

Endothelium serves as a sensor of signals with in the circulatory system such as pressure, shear stress and vasoactive substances and thus it has an important role in maintaining the homeostatic balance of vessels and associated organs [4]. Endothelial cells are reservoirs of different agonist and antagonist molecules such as vasodilators and vasoconstrictors, procoagulants and anticoagulants, inflammatory and anti-inflammatory molecules, fibrinolytics and antifibrinolytics as well as oxidizing and antioxidizing agents [Table-1] [2, 5].

Endothelium can be injured by factors such as oxidative stress, endoplasmic reticulum stress, metabolic stress, genotoxic stress and pathways mediated by immune system. Endothelium derived NO (a potent vasodilator, inflammatory and hemostatic modulator) is recognized to be the central point of a number of pathologic processes that are critical to the development of diseases which result from endothelial dysfunction.

Among the several factors that damage endothelium, reactive oxygen species (ROS) are increasingly acknowledged as the key culprits which are responsible for altering normal endothelial cell functions. Hyperstimulation of mechanisms that produce free radicals and oxidative change of signaling molecules have an influence on intracellular signaling pathways leading to endothelial dysfunction and how development of disease conditions such as hypertension, atherosclerosis, and diabetes. Mechanisms of redox regulation of endothelial function how different pharmacological agents affect the endothelial function adversely through redox mechanisms are discussed in this article.

Table: 1. Agonist and antagonist molecules produced by endothelial cells

Physiological function	Molecules
Vasodilators	Nitric oxide (NO), C-type Natriuretic Peptide, Prostacyclin (PGI ₂), PGE ₂ , Endothelium Derived Hyperpolarization Factor (EDHF),
Vasoconstrictors	Endothelins 1, 2 and 3, Angiotensin II, Reactive Oxygen Species (ROS), Thromboxane A ₂ , Endothelium Derived Constriction Factor (EDCF)
Inflammatory modulators	NO, Intercellular Adhesion Molecule-1 (ICAM-1), Vascular Adhesion Molecule-1 (VCAM-1), Selectins, NFκB
Hemostasis modulators	Plasminogen Activator, Tissue Factor Inhibitor, von Willebrand Factor, NO, Prostacyclin, Thromboxane A ₂ , Plasminogen Activator Inhibitor-1, Fibrinogen
Growth factors	Vascular Endothelial Growth Factor (VEGF), Basic Fibroblast Growth Factor (bFGF), Platelet Derived Growth Factor (PDGF), Transforming Growth Factor β (TGF β)
Cell proliferative agents	Endothelin 1, Angiotensin II
Other proteins	B type Natriuretic Peptide, Adrenomedulin, Interleukins, Endoadenosine Diphosphatase, Thrombomodulin, Tissue Factor, Vascular Cell Adhesive Molecules, Intracellular Adhesive Molecules, Integrins, α-urokinase, Protein S

[II] REDOX REGULATION OF ENDOTHELIAL FUNCTION

Reactive oxygen species (ROS) are well recognized to function as signaling molecules. Be that as it may, at higher concentrations they can induce cell injury and death by oxidant modification of proteins and carbohydrates, lipid peroxidation, and DNA strand nicks [6]. ROS can modulate phenotypes in vascular endothelial cells [7]. Homeostatic mechanisms in ROS generation in endothelial cells are depicted in [Figure-1]. Endothelial cells can be challenged by ROS that are produced by activated inflammatory cells, smooth muscle cells and endothelial cells themselves. ROS contribute to endothelial dysfunction and remodeling through oxidative damage by reducing the bioavailability of NO, impairing endothelium dependent vasodilatation and inducing apoptosis, stimulating endothelial cell migration and activating adhesion molecules and inflammatory reaction [8]. Sources of ROS anion in the vascular wall under both normal and pathophysiological conditions involve mitochondria, cytochrome P450-type enzymes, cyclooxygenase, lipoxygenase, NAD(P)H oxidase, xanthine oxidase, and nitric oxide synthase (NOS) [9]. Findings in animal models imply that, in hypertension, chronic renal failure and in diabetes, enhanced production of ROS leads to decreased NO bioavailability and endothelial dysfunction [10-12]. The major endothelial ROS is superoxide anion (O₂⁻), which inactivates nitric oxide (NO), thus impairing vascular relaxation [13]. Dismutation of superoxide anion by superoxide dismutase (SOD)

produces hydrogen peroxide (H₂O₂), a more stable ROS, which in turn is converted to water by catalase and glutathione peroxidase. Under certain circumstances, ·O₂⁻ can be produced by nitric oxide synthase (NOS) athrough 'NOS uncoupling'. Finally, the reaction product peroxynitrite (OONO⁻), from ·O₂⁻ and NO, is a strong oxidant molecule. High levels of ·O₂⁻ and subsequent accumulation of H₂O₂ result in decreased NO bioavailability and play a critical role in endothelial remodeling [14]. A schematic representation of ROS mediated endothelial dysfunction is given in [Figure-2].

[III] OXIDATIVE STRESS AND ENDOTHELIAL DYSFUNCTION

There is equilibrium between reactive oxygen species (ROS) formation and endogenous anti oxidant defense mechanisms. But when this balance is disturbed, it can lead to oxidative stress. This state of oxidative stress can result in injury to all the important cellular components such as proteins, DNA and membrane lipids which can lead to cell death. Endothelial dysfunction is a common accompaniment in several diseases [6]. Some of these oxidation-linked diseases can be worsened by numerous pro-oxidant drugs, which are used in the treatment of these diseases.

3.1. Anti-cancer drugs

Drug induced toxicity to vascular endothelium has received much attention in recent times for the reason that tumour cells require a functioning endothelium for growth and proliferation.

Interestingly, some of the anti cancer drugs are more toxic to endothelial cells than to tumour cells [15]. Drug induced endothelial dysfunction occurs through oxidant stress.

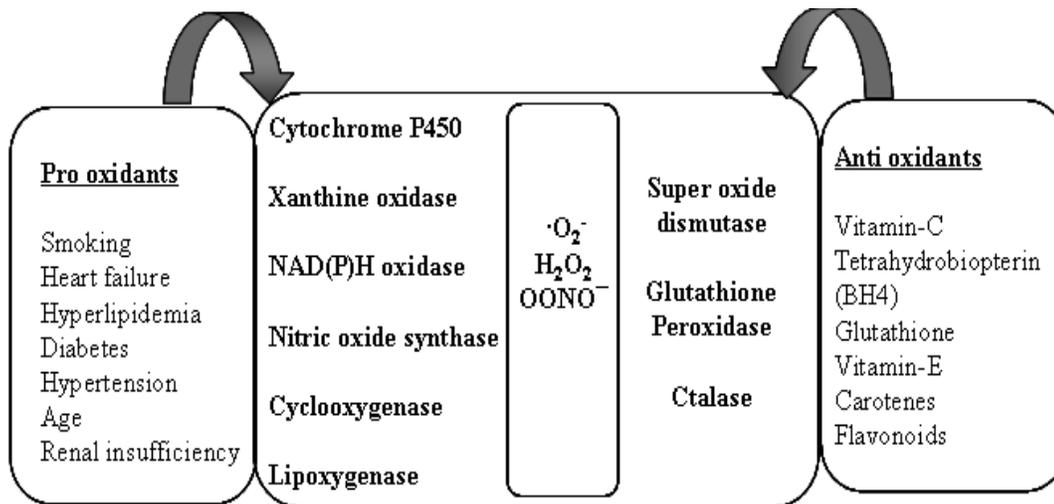


Fig: 1. Reactive oxygen species (ROS) homeostasis in endothelial cells. ($\cdot\text{O}_2^-$) Super oxide anion; (H_2O_2) Hydrogen peroxide; (OONO^-) Peroxy nitrite anion

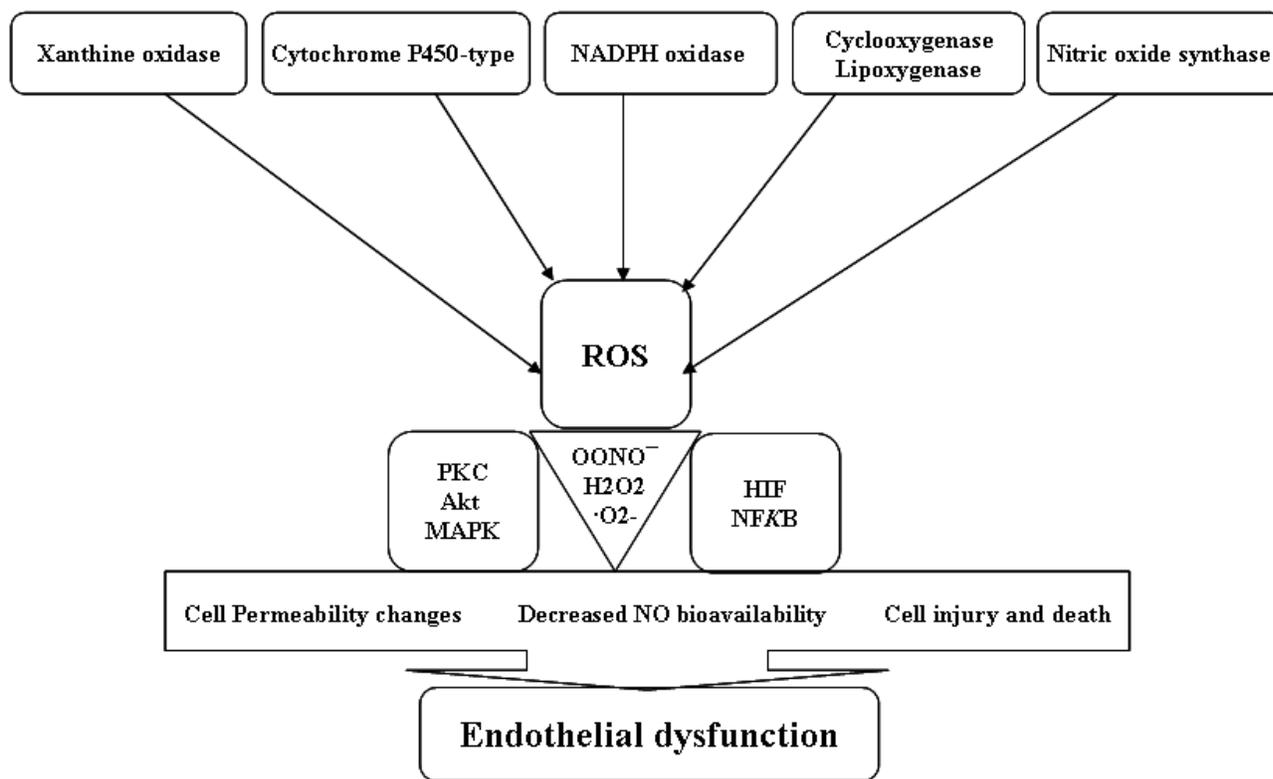


Fig: 2. Production of reactive oxygen species (ROS) and endothelial dysfunction. (PKC) Protein Kinase C; (MAPK) Mitogen Activated Protein Kinase; (HIF) Hypoxia Inducing Factor; (NFκB) Nuclear Factor Kappa B

Quinones are one class of drugs which are used very early in cancer treatment. Doxorubicin (DOX) is an anthracycline antibiotic member of this class and is commonly used in the treatment of cancers of breast, endometrium, ovary, testicle, thyroid and lung. DOX and other quinones cause topoisomerase II inhibition resulting in chemical and oxidative damage to DNA and thereby target cell damage [16]. Even though most studies on the mechanism of action of DOX have focussed on damage to the tumour cells, many studies have shown that DOX induces production of H₂O₂ causing toxicity to both endothelial cells and cardiomyocytes [17]. As a result, a broader clinical use of DOX is restrained. DOX is converted into a semiquinone radical after univalent reduction on mitochondrial Complex I or NADH dehydrogenase. In the presence of oxygen, the semiquinone can directly transfer its unpaired electron to oxygen, generating superoxide anion and regenerating DOX in the process. If it is not counter-balanced by anti-oxidants, ROS produced in this redox cycle can prop up lipid and protein oxidation in mitochondrial membranes along with mtDNA oxidation. This will set up the background for oxidative injury [18].

Menadione is another anti-cancer drug of the quinone family and experiments with the drug has provided insights into the quinone toxicity in endothelial cells [19, 20]. Menadione reacts directly with reduced glutathione (GSH) [21] and irreversibly obstructs key thiol-enzymes involved in GSH and ATP metabolism, glucose 6-phosphate dehydrogenase (G6PDH) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). GSH exhaustion compromises anti oxidant defenses, leading to ROS induced barrier failure [6, 22], necrosis and apoptosis [23]. Menadione toxicity is mediated by poly (ADP-ribose) polymerase activation *via* hydrogen peroxide formation and oxidative stress in endothelial cells. Lethal cell injury seems to be initiated by H₂O₂-mediated activation of PARP, presumably as a result of NAD⁺ and ATP depletion [6, 19, 24].

DOX and a number of other cancer chemotherapeutic drugs have also been shown to cause oxidant stress-induced endothelial injury and progressive peripheral oedema [25].

Arsenic trioxide (Trisenox) is used to treat leukemia that is unresponsive to first line agents. It is suspected that arsenic trioxide induces cancer cells to undergo apoptosis [26, 27]. Recently, arsenite has been found to be associated with generation of reactive oxygen species as well [28, 29]. In addition to producing glycolytic stress and depleting energy metabolism, high concentrations of arsenite increases phosphorylation of endothelial heat shock proteins [30, 31]. Data from experimental studies suggest that superoxide and H₂O₂ are the predominant reactive species produced by endothelial cells after arsenite exposures leading to cell mal functioning. H₂O₂ is the main reactive oxygen species released by human endothelial cells stimulated by arsenite. DNA strand breaks are introduced by arsenite *via* reactive oxygen species [32]. Mutagenesis, carcinogenesis, aging, and apoptosis are the outcome of this event. In addition, free radicals and oxidants bring on the release of metal ions, which in turn generate more reactive species (eg:

heme-containing compounds, iron storage proteins) which can also contribute to oxidative DNA damage [33, 34]. Studies also suggest that arsenite may trigger oxidative stress through multiple pathways such as inhibition of catalase and glutathione peroxidase and also stimulation of superoxide dismutase and NADPH oxidase [32, 35].

3.2. Immunosuppressive drugs

Immunosuppressive drugs can also cause endothelial dysfunction through redox pathway. Trapp and colleagues studied the effect of therapeutic concentrations of methylprednisolone, mycophenolic acid, cyclosporine A, rapamycin and tacrolimus to find out the resultant generation of oxidative stress, apoptosis, metabolic activity and proliferation in human microvascular endothelial cells (HMEC-1). Mycophenolic acid, cyclosporine A and rapamycin are stronger inducers of oxidative stress in endothelial cells compared with methylprednisolone and tacrolimus. Cyclosporine A produced the strongest increase in oxidative stress, metabolic activity and apoptosis [36-38]. Immunosuppressive drugs induce NADPH oxidase enzyme. In vascular cells they target Nox1, 2 and 4. Further, immunosuppressant mediated induction of oxidative stress, metabolic activity, and apoptosis are strongly linked. The molecular mechanisms however are yet to be clarified.

3.3. Anti-retroviral drugs

Antiretroviral Therapy (ART) has been reported to induce significant endothelial dysfunction in patients with HIV. This factor also contributes to the increase in cardiovascular diseases associated with ART [39]. In patients undergoing treatment with antiretroviral drugs, cardiovascular diseases are a key contributor to non-HIV related deaths [40]. Also, HIV-associated atherosclerosis is observed in comparatively younger patients with HIV, taking antiretroviral agents [41].

Indinavir, a protease inhibitor against HIV has been convincingly shown to directly induce endothelial dysfunction. Atazanavir, another protease inhibitor, though has lesser side effects, has been found not to improve endothelial function [42]. Prolonged treatment with azidothymidine (AZT), a reverse transcriptase inhibitor also results in endothelial mitochondrial dysfunction and subsequently cardiovascular alterations. This has been demonstrated in human umbilical vein endothelial cells treated with azidothymidine [43]. During *in vivo* studies, it has been observed that AZT treatment alters cardiac mitochondrial ultrastructure and the expression of mitochondrial cytochrome B mRNA in a dose and time-dependent manner [44]. Although these results suggest a direct effect on the mechanisms of DNA replication, it should also be taken into account that direct effects on the mitochondrial oxidative phosphorylation machinery can generate more oxygen-free radicals, alterations in the mitochondrial structure and thus cell function.

One hypothesis for the initiation of ART induced endothelial dysfunction is that oxidative stress negatively modulates endothelial nitric oxide synthase dependent vasodilation and increases the release of the vasoconstrictive factor ET-1. AZT and indinavir can provoke direct endothelial dysfunction by increased release of endothelin-1 (ET-1) with increased ROS production leading to decreased endothelium-dependent vasodilation [45]. Endothelial dysfunction may be mediated by mitochondrial dysfunction since mitochondrion is a major source of cellular ROS and is a common target for many toxicants. ART treatment can significantly induce cellular mitochondrial dysfunction at a very early time point as seen in gene transfer experiments, over expressing superoxide dismutase [46]. In experiments involving gene transfer of a mitochondria-targeted versus a cytosolic catalase, overexpression of mitochondrial catalase not only abrogated ART-induced ROS production in HUVECs, but also diminished ET-1 release, indicating that mitochondrial dysfunction and mitochondria-derived ROS production may be responsible for anti retroviral drug induced endothelial dysfunction. Mitochondria-derived ROS may be a factor responsible for ART-induced endothelial dysfunction and maybe, atherosclerosis in HIV patients [47].

Surprisingly, anti retroviral agents of two completely different categories lead to similar toxicities in endothelial cells. When administered alone, both drugs compromise cellular mitochondrial function and induce mitochondria-derived ROS production in endothelial cells. Though the precise mechanisms for mitochondrial damage may not be the same, mitochondrial dysfunction mediated oxidative stress represent a common pathway by which these two drugs initiate endothelial dysfunction.

3.4. Aldosterone and aldosterone antagonists

It is well recognized that aldosterone induces endothelial dysfunction and perivascular fibrosis, but the exact mechanisms of these effects are not well established. Nagata et al [48] reported that eNOS function is negatively regulated by aldosterone. They proposed that the mechanism is through the oxidation of Tetrahydrobiopterin (BH4) and uncoupling of eNOS because of a deficiency in its cofactor. Aldosterone possibly has an action similar to that of angiotensin II, which also suppress eNOS function *via* NO synthase uncoupling, through ROS production and Ser 1177 dephosphorylation [48, 49].

Spironolactone is an aldosterone antagonist used in the treatment of chronic heart and kidney diseases. In patients with Type 2 diabetes, spironolactone worsens endothelial function [50]. This may be because of increase in plasma angiotensin II associated with spironolactone treatment. Angiotensin II is a pro oxidant and leads to increase in the quantity of ROS and thus oxidative stress to the cells. AngII causes increase in cortical NADPH and NADH oxidase dependent production of superoxide radicals [51]. Superoxide dismutase mimetic tempol, diminishes oxidative stress in experimental animal models of diabetes mellitus [52]

and abates vascular dysfunction in kidney of AngII infused rats [53]. Eventhough the mechanisms are unclear, a cautious approach is indicated in prescribing spironolactone to patients with diabetes.

3.5. Diethyldithiocarbamate

Diethyldithiocarbamate (DDTC) is a sulfhydryl-containing carbamate that is the primary *in vivo* metabolite of disulfiram. Clinically it is used to induce alcohol aversion as DDTC inhibit aldehyde dehydrogenase. The drug is also considered to block cancer metastasis and angiogenesis. DDTC has been shown to be toxic to endothelial cells by way of oxidative shift in the intracellular redox state. DDTC potentiates oxidative damage by also inhibiting superoxide dismutase [54]. DDTC-induced cytotoxicity and apoptosis are enhanced by depletion of intracellular GSH [55]. There are also reports suggesting that DDTC blocks oxidoreductase enzymes such as xanthine oxidase, thus inhibiting vascular super oxide production [56].

3.6. Nanoparticle drugs and drug carriers

Nanoparticles are increasingly being employed for drug delivery [57, 58] and recently toxicity from nanoparticles has raised serious concerns with respect to their use. Among the possible mechanisms of action postulated for toxicity of nanoparticles, at the cellular level, oxidative stress is considered to be an important one [59].

Silica nanoparticles are accepted drug carriers for various therapeutic agents. Once silica nanoparticles enter the bloodstream, endothelial cells in the lumen of blood vessels and the heart are in direct contact with them. Endothelial cells negotiate the clearance of nanoparticles [60]. Experiments by Napierska *et al.* demonstrated that smaller silica nanoparticles elicit a higher cytotoxic response and cause endothelial cell necrosis [61]. Xin Liu and Jiao Sun have also confirmed that exposure to silica nanoparticles is a source for ROS generation in endothelial cells and that the ROS generated can provoke apoptosis *via* JNK/p53 dependent mitochondrial pathways. Exposure to silica nanoparticles at elevated concentrations also causes activation of NF- κ B because of oxidative stress in endothelial cells. The outcome is upregulation of CD54, CD62E, TF, IL-6, IL-8 and MCP-1 with a possible risk for development of cardiovascular diseases [62].

Inhibition of tumour angiogenesis by chitosan nanoparticles (CNP) is associated with impaired levels of vascular endothelial growth factor receptor 2 (VEGFR2) [63], which can affect paracrine activities of endothelial cells through ROS pathway.

When intravenously administered, nanoparticles can also be entrapped by mononuclear phagocytic system in liver and spleen [64]. In addition to a reduction of therapeutic efficacy, liver entrapment also may affect liver function mainly through depletion of anti oxidant defense as a result of release of oxidative

species in hepatocytes and vascular endothelial cells. Nanoparticles induce a temporary depletion of GSH and GSSG levels and inhibit SOD activity [65].

One of the advantages of the use of nanoparticles is the ability of these particles to cross the blood brain barrier (BBB). This factor

may also be the key drawback for systemic administration of nanoparticles as they may cause brain toxicity through endothelial redox injury [66]. Experiments with MnO_2 particles have revealed that nanoparticles generate ROS and oxidative stress in the brain [67].

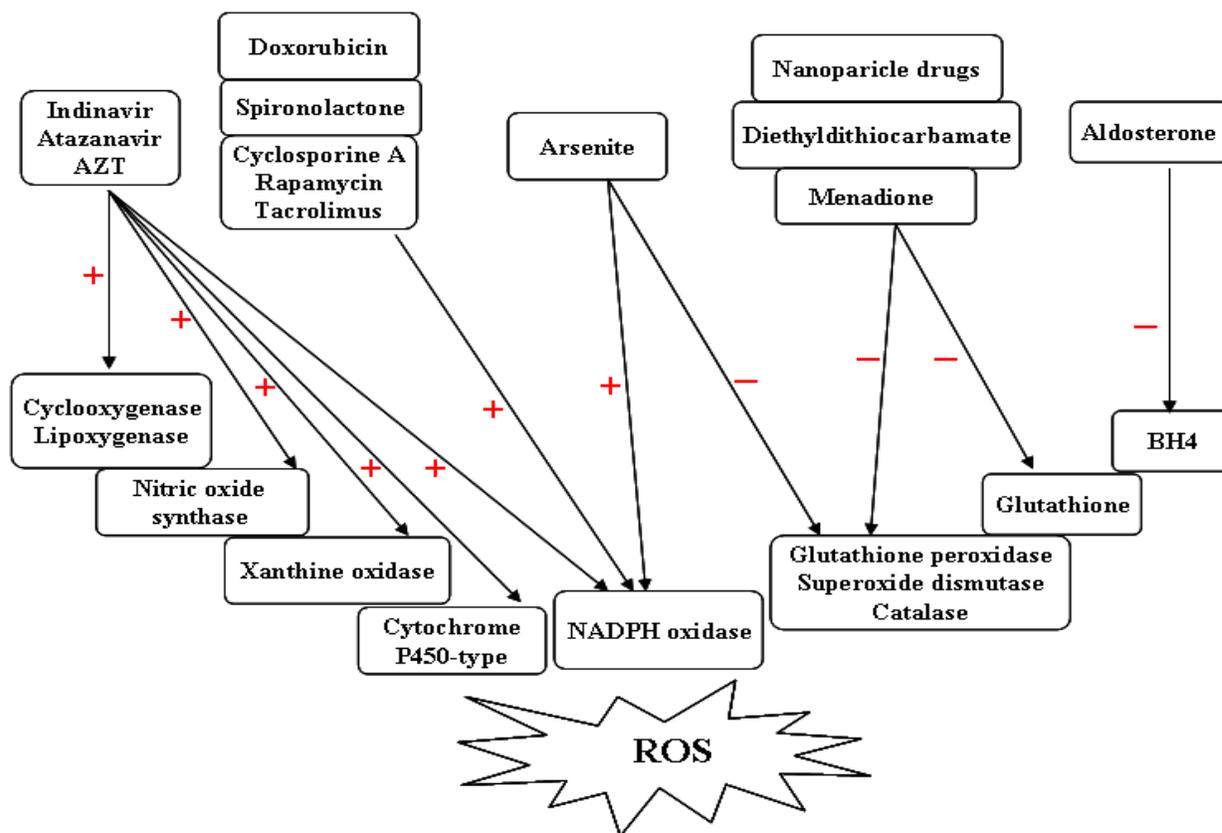


Fig. 3. Pathways of drug induced (ROS) production. (AZT) Azidothymidine; (BH4) Tetrahydrobiopterin

[IV] ROLE OF ANTI OXIDANTS IN THE TREATMENT OF DRUG INDUCED ENDOTHELIAL DYSFUNCTION

Administration of gamma-glutamylcysteine ethyl ester (GCEE) [68, 69] and N-acetylcysteine (NAC), which are anti oxidants as well as glutathione precursors have been shown to abate adriamycin induced endothelial dysfunction in rats [70]. *Phyllanthus maderaspatensis* has also been used as a dietary supplement for reduction of adriamycin-induced toxicity and oxidative stress in mice [71]. Thioredoxin is reported to have ROS scavenging effect and can possibly protect endothelial cells from redox injury [72]. Beta carotene administration is advantageous against cyclosporine induced oxidant injury. A disadvantage is that beta carotene decreases the plasma concentration of cyclosporine, thus diminishing the action of the drug [73]. Probulcol is also known to attenuate oxidative stress and endothelial injury [74].

Vitamin E exerts potent anti oxidant activity against oxidative stress induced by peroxynitrite, but it has only a modest effect on oxidative stress induced by hypochlorite [75]. On the other hand, carotenoids are efficient anti oxidants when the oxidizing species is singlet oxygen [76]. Their effectiveness against peroxynitrite is not confirmed. Given that efficacy of anti oxidants has been demonstrated only in vitro and animal studies, the role of anti oxidant therapy in patients in abating endothelial dysfunction caused by drugs is unclear.

Some of the pharmacologically important medicinal plants/extracts have been found to reverse the oxidant injury induced by different drugs. These plant materials include Dandelion (*Taraxacum officinale*) leaves [77], Amla (*Emblia officinalis*) fruit [78], Coriander (*Coriandrum sativum*) seeds [79], Amaranth (*Amarantus of Alexandria*) leaves [80] and Pigeon pea (*Cajanus indicus*) leaves [81]. But their usefulness in human patients is debatable.

[V] SUMMARY

A significant number of drugs alter the redox balance in endothelial cells either directly or indirectly. While a short term use of the drugs that can alter the redox path may not cause adverse effects, long term use can invariably lead to a decompensatory phase and oxidant injury. Understanding the mechanisms of regulated generation of ROS in endothelial cells and its downstream effects is necessary to tackle the undesirable condition and to prevent the progression of adverse side effects. Whether anti oxidant therapies would really be beneficial to prevent the endothelial oxidative stress associated drugs is unclear. Pathological conditions which are linked to increased oxidative stress are not always because of high free radical generation such as HO[•] and O₂^{•-} but linked to the disruption of the function of redox circuits. So the target for anti oxidant therapy cannot solely be scavenging the free radicals. In addition to the uncontrolled generation of ROS associated with drug induced toxicity, abnormal activation of inflammatory pathway is also a cause for injury to endothelial cells. One of the strategies for decreasing endothelial injury by drugs is possibly to identify specific and sensitive markers for early detection of oxidative stress in endothelial dysfunction.

[VI] FUTURE PERSPECTIVES

Redox biology of drug induced endothelial dysfunction is complex as it involves diverse host and different pathophysiological mechanisms. Identifying a target for prevention of endothelial dysfunction caused by drugs is a hard task. Future research should also address how ROS mediated remodeling events affect endothelial stability and its paracrine secretions, whether the type or the proportion of different ROS is a significant factor for progression of secondary effects, whether regional location of endothelial cells (specific to different organs) has any effect on ROS mediated adverse effects and whether age, as it influences both pharmacokinetic and pharmacodynamic properties of drugs, is a determinant factor in increasing the risk for endothelial dysfunction that results from drug toxicity.

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