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Drug Metabolism and Oxidative Stress: Cellular Mechanism and New Therapeutic Insights

Sharmistha Banerjee[1], Jyotirmoy Ghosh[2] and Parames C Sil[1]*

[1]*Division of Molecular Medicine, Bose Institute, P-1/12, CIT Scheme VII M, Kolkata 700054, India*
[2]*Chemistry Department, Banwarilal Bhalotia College Asansol, Ushagram, Asansol-713303, West Bengal, India*

Abstract

Oxidative stress, generated during drug metabolism, acts as a source of origin and progression of many dreadful diseases. Reactive metabolites formed during this process cause oxidative stress and can impair the function of drug-metabolizing enzymes leading to toxicity. It is, therefore, important to investigate the mechanism of drug-induced toxicity and to find a remedy so that cellular toxicity can be minimized. This review highlights the mechanism of reactive oxygen species generation during cytochrome P450 mediated metabolism of various drugs and endogenous molecules, cytochrome mediated metabolism of arachidonic acid and the role of its metabolite, 20-Hydroxy-5,8,11,14-eicosatetraenoic acid in cardiovascular diseases. This review aims to provide updated knowledge on the mechanism of reactive oxygen species generation during drug metabolism, association of drug metabolizing enzyme in diseases and the role of antioxidant therapy that helps to minimize cellular toxicity. The most significant challenges in drug discovery are the unpredictable nature of drug toxicity due to the oxidative stress in drug metabolism. These difficulties can be overcome by inhibiting toxic metabolites formation or by the modification of the structure of the original compounds for the amelioration of toxicity of the toxic metabolites. Another aspect of reducing drug toxicity is the inhibition of the drug metabolizing enzymes.

Keywords: Acetaminophen; Aminochrome; Doxorubicin; L-DOPA; Oxidative Stress; Quinone

Abbreviations: APAP: Acetaminophen; Ahr: Aryl Hydrocarbon Receptor; CYP: Cytochrome; DMA: Desmethylarzoxifene; GST: Glutathione-S-Transferase; 20 HETE: 20-Hydroxy-5,8,11,14-Eicosatetraenoic Acid; 2-OHE: 2-Hydroxyestrogen; L-DOPA: L-Dihydroxyphenylalanine; MPTP: 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydro Pyridine; NRF2: NF-E2-Related Factor 2; NAPQI: N-Acetyl-P-Benzoquinone Imine; 2,3-QE: 2,3-Quinone Estrogen; 3.4-QE: 3,4-Quinone Estrogen; ROS: Reactive Oxygen Species; SERM: Selective Estrogen Receptor Modulators; SNP: Single Nucleotide Polymorphism.

Introduction

Reactive Oxygen Species (ROS), are formed as a byproduct of normal metabolism. These molecules play an important role in cell-signaling pathways and likely to act as key players in many diseases and homeostasis. Species include oxygen radicals and reactive non-radicals. Apart from normal physiological roles, excess ROS formation can also occur during stress such as toxicant exposure, radiation damage, and disease that subsequently produces oxidative stress [1]. This oxidative stress plays an important role in drug metabolism. Most of the drugs administered to patients are lipophilic in nature [2] and can enter into the cell easily through plasma membrane. However, to reach the target site for proper adsorption, distribution, and excretion, these drugs must be converted to hydrophilic molecules. The conversion of these non-polar compounds into polar compounds is termed as drug metabolism. Drug and other xenobiotic metabolism occur through three phases, namely, phase I, phase II and phase III. The major enzyme that play a pivotal role in phase I is Cytochrome P450s (CYP families) [3]. CYP contains heme moieties and helps to oxidize lipophilic drugs for their conversion to the hydrophilic molecule. CYP is a part of microsomal monooxygenase system present in the endoplasmic reticulum. Usually, the poor coupling of the P450 catalytic cycle results in the continuous production of ROS which contributes to the oxidative stress. This stress has profound effect on signaling pathways and other cellular functions [4,5]. Phase II of drug metabolism mainly involves glucuronidation catalyzed by UDP glucuronosyltransferase (UGT). In this process, glucuronic acid binds to a substrate having suitable functional group via covalent linkage. Detoxification of xenobiotics also involves the activity of glutathione-S-transferase and NAD(P)H: quinone oxidoreductases NQO1 and NQO2 to counteract oxidative stress during drug metabolism [2,6]. Phase III involves the participation of various transporters involved in drug metabolism. Since ROS is produced mainly in phase I of drug metabolism, so phase III is not that much important in this context.

Biotransformation of the drug is a complicated process consisting of several steps that lead to the production of ROS. Living beings have evolved with the ability to maintain homeostasis under normal physiological conditions. There exists a fine balance between production, sequestration and neutralization of ROS [7,8]. Oxidative stress occurs when the balance between pro and the antioxidant system gets disrupted. Most of the drugs and xenobiotics generate reactive quinone metabolites in the intermediate stage of their metabolism [6]. This ultimately results in the formation radicals and ortho quinone that reacts with nitrogen bases of DNA forming adducts [9]. These DNA adducts hinder DNA replication, alters epigenetic phenomena (like promoter methylation), and leads to accumulation of mutation and eventually cancer [10]. ROS, generated during biotransformation, can also help cytochrome P450 inhibition [11] and leads to cellular

***Corresponding author:** Parames C Sil, Division of Molecular Medicine, Bose Institute, Kolkata, India, Tel: 9133-25693243; Fax: 9133-2355-3886; Email: parames@jcbose.ac.in

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stress and finally cell death. This review intends to provide updated knowledge on the mechanism of ROS generation during metabolism of various drugs, an endogenous defence mechanism to combat oxidative stress, association of drug metabolizing enzyme in diseases and antioxidant therapy.

Reactive Oxygen Species Generation During Drug Metabolism

Cytochrome P450 is involved in Reactive Oxygen Species (ROS) generation during phase I of drug metabolism. Substrate binding to CYP450 occurs via the combination of one molecule of oxygen to the enzyme leading to the formation of an oxy complex. The oxy complex thus formed is again reduced to peroxy complex that accepts two protons and produce water through intermediate reactions. Since ROS is generated during the intermediate stages of CYP-mediated biotransformation of drugs, their continuous production results in NADPH consumption by the CYP molecules [4]. Although it was mentioned earlier that ROS is generated during the reaction of CYP P450 with its substrate, the electron-transfer chain of microsome continues to oxidize NADPH and produce ROS even in the absence of any substrate [12]. This excessive ROS generation leads to repression of CYP gene expression (specifically CYP1A1 gene expression) is through the inactivation of the transcription factor, nuclear factor 1 [11] (Figure 1). Enzymes like lipooxygenase, cyclooxygenase and xanthine oxidase can also contribute to ROS production.

ROS generated during biotransformation of drugs consists of hydrogen peroxide, hydroxyl and superoxide radical. The hydroxyl radical is very reactive and can modify nitrogenous bases of DNA leading to DNA strand breakage. These ROS particularly superoxide radical gets protonated to form perhydroxyl radical. This radical plays a significant role in lipid peroxidation and membrane destabilization. These reactive species also reacts with nitric oxide (NO) forming peroxynitrite ion (ONOO) and exerting deleterious effect on DNA, protein and lipid molecule [8]. Peroxisome is also a source of hydrogen peroxide but catalase present in this organelle decomposes it into water and oxygen creating a fine balance at physiological condition.

Oxidative Stress Generated by Drug Metabolites

Most of the drugs and xenobiotics, to which human beings are exposed, generate quinone metabolites. Quinones are involved in electron transport. Quinone-quinol cycle leads to oxidative stress leading to devastating effect. Many drugs are converted to quinone metabolites during biotransformation. These quinone metabolites are reactive molecular species which forms adducts with macromolecules, anti-oxidant molecules like GSH and deplete the pool of antioxidant molecule like GSH thereby generating more ROS. Excessive ROS generation and sequestration of endogeneous antioxidant species lead to oxidative stress. Prolonged persistence of cellular stress causes sustained activation of stress responsive MAPKs ultimately manifesting into cell death (Figure 2).

Acetaminophen

Acetaminophen is a widely used as analgesic and antipyretic drug. This drug exerts pharmacological action through inhibition of cyclooxygenase 3 in the central nervous system and prostaglandin E_2. It has been established that both the liver and extrahepatic tissues are primarily affected because of toxicity of Acetaminophen (APAP). In adults, very little amount (~1%) of the therapeutic dosing has been reported to be excreted in the urine; most of it (approximately 63%) is metabolized via glucuronidation and the rest (~34%) by sulfation. Primarily, water-soluble metabolites are formed in these phase II reactions in the liver and are easily excreted via the kidney. Less than 5% of APAP, at the therapeutic doses, is oxidised to the reactive intermediate, electrophilic N-Acetyl-P-Benzoquinone Imine (NAPQI), by the microsomal P-450 enzyme system which, in turn, gets reduced by glutathione and subsequently excreted as a relatively benign compound, mercapturic acid. However, in the presence of excess APAP (beyond the therapeutic doses), stores of sulfate and glutathione are depleted, more of the APAP is alternatively routed to the CYP-450 mixed function oxidase system and more reactive intermediates (NAPQI) is generated. In other words, more severe glutathione depletion as well as massive production of metabolites occurs in this scenario. These free electrophilic intermediates form adduct with sulfhydryl and glutathione moieties on cellular proteins as a result of which cellular homeostasis is disrupted and ultimately initiates cell death. This has been reported for both the liver and kidney tissues in animal models [13].

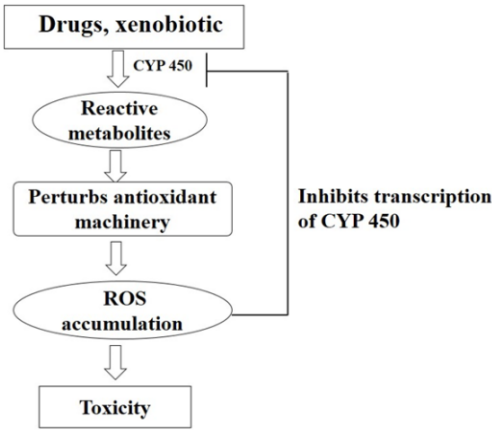

Figure 1: Flow chart depicting CYP mediated ROS generation and transcriptional repression of CYP.

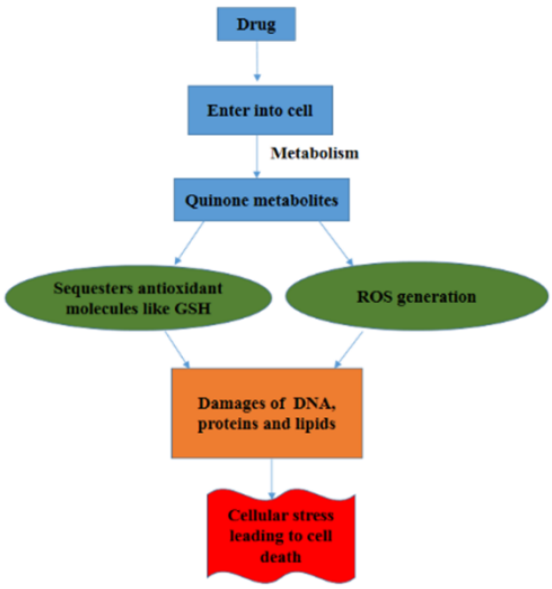

Figure 2: Flowchart depicting mechanism of drug metabolism induced cellular stress.

Recent investigations clearly demonstrated the complex interplay between the pathways of toxin metabolism, intracellular signaling and the host inflammatory response. Among the various signaling molecules involved in the acetaminophen-induced hepatic damage, the signal transduction pathway of MAP kinase family proteins, JNK, is reported to be stimulated by both extracellular and intracellular stimuli including Reactive Oxygen Species (ROS), UV radiation and pro-inflammatory cytokines. This unique molecule can control many basic mammalian physiological processes including phosphorylation of specific subunits (c-Jun, JunB, JunD and ATF 2) of the activating protein 1 (AP-1) transcription factor. Besides, it can also activate the genes which regulate various types of functions including cell proliferation, survival and death [14] (Figure 3).

Micro RNA also plays a major role in APAP-induced hepatotoxicity. Micro RNA-122 is reported to be a novel biomarker of APAP-induced hepatotoxicity in adults. Search for potential micro RNA biomarkers of APAP-induced hepatotoxicity in children was also carried out. Global level of serum and urine micro RNAs were examined in three groups of children: 1) healthy children, 2) Hospitalized children who were receiving therapeutic doses of APAP, 3) hospitalized children receiving overdose of APAP. 147 miRNAs were detected in APAP overdose group. Out of 147, 8 miRNAs were significantly increased in serum (miR-122, -375, -423-5p, -30d-5p, -125b-5p, -4732-5p, -204-5p, and -574-3p) with respect to other 2 groups. Urine samples from the patients showed elevation in 4 miRNAs (miR-375, -940, -9-3p and -302a). Correlation of peak APAP-protein adduct level (indicator of NAPQI mediated oxidative stress) with serum miR-122 ($R = 0.94$; $p = 0.01$) was observed followed by miR-375 ($R = 0.70$; $p = 0.05$) [15].-

Selective Estrogen Receptor Modulators (SERM)

Many SERM are converted to quinone methides during bioactivation that accounts for cellular toxicity. Tamoxifen, a well-known anticancer agent, undergoes hydroxylation to 4-Hydroxytamoxifen catalyzed by CYP2D6. This 4-Hydroxytamoxifen gets oxidized via P450 mediated oxidation to para-quinone methide that forms stable adducts with nitrogen bases of DNA [16]. SERM, raloxifene, Desmethylarzoxifene (DMA) and bazedoxifene inhibit chemical carcinogenesis. Raloxifene and DMA up-regulate sulfotransferase (SULT 1E1) and glucuronidase

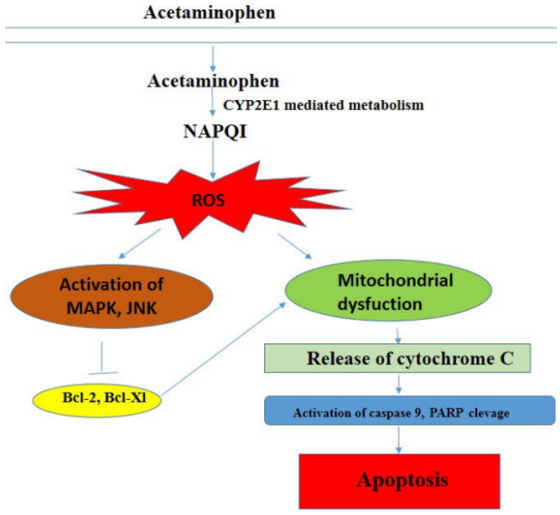

Figure 3: Mechanism of generation of ROS and cellular toxicity by acetaminophen.

(UGT 1A1). Up-regulation of phase II enzymes involved in drug metabolism helps in detoxification of catechol estrogen metabolites ultimately leading to attenuation of ROS formation. This reduced ROS formation inhibits malignant transformation caused by a subset of SERMs [17]. Estrogen is a hormone and is used in hormone replacement therapy (HRT) to improve menopause disorder. Its metabolism plays a crucial role in inducing oxidative stress and cellular toxicity. Estrogen gets converted to 2-Hydroxyestrogen (2-OHE) and 4-Hydroxyestrogen (4-OHE) during metabolism. 2-OHE gets converted to 2, 3 Semiquinone Estrogen that gets converted to 2,3-Quinone Estrogen (2,3-QE). 4-OHE gets converted to 3, 4-Semiquinone Estrogen that ultimately enters into redox cycle to generate 3, 4-Quinone Estrogen (3.4-QE). These two metabolites (2, 3-QE and 3, 4-QE) cause cellular toxicity and build a smooth road towards carcinogenesis by activating NRF2 and inducing Heme Oxygenase 1 (HO-1) expression [18].

Antipsychotic Drug

Clozapine is a frequently prescribed antipsychotic drug that causes a severe adverse reaction. Oxidative bioactivation of this drug by CYP450 families generates reactive nitrite ion, clozapine-N-oxide. These reactive intermediates generated during metabolism, cause ROS formation and impose oxidative stress. CYPs, particularly CYP3A4 and CYP2D6 (having highest specific activity), convert clozapine to GSH reactive nitrenium ions. Between these two, CYP3A4 is more important because it is highly expressed in the liver as compared to CYP2D6. Studies on bioactivation of clozapine from liver microsomes of one hundred individual samples showed eight-fold variability in the activity of CYP3A4. Inter individual differences and drug interactions at CYP3A4 level determine the exposure of the hepatic tissue to reactive metabolites [19]. Recently, use of clozapine has been restricted because of its life-threatening risk of inducing neutropenia and agranulocytosis [20]. So efforts have been made to modify the structure of clozapine to minimize the nitrenium ion formation. Quetiapine is one such clozapine inspired drug used in the treatment of schizophrenia [21,22]. This antipsychotic drug undergoes bioactivation by cytochromeP450 D26 and gets converted to 7-hydroxyquitiapiene. This 7-hydroxyquitiapine is further oxidized by myeloperoxidase to generate reactive quinone imine that accounts for neurotoxicity [20].

Chlorpromazine is a member of the largest class of antipsychotic drug. This drug is known to cause hepatotoxicity in some patients. This molecule is converted to toxic quinone imine metabolite by CYP2D1 and CYP1A2. It also undergoes peroxidase catalyzed oxidation to generate toxic radicals that account for hepatic pathophysiology [23].

Relation of Toxicity with Chemical Structure

Phenothiazine derivatives (PTZs) are used for the treatment of schizophrenia and anxiety [24]. These derivatives are a class of heterocyclic compounds having a tricyclic phenothiazine nucleus (PHT) with the central ring containing sulfur and nitrogen atoms. Phenothiazine derivatives (PTZs) are substituted at position 2 and 10; nature of the ligand at position 10 determines the pharmacological antipsychotic activity and the presence of electron withdrawing groups at position 2 increases therapeutic efficiency of these drugs. PTZs can be classified as aliphatic, piperazine, or piperidine derivatives based on 10-position substituents [25]. These drugs exert a toxic effect on liver including acute intrahepatic cholestasis [25] and hepatitis [26]. De Faria et al. has investigated the structural features of drugs (chlorpromazine, CPZ; fluphenazine, FP; thioridazine, TR; trifluoperazine, TFP; and trifluprom azine, TFPZ), responsible for hepatotoxicity of these PTZs [24]. De Farria et al. demonstrated that the cytotoxic effect of IMP was

very low compared to that of CPZ. The chemical structure of both the drugs are quite similar to each other except that the sulphur atom of the PHT ring is substituted by two methylene groups, leading to the decrease in reactivity of the sulphur atom and resonance of the tricyclic structure [24]. The toxic effect of PTZs also depends on the side chains of PTZs. Derivatives of PTZs containing piperazine or piperidine amine groups linked to a small chain from the phenothiazinic nitrogen accounts for toxicity. De Faria et al. demonstrated that piperidine derivative containing TR was the most cytotoxic in HTC cells among all the drugs used in the study. Piperazine and aliphatic derivatives follow piperidine, group. The cytotoxicity increases in the following order TR > TFP > FP > TFPZ > CPZ. The order of potencies based on substituents at C2 is SCH_3 > CF_3 > Cl, and that of at N10 is piperidine > piperazine > aliphatic. The cytotoxic effect of these drugs varies from one cell to another. In leukemia cell lines, PTZ with CF_3 substituent at C2 position was found to be the most toxic [27]. From the above information, it can be inferred that the substitution at N10 position seems to have a more profound effect than that of C2 position in the manifestation of cytotoxicity in HTC cell line. Formation of cation derived species may handle PTZ induced cytotoxicity and cell death. HOMO energy is the energy required to remove one electron from a molecule. Formation of cation derived species depends on HOMO energy. Both TR and TFP have electron donating group (SCH_3) and TFPZ has electron withdrawing group (CF_3). Therefore, the HOMO energy of the former are lower than that of the later. This indicates that there is a possibility of cation-radical formation in PTZs which may account for cytotoxicity [24]. Further research is needed to elucidate the participation of PTZ cation-radical species**.** PTZs induced the reduction of mitochondrial membrane potential in HTC cells followed the same order of potency as PTZs exhibited for the toxicity that indicates that the cytotoxicity resulted in mitochondrial-mediated cell death [24].

L-Dihydroxyphenylalanine (L-DOPA) is administered to patients suffering from Parkinson's diseases. Neurotoxicity associated with Parkinson's disease is intricately related to dopamine oxidation giving rise to free radicals and quinone species [28]. This leads to oxidative stress and can induce various forms of mitochondrial dysfunctions [29,30]. Dopamine is first converted to dopamine quinone that in turn undergoes one electron oxidation to form dopamine o-semiquinone (Figure 4). The semiquinone form can disproportionate to generate dopamine o-quinone that ultimately cyclizes to generate aminochrome [31]. Aminochrome forms adducts with α-synuclein leading to the formation of neurotoxic protofilaments [32]. These dopamine derived quinones have high reactivity towards NADH and GSH and contribute to the underlying mechanism of oxidative stress-induced mitochondrial dysfunction in the pathophysiology of Parkinson's disease [33].

1-Methyl-4-Phenyl-1,2,3,6-Tetrahydro Pyridine (MPTP), present in the synthetic heroin, is a potent inducer of Parkinson's disease [34]. It undergoes oxidation and is converted to a potent neurotoxin, MPP [35]. MPP inhibits complex I in mitochondria of the dopaminergic neuron [36], leading to depletion of ATP [37] and increases production of ROS [38]. These phenomena ultimately lead to neuronal cell death [39]. Mitochondrial-targeted cytochrome P4502D6 (CYP2D6) along with adrenodoxin and adrenodoxin reductase plays a crucial role in the metabolism of MPTP to MPP+. MPTP treatment induces mitochondrial translocation of Parkin, an autophagic marker in differentiated neurons expressing mitochondrial targeted CYP2D6. This mitochondrial targeted CYP2D6 plays a pivotal role in the pathophysiology of Parkinson's disease [40].

Berberine

Berberine is the most abundant protoberberine alkaloid [41]. CYP enzymes, namely CYP2D6, CYP1A2 and CYP3A4, contribute to oxidative metabolism of this alkaloid and play a major role in the generation of berberine metabolites like demethyleneberberine, thalifendine. Demethyleneberberine is one of the most abundant metabolites generated through NADH-dependent oxidation of berberine with the help of liver microsomal enzymes and hepatic P450s [42]. Palmatine, jatrorrhizine, demethyleneberberine, thalifendine and berberrubine, the metabolites of berberine cause metabolism-dependent irreversible inactivation of CYP1B1 [43].

Doxorubicin

Doxorubicin is a widely used anticancer drug. This drug exerts its pharmacological effect in three ways. Firstly, the drug may insert into DNA strand of cancer cell and prevent DNA replication. Secondly, it may interfere with topoisomerase II, an enzyme involved in DNA replication. Finally doxorubicin can also generate free radical formation which generates oxidative stress in cancer cell. All these three mode of action of the drug ultimately lead to death of cancer cell.

During the metabolism of doxorubicin, ROS is produced in more than one ways. In the presence of NADH, the quinone moiety of doxorubicin molecule is transformed into a semiquinone moiety via one-electron reduction mechanism by the complex I of the mitochondrial electron transport chain system [44]. The semiquinone moiety thus produced reacts with molecular oxygen to form a superoxide radical (O^{2-}), and doxorubicin molecule returns to its original quinone form. The cycling of this process between quinone and semiquinone forms generates a huge amounts of superoxide radical (O^{2-}). This in turn produces a variety of active ROS/RNS species, including H_2O_2, •OH, and $ONOO^-$ [45]. Some endogenous reductases and the endothelial isoform of nitric oxide synthase, present in heart tissue, help in catalyzing the redox cycling of doxorubicin.

Doxorubicin also interferes with iron used for normal metabolic reactions and produces ROS. Doxorubicin semiquinone moiety, O^{2-}, and their byproduct H_2O_2 helps in the release of iron from ferritin, which is an useful iron-storage protein [46] and cytoplasmic aconitase, where [4Fe-4S] cluster is present. Due to the loss of [4Fe-4S] cluster cytoplasmic aconitase is converted into iron regulatory protein (IRP)-1 which subsequently binds with strong affinity to conserve iron-responsive elements (IRE) in the un-translated regions of transferrin receptor (TfR) [45]. As a result of this, iron uptake exceeds iron sequestration and increase in cellular levels of free iron take place which leads to the more production of •OH via Fenton chemistry reaction, ultimately leading to the oxidative stress and cytotoxicity.

Another important factor which contributes in ROS generation is the metabolic turnover of doxorubicin itself. The carbonyl group at C13 of aldoketo reductases is converted to a hydroxyl group, thereby

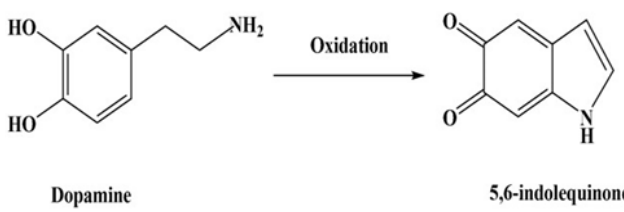

Figure 4: Formation of reactive metabolite from L-DOPA.

inserting a secondary alcohol function in doxorubicin. This particular derivative is highly efficient in releasing iron from the [4Fe-4S] cluster of cytoplasmic aconitase, causing further imbalance in iron metabolism and impose oxidative stress. Doxorubicin can also be metabolized via the cleavage of the bond containing sugar residue of the parent compound into its aglycone form. Its lipophilic aglycone metabolite possesses a higher membrane diffusion capacity than doxorubicin moiety and can easily be accumulated in the inner mitochondrial membrane which leads to ROS formation and deterioration of the functional integrity of the respiration chain [45].

Atorvastatin

Atorvastatin, a member of statin drug family, is the most popularly used 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor [47]. It is an important enzyme in the synthesis of cholesterol and is considered to take part in the rate limiting step of this particular synthesis. High level of cholesterol is a risk factor for coronary disease and atherosclerosis [48]. Atorvastatin undergoes metabolism in hepatic tissue and generates reactive toxic metabolites [49]. Oxidative metabolism of this drug through CYP3A4 leads to the formation of hydroxylated acid metabolites, 4-hydroxy-atorvastatin acid and 2-hydroxy-atorvastatin acid [47,50]. The hydroxyl derivatives are converted to lactone metabolites through CYP3A4 mediated metabolism. The lactone and hydroxylated metabolites can interconvert themselves through hydrolysis and lactonization [51]. The formation of atorvastatin lactone is regulated via UGT1A1 and UGT1A3 mediated metabolism and that of 2-hydroxy-atorvastatin acid is regulated by CYP3A4 and CYP2C8 mediated metabolism [52]. Statins are often administered with other drugs because many patients, suffering from hyperlipidemia, also suffer from coronary heart diseases and diabetic complications. So there is a high chance of adverse drug-drug interaction [53,54].

The oxidative metabolism of atorvastatin and generation of reactive metabolites must be considered for a reliable prediction of drug disposition, especially when this drug is administered with other drugs. The inter conversion among hydroxylated and lactone form plays an integral role in drug-drug interaction [55,47].

Gene expression profiling of two statin drugs (fluvastatin and atorvastatin), having the ability to form toxic reactive p-quinoneimine intermediates, showed changes in the expression of 857 and 1091 transcripts, respectively. However, only 102 transcripts were differentially expressed in case of simvastatin because this drug cannot form aminophenol intermediate due to lack of nitrogen and an arene ring. The differentially expressed targeted genes related mostly to cell death, cell cycle regulation, metabolism and cell defense [56,57].

Pal et al. evaluated the toxic mechanism of atorvastatin in mouse liver. Atorvastatin has been shown to enhance the level of ALP and ALT in hepatic tissue indicating hepatic damage. ROS and oxidative stress played the pivotal role in this hepatotoxicity. Administration of the drug led to the phosphorylation of stress responsive MAPKinse, JNK, p38 and ERK. Also, oxidative stress induced endoplasmic-reticulum stress mediated apoptosis through caspase 12 and mitochondria mediated intrinsic pathway of apoptosis (Figures 5 and 6) [58].

Role of Drug Metabolizing Enzymes in Disease

Cytochrome P450 belongs to the largest superfamilies of enzymes involved in the biotransformation of drugs, xenobiotics and endogenous molecules [59]. Metabolites generated via CYP-mediated metabolism of endogenous substances play crucial roles in the origin and progression of many diseases. Oxidative stress mediated metabolic dysfunction of adipose tissue leads to obesity and cardiovascular diseases [60,61].

Arachidonic acid metabolism is a very important phenomenon in vascular tone [62]. 20-Hydroxy-5, 8, 11, 14-eicosatetraenoic acid (20 HETE) is formed by omega-hydroxylation of this molecule, and this reaction is catalyzed by cytochrome P450. 20 HETE induces oxidative stress via the stimulated generation of superoxide radicals. It inhibits the expression of endothelial nitric oxide synthase (eNOS) and increases the expression of pro-inflammatory cytokines [63,64].

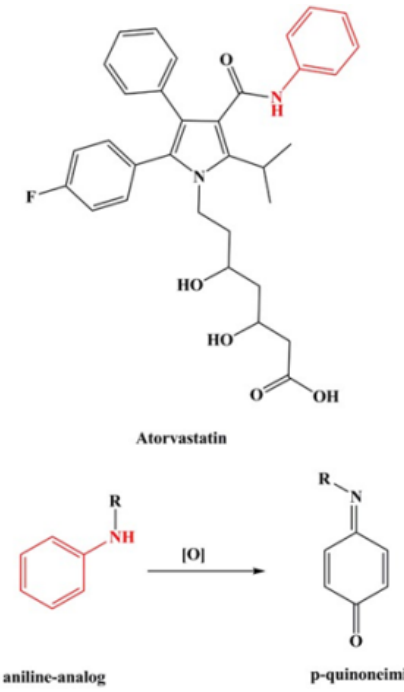

Figure 5: Structure of atorvastatin and generation of quinine metabolite.

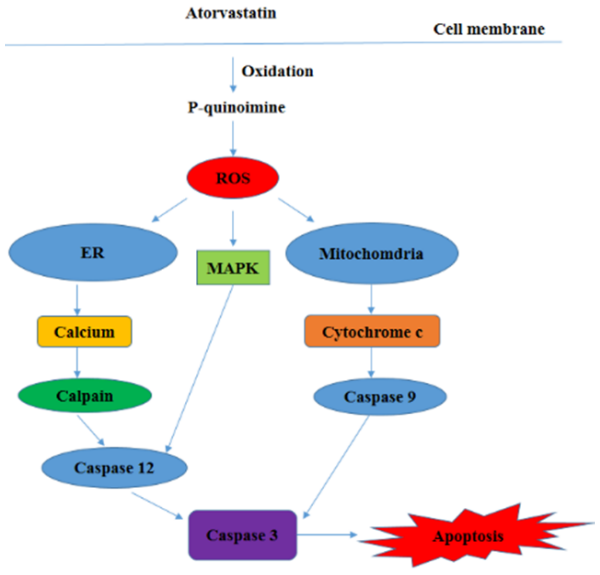

Figure 6: Mechanism of cellular toxicity by atorvastatin.

Kim et al. investigated the effect of 20 HETE on mesenchymal stem cells (MSC) as these cells differentiate into adipocytes. Expression of two major 20 HETE synthase enzymes, CYP4A11, and CYP4E2, are found to be decreased in MSC at the time of adipocyte differentiation. Exogenous administration of 20 HETE increases the differentiation of MSC in a dose-dependent manner in association with increased expression of PPARγ and β catenin. This study clearly demonstrates that 20 HETE increases mature inflamed adipocyte hypertrophy [65].

The rennin-angiotensin system plays a key role in maintaining cardiac homeostasis by generating angiotensin II (ANG II) [66]. Excess production of ANG II leads to hypertension [67], inflammation, ROS production and endothelial dysfunction [68]. The arachidonic acid metabolite, 20 HETE also helps to mediate ANG II-induced vasoconstriction [69], and CYP4A has been reported to be involved in this mechanism [70,71]. CYP1E1, expressed highly in cardiovascular tissue, can also generate 20 HETE from arachidonic acid and generate ROS [72]. A recent report suggests that ANG II infusion increases renal CYP1B1 activity and endothelial dysfunction in CYP1B1+/+ mice compared to CYP1B1-/- mice [73] and CYP1B1 activity in renal tissue. This infusion with ANG II also increases ROS production, NADPH oxidase activity and the levels of ERK1/2 and p38 MAPK. CYP1B1 can be used as a therapeutic target to treat hypertension associated renal disease.

Endogenous Antioxidant Mechanism

It is apparent from the above-mentioned points that the reactive molecules generated during CYP450 mediated metabolism of drugs cause oxidative stress and led to a pathophysiological response. The balance between pro and anti-oxidants get disrupted during oxidative stress. To resume the fine balance and to restore homeostasis, molecules related to stress response get activated. These molecules play a key role in detoxification of toxic substances. One such endogenous molecule is NF-E2-related factor 2 (NRF2) transcription factor [74,75]. Under normal physiological conditions (unstressed conditions), this transcription factor is sequestered in the cytosol by a molecule called keap1. keap1 is an ubiquitin ligase that degrade NRF2 under normal conditions [76,77]. During oxidative stress, excessive ROS production leads to oxidation of distinct cysteine residue of keap1 that leads to conformational changes of the protein such that it can no longer bind to NRF2, and proteasomal degradation of NRF2 is prevented [78]. NRF2 then translocates to the nucleus and binds to DNA sequence of antioxidant response genes for transcription and subsequently involves the activation of other antioxidant response genes like NQO-1, NQO-2, Heme oxygenase 1(HO-1), etc.

Quinone metabolites [such as 1,4-Benzoquinone (BQ) and aminochrome] have been reported to induce endoplasmic reticulum stress (ER stress) and autophagy in dopaminergic N27 cells; however, overexpression of NQO1 can protect the cells against apoptosis and ER stress [79]. NRF2 plays a major role in protecting neuronal cells against 6-hydroxydopamine toxicity [80]. A β-lapachone analogue (3,4-dihydro-2,2-dimethyl-9-chloro-2H-naphtho[1,2b] pyran-5,6-dione) (9-chloro β-lapachone), named CGQ is known to possess anti-tumour and anti- viral activities. This compound causes hepatic toxicity by depleting GSH and producing more superoxide radicals resulting oxidative stress. NQO1 helps in detoxification of toxic metabolites and rescues the hepatic cells from oxidative stress [81].

Aryl hydrocarbon Receptor (AhR) is a transcription factor that has pleiotropic activities and is involved in detoxification of endo and xenobiotics [82]. AhR translocates to the nucleus after ligand binding and association with aryl hydrocarbon receptor nuclear translocator (Arnt). This heterodimer then binds to xenobiotic response elements and transcribes genes responsible for detoxification of toxic metabolites. AhR is also reported to be involved in xenobiotic detoxification in lung tissues [83].

Antioxidant Therapy

From the above-mentioned points, it is transparent that reactive metabolites formed during drug metabolism generate oxidative stress. Though endogenous defense molecular mechanism plays a big role to circumvent oxidative stress-mediated damage, but excessive ROS generation can overcome endogenous defense mechanism. For this reason, administration of antioxidant molecules can prove to be beneficial.

Resveratrol, a natural antioxidant phytoestrogen exerts protective effect estrogen induced breast cancer through NRF2 mediated upregulation of antioxidant molecules like NQO1, superoxide dismutase 3 (SOD3) [84].

Mangiferin (2-C-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone), a naturally occurring polyphenol is present in leaves, roots and barks of plants belonging to Anacardiaceae and Gentianaceae families (e.g. *Mangifera indica*, mango) [85]. This molecule was found to ameliorate galactosamine-induced hepatic toxicity, reduced ROS and nitric oxide (NO) formation. Expression of NADPH:quinone oxidoreductase-1, heme oxygenase-1, GSTα and NRF2 were restored by mangiferin treatment [86] This natural antioxidant also attenuated lead induced hepatic toxicity and cell death via modulation of MAPKinase pathway and antioxidant machinery [87].

Taurine (2-aminoethanesulfonic acid), ameliorated alloxan induced diabetic nephropathy by decreasing blood glucose level, the level of pro-inflammatory cytokines. It also reduced renal oxidative stress by reducing activity of xanthine oxidase and inhibition of p47phox/CYP2E1 pathways [88]. This molecule protects testis from doxorubicin-induced damage. Taurine restored the activities of catalase, superoxide dismutase, glutathione reductase and glutathione S-transferase thereby reducing oxidative stress. Also it also ameliorated endoplasmic reticulum stress mediated testicular cell death [89]. Taurine has shown to exert a protective effect in the liver against acetaminophen-induced oxidative damage [90]. This molecule also protects against arsenic mediated hepatic and cardiac damage, also sodium fluoride mediated toxicity in murine hepatocytes [91-93]. Curcumin and quercetin treatment decreased ROS formation, activities of GST, SOD. Expression and activities of drug metabolizing enzymes cytochrome P450 and b5 were also reduced by treatment with these two polyphenols in benzo(a)pyrene induced lung carcinogenesis [94]. Quercetin has been proved to be effective against fluoride-induced oxidative stress in multiple organs like liver, kidney, testis and brain [95]. Curcumin protects microglial cells by induction of antioxidant gene HO-1 and reducing inflammation [96]. This polyphenol also ameliorates diabetes induced multiple organ pathophysiologies [97-100]. Morin, another polyphenol ameliorates NSAID-induced gastropathy [101]. A novel protein isolated from a plant Phyllanthus niruri ameliorated aspirin-induced toxicity in liver and spleen [102]. Arjunolic acid attenuated sodium nitrite-induced cardiac pathophysiology by modulating pro and inflammatory cytokines, extrinsic and intrinsic apoptotic pathways and also ameliorated sodium fluoride induced renal and hepatic oxidative stress [103,104]. This molecule ameliorates doxorubicin-induced cardiac toxicity [105]. Arjunolic acid also exerts its beneficial role in type I diabetes [106-108]. This molecule also protects liver and

kidney from atorvastatin mediated toxicity [109]. Studies suggest that antioxidant therapy can be effective in oxidative stress-mediated cellular toxicity during drug metabolism.

Studies conducted on animal models have shown that antioxidant therapy can be effective in combating oxidative stress mediated cytotoxicity, however, there are some side effects associated with antioxidant therapy. Curcumin is a well-known anti-oxidant and anti-inflammatory molecule, but long time administration of curcumin may chelate dietary trace elements leading to adverse effects. Six months of dietary supplementation with 0.2% curcumin lead to iron deficiency in C57BL/6J mice. Reduced expression of ferritin and hepcidin in the liver of curcumin supplemented animals were also observed.

Chin et al. have demonstrated that long-term supplementation of curcumin with western diet (20% fat and 10% sugar) aggravate iron deficiency probably through chelation of iron [110]. The major problem of curcumin relates to its poor bioavailability. To improve the bioavailability of curcumin, nano-curcumin and nano-vector for delivery of hydrophobic molecules have come into scenario [105]. The clinical trial was carried out with THERACURMIN, a nanoparticle-based curcumin in pancreatic cancer patients. Sixteen cancer patients (14 patients having pancreatic cancer and two patients having biliary tract cancer) were enrolled for the clinical trial. These patients failed to respond to standard gemcitabine-based chemotherapy. Two patients showed abdominal pain on administration of THERACURMIN [111]. Epelbaum et al. also reported that patients suffer from abdominal fullness and abdominal pain following curcumin administration [112]. Genistein is a phytoestrogen that possess pleiotropic activities like anti-oxidant, anti-inflammatory, anti-diabetic, anti-cancer, etc [113]. Reports suggest that genistein causes altered ovarian development, ovulation and estrous cycle [114,115].

Recent Trends and Conclusion

Pharmaceutical industry, now-a-days, is trying to deliver safe and effective medicines by reducing operating costs and cycle times for drug development to improve the existing therapies in terms of efficacy and safety. One of the most significant challenges to face these demands is the unpredictable nature of the most forms of drug toxicity which arises due to the oxidative stress during drug metabolism and frequently leads to the failure of new drug candidates preclinical toxicity testing. This failure is directly related to the acute, sub chronic, or chronic toxicity testing stages. Many target organ toxicities can be identified in the preclinical stage of safety evaluation; some adverse effects mediated via oxidative stress mechanism, practically remain unknown during animal experiments. These idiosyncratic toxicities do not usually occur and can only be identified during large scale clinical trials. However, this situation creates a negative impact as a significant amount of money has already been spent into the drug candidate. The scenario becomes even worse, if a compound needs to be withdrawn after it has been introduced into the market. From the point of view of both the patient safety and economic loss to the sponsor company, this unpredictable nature of the drug toxicity is, therefore, a great concern [116]. Researchers are actively investigating to get rid of this situation. It may be avoided by the inhibition of toxic metabolites formation or by some structural modification that could ameliorate the toxicity induced by the metabolites. For example, carbamazepine is an anticonvulsant and analgesic drug. Its derivative, oxcarbazepine, developed by the structural modification of carbamazepine to avoid the oxidative-metabolism induced side effects, is also an anticonvulsant and mood-stabilizing drug, primarily used for the epilepsy treatment [117]. Carbamazepine is oxidised by CYP system and oxcarbazepine undergoes reduction of the keto moiety and forms 10-monohydroxy derivative. Another such example is aflatoxin. Aflatoxin B1 is well established mycotoxin. Guengerich et al. has established the mechanism for the metabolism of the mycotoin via CYP mechanism. It was suggested that during the metabolism, the mycotoxin is converted into an exo epoxide and an endo epoxide in which the exo compound is mostly toxic but it could be modulated by conjugation with GST [5,118].

Another aspect to reduce the drug toxicity is the inhibition of the drug metabolizing enzymes. Inhibition of the drug metabolizing enzymes is an interest field of interest to most of the pharmacologists, enzymologists and clinicians. Two main practical applications on the knowledge of inhibition of drug metabolizing enzymes are required in the pharmaceutical industry. First one is drug-drug interaction, i.e., one drug may interact and inhibit the biotransformation of another when these two are taken simultaneously**.** In pharmacotherapy, these drug interactions are of major concern. Because of fatal interactions, several important drugs have been withdrawn from the market as those have been reported to cause serious adverse effects related to drug interactions [117-121]. Probably, the most common cause for documented drug interactions is either induction or inhibition of cytochrome P450 enzymes [121]. Unsafe elevations in the plasma concentrations of drugs could be the result of the inhibition of metabolism due to the competition of the drugs for the same enzyme and this can lead to serious adverse effects as well as toxicities [121]. On the other hand, because of the enzyme induction, the rate of drug elimination and attenuation of its pharmacological effect can be increased as a result of the decreased plasma concentration [121]. Here it is worth mentioning that except CYP2D6, all other isoforms of the CYPs involved in drug metabolism, are inducible enzymes and can be induced by exogenous chemicals and even some endogenous factors [116].

Drug metabolism issues also have a close relation with the clinical trial of drugs. The aim of the clinical trials is to determine the toxicity and the efficacy of certain drugs in the cellular system [5]. New information related to the interaction of drugs with other molecules, formation of reactive metabolites, also the nature of its toxicity can be obtained from these studies. Undesirable toxic reactions reduce the efficacy of the drugs; elimination or at least reduction of those can enhance the same. In addition, the diversity of drug metabolizing enzymes has been reported to play a significant role in drug toxicity. For example, allelic variants with different catalytic activities of CYP enzymes can be expected to be responsible for the drug toxicity. Although the degrees of toxicity of all the isoforms are not the same, studies suggest that certain drugs are highly toxic in some populations due to a heritable deficiency in the CYP450 enzymes. Both CYP2D6 and CYP2C19 are expressed in a polymorphic manner. However, CYP2C19 has at least 70 single nucleotide polymorphisms (SNPs) [5]. It can, therefore, be said that an individual obviously suffers from poor metabolism of certain drugs because of the lacking a particular CYP gene and face the consequences of drug toxicity. Now-a-days, combination of pharmacogenetics and human genomics is providing sufficient knowledge on genetic variations and helps controlling the variability in drug response as well as the associated toxicity [122]. The sequence variation in the proteins targeted by any drug has pronounced effect on the potency as well as side effects of that drug and is strongly regulated by their drug-metabolizing enzymes and transporters. Combining, we say, these factors play the most crucial role for the variable drug responses in individuals [123-129].

Both the drug efficacy and toxicity depend on the optimum dose of a particular drug. Sometimes it has been observed that the optimum treatment necessary for productive and safe therapy diverges significantly from patient to patient. Therefore, if the dose of the drug is not applied accordingly, the clinical result may change. On the other hand, a particular drug response in an individual, both the genetic and non-genetic factors are responsible for this effect [5]. Genetic polymorphisms of proteins are considered to be the major determinants of the metabolism of drugs. In case of non-genetic factor, the normal condition may return whenever the factor is removed [123]. Drug target is responsible for adverse drug reaction, which results in two types of side effects i.e. on-target as well as off-target side effects. Pharmacokinetics variations sometimes alter the concentration of a toxic drug or metabolite in the target tissue [123] and which ultimately results in variable toxicity. Some genetic changes have direct or indirect effect on both drug efficacy and drug safety and this type of divergence may modulate the biological context in which a particular drug reaction occurs [5].

The formation of the active metabolites during drug metabolism mainly depends on the balance among the various CYP enzymes. For example, the bio-activation process of clopidogrel (to date, second most prescribed drug which is used to inhibit blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease [5,129] depends on the balance of many CYP enzymes (CYP2C19, CYP1A2, CYP2B6, CYP3A4 and CYP2C9). Similarly, Jin et al. showed that the bio-activation of tamoxifen also mediated via the co-operation among the CYP enzymes. Combining, we would like to say that pharmacogenomics guided drug development is essential for promoting efficient, safe, widely applicable and cost-effective drug therapy.

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