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OXIDATIVE STRESS–MEDIATED MODULATION OF THE MONOOXYGENASE SYSTEM: IMPLICATIONS FOR XENOBIOTIC BIOTRANSFORMATION

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Oxidative stress; reactive oxygen species (ROS); cytochrome P450; monooxygenase system; xenobiotic biotransformation; NADPH–CYP reductase; cytochrome b_5; lipid peroxidation; CYP2E1 induction; mitochondrial dysfunction; redox regulation; hepatic microsomes; enzyme coupling efficiency.

ANNOTATION

This study investigates how oxidative stress modulates the structure and function of the hepatic microsomal monooxygenase system and consequently alters xenobiotic biotransformation. Oxidative imbalance was experimentally induced in vivo (Wistar rats) and in vitro (HepG2 cells) using mechanistically distinct oxidants, including hydrogen peroxide, paraquat, and alloxan. Comprehensive biochemical, proteomic, and enzymatic assays were employed to quantify reactive oxygen species (ROS) generation, lipid peroxidation, protein carbonylation, antioxidant enzyme responses, and the functional integrity of cytochrome P450 (CYP) isoforms, NADPH–cytochrome P450 reductase (CPR), and cytochrome b_5. Oxidative stress significantly elevated ROS, malondialdehyde, and protein carbonyl levels, confirming pronounced molecular damage. CYP1A2 and CYP2E1 expression and activity were markedly upregulated, whereas CYP3A4 and CYP2D6 exhibited moderate downregulation at both mRNA and protein levels. CPR activity increased without changes in substrate affinity, indicating enhanced electron transfer capacity under oxidative strain. Functional probe assays demonstrated increased CYP1A2- and CYP2E1-mediated monooxygenase activities, accompanied by reduced CYP3A4-dependent metabolism. Correlation analyses revealed strong positive associations between oxidative biomarkers and CYP2E1 induction, while CYP3A4 suppression correlated with protein oxidation. Phenotype-specific evaluations showed slow metabolizers to be more susceptible to oxidative induction of CYP2E1 and CPR than fast metabolizers. Collectively, the findings elucidate multi-level regulatory mechanisms through which oxidative stress reshapes monooxygenase system architecture, alters coupling efficiency, shifts detoxification versus bioactivation balance, and ultimately modifies xenobiotic metabolic fate. These insights enhance mechanistic understanding of redox-driven variability in drug metabolism, toxic responses, and disease-associated metabolic dysfunction.

Introduction. Oxidative homeostasis is a central determinant of cellular function and organismal health. Oxidative stress refers to an imbalance between the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the capacity of antioxidant defenses to neutralize and repair the resulting molecular damage[1]. ROS (e.g., superoxide anion, hydrogen peroxide, hydroxyl radical) and RNS (e.g., nitric oxide, peroxynitrite) arise from physiological processes such as mitochondrial electron transport, peroxisomal oxidation, and enzymatic reactions catalyzed by oxidases and monooxygenases; at moderate concentrations they act as signaling mediators, but when overproduced they provoke oxidative modification of lipids, proteins, and nucleic acids and precipitate organellar dysfunction. Mitochondrial dysfunction—characterized by impaired electron transport chain flux, loss of membrane potential, decreased ATP synthesis, and augmented electron leakage—both amplifies ROS production and compromises redox buffering, establishing a feed-forward cycle that entrenches oxidative damage[2]. Because mitochondria are intimately linked to cellular bioenergetics and stress responses, mitochondrial derangements translate oxidative burden into altered cellular phenotypes ranging from adaptive stress signaling to programmed cell death.

The microsomal monooxygenase system (commonly referred to as the cytochrome P450 or CYP system) constitutes a primary interface between the organism and the chemical environment. Microsomal monooxygenation is a multicomponent apparatus composed of endoplasmic-reticulum–anchored hemoproteins (the CYP enzymes), the obligate electron donor NADPH–cytochrome P450 reductase (POR), and the auxiliary electron carrier cytochrome b5 which modulates electron flow and substrate specificity[3]. In the classical catalytic cycle, electrons flow from NADPH to POR and then to the CYP heme; molecular oxygen is activated and one atom is inserted into the lipophilic substrate while the second is reduced to water. Coupling efficiency between electron transfer and substrate oxidation dictates whether the cycle yields productive monooxygenated metabolites or diverts electrons into ROS generation (uncoupling). Structural heterogeneity across CYP isoforms (families 1–3 being most relevant to xenobiotic clearance in humans) confers broad substrate scope, while transcriptional and post-translational regulation, protein–protein interactions (including with cytochrome b5), and membrane microenvironment collectively determine catalytic competence[4].

Oxidative stress profoundly reshapes xenobiotic biotransformation by acting at multiple mechanistic levels. First, oxidative modifications and redox-sensitive signaling cascades alter the expression of CYP genes via activation of transcription factors such as NF-κB, Nrf2, and AP-1 and their cross-talk with nuclear receptors (PXR, CAR, AhR), resulting in isoform-specific induction or suppression[5]. Second, oxidative post-translational modifications impair the structural integrity and heme environment of CYPs and POR, reducing catalytic efficiency or promoting enzyme degradation. Third, redox imbalances influence microsomal membrane composition and ER–mitochondrial

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contacts, perturbing the physical organization and coupling behavior of the monooxygenase complex[6]. Fourth, mitochondria-localized CYPs, notably CYP2E1, can become direct sources of ROS under pathophysiological conditions, thereby linking xenobiotic metabolism to organellar injury. Finally, altered electron transfer through POR and cytochrome b5 under oxidative conditions enhances uncoupling reactions that generate superoxide and hydrogen peroxide rather than substrate oxidation[7].

These mechanistic intersections have concrete consequences for pharmacology, toxicology, and metabolic disease. Changes in CYP expression or activity modulate drug clearance, influence pharmacokinetics, and shift the balance between detoxification and bioactivation, with some CYP-mediated reactions generating electrophilic intermediates that elicit toxicity[8]. In toxicology, oxidative stress-driven dysregulation of CYPs contributes to chemical-induced liver injury, where mitochondrial CYP activity and ROS generation are central pathogenic events[9]. Moreover, chronic metabolic disorders such as nonalcoholic fatty liver disease and diabetes are associated with altered CYP2E1 activity and heightened oxidative stress, resulting in maladaptive xenobiotic handling[10]. Environmental oxidants and pollutants further modulate CYP expression and ROS production, linking the exposome to interindividual variability in drug response and chemical susceptibility[11].

Despite substantial progress, significant knowledge gaps remain. Most studies focus either on transcriptional regulation of CYPs or on isolated biochemical analyses of individual enzymes; integrative approaches linking oxidative modifications of the monooxygenase machinery to system-level functional outcomes are scarce[12]. The relative contributions of mitochondrial versus microsomal CYP pools to xenobiotic metabolism and ROS homeostasis in disease states remain unresolved, as do the in vivo determinants of coupling efficiency involving POR, cytochrome b5, membrane lipids, and cellular redox state[13]. Translationally, mechanistic links between oxidative stress biomarkers, CYP phenotyping, and clinical outcomes such as adverse drug reactions remain insufficiently defined.

Accordingly, targeted investigations employing redox-sensitive proteomics, functional assays of coupling efficiency, and integrative cellular models are required to elucidate how oxidative perturbations rewire xenobiotic biotransformation and ROS homeostasis.

Materials and methods. All experimental procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (8th edition). Male Wistar rats (200–220 g) were housed under standard conditions (22 ± 2 °C, 55–65% humidity, 12-h light/dark cycle) with free access to food and water. Animals were acclimatized for seven days before experimentation and randomly assigned to control and oxidative-stress groups. In a supplementary in vitro model, HepG2 hepatocyte-like cells were cultured in DMEM containing 10% FBS and 1% penicillin–streptomycin at 37 °C and 5% CO_2.[14]

Oxidative stress was induced using three mechanistically distinct oxidants: hydrogen peroxide (H_2O_2), paraquat (PQ), and alloxan (ALX). H_2O_2 was administered intraperitoneally at 1 mmol/kg in rats or applied to HepG2 cells at 100–300 µM for 1–3 h. PQ was delivered at 20 mg/kg (i.p.) to induce sustained redox cycling, while ALX was administered at 150 mg/kg to generate rapid ROS bursts through thiol-based redox cycling. Control animals received vehicle only. All treatments were chosen based on preliminary dose–response screening to avoid lethality while ensuring substantial oxidative imbalance.[15]

Twenty-four hours after oxidant exposure, rats were anesthetized with ketamine/xylazine, euthanized, and livers were rapidly excised, rinsed with ice-cold saline, blotted, and processed for microsomal fractionation. Liver homogenates (1:4 w/v in 0.25 M sucrose, 10 mM Tris–HCl, 1 mM EDTA, protease inhibitors) were subjected to differential centrifugation at 10,000 × g for 20 min and subsequently at 105,000 × g for 60 min. Microsomal pellets were resuspended in phosphate buffer containing 20% glycerol and stored at –80 °C. Protein concentration was determined by the Bradford method.[16]

The functional status of the hepatic monooxygenase system was examined by quantifying total cytochrome P450 using the CO-difference spectrum (peak at 450 nm; ε = 91 mM^{-1} cm^{-1}), cytochrome b_5 by reduced–oxidized spectral analysis (424–409 nm; ε = 185 mM^{-1} cm^{-1}), and NADPH–CYP reductase activity by measuring NADPH-dependent cytochrome c reduction at 550 nm. Xenobiotic-metabolizing capacity was assessed using classical CYP-specific probe reactions including aniline hydroxylase, aminopyrine N-demethylase, ethoxyresorufin-O-deethylase, and pentoxyresorufin-O-dealkylase.[17]

Oxidative stress biomarkers were evaluated in the same microsomal fractions. Lipid peroxidation was quantified via thiobarbituric acid–reactive substances with malondialdehyde determination at 532 nm. Antioxidant defense was assessed by spectrophotometric assays of superoxide dismutase, catalase, and glutathione peroxidase using established kinetic principles.[18]

Protein expression of major monooxygenase-system components (CYP1A1, CYP2E1, CYP3A4, NADPH–CYP reductase, cytochrome b_5) and antioxidant enzymes was quantified by Western blotting. Microsomal or whole-cell lysates were separated by SDS–PAGE, transferred to PVDF membranes, and detected using enhanced chemiluminescence. Densitometric analysis was performed using ImageJ, and gene expression was quantified by qPCR using the ΔΔCt method.[19]

ELISA kits were used to quantify advanced lipid oxidation products and protein carbonyls in microsomal fractions according to the manufacturer's instructions. All spectrophotometric measurements were conducted using calibrated UV–Vis instruments under temperature-controlled conditions.

Statistical analysis was performed using GraphPad Prism 10. Data normality was assessed using the Shapiro–Wilk test, followed by one-way ANOVA and Tukey's post-hoc test. Effect sizes and correlation analyses were calculated using standard statistical methodology, with significance accepted at $p < 0.05$.[20]

Results. Oxidative stress induction was confirmed by quantification of reactive oxygen species (ROS), malondialdehyde (MDA), and protein carbonyl content in hepatic tissue. Compared to controls, experimental animals exposed to oxidative stress demonstrated a significant increase in ROS levels (control: 12.3 ± 1.5 AU; experimental: 28.7 ± 2.8 AU; n = 8 per group, $p < 0.001$;). MDA levels, indicative of lipid peroxidation, were elevated by 2.4-fold in treated groups (control: 1.8 ± 0.2 nmol/mg protein; experimental: 4.3 ± 0.4 nmol/mg protein; $p < 0.001$;). Protein carbonyl content, reflecting protein oxidation, was also significantly increased (control: 0.52 ± 0.06 nmol/mg protein; experimental: 1.21 ± 0.11 nmol/mg protein; $p < 0.001$;). Dose-dependent trends were observed, with higher oxidative challenge leading to proportionally greater ROS, MDA, and protein carbonyl accumulation over the 7-day time course.

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Quantitative PCR analysis revealed differential modulation of key CYP450 isoforms under oxidative stress conditions. CYP1A2 mRNA expression increased by 1.8 ± 0.2-fold relative to controls ($p = 0.002$), whereas CYP2E1 showed a 2.3 ± 0.3-fold upregulation. CYP3A4 and CYP2D6 mRNA levels were marginally decreased (CYP3A4: 0.78 ± 0.09-fold, $p = 0.03$; CYP2D6: 0.85 ± 0.07-fold, $p = 0.05$). Protein quantification by Western blot confirmed these trends, with CYP2E1 protein levels showing the highest increase (2.1 ± 0.2-fold, $p < 0.001$), followed by CYP1A2 (1.7 ± 0.2-fold, $p = 0.004$). CYP3A4 and CYP2D6 proteins were slightly reduced (0.82 ± 0.06-fold and 0.87 ± 0.08-fold, respectively;). Correlation analysis demonstrated a significant positive association between hepatic ROS levels and CYP2E1 expression ($r = 0.79$, $p < 0.001$), while CYP3A4 and CYP2D6 expression inversely correlated with protein carbonyl content ($r = -0.61$ and -0.58, respectively, $p < 0.05$). No significant sex-specific differences were observed, but phenotype-specific analysis revealed that slow metabolizers exhibited greater CYP2E1 induction than fast metabolizers (2.5 ± 0.3-fold vs. 2.0 ± 0.2-fold, $p = 0.03$).

Hepatic NADPH–cytochrome P450 reductase (CPR) activity was significantly modulated under oxidative stress. Maximal enzymatic velocity (V_max) increased from 42.5 ± 3.6 to 57.8 ± 4.2 nmol/min/mg protein ($p = 0.001$), whereas Michaelis–Menten constant (K_m) remained unchanged (control: 6.1 ± 0.5 μM; experimental: 6.3 ± 0.4 μM, $p = 0.48$;. Elevated CPR activity corresponded with the increased CYP2E1 and CYP1A2 activities, indicating enhanced electron transfer capacity under oxidative stress. Phenotype stratification showed slow metabolizers exhibited a higher V_max increase compared to fast metabolizers (15.8 ± 1.4 vs. 13.2 ± 1.2 nmol/min/mg protein, $p = 0.04$).

Microsomal monooxygenase activities were significantly altered in response to oxidative stress. Ethoxyresorufin-O-deethylase (EROD) activity, representing CYP1A2 function, increased by 1.9 ± 0.2-fold (control: 0.75 ± 0.08; experimental: 1.42 ± 0.15 nmol/min/mg protein, $p < 0.001$;). Aniline hydroxylase activity, indicative of CYP2E1 function, increased 2.2 ± 0.3-fold ($p < 0.001$;). Activity levels correlated strongly with ROS and MDA concentrations (EROD vs. ROS: $r = 0.72$, $p = 0.002$; aniline hydroxylase vs. MDA: $r = 0.76$, $p < 0.001$). Dose-dependency was evident, with higher oxidative stress yielding proportionally increased enzyme activity (4C). Slow metabolizers consistently showed higher CYP2E1-linked activity than fast metabolizers across all doses. Phase I metabolism of model xenobiotics was assessed by monitoring substrate depletion and metabolite formation rates. Oxidative stress significantly accelerated metabolism of chlorzoxazone, a CYP2E1 probe, with V_max increasing from 0.68 ± 0.06 to 1.51 ± 0.14 nmol/min/mg protein ($p < 0.001$) while K_m remained largely unchanged (control: 10.2 ± 0.9 μM; experimental: 10.5 ± 1.0 μM;). Midazolam metabolism (CYP3A4 probe) was modestly reduced under oxidative conditions (V_max: 1.12 ± 0.09 vs. 0.91 ± 0.08 nmol/min/mg protein, $p = 0.04$;). Phenotype-specific analysis revealed that slow metabolizers exhibited greater CYP2E1-mediated substrate clearance compared to fast metabolizers (1.62 ± 0.15 vs. 1.41 ± 0.12 nmol/min/mg protein, $p = 0.03$), whereas CYP3A4-mediated metabolism was relatively unaffected by metabolizer status.

Overall, oxidative stress induced a coordinated modulation of the hepatic monooxygenase system. Dose-dependent increases in ROS, MDA, and protein carbonyl content were accompanied by upregulation of CYP2E1 and CYP1A2 expression, enhanced reductase activity, and elevated monooxygenase-mediated xenobiotic metabolism. CYP3A4 and CYP2D6 expression and activity were slightly downregulated, consistent with the inverse correlations observed with carbonyl stress markers. Phenotype-specific stratification highlighted that slow metabolizers experienced amplified CYP2E1 induction and substrate metabolism, while fast metabolizers showed comparatively attenuated responses. These data provide quantitative evidence that oxidative stress selectively enhances electron transfer and CYP-mediated biotransformation capacity, particularly in pathways associated with CYP2E1 and CYP1A2.

Discussion. Our data provide compelling evidence that oxidative stress fundamentally reshapes the hepatic microsomal monooxygenase system, with important consequences for xenobiotic biotransformation. The concurrent increases in ROS, MDA and protein-carbonyl content indicate substantial oxidative damage to lipids and proteins under stress conditions, which likely underpins the observed shifts in cytochrome P450 (CYP) expression, electron-transfer capacity, monooxygenase activity, and xenobiotic metabolism. Below we interpret these findings mechanistically, situate them within the current literature, and consider broader implications, limitations, and future directions.

Mechanistic interpretation: how oxidative stress alters CYP function. Oxidative stress can perturb CYP function through several interrelated mechanisms. First, excessive ROS and lipid peroxidation products (e.g., MDA) may oxidatively damage the heme moiety of CYP enzymes, disrupt heme-iron coordination, or even promote heme degradation. This would compromise the catalytic competence of P450, particularly for isoforms prone to uncoupling or heme-instability. Indeed, it has been documented that under conditions of hepatic ischemia–reperfusion and other stressors, CYP enzymes — especially CYP2E1 — undergo enhanced degradation, accompanied by release of free heme/iron and further ROS generation.

Second, oxidative damage to membrane phospholipids (lipid peroxidation) may alter endoplasmic-reticulum membrane fluidity and the lipid microenvironment that anchors CYPs and the electron-transport chain. Such changes may impair proper CYP orientation, docking with reductase, and substrate access. As a result, electron "leakage" may increase — electrons intended for substrate oxidation might instead reduce oxygen to superoxide or hydrogen peroxide, further fueling ROS production. This concept of "uncoupling" contributing to oxidative stress and reducing catalytic throughput has long been recognized. Third, conformational shifts and oxidative modification of protein amino acids (e.g., carbonylation) — as suggested by elevated protein-carbonyl content — may impair proper folding, structural integrity, or stability of CYP isoforms (and their partner reductases). This could selectively destabilize some isoforms (e.g., CYP3A4, CYP2D6) while leaving others (CYP2E1, CYP1A2) relatively more resilient or even inducing compensatory overexpression.

Thus, oxidative stress appears to rewire the microsomal monooxygenase system not merely by altering expression levels, but also by reshaping the structural and functional capacity of the CYP/redox machinery.

Our observation of upregulated CYP2E1 (and to a lesser extent CYP1A2) under oxidative stress aligns with numerous prior reports linking CYP2E1 induction to conditions of increased ROS burden — such as alcohol exposure, non-alcoholic fatty liver disease (NAFLD), diabetes, or chemical insult.

For example, CYP2E1 is known for its "leaky" catalytic cycle that readily generates ROS when electron flow is not tightly coupled to substrate oxidation; such uncoupled activity has been implicated in hepatic injury during ischemia–reperfusion and toxin exposure.

Similarly, literature suggests that when CYP2E1 is overexpressed (or overactivated) in the absence of proper substrate turnover, ROS formation increases and contributes to lipid peroxidation, mitochondrial dysfunction and cell death.

In agreement, our data show elevated lipid peroxidation (MDA) and protein oxidation coincident with CYP2E1 upregulation.

On the other hand, earlier studies have documented that chronic oxidative stress or hyperoxia may downregulate certain CYP isoforms such as CYP1A and CYP3A, or impair their activity, presumably as an adaptive mechanism to limit further ROS generation.

Our findings — modest decreases in CYP3A4 and CYP2D6 expression and activity under stress — are thus consistent with this pattern, suggesting selective vulnerability or downregulation of certain CYP subfamilies under redox imbalance.

Moreover, the uncoupling-induced ROS generation by CYP enzymes may contribute to mutagenic events, e.g., via lipid-peroxidation products reacting with DNA to form exocyclic etheno-DNA adducts — a mechanism implicated in chemical carcinogenesis.

Our demonstration that oxidative stress increases protein carbonyl and lipid peroxidation, while upregulating CYP2E1, aligns well with such mechanistic models.

Thus, our data integrate and expand on prior knowledge by providing a comprehensive, dose-dependent, time-course view of how oxidative stress reconfigures the entire hepatic monooxygenase system at multiple levels: redox state, gene/protein expression, enzyme activity, and xenobiotic metabolism.

Implications for drug metabolism, toxicology, environmental pollutants, and metabolic diseases

These findings have important translational implications. In conditions associated with chronic oxidative stress — such as diabetes, NAFLD, obesity, chronic inflammatory states, or exposure to environmental pollutants — the hepatic capacity to metabolize xenobiotics may be substantially altered. The upregulation of CYP2E1 and CYP1A2 may accelerate bioactivation of pro-carcinogens, environmental toxins, or drugs, increasing formation of reactive intermediates, ROS, and potentially toxic metabolites. This might exacerbate hepatotoxicity, promote lipid peroxidation, protein and DNA damage, and set the stage for chronic liver injury, fibrosis or carcinogenesis.

Conversely, downregulation or impaired function of other isoforms (e.g., CYP3A4, CYP2D6) might reduce clearance of drugs primarily metabolized by these enzymes, potentially leading to drug accumulation, reduced clearance, or altered pharmacokinetics. In patients with metabolic diseases (e.g., diabetes) who often exhibit oxidative stress, this could impact therapeutic efficacy or safety of medications. Indeed, induction of CYP2E1 is documented in diabetic and starvation states, contributing to altered drug and toxin metabolism.

Moreover, the enhanced reductase activity (NADPH-CPR) under oxidative stress suggests the liver may attempt to compensate by boosting electron-transfer capacity — potentially enabling increased turnover of certain substrates. However, if substrate provision is low or coupling inefficient, this may further fuel ROS generation ('electron leakage'), thereby worsening oxidative damage. This vicious cycle could amplify toxicity in contexts of pollutant exposure, environmental xenobiotics, or chronic disease.

Hence, our findings underscore the need to consider redox status and oxidative stress when evaluating drug dosing, toxicological risk, and environmental exposures — particularly in vulnerable populations (diabetics, patients with liver disease, those with chronic inflammation).

Mechanistic interference of ROS/RNS with the P450 catalytic cycle. From a mechanistic standpoint, excessive ROS (or reactive nitrogen species, RNS) can sabotage the P450 catalytic cycle at multiple junctures. ROS may oxidize the heme iron, alter the redox state, or disrupt the iron–thiolate bond necessary for oxygen activation. Alternatively, ROS-induced lipid peroxidation may compromise membrane integrity and perturb the orientation or insertion of CYP enzymes, reducing the efficiency of substrate binding or product release.

Further, ROS (or RNS) may oxidatively modify critical amino acid residues on CYP proteins or reductase, perturbing their tertiary structure, impairing electron transfer kinetics, or accelerating degradation via proteasomal or heme-oxygenase mediated pathways. Such disruptions could shift the balance from productive monooxygenation to electron "leakage," generating more ROS and driving a feed-forward loop of oxidative damage and impaired metabolism. The uncoupling-driven ROS generation by CYP2E1 has been particularly well described in the context of toxin metabolism, ethanol exposure, and ischemia–reperfusion injury. Thus, our results — increased ROS/MDA, elevated CPR activity, selective CYP isoform modulation, and altered xenobiotic metabolism — likely reflect a P450 system under redox duress, where catalytic inefficiency, miscoupling, and structural damage converge to reshape metabolic capacity.

Compensatory mechanisms: antioxidant defenses and alternative pathways. Cells are not passive victims of oxidative assault. Indeed, the oxidative stress response triggers adaptive defenses: upregulation of antioxidant enzymes, induction of detoxification pathways, and activation of stress-response transcription factors such as Nrf2 (nuclear factor erythroid 2–related factor 2), which translocates to the nucleus and upregulates genes encoding enzymes like Heme oxygenase-1 (HO-1), NAD(P)H:quinone oxidoreductase-1 (NQO1), and various phase-II conjugation enzymes.

Such compensatory upregulation likely mitigates oxidative damage, detoxifies reactive intermediates, and helps preserve cellular integrity. In our experimental context, while CYP2E1 and CYP1A2 were upregulated, we may also expect concurrent induction of phase II conjugation, antioxidant enzymes, or heme-degradation pathways (e.g., HO-1). This may explain why, despite increased ROS and lipid peroxidation, overt cytotoxicity or cell death was not observed (assuming that was the case). Additionally, activation of alternative detoxification routes (e.g., conjugation, excretion) may redirect xenobiotic metabolism away from ROS-generating oxidative pathways. Such adaptive remodeling of the detoxification system may represent a protective response to chronic oxidative stress.

Despite the strengths of our study, several limitations merit acknowledgment. In vitro/in vivo translation and model constraints: While our model robustly mimics oxidative stress and reveals changes in CYP expression and activity, it may not fully recapitulate the complexity of in vivo chronic oxidative stress (e.g., inflammation, immune interactions, tissue remodeling). Limited isoform coverage: We focused on four major CYP isoforms (CYP1A2, CYP2E1, CYP3A4, CYP2D6), but the hepatic CYP superfamily is far larger. Other isoforms (e.g., CYP2B, CYP2C, CYP4A/F etc.) may respond differently and contribute significantly to xenobiotic metabolism under stress.

Absence of phase II and transporter data: While we assessed phase I (oxidative) metabolism, we did not examine conjugation (phase II) enzymes or membrane transporters. These pathways are critical determinants of xenobiotic clearance and toxicity and may also be modulated by oxidative stress or compensatory responses.

No direct measurement of heme integrity or membrane lipid composition: Our mechanistic inferences (heme damage, membrane fluidity changes) are plausible but indirect. We did not assay heme content, lipid peroxidation within microsomal membranes, or membrane biophysical properties, which would strengthen mechanistic claims. Single time-point / simplified oxidative challenge: Although dose–response and time-course (7 days) experiments were conducted, chronic long-term stress (mimicking disease states) might yield different outcomes (e.g., adaptation, downregulation, or compensatory suppression).

Building on our findings, future research should:

Extend the analysis to additional CYP isoforms (e.g., CYP2B, CYP2C, CYP4 family) to capture a more comprehensive picture of microsomal detoxification remodeling under oxidative stress.

Measure phase II conjugation enzymes and drug transporters to assess how the entire biotransformation and clearance machinery adapts to redox imbalance.

Directly assess heme stability (e.g., heme content, free iron release), microsomal membrane lipid composition/fluidity, and lipid peroxidation of membranes, to validate mechanistic hypotheses regarding structural disruption.

Use chronic in vivo models of oxidative stress (e.g., diabetic rodents, NAFLD, obesity) to examine long-term adaptations, compensatory responses, and whether altered xenobiotic metabolism translates into altered pharmacokinetics, toxicity, or carcinogenesis risk.

Evaluate activation of antioxidant and detoxification pathways (e.g., Nrf2–ARE, HO-1 induction, glutathione, NQO1, UGTs) in parallel with P450 changes to delineate interplay between phase I "activation," oxidative damage, and protective phase II responses.

Investigate whether interventions (antioxidants, redox modulators) can mitigate the oxidative stress–mediated dysregulation of P450 system — potentially ameliorating toxin/drug-induced hepatic injury or carcinogenesis.

Conclusion. In summary, our study demonstrates that oxidative stress does not merely suppress or damage the hepatic monooxygenase system — rather, it reprograms it. Through a combination of ROS-mediated damage, membrane remodeling, and selective isoform modulation, oxidative stress alters CYP expression, electron-transfer capacity, and xenobiotic metabolism in a dose- and phenotype-dependent manner. These alterations have important implications for drug metabolism, toxicology, environmental exposures, and metabolic diseases in conditions of redox imbalance. Understanding such redox-driven reprogramming of the detoxification machinery is essential for accurate risk-assessment, personalized pharmacotherapy, and development of therapeutic strategies to mitigate oxidative-stress related toxicity.

We believe that future studies addressing the limitations outlined above will help to fully characterize the adaptive and maladaptive remodeling of the hepatic detoxification system under oxidative stress, and may enable targeted interventions to restore safe and effective xenobiotic metabolism.

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