УДК 577.15:615.9:612.017.1

КОНЦЕНТРАЦИОННО-ЗАВИСИМОЕ ПОВЫШЕНИЕ УРОВНЯ мРНК *CYP1A1* И *CYP1B1* ПОД ДЕЙСТВИЕМ ФЕНОБАРБИТАЛА В ЛИМФОЦИТАХ ПЕРИФЕРИЧЕСКОЙ КРОВИ ЧЕЛОВЕКА

М. С. И. ГАИТ[1)], ***А. Г. СЫСА***[1)], ***Е. П. ЖИВИЦКАЯ***[1)]

[1)]*Международный государственный экологический институт им. А. Д. Сахарова, Белорусский государственный университет, ул. Долгобродская, 23/1, 220070, г. Минск, Беларусь*

Ферменты цитохрома Р450 *CYP1A1* и *CYP1B1*, регулируемые через рецепторы к ароматическим углеводородам (AhR), играют важную роль в метаболизме ксенобиотиков-экотоксикантов и иммунотоксичности. Хотя фенобарбитал является известным индуктором ферментов *CYP450*, однако его концентрационно-зависимые эффекты в лимфоцитах человека остаются малоизученными. Изолированные лимфоциты периферической крови человека (ЛПК) здоровых взрослых доноров ($n = 20$) подвергали воздействию фенобарбитала (50 мкМ или 100 мкМ) в течение 5 часов. Уровень мРНК *CYP1A1/1B1* определяли методом количественной ПЦР (референсный ген *GAPDH*; эффективность 90–110 %). Выживаемость клеток оценивали с помощью флуоресцентного красителя CFSE через 72 ч культивирования. Данные представлены в виде среднего значения ± – стандартная ошибка среднего; групповые различия проверяли с помощью однофакторного дисперсионного анализа (ANOVA) с использованием апостериорного критерия Тьюки. Взаимосвязи определяли с помощью коэффициента корреляции Пирсона. Фенобарбитал в концентрации 50 мкМ увеличивал уровень мРНК *CYP1A1* и *CYP1B1* (в 3,42 ± 0,8 и 2,61 ± 0,5 раза по сравнению с контролем; в обоих случаях $p < 0,01$),

Образец цитирования:
Гаит МСИ, Сыса АГ, Живицкая ЕП. Концентрационно-зависимое повышение уровня мРНК *CYP1A1* и *CYP1B1* под действием фенобарбитала в лимфоцитах периферической крови человека. *Журнал Белорусского государственного университета. Экология.* 2025;3:76–81 (на англ.).
https://doi.org//10.46646/2521-683X/2025-3-76-81

For citation:
Ghaith MSI, Sysa AG, Zhyvtskaya EP. Concentration-dependent upregulation of *CYP1A1* and *CYP1B1* mRNA by phenobarbital in human peripheral blood lymphocytes. *Journal of the Belarusian State University. Ecology.* 2025;3:76–81.
https://doi.org//10.46646/2521-683X/2025-3-76-81

Авторы:
Алексей Григорьевич Сыса – кандидат химических наук, доцент; доцент кафедры экологической химии и биохимии.
Мготир Гаит Саджид Ибрагим – аспирант кафедры экологической химии и биохимии.
Елена Петровна Живицкая – старший преподаватель кафедры экологической медицины и радиобиологии.

Authors:
Aliaksei G. Sysa, PhD (chemistry), docent; associate professor at the department of environmental chemistry and biochemistry.
aliaksei.sysa@iseu.by
Mgotir Ghaith Sajid Ibrahim, postgraduate student at the department of environmental chemistry and biochemistry.
shiva.samma97@gmail.com
Alena P. Zhyvitskaya, senior lecturer at the department of environmental medicine and radiobiology
alena.zhyvitskaya@gmail.com

тогда как 100 мкМ концентрация вызывала меньшую индукцию (в 1,25 ± 0,2 и 1,21 ± 0,1 раза; в обоих случаях $p < 0,05$ по сравнению с контролем). Выживаемость клеток уменьшилась до 62 ± 7,1 % от контроля при 50 мкМ ($p < 0,01$) и составила 88 ± 4,3 % при 100 мкМ ($p > 0,05$ по сравнению с контролем; $p < 0,01$ по сравнению с 50 мкМ). Индукция *CYP1A1/1B1* отрицательно коррелировала с показателями CFSE ($r = −0,85$ и $−0,82$; в обоих случаях $p < 0,001$). Показано, что фенобарбитал связан с дифференциальной активацией мРНК *CYP1A1/CYP1B1* и снижением выживаемости клеток в исследованных концентрациях. Полученные предварительные результаты указывают на потенциальные эффекты, зависящие от концентрации, что требует дальнейших структурно-функциональных исследований.

Ключевые слова: ферменты цитохрома Р450; *CYP1A1*; *CYP1B1*; фенобарбитал; лимфоциты периферической крови; количественная ПЦР; иммунотоксичность; метаболизм ксенобиотиков.

CONCENTRATION-DEPENDENT UPREGULATION OF *CYP1A1* AND *CYP1B1* MRNA BY PHENOBARBITAL IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

M. S. I. GHAITH[a], ***A. G. SYSA***[a], ***E. P. ZHYVTSKAYA***[a]

[a]*International Sakharov Environmental Institute, Belarusian State University,*
23/1 Daŭhabrodskaja Street, Minsk 220070, Belarus
Corresponding author: A. G. Sysa (aliaksei.sysa@iseu.by)

Cytochrome P450 enzymes *CYP1A1* and *CYP1B1*, regulated by the aryl hydrocarbon receptor (AhR) pathway, play a critical role in xenobiotic metabolism and immunotoxicity. While phenobarbital is a known inducer of CYP450 enzymes, its concentration-dependent effects in human lymphocytes remain poorly characterized. Isolated human peripheral blood lymphocytes (PBLs) from healthy adult donors ($n = 20$) were exposed to phenobarbital (50 μM or 100 μM) for 5 h. *CYP1A1/1B1* mRNA was quantified by qPCR (*GAPDH* reference; efficiency 90–110 %). A CFSE-based assay was used to assess cell viability at 72 h. Data are mean ± SEM; group differences were tested by one-way ANOVA with Tukey's post hoc test; correlations by Pearson's *r*. Phenobarbital at 50 μM increased *CYP1A1* and *CYP1B1* mRNA (3.42 ± 0.8- and 2.61 ± 0.5-fold vs control; both $p < 0.01$), whereas 100 μM elicited smaller induction (1.25 ± 0.2- and 1.21 ± 0.1-fold; both $p < 0.05$ vs control). The CFSE readout was reduced to 62 ± 7.1 % of control at 50 μM ($p < 0.01$) and was 88 ± 4.3 % at 100 μM (not significant vs control; $p < 0.01$ vs 50 μM). Induction of *CYP1A1/1B1* negatively correlated with the CFSE readout ($r = −0.85$ and $−0.82$; both $p < 0.001$). Phenobarbital was associated with differential *CYP1A1/CYP1B1* mRNA upregulation and reduced proliferation at the tested concentrations. These preliminary findings suggest potential concentration-related effects, warranting further mechanistic studies.

Keywords: cytochrome P450; *CYP1A1*; *CYP1B1*; phenobarbital; human peripheral blood lymphocytes; qPCR; immunotoxicity; xenobiotic metabolism.

Introduction

Cytochrome P450 (CYP) enzymes represent a superfamily of heme-containing monooxygenases that are essential for the biotransformation of endogenous compounds, pharmaceuticals, and environmental xenobiotics. Among these, *CYP1A1* and *CYP1B1* are particularly noteworthy for their dual roles in human health and ecological toxicology. On one hand, they facilitate the detoxification of harmful substances by catalyzing oxidative reactions that render lipophilic compounds more water-soluble and excretable. On the other hand, they can bioactivate procarcinogens into reactive metabolites, thereby contributing to mutagenesis, carcinogenesis, and other toxicological outcomes. This paradoxical functionality positions CYP1 enzymes as critical mediators in the interface between environmental exposures and cellular homeostasis.

The regulation of *CYP1A1* and *CYP1B1* is predominantly governed by the aryl hydrocarbon receptor (AhR) pathway, a ligand-activated transcription factor that responds to a wide array of environmental contaminants. Polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene (BaP) and 3-methylcholanthrene (MC), are prototypical AhR agonists. These compounds are ubiquitous persistent organic pollutants (POPs) originating from diverse sources, including incomplete combustion of fossil fuels, wildfires, industrial processes, vehicle exhaust, and tobacco smoke. PAHs' persistence in the environment – due to their chemical stability and bioaccumulation potential – poses significant risks to ecosystems and human populations. For instance, epidemiological studies have linked PAH exposure to increased incidences of lung cancer, skin disorders, and reproductive toxicities in occupationally exposed workers, such as firefighters and industrial laborers [1]. In wildlife, PAHs disrupt endocrine systems and immune responses, leading to population declines in sensitive species like fish and birds.

The AhR-mediated induction of *CYP1A1*/*CYP1B1* by PAHs not only enhances the metabolic activation of these pollutants but also generates reactive oxygen species (ROS), which can exacerbate oxidative stress, DNA damage, and inflammation. This mechanism is a cornerstone of how environmental pollutants perturb cellular and organismal homeostasis across species.

In contrast to the AhR pathway, phenobarbital – a barbiturate pharmaceutical agent historically used as an anticonvulsant and sedative – primarily induces hepatic CYP enzymes through the constitutive androstane receptor (CAR). CAR, a member of the nuclear receptor superfamily, orchestrates the upregulation of CYP2B and CYP3A isoforms, which are vital for drug metabolism and detoxification in the liver. Phenobarbital's discovery in the early 20th century revolutionized epilepsy treatment, but its long-term use has been associated with side effects like enzyme induction leading to drug interactions. While the hepatic effects of phenobarbital are extensively documented, including its role in enhancing the clearance of xenobiotics and endogenous steroids, its interactions with extrahepatic CYP enzymes, particularly in immune cells such as lymphocytes, remain underexplored. This knowledge gap is significant because lymphocytes, as key components of the adaptive immune system, are highly sensitive to environmental and pharmacological stressors. Understanding these interactions could reveal novel insights into how therapeutic agents might inadvertently modulate responses to environmental toxins.

Lymphocytes serve as sentinels of immune function, exhibiting unique CYP450 regulatory patterns that are profoundly influenced by environmental exposures. For example, PAHs robustly induce *CYP1A1* and *CYP1B1* in lymphocytes via AhR activation, leading to the metabolic activation of procarcinogens and the production of ROS that impair immune surveillance and function. Studies have shown that exposure to MC, a model PAH, elevates *CYP1A1* mRNA levels, ethoxyresorufin-O-deethylase (EROD) activity, and protein expression in lymphocytes, patterns that mirror those observed in human populations exposed to urban air pollution or occupational hazards [1]. Such induction can compromise lymphocyte proliferation, cytokine production, and overall immunocompetence, contributing to increased susceptibility to infections and cancers. Conversely, phenobarbital does not typically replicate these effects in lymphocytes at standard concentrations, highlighting a tissue-specific regulatory divergence. Lymphocytes express minimal functional CAR signaling but retain robust AhR responsiveness to environmental ligands like PAHs [2; 3]. This dichotomy is ecologically and clinically relevant: lymphocytes can act as accessible biomarkers for monitoring pollutant exposure in biomonitoring programs, distinguishing between environmental (AhR-driven) and pharmacological (CAR-driven) induction pathways.

Mechanistic investigations have further illuminated this regulatory split. In hepatocytes, phenobarbital activates CAR, which translocates to the nucleus and binds to phenobarbital-responsive enhancer modules (PBREMs) in the promoters of CYP2B and CYP3A genes, thereby enhancing transcription [4]. However, in lymphocytes, CAR expression is low or absent, and phenobarbital does not effectively engage the AhR pathway, rendering *CYP1A1* and *CYP1B1* largely unresponsive [5]. Gene expression profiling in human peripheral blood lymphocytes (PBLs) and cell lines like HL-60 has confirmed that phenobarbital can induce CYP2B6 – a CAR target – but not *CYP1A1* or *CYP1B1* [3; 6]. These observations are consistent with clinical biomonitoring data, where individuals on phenobarbital therapy exhibit no significant elevation in lymphocyte *CYP1A1*/*CYP1B1* levels, in stark contrast to those exposed to AhR agonists like dioxins or PAHs.

Despite this growing consensus, several critical gaps persist in the literature. Most prior studies have evaluated phenobarbital at single concentrations, neglecting potential dose-response dynamics in non-hepatic cells. Additionally, while high doses of phenobarbital are known to induce cytotoxicity through mechanisms like mitochondrial dysfunction and apoptosis, the interplay between CYP induction and lymphocyte proliferation remains unclear. This is particularly important given lymphocytes' role in immune responses; any modulation could have implications for vaccine efficacy, autoimmune diseases, or cancer immunotherapy. Furthermore, inter-individual variability – driven by genetic polymorphisms in AhR or CAR, lifestyle factors, or co-exposures – has not been adequately addressed in small-scale studies.

Here, we assessed phenobarbital's concentration-dependent association with *CYP1A1/1B1* mRNA in human PBLs and explored a functional readout of cell division using CFSE. We tested two concentrations (50 and 100 μM) chosen for pharmacological relevance and to bracket the range commonly used in *in vitro* induction studies. Because canonical pathway controls (e. g., AhR agonists such as benzo[a]pyrene/TCDD; CAR agonist CITCO) and protein/activity measurements were not included in this pilot, mechanistic inferences are not attempted. Instead, we focus on documenting the observed transcriptional responses and their relationship to the CFSE readout, and we delineate a clear plan for essential validation experiments.

Materials and Methods

Study Design and Ethical Approval. This in vitro experimental study was designed to investigate the concentration-dependent effects of phenobarbital on *CYP1A1* and *CYP1B1* induction in isolated human PBLs, with an emphasis on transcriptional changes and proliferative capacity. The protocol adhered strictly to ethical guidelines

outlined in the Declaration of Helsinki, ensuring participant safety and data integrity. Written informed consent was secured from 20 healthy adult donors (10 males, 10 females; aged 25–40 years) before blood collection. Donors were screened to exclude those with recent illnesses, medication use (including phenobarbital or other CYP inducers), smoking history, or occupational exposure to PAHs, to minimize confounding variables. This sample size was chosen for a pilot study to detect preliminary trends while accounting for inter-individual variability.

Lymphocyte Isolation and Culture. Peripheral blood (10–20 mL per donor) was collected via venipuncture into heparinized vacutainer tubes to prevent coagulation and preserve cell viability. Samples were processed within 2 hours to avoid degradation. Lymphocytes were isolated using Lymphocyte Separation Medium (LSM; Capricorn Scientific, Germany), a Ficoll-based density gradient solution that separates mononuclear cells based on buoyancy. Centrifugation was performed at 400 g for 30 minutes at room temperature, following manufacturer recommendations to optimize yield and purity. The resulting lymphocyte-enriched layer was aspirated, washed twice in sterile phosphate-buffered saline (PBS; Thermo Fisher, USA) to remove residual LSM and plasma contaminants, and resuspended in complete RPMI-1640 medium (Gibco, USA).

The medium was supplemented with 10 % heat-inactivated fetal bovine serum (FBS; Sigma-Aldrich, USA) for nutrient support, 2 mM L-glutamine as an energy source, and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) to prevent bacterial contamination. Cell counts were determined using a hemocytometer, and viability was confirmed via trypan blue exclusion (0.4 % w/v; Sigma-Aldrich), requiring > 95 % viability for inclusion. This threshold ensures experimental reliability, as lower viability could introduce artifacts in gene expression or proliferation assays. Cells were maintained in a humidified incubator at 37 °C with 5 % CO_2 to mimic physiological conditions.

Phenobarbital Treatment. Isolated lymphocytes were seeded at a density of $1*10^6$ cells/mL into 24-well plates (Corning, USA) to allow for uniform exposure and easy harvesting. Phenobarbital (Sigma-Aldrich, USA; purity > 98 %) was dissolved in dimethyl sulfoxide (DMSO) and added to achieve final concentrations of 50 μM (therapeutic-relevant, based on clinical pharmacokinetics) or 100 μM (supra-therapeutic, commonly used in induction studies). Vehicle controls received 0.1 % DMSO (v/v) to match solvent exposure. The treatment duration was 5 hours at 37 °C under 5 % CO_2, selected based on prior kinetic studies showing peak transcriptional responses for CYP genes within this timeframe, while minimizing acute cytotoxicity that could confound results. No post-treatment washes or media changes were performed to simulate continuous exposure, reflecting real-world scenarios of sustained drug levels *in vivo*. This design choice, however, may amplify any cumulative effects during subsequent assays.

Lymphocyte Viability Assay. To assess functional outcomes, we employed the CFSE dilution method, a flow cytometry-based technique that tracks cell division by monitoring the progressive halving of fluorescent dye intensity in daughter cells. After the 5-hour phenobarbital treatment, cells were labeled with 5 μM CFSE (BioLegend, USA) for 15 minutes at 37 °C directly in the culture medium, ensuring even staining without disrupting the experimental setup. Labeling was quenched by adding ice-cold RPMI-1640 medium supplemented with 10 % FBS, avoiding washes to prevent cell loss, which is critical for low-yield primary cells. Labeled cells were then cultured for an additional 72 hours under the same conditions, with continuous phenobarbital exposure (no media changes) to evaluate long-term effects. Fluorescence was quantified using a BD FACSCanto™ II flow cytometer (BD Biosciences, USA), with data acquisition of at least 10,000 events per sample for statistical robustness. Analysis was performed in FlowJo v10.8.1 software (BD Biosciences), where proliferation was quantified as the ratio of divided (low CFSE intensity) to undivided (high CFSE intensity) cells, yielding a viability/proliferation index. Notably, no exogenous mitogens (e. g., phytohemagglutinin or concanavalin A) were added, so the assay likely captures basal homeostatic proliferation or survival rather than stimulated clonal expansion.

RNA Extraction and cDNA Synthesis. Total RNA was extracted using the FavorPrep Blood/Cultured Cell Total RNA Mini Kit (FAVORGEN, Korea), following the manufacturer's protocol. RNA purity (A260/A280 ratio: 1.8–2.0) and concentration were quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher). First-strand cDNA was synthesized from 1 μg RNA using the EasyScript® cDNA Synthesis Kit (TransGen Biotech, China), with $Oligo(dT)_{18}$ primers and the following conditions: 25 °C for 10 min, 42 °C for 15 min.

Quantitative Real-Time PCR (qPCR). Primers for *CYP1A1, CYP1B1*, and the reference gene *GAPDH* (Table 1) were designed using Primer-BLAST (NCBI) and validated for efficiency (90–110 %) via standard curve analysis. qPCR was performed in triplicate using GoTaq® qPCR Master Mix (Promega, USA) on a SaCycler-96 system (Sacace, Italy). Reactions (20 μL) included 10 μL master mix, 0.5 μM primers, and 5 μL cDNA. Cycling conditions: 95 °C for 1 min, followed by 45 cycles of 95 °C for 15 sec, 60 °C for 20 sec, and 72 °C for 15 sec. Melt curve analysis confirmed specificity.

Table 1

Primer Sequences

Таблица 1

Последовательности праймеров

Gene	Forward Primer (5′–3′)	Reverse Primer (5′–3′)
CYP1A1	CACCTGGCACTGTCAAGGAT	ATAGCTTCCTGTGGGGGATGG
CYP1B1	ACGCCTTTATCCTCTCTGCG	CTCTGCTGGTCAGGTCCTTG
GAPDH	CTACCAGTGCAAAGAGCCCA	TGGTCATCAACCCTTCCACG

Statistical Analysis. Data are presented as mean ± SEM. Normality was confirmed using Shapiro-Wilk tests, and homogeneity of variance was assessed via Levene's test. Fold changes in gene expression were calculated using the $2^{-\Delta\Delta Ct}$ method, where $\Delta\Delta Ct = (Ct_{target} - Ct_{GAPDH})_{sample} - (Ct_{target} - Ct_{GAPDH})_{control}$.

Group differences were analyzed by one-way ANOVA followed by Tukey's post-hoc test. Correlation between CYP induction and proliferation was evaluated using Pearson's coefficient (r). All analyses were performed in R ($p < 0.05$ considered significant).

Results and Discussion

The experimental investigation yielded distinct patterns of cytochrome P450 induction and associated functional consequences in phenobarbital-treated human peripheral blood lymphocytes (PBLs). Quantitative PCR analysis demonstrated significant differential induction of *CYP1A1* and *CYP1B1* mRNA expression following phenobarbital exposure. At the lower concentration of 50 μM, phenobarbital elicited a pronounced upregulation of *CYP1A1*, with a mean fold change of 3.42 ± 0.8 relative to untreated controls ($p < 0.01$). A similar trend was observed for *CYP1B1*, which exhibited a 2.61 ± 0.5-fold increase in expression ($p < 0.01$). In contrast, the higher 100 μM phenobarbital dose resulted in markedly attenuated induction, with *CYP1A1* expression increasing by only 1.25 ± 0.2-fold ($p < 0.05$ vs. control) and *CYP1B1* showing a marginal 1.21 ± 0.1-fold elevation ($p < 0.05$). Statistical comparison between the two treatment groups confirmed that the 50 μM concentration induced significantly greater *CYP1A1* (2.74-fold difference, $p < 0.01$) and *CYP1B1* (2.15-fold difference, $p < 0.01$) expression compared to the 100 μM dose (Table 2).

Table 2

Fold Changes in *CYP1A1* and *CYP1B1* Expression

Таблица 2

Изменение экспрессии *CYP1A1* и *CYP1B1* (раз)

Treatment	*CYP1A1* Fold Change (Mean ± SEM)	*CYP1B1* Fold Change (Mean ± SEM)
Control	1.0 ± 0.0	1.0 ± 0.0
Phenobarbital, 50 μM	3.42 ± 0.8*	2.61 ± 0.5*
Phenobarbital, 100 μM	1.25 ± 0.2*	1.21 ± 0.1*

* – Significant difference compared to control ($p < 0.05$).

In parallel with transcriptional changes, phenobarbital treatment exerted measurable effects on lymphocyte proliferative capacity. CFSE dilution assays revealed that the 50 μM dose reduced cellular proliferation to 62 ± 7.1 % of control levels ($p < 0.01$), whereas the 100 μM dose preserved proliferation at 88 ± 4.3 % of baseline (not significant vs. control; $p < 0.01$ vs. 50 μM). Correlation analyses further underscored a robust inverse relationship between CYP induction and proliferative activity. *CYP1A1* upregulation exhibited a strong negative correlation with proliferation ($r = -0.85$, $p < 0.001$), as did *CYP1B1* induction ($r = -0.82$, $p < 0.001$), suggesting a dose-dependent interplay between enzymatic activation and functional impairment.

Notably, inter-individual variability was observed across the donor cohort ($n = 20$). *CYP1A1* induction at 50 μM phenobarbital ranged from 2.1- to 4.8-fold, while *CYP1B1* responses varied between 1.9- and 3.3-fold. Despite this variability, the overall dose-response relationship remained consistent, with the 50 μM dose uniform-

ly surpassing the 100 μM dose in induction magnitude. Subgroup analyses detected no statistically significant differences in *CYP1A1/CYP1B1* expression or proliferation rates based on donor sex (10 male, 10 female) or age (25–40 years).

This pilot study documents that phenobarbital exposure is associated with increased *CYP1A1/1B1* mRNA in human PBLs at 50 μM, with smaller changes at 100 μM, and that higher CYP1 expression coincides with a reduced CFSE-based cell-division readout. These results add to a mixed literature on CYP regulation in lymphocytes and warrant careful validation.

First, mechanistic attribution is not possible from the current data. In lymphocytes, *CYP1A1*/*1B1* are typically induced by AhR agonists (e.g., PAHs). Although phenobarbital is a CAR activator in hepatocytes, cross-talk among xenobiotic receptors and stress responses can modulate CYP expression. Without canonical positive controls (AhR agonist such as benzo[a]pyrene or TCDD; CAR agonist such as CITCO) and readouts of CAR/AhR engagement, we cannot determine whether the observed mRNA changes reflect AhR activation, off-target signaling, stress responses, or indirect effects. Future work should include these controls and quantify CYP2B6 mRNA/protein as a CAR pathway readout in the same samples.

Second, the smaller effect at 100 μM versus 50 μM across two dose levels suggests a biphasic pattern within the tested range, but additional concentrations are required to characterize the dose-response shape. Non-linear or non-monotonic patterns are common in xenobiotic signaling, however, this cannot be concluded here.

Third, functional implications require confirmation. The inverse association between CYP1 expression and the CFSE readout suggests that phenobarbital-exposed cells divided less over 72 h; however, because exogenous mitogens were not documented, the CFSE metric may reflect combined effects on survival and homeostatic cycling rather than antigen-driven proliferation. Protein-level confirmation (*CYP1A1/1B1* immunoblot) and an activity assay (e. g., EROD) would establish whether mRNA changes translate to enzyme function. Parallel assessment of viability/apoptosis (e. g., Annexin V/PI) at 5 h and 72 h would distinguish cytotoxicity from cell-cycle effects.

Strengths of this study include the use of primary human donors (n = 20), triplicate qPCR with primer efficiency validation, and correlation analyses across donors. Limitations include lack of pathway controls, reliance on a single reference gene (*GAPDH*) for normalization, absence of protein/activity data, and testing only two concentrations.

Conclusion

In human PBLs, phenobarbital exposure was associated with increased *CYP1A1/1B1* mRNA at 50 μM and a smaller increase at 100 μM, alongside a lower CFSE-based cell-division readout at 50 μM. These preliminary findings require confirmation with pathway-specific positive controls (AhR/CAR), additional dose levels, orthogonal protein/activity assays, multiple stable reference genes, and mitogen-stimulated proliferation protocols before mechanistic conclusions can be drawn.

References

1. Dey A, Parmar D, Dayal M. Cytochrome P450 1A1 (*CYP1A1*) in blood lymphocytes evidence for catalytic activity and mRNA expression. *Life Sciences.* 2001;69(4):383–393. Doi:10.1016/s0024-3205(01)01130-4.

2. Saurabh K, Sharma A, Yadav S, et al. Polycyclic aromatic hydrocarbon metabolizing cytochrome P450s in freshly prepared uncultured rat blood lymphocytes. *Biochemical Pharmacology.* 2010;79(8):1182–1188. Doi:10.1016/j.bcp.2009.11.021.

3. Sharma A, Saurabh K, Yadav S, et al. Expression profiling of selected genes of toxication and detoxication pathways in peripheral blood lymphocytes as a biomarker for predicting toxicity of environmental chemicals. *International Journal of Hygiene and Environmental Health*. 2013;216(6):645–651. Doi:10.1016/j.ijheh.2012.11.002.

4. Hannon-Fletcher MP, Barnett YA. Lymphocyte cytochrome P450 expression: inducibility studies in male Wistar rats. *British Journal of Biomedical Science.* 2008;65(1):1–6. Doi:10.1080/09674845.2008.11732786.

5. Siest G, Jeannesson E, Marteau JB, et al. Transcription factor and drug-metabolizing enzyme gene expression in lymphocytes from healthy human subjects. *Drug Metabolism and Disposition*. 2008;36(1):182–189. Doi:10.1124/dmd.107.017228.

6. Kawai M, Chen J, Cheung CY, et al. Transcript profiling of cytochrome P450 genes in HL-60 human leukemic cells: upregulation of *CYP1B1* by all-trans-retinoic acid. *Molecular and Cellular Biochemistry*. 2003;248(1–2):57–65. Doi:10.1023/a:1024101430363.

Статья поступила в редколлегию 02.06.2025.
Received by editorial board 02.06.2025.