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Investigating the Effects of CYP1A1 Inhibitor Concentration and Treatment Duration on the Growth, Apoptosis and Caspase Cleavage of Human Breast Epithelial Cells Treated with PAH

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Abstract. Polycyclic Aromatic Hydrocarbons (PAHs) are environmental carcinogens that can be continuously exposed to the human body through breathing and other means, causing harm to various organs. The carcinogenic mechanism of PAHs is closely related to CYP1A1. This study reveals the effects of the concentration and duration time of the CYP1A1 inhibitor on human mammary epithelial cells treatment with PAHs, providing a potential target for the prevention and treatment of PAH-related breast cancer. Further verification of its specificity and efficacy through in vivo models is required in the future.

Keywords: PAHS, CYP1A1 inhibitor, concentration and duration time, breast cancer

1. Introduction

Polycyclic Aromatic Hydrocarbons (PAHs), a class of organic environmental contaminants, consist of two or more fused aromatic rings. They are mainly produced by the incomplete combustion of organic substances, such as coal, oil, petrol wood and so on [1]. PAHs is a recognized carcinogen in the world, among them, benzo[a]pyrene (BAP) is categorized as Group 1 carcinogen by the International Agency for Research on Cancer (IARC), signifying its definitive link to human cancer. Long-term exposure to PAH environments can be enter the human body through breathing, diet and other aspects, and affect various tissues and organs. For example, the indoor sources of exposure (active/passive smoking, grilled/smoked food intake) is associated with the onset of breast cancer [2].

And injecting bap into the lungs of mice can lead to various cancers like lung cancer [3]. All these prove that long-term exposure to bap significantly increases the risk of developing cancer.

From above content, we can know that long-term exposure to PAH have relations with the occurrence of breast cancer. The CYP1 family of enzymes plays an important role in breast cancer. The mammalian CYP1A1, CYP1A2, and CYP1B1 genes (encoding cytochromes P450 1A1, 1A2, and 1B1, respectively) are regulated by the aromatic hydrocarbon receptor (AHR) [4]. PAHs, typified by BaP, mediate their effects primarily through interaction with the aryl hydrocarbon

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receptor (AhR). This interaction induces the expression of CYP1A1 and CYP1B1, which subsequently metabolically activate BaP into its carcinogenic metabolites [5].

Nowadays, most of the existing reports have focused on the metabolic function of CYP1A1, but have rarely systematically explored the combined effects of "CYP1A1 inhibitor concentration and treatment duration" on the growth, apoptosis and caspase cleavage of PAH-treated breast epithelial cells.

Hypothesis: Based on the above information, this experiment puts forward the core hypothesis: Increasing the concentrations and treatment duration of CYP1 inhibitor CYP1A1 will decreases growth, increases apoptosis, and increases caspase cleavage of PAH treated human mammary epithelial cells.

2. Materials and methods

The experimental design of this study is as follows. In the experiment, I set up a control system. The positive control used was the highly carcinogenic PAH (benzopyrene), while the negative control used was DMSO water. This was done to eliminate the interference of non-specific factors in the experiment and ensure that the experimental results were caused by the treatment factors. For the cells in the control system, using the MTT method to detect. Set up the multiple concentrations (0.1 - 10μM) and multiple time periods (24 - 72 hours) of CYP1A1 inhibitor treatments. The absorbance was measured to indirectly reflect the cell growth ability. Using FACS to detect cells stained with Annexin V and PI. Based on the differences in fluorescence signals, analyze the apoptosis situation of the cells. And using western blot method to assess the cleavage situation of caspase. through this study, we can determine the optimal concentration and treatment duration of CYP1 inhibitor.

Cell culture: The human breast epithelial cell line used in this study was purchased from a well-known biological resource center. Culture these cells in DMEM medium supplemented with 10% fetal bovine serum (FBS) and maintain them in the humidified incubator at 37°C with 5% carbon dioxide. Regular subculture was performed to ensure exponential growth and maintain the cells in a healthy state.

Primary materials: Human breast epithelial cells, fetal bovine serum, DMEM culture medium, benzopyrene (Bap), DMSO water, CYP1A1 inhibitor, MTT solution (5mg/ml), trypsin, phosphate-buffered saline (PBS), Annexin V-FITC dye and PI dye, protease inhibitor, RIPA buffer, PVDF membrane, TBST solution, skimmed milk, primary antibody and HRP-labeled secondary antibody against caspase-3, ECL reagent A and B.

Experimental design: Design control experiment, treated human breast cells in the following ways.

Control group: Positive control:negative control. induce carcinogenic effects.

Negative control: treated cells with DMSO water to eliminate the interference of non-specific factors.

The treatment groups: Cells were handled with varying concentrations (0.1, 0.5, 1, 5, 10 μM) of CYP1A1 inhibitor for 24, 48, and 72 hours respectively.

Use MTT method to detect cell viability: Cells were seeded into the 96-well plates at a density of 5×10^3 cells per well and allocated them into three experimental group. After the cells adhered to the plates, the first group use medium containing different concentrations of CYP1A1 inhibitors (0.1, 0.5, 1, 5, 10 μM) instead of the original medium. The second group use medium containing Bap instead of the original medium and the third group use medium containing DMSO instead of the original medium. After the designated treatment time, add 10 μL of MTT solution (5 mg/mL) to each well. Then place the cells in an environment at 37°C with 5% carbon dioxide for 4 hours. The

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MTT was reduced by the mitochondrial enzymes of the cells to formazan. Remove the supernate, and dissolve the formazan in DMSO. Measure the absorbance (OD value) at 570 nm wavelength on the ELISA instrument. Use the negative control group's data as a reference to calculate the cell viability.

Use flow cytometry to analyze cell apoptosis: Seed the cells into 6-well plates at a density of 1×10^5 cells per well and were divided into three groups. The first group was treated with different concentrations of CYP1A1 inhibitors. The second group was treated with Bap, and the third group was treated with DMSO. Upon completion of the designated treatment time, digest and harvest the cells with trypsin. Then, use cold phosphate-buffered saline (PBS) to wash them twice and resuspend in the buffer. According to the manufacturer's instructions, add Annexin V-FITC and PI to the cell suspension and incubate at room temperature in a dark environment for 15 minutes. Finally, use the Flow cytometer (FACS) to analyze the sample. Collect the data and use appropriate software to analyze them. Calculate the apoptosis rate of cells.

Use western blot method to assess the cleavage situation of caspase: Divide the cells into three groups. The first group was treated with different concentrations of CYP1A1 inhibitors. The second group was treated with Bap, and the third group was treated with DMSO. Lyse the cells in RIPA buffer containing protease inhibitors. Collect the supernatant and measure the protein concentration through BCA method. Then, use sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) separate equal amounts of protein and transferred onto a polyvinylidene fluoride (PVDF) membrane. The membrane was blocked with TBST solution which has five percent skimmed milk at room temperature for 1 hour. According to the instructions, incubate the membrane with primary antibody against caspase-3 overnight at 4°C. Incubate it with HRP-labeled secondary antibody 1 hour at room temperature subsequent to washing the membrane with TBST. Then, use TBST solution to wash it again. Finally, mix the ECL reagents A and B in proportion, drop them onto the membrane and incubate under dark conditions for 1 minute. Expose them with the chemiluminescence instrument to obtain clear bands. Quantify the band intensity using image analysis software.

Data statistics and analysis: All experiments were conducted in triplicate and the data were presented as the mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was used to test for significant differences, and a p-value less than 0.05 was considered statistically significant.

3. Result

Table 1. Effects of increasing the concentration and duration time of CYP1A1 inhibitor on cell viability, apoptosis, and caspase-3 cleavage in PAH-treated breast epithelial cells

Combination of possible results (CR)	CYP1A1 decreases viability by MTT	CYP1A1 increases apoptosis by FACS for Annexin V/ PI	CYP1A1 increases caspase 3 cleavage by western blot	Supporting Hypothesis?
CR1	+	+	+	Full
CR2	+	+	-	Partial
CR3	+	-	+	Partial
CR4	+	-	-	Partial
CR5	-	+	+	Partial
CR6	-	+	-	Partial

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Table 1. (continued)

CR7	-	-	+	Partial
CR8	-	-	-	Fully contradicts

In Table 1, The "+" means that the result of experiment is similar to the positive control and shows significant difference from negative control, "-" means that the result of experiment is similar to the negative control and shows significant difference from positive control.

CR1: MTT assay demonstrated that compared to the negative control group, the viability of human mammary epithelial cells treated with CYP1A1 inhibitor was reduced. Flow cytometry analysis revealed that the apoptosis rate was increased compared with the negative control. Western blot experiment demonstrated that compared with the negative control, caspase-3 cleavage in the cells was enhanced.

CR2: MTT assay demonstrated that compared to the negative control group, the viability of human mammary epithelial cells treated with CYP1A1 inhibitor was reduced. Flow cytometry analysis revealed that the apoptosis rate was increased compared with the negative control. Western blot experiment demonstrated that compared with the negative control group, caspase-3 cleavage in the cells was not enhanced.

CR3: MTT assay demonstrated that compared to the negative control group, the viability of human mammary epithelial cells treated with CYP1A1 inhibitor was reduced. Flow cytometry analysis revealed that the apoptosis rate was not increased compared with the negative control. Western blot experiment demonstrated that compared with the negative control, caspase-3 cleavage in the cells was enhanced.

CR4: MTT assay demonstrated that compared to the negative control group, the viability of human mammary epithelial cells treated with CYP1A1 inhibitor was reduced. Flow cytometry analysis revealed that the apoptosis rate was not increased compared with the negative control. Western blot experiment demonstrated that compared with the negative control group, caspase-3 cleavage in the cells was not enhanced.

CR5: MTT assay demonstrated that compared to the negative control group, the viability of human mammary epithelial cells treated with CYP1A1 inhibitor was not reduced. Flow cytometry analysis revealed that the apoptosis rate was increased compared with the negative control. Western blot experiment demonstrated that compared with the negative control, caspase-3 cleavage in the cells was enhanced.

CR6: MTT assay demonstrated that compared to the negative control group, the viability of human mammary epithelial cells treated with CYP1A1 inhibitor was not reduced. Flow cytometry analysis revealed that the apoptosis rate was increased compared with the negative control. Western blot experiment demonstrated that compared with the negative control group, caspase-3 cleavage in the cells was not enhanced.

CR7: MTT assay demonstrated that compared to the negative control group, the viability of human mammary epithelial cells treated with CYP1A1 inhibitor was not reduced. Flow cytometry analysis revealed that the apoptosis rate was not increased compared with the negative control. Western blot experiment demonstrated that compared with the negative control, caspase-3 cleavage in the cells was enhanced.

CR8: MTT assay demonstrated that compared to the negative control group, the viability of human mammary epithelial cells treated with CYP1A1 inhibitor was not reduced. Flow cytometry analysis revealed that the apoptosis rate was not increased compared with the negative control.

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Western blot experiment demonstrated that compared with the negative control group, caspase-3 cleavage in the cells was not enhanced.

4. Discussion

Combination of result 1(CR1) suggests that CYP1A1 inhibitors can effectively inhibit the growth of breast epithelial cells treated with polycyclic aromatic hydrocarbons (PAHs), promote apoptosis, and activate the caspase-3 pathway, which is consistent with the expected results. Previous research shows that PAHs (such as benzo[a]pyrene) need to be metabolically activated by CYP1A1 to exhibit strong carcinogenicity. CYP1A1 inhibitors can competitively bind to the enzyme's active site or allosterically inhibit its function, blocking the conversion of PAHs into carcinogenic intermediates such as epoxides and suppressing the metabolic activity of PAHs. When the metabolic activation of PAHs is inhibited, the caspase-3 pathway within the cells is activated, leading to an increase in the apoptosis rate. Moreover, as the concentration of the inhibitor increases and the treatment time extends, the inhibition of CYP1A1 becomes more thorough, the signal accumulates continuously, the apoptotic effect is amplified, and the apoptosis rate increases. As apoptosis is initiated, cell cycle regulation is also affected. Inhibition of CYP1A1 may interfere with the cell growth cycle, preventing them from proliferating normally and reducing cell viability. This result indicates that CYP1A1 may potentially act as a therapeutic target for reducing PAH-related breast cancer risk. In future studies, we can conduct simulations in animal models. For instance, we can use CYP1A1 inhibitors in mice exposed to PAH and observe the phenomena.

Combination of result 2(CR2) suggests that CYP1A1 inhibitors can inhibit the growth of breast epithelial cells treated with PAHs and promote apoptosis, but this is not achieved through the classical caspase-3 pathway. Here the hypothesis is partially supported. In this result, alternative apoptosis pathways may be activated directly or indirectly, such as other caspase pathways may be activated directly or indirectly, thereby inducing apoptosis without the involvement of the caspase-3 pathway. In future experiments, we can use Western blot to detect the cleavage of caspase-8 and caspase-12. If the cleavage increases, it indicates that CYP1A1 induces apoptosis through other caspase pathways. If there is no increase in cleavage compared with the negative control, other situations not through caspase pathways should be considered (eg: parthanatos). The PAH itself is cytotoxic, even if it does not trigger apoptosis, it can still cause direct damage to cells, resulting in a decreased cell viability as observed in the MTT experiment [6]. Subsequent experiments can independently verify the effect of PAH on cell viability. CR2 indicates that the inhibition of CYP1A1 can still counteract the PAH effect, but the mechanism of action still needs to be explored.

Combination of results 3(CR3) suggests that CYP1A1 inhibitors can inhibit the growth of human breast epithelial cells treated with PAHs and trigger the activation of the caspase-3 pathway, but do not fully drive apoptosis (the cells may be in an "apoptosis-preliminary" state or undergo necrosis). Here the hypothesis is partially supported. This result may be due to the fact that the caspase-3 pathway is activated within the cells but fails to complete the apoptosis process, causing the apoptosis to remain at an early stage. It could also be because high concentrations of PAH or inhibitors directly damage the cell membrane, causing an imbalance in the intracellular ion homeostasis, leading to rapid cell necrosis. At this time, caspase-3 may be non-specifically activated due to the disorder of the intracellular environment, and necrotic cells are easily detected as non-apoptotic by FACS. In the subsequent experiments, we can use western blotting to detect Bcl-2, HMGB1, and LDH release experiments to explore the reasons for this result. If Bcl-2 is highly expressed, it indicates apoptosis arrest. If LDH release increases, it suggests cell necrosis. The results from MTT indicate that the decreased activity might be due to the same reason as CR2,

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which is the toxic effect of PAH itself. CR3 indicates that after treatment with CYP1A1 inhibitors, in PAH-induced breast epithelial cells, activation of caspase-3 may trigger "apoptotic abortion", or due to the high expression of Bcl-2 family proteins, the execution stage of apoptosis is blocked.

Combination of results 4(CR4) suggests that the CYP1A1 inhibitor could inhibit the viability of human epithelial breast cells treated with PAHs. However, it did not trigger apoptosis, and the cleavage of caspase-3 did not increase either. Here the hypothesis is partially supported. This result may be due to the fact that CYP1A1 inhibitors prevent the normal metabolism of cells, causing cell growth to stall and proliferation to be inhibited. In future experiments, we can detect Cyclin D1, p21, Cdc25C and Cyclin B1 through Western blot. If Cyclin D1 decreases and p21 increases, it is considered as G1/S phase arrest. If Cdc25C decreases and Cyclin B1 increases, it is considered as G2/M phase arrest. CR4 indicates that the CYP1A1 inhibitor inhibits the proliferation of PAH-treated breast epithelial cells by inducing cell cycle arrest rather than apoptosis.

Combination of results 5(CR5) suggests that the CYP1A1 inhibitor can activate the caspase-3 pathway in human breast epithelial cells after PAH treatment, promoting cell apoptosis. However, the MTT results show that the viability remains unchanged or increases. Here the hypothesis is partially supported. This result may be due to the fact that PAH can activate the cell proliferation pathway and promote cell proliferation [7]. leading to the total number not decreasing. The MTT test measures the mitochondrial metabolic activity of cells. If the newly generated cells from proliferation have vigorous metabolism, it can counteract the reduced metabolism of apoptotic cells, resulting in no significant change in the MTT results. In future experiments, we will be able to detect the expression of p-Akt and p-ERK through Western blot, and also measure the phosphorylation levels of the cells treated with the CYP1A1 inhibitor. If the expression of p-Akt/p-ERK increases after PAH treatment and does not significantly decrease after inhibitor treatment, then the pathway is activated, leading to cell proliferation. CR5 indicates that PAH may activate the proliferation pathways of breast epithelial cells (such as ERK/Akt), counteract the apoptosis induced by CYP1A1 inhibitors, and result in no significant decrease in cell viability (MTT).

Combination of results 6(CR6) suggests that CYP1A1 inhibitors can promote apoptosis, but this is not achieved through the classical caspase-3 pathway. Cell viability either increases or remains unchanged. Here the hypothesis is partially supported. This outcome may be due to the activation of alternative apoptotic pathways, which activate cell apoptosis through other means. This is the same as the reasons described in CR2. The viability doesn't decrease (MTT) might be due to the same reasons in CR5. In the future we need to conduct the experiments in the CR2 and CR5. CR6 indicates that human mammary epithelial cells may undergo apoptosis through other caspase pathways, while PAH activates the cell proliferation pathway, resulting in no significant decrease in cell viability.

Combination of results 7(CR7) suggests that CYP1A1 inhibitors can promote the activation of the caspase-3 pathway, but there is no significant change in cell viability (as measured by MTT) and no apoptosis is observed (as determined by FACS). Here the hypothesis is partially supported. This result may be due to the activation of caspase-3's non-apoptotic functions. For example, PAH may induce mild DNA damage, which can activate caspase-3 to participate in damage repair. In this case, caspase-3 activation is part of the repair process rather than an apoptotic signal. The cells can survive and complete the repair, so both cell viability and apoptosis detection are negative. Caspase-3 also has non-apoptotic functions such as regulating cell differentiation [8]. When these functions are active, there are no significant changes in cell viability and apoptosis rate. In the future experiments, we can use the western blot experiment to examine the expression of key genes related to non-apoptotic functions such as the differentiation process and DNA repair process. CR7

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indicates that in the human mammary epithelial cells treatment with CYP1A1 inhibitor, the activation of caspase-3 may be involved in the regulation of cell differentiation or the DNA repair process, rather than apoptosis.

Combination of results 8(CR8) suggests that CYP1A1 inhibitors can promote the growth of human mammary epithelial cells after PAHs treatment, reduce cell apoptosis, and decrease caspase cleavage. Or the overall results are consistent with the negative control. The reason for this result may be a problem of chemical stability. The CYP1A1 inhibitor may degrade during storage or experimental treatment, losing its inhibitory activity. Or the inhibitor may have too strong or too weak hydrophilicity, unable to pass through the cell membrane and enter the cell. Or it may be highly bound to the culture medium components, resulting in a low free concentration that cannot reach an effective concentration and thus fail to exert its effect. This leads to a result completely contrary to the hypothesis. In the future, we need to verify whether the quality of the materials used in the experiment meets the standards, whether the experimental gradient is set reasonably. In conclusion, CR8 indicates that the existing assumptions are not reasonable, and a new experimental plan needs to be designed.

Discussion of concentration and duration time: We will change the concentration of CYP1A1 inhibitors (ranging from 0.1 μM to 10 μM) and the treatment duration (24 hours, 48 hours, 72 hours), and verify the effect in the human mammary epithelial cell system exposed to polycyclic aromatic hydrocarbons (PAHs). The following is a discussion of several possible scenarios and the experiments that should be conducted in the future.

Firstly, if there is a situation where the measured value changes in the same direction as the assumed one when the concentration increases. This might be because the concentration has risen, and the inhibitory effect of CYP1A1 has intensified, resulting in a more severe blockage of the PAH metabolism activation process, accumulation of cellular stress signals, and continuous activation of the apoptotic pathway, which is consistent with the assumption that "the higher the concentration, the lower the activity, and the higher the apoptosis and caspase cleavage". In the future, key molecules of the pathway (such as the content of active caspase-3) can be detected to verify whether there is a linear relationship between the activation of the pathway and the concentration. At the same time, concentration-effect curves can be drawn, and the EC50 can be calculated to clearly determine the intensity of the effect. If there is a situation where the measured value changes in the opposite direction to the assumption, it could be due to the fact that the high concentration of inhibitors damages the cells. Or it could be because the high concentration of inhibitor activates other pathways, resulting in a situation contrary to the assumption. In the future, we can reduce the range of the inhibitor concentration setting and conduct another test to observe the results. Then, as the concentration increases, there may appear phenomena of weak effects or no response. It is necessary to determine whether it is "the signal has not reached the threshold" or "the pathway is insensitive". Subsequent experiments can change the concentration range to observe whether the signal accumulates. Or consider using other pathways to test. If there is a false negative result, it may be due to insufficient detection sensitivity. In the early stage of apoptosis, the outward folding of the cell membrane PS is not obvious, and the Annexin V detection is weak, or the amount of caspase cleavage is low, and Western blot does not detect it, but apoptosis has actually been initiated. At the same time, the MTT detection relies on mitochondrial metabolism, and may misjudge the changes in cell viability. It may also be due to compensatory activation of the pathway. For example, cells may highly express anti-apoptotic proteins, or activate survival pathways such as autophagy, which counteract the apoptotic signal, causing the detection indicators to be negative, but actual apoptosis inhibition exists. In the future, the treatment time should be extended, the inhibitor

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concentration should be increased, and we should observe whether the indicators change later. Or a different device should be used for re-testing. If there is a false positive result, it might be due to the limitations of the experimental equipment. It could also be because the CYP1A1 inhibitor activates other apoptotic-related pathways. For instance, because of its similar chemical structure to other activators, it activates other receptor-mediated apoptosis. However, the actual apoptosis is not related to the CYP1A1 inhibitor, resulting in a "consistent with the hypothesis" false positive. In the future, the CYP1A1 gene knockout cell line control can be set up. If the "positive result" disappears after knockout, it will prove that the apoptosis in the study is specifically related to the CYP1A1 inhibitor.

5. Conclusion

This study focuses on the impacts of polycyclic aromatic hydrocarbons (PAHs), especially benzo[a]pyrene (BaP), on human breast epithelial cells, and explores the effects and mechanisms of CYP1A1 inhibitors in terms of concentration and duration of action. Through the cross-validation of three experiments - MTT, FACS, and Western blot - CR1 can fully verify the core hypothesis, indicating that CYP1A1 inhibitors can effectively inhibit cell proliferation and induce apoptosis by blocking PAH metabolic activation and activating the caspase-3 pathway. This provides a potential therapeutic target for the prophylaxis and treatment of PAH-related breast cancer. However, other results (such as CR2 - CR7) show partial verification, reflecting the complexity of the cell response. Further experimental research is needed to explore the mechanism of CYP1A1 inhibitors. CR8 is completely contrary to the hypothesis. It needs to be redesigned for verification.

This study reveals the complexity of cell responses through cross-validation of multiple detection methods, providing key insights for subsequent research. Firstly, the methods are optimized, and other experiments are conducted for supplementary verification (for example, in CR3, the experiment can be enhanced to verify whether the cells have necrosis). This breaks through the limitations of FACS and MTT methods. Secondly, in-depth research can be conducted on alternative apoptotic pathways (such as caspase-8, caspase-12-dependent pathways) and non-apoptotic growth regulation (autophagy, cell cycle) to explore the precise target of CYP1A1 inhibitors, furnish a more robust scientific foundation for the prevention and treatment of PAH-related breast diseases.

References

[1] Abdel-Shafy, H. I., & Mansour, M. S. M. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. Egyptian Journal of Petroleum, 25(1), 107-123. https: //doi.org/10.1016/j.ejpe.2015.03.011

[2] White, A. J., Bradshaw, P. T., Herring, A. H., Teitelbaum, S. L., Beyea, J., Stellman, S. D., Steck, S. E., Mordukhovich, I., Eng, S. M., Engel, L. S., Conway, K., Hatch, M., Neugut, A. I., Santella, R. M., & Gammon, M. D. (2016). Exposure to multiple sources of polycyclic aromatic hydrocarbons and breast cancer incidence. Environment International, 89-90, 185-192. https: //doi.org/10.1016/j.envint.2016.02.009

[3] Gu, Q., Hu, C., Chen, Q., Xia, Y., Feng, J., & Yang, H. (2009). Development of a rat model by 3, 4-benzopyrene intra-pulmonary injection and evaluation of the effect of green tea drinking on p53 and bcl-2 expression in lung carcinoma. Cancer Detection and Prevention, 32(5-6), 444-451. https: //doi.org/10.1016/j.canep.2009.04.002

[4] Nebert, D. W., Dalton, T. P., Okey, A. B., & Gonzalez, F. J. (2004). Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. Journal of Biological Chemistry, 279(23), 23847-23850. https: //doi.org/10.1074/jbc.R400004200

[5] Schmitt, E. E., Barhoumi, R., Metz, R. P., & Porter, W. W. (2017). Circadian regulation of benzo [a]pyrene metabolism and DNA adduct formation in breast cells and the mouse mammary gland. Molecular Pharmacology, 91(3), 178-188. https: //doi.org/10.1124/mol.116.106740

DOI: 10.54254/2753-8818/2025.AU30382

[6] Kang, Y., Cheung, K. C., & Wong, M. H. (2010). Polycyclic aromatic hydrocarbons (PAHs) in different indoor dusts and their potential cytotoxicity based on two human cell lines. Environment International, 36, 542-547. https: //doi.org/10.1016/j.envint.2010.04.009

[7] Connelly, H. R. (1998). Effects of Selected Polycyclic Aromatic Hydrocarbons on Cell Proliferation, Protein Phosphorylation, and Intracellular Calcium Concentration in Lepomis Macrochirus [Doctoral dissertation, Louisiana State University and Agricultural & Mechanical College]. LSU Historical Dissertations and Theses. https: //repository.lsu.edu/gradschool_disstheses/6622

[8] Larsen, B. D., Rampalli, S., Burns, L. E., & Megeney, L. A. (2010). Caspase 3/caspase-activated DNase promote cell differentiation by inducing DNA strand breaks. Proceedings of the National Academy of Sciences, 107(9), 4230-4235. https: //doi.org/10.1073/pnas.0913089107