ISSN: 1675-7785
eISSN: 2682-8626

Available online at
https://scilett-fsg.uitm.edu.my/

Science Letters 18(1) 2024, 1 – 9.

Science Letters

The Role of Bilirubin in Preventing the Development of Tobacco-Induced Lung Cancer: A Review

Nurnadia Majid[1], Hasseri Halim[1,2], Salfarina Ramli[1,2*]

[1]*Faculty of Pharmacy, Universiti Teknologi MARA Cawangan Selangor Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia*
[2]*Integrative Pharmacogenomics Institute (iPROMISE), Universiti Teknologi MARA Cawangan Selangor Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia*

ARTICLE INFO

Article history:
Received 13 February 2023
Revised 13 August 2023
Accepted 1 September 2023
Online first
Published 22 January 2024

Keywords:
Bilirubin
Tobacco-Specific Nitrosamines
CYP2A6
CYP2A13
Lung Cancer

DOI:
10.24191/sl.v18i1.21163

ABSTRACT

The potent antioxidant properties of bilirubin and the inverse relationship between serum bilirubin level and cancer risk support the protective function of bilirubin, particularly in tobacco-induced lung cancer. 4-(Methylnitrosamino) -1-(3-pyridyl)-1-butanone (NNK) is one of the procarcinogens in tobacco smoke that requires metabolic activation to form its metabolite; 4-(methylnitrosamino) -1-(3-pyridyl) -1-butanol (NNAL). The activation produces free radicals with DNA-damaging properties. From the findings of previous studies, it can be deduced that bilirubin prevents the development of tobacco-induced lung cancer by directly scavenging free radicals derived from tobacco product smoke and inhibiting cytochrome P450 2A6 and 2A13-mediated NNK metabolism. Furthermore, as an endogenous substrate of CYP2A6, bilirubin affects the expression and hydroxylation activity of CYP2A6, but the impact of bilirubin on CYP2A13 has yet to be investigated in detail. More research is necessary to elucidate the impact of bilirubin on NNK and NNAL metabolism mediated by both enzymes. Consequently, this review describes the current status and potential capabilities of bilirubin. The ongoing research gaps and areas for future research are highlighted.

INTRODUCTION

Bilirubin is known to be the pigment that gives bile its color. Initially, haem in haemoglobin is converted to green biliverdin by haem oxygenase during the breakdown of senescent red blood cells by phagocytes. This is then broken down into orange bilirubin by biliverdin reductase. Later, serum albumin is transported to the liver, where most bilirubin is conjugated with glucuronide before being excreted into the bile. At the same time, the unconjugated fraction remains bound to albumin. Both conjugated and unconjugated fractions produced total serum bilirubin [1][2]. The unconjugated bilirubin is stored in the gallbladder and will be released into the small intestine to break down fats from food [3]. Increased production, reduced uptake, and low glucuronidation capacity increase plasma unconjugated bilirubin levels [4].

* Corresponding author. *E-mail address*: salfarina2892@uitm.edu.my

https://doi.org/10.24191/sl.v18i1.21163

The normal levels of total bilirubin and conjugated bilirubin ranged from 0.1 to 1.2 mg/dl and 0.1 to 0.3 mg/dl, respectively. Total bilirubin above 1.2 mg/dL is considered elevated, while a higher concentration in serum (>2.5 mg/dL) would lead to hyperbilirubinemia and jaundice [5]. A rapid and reliable method is important for measuring serum bilirubin levels as an early diagnosis of conditions such as hyperbilirubinemia [6]. Bilirubin levels have become one of the parameters monitored in toxicity and safety studies of chemicals such as drugs, herbs, and foods. For example, the toxicity of herbal extracts can be inferred from the high serum bilirubin levels observed in subjects after consumption [7]. In contrast, bilirubin levels have been monitored to indicate hemooxygenase activity or the status of endogenous antioxidants in the patients [8]. However, serum bilirubin is often monitored after exposure to chemicals.

In addition to nicotine and polyaromatic hydrocarbons (PAH), tobacco products such as cigarettes and electronic cigarettes contain toxicants collectively called tobacco-specific nitrosamines (TNSA). 4-(Methylnitrosamino) -1-(3-pyridyl)-1-butanone (NNK) is one of the tobacco-specific nitrosamines (TSNA). Cytochrome P450 2A6 and 2A13 metabolize NNK to form 4-(methylnitrosamino)-1-(3-pyridyl) -1-butanol (NNAL) [9]. A proportion of NNAL is detoxified by glucuronidation and excreted in human urine. However, NNAL also has carcinogenic properties as NNK. The methyl DNA adducts and aldehyde DNA adducts produced during the metabolism of NNK and NNAL by CYP2A6 and 2A13 induce oxidative stress, and the general tumorigenic properties of tobacco smoke in humans have previously been extensively discussed [10].

Low levels of unconjugated bilirubin have been hypothesized, indicating a lack of antioxidant protection and the progression of cell injury [11]. Therefore, it is likely that people with genetically raised serum bilirubin, although exposed to high levels of smoke oxidants, could be protected from lung cancers [12]. Also, bilirubin is a better antioxidant than vitamin E in breaking down chains of peroxyl radical chains. As a circulating antioxidant in the body, bilirubin production and metabolism are likely to profoundly impact critical steps in cellular pathways and homeostasis [5]. More research is needed to elucidate this endogenous antioxidant's biological purpose, particularly in protecting against lung cancer. Therefore, this review discusses the current findings, the potential role of bilirubin, and the knowledge gap that can be addressed for future research.

SERUM BILIRUBIN LEVEL IN SMOKERS

Smoking tobacco products, such as cigarettes and electronic cigarettes, or exposure to environmental tobacco smoke (ETS) are among the many ways a person can be exposed to free radical sources. When a smoker inhales a lit cigarette, two types of smoke are mainstream tobacco smoke (MTS) and side-stream tobacco smoke (SSS), which results from the passive burning of the lit cigarette [13]. Direct decomposition of tobacco components produces primary free radicals in tobacco smoke. They are affected by the constituents' chemical structure and pyrolysis temperature [14]. The free radicals in conventional cigarette smoke were oxygen-centered, most likely alkoxy radicals. In contrast, carbon-centered radicals were found in the aerosol of the heat-not-burn product and electronic cigarettes [15].

Generally, free radical inhalation or intake overwhelms endogenous antioxidant defense. Reduced serum levels of bilirubin in smokers compared to non-smokers are due to an antioxidant response to oxidative stress caused by chronic smoking [16]. On the other hand, the bilirubin level increased in both smokers and non-smokers after alcohol consumption. The study postulated that the increase was caused by slight subclinical hemolysis, but the mechanism responsible remains uncertain. However, an increase in total bilirubin among non-smokers could be due to competitive inhibition of bilirubin conjugation by alcohol. This finding was not observed in smokers, suggesting that smokers may not receive the same benefit from moderate drinking as non-smokers [17].

Bilirubin levels have also been associated with cancer risk in female and male smokers. For every 0.1 mg/dL decrease in bilirubin, the risks of lung cancer incidence and mortality increased by 5% and 6%, respectively, in male smokers [18]. In contrast, the hazard ratio of coronary heart disease incidents decreased with elevated bilirubin in females but not men [19]. Furthermore, statistical evaluations on 10-

https://doi.org/10.24191/sl.v18i1.21163

year electronic medical records using a combination of machine learning and classical statistical approaches found an association between low bilirubin levels (< 0.6 mg/dL) with the cause of death related to bilirubin levels such as cancer and cardiovascular diseases in men. However, no significant association was found between serum bilirubin levels and lung cancer risk in women [20].

Bilirubin protects against prolonged oxidative stress to prevent the onset and progression of chronic diseases such as cancer [21]. This is supported by data that show a prevalence of cancer and cardiovascular disease among individuals with elevated circulating bilirubin concentrations lower than in normal populations [22]. So, it is no surprise that low bilirubin levels associated with smoking increase the risk of lung cancer [23]. Furthermore, lower serum bilirubin levels have been frequently observed in patients with lung cancer compared to healthy subjects. Therefore, monitoring serum bilirubin levels has been proposed as a clinically reliable marker for predicting lung cancer [24].

Interestingly, smoking cessation has an impact on serum bilirubin levels as well. Smokers who abstained from smoking for an extended period showed a significant increase in their indirect (unconjugated) and total bilirubin levels [11]. Bilirubin concentration increased with time after smoking cessation in former smokers. According to other studies above, increased daily and cumulative tobacco consumption was associated with lower concentrations of plasma bilirubin [25].

The results of previous studies draw an association between bilirubin concentration and oxidative stress levels and the risk of developing lung cancer. Smoking is associated with instant gratification provided by inhaled nicotine and DNA damage induced by more than 70 carcinogens in lung tissue. Therefore, lung cancer is the most prevalent cancer induced by tobacco smoke. Tobacco smoke compositions such as benzo(a)pyrene (BP) and aldehydes, as well as their ability to induce DNA damage and their effects on DNA repair, have been well studied [26]. The metabolic activation of tobacco-specific nitrosamines (TNSA) is the subject of this review.

Activation of Tobacco-Specific Nitrosamines (TSNA) by Enzymes CYP2A6 and CYP2A13.

Essentially, freshly harvested tobacco contains a negligible amount of TSNAs. However, there is an increase in the amounts at the late yellowing and early browning stage of tobacco leaves during the final curing of cigarette production. There have been efforts to reduce harmful carcinogens, including NNK, in new smokeless tobacco products through modified agricultural production, manufacturing, and storage practices [27]. Compared to conventional cigarettes, free radical levels are substantially reduced in electronic cigarettes and heat-not-burn products [15]. However, NNK is present in both conventional cigarettes and electronic cigarettes and has been established as one dangerous nitrosamine with toxicological implications [28]. A study reported that four weeks of exposure to NNK influenced the expression of the Twist protein, which plays a vital role in tumor cell invasion, migration, and metastasis, suggesting an increased risk of lung cancer metastasis among the population exposed to NNK [29].

Inhalation exposure in the population exposed to tobacco smoke accounts for a predominant portion of daily uptake for cigarette users. At the same time, oral ingestion could be the primary source of NNK exposure for oral tobacco product users. Once in the body, 89% of NNK is rapidly converted by carbonyl reductase to NNAL [9], which is then glucuronidated by UDP-glucuronosyltransferases (UGT) and excreted in urine [30]. As a result, the urinary concentration of NNAL has been used to quantify exposure to NNK. Determining NNAL concentrations in urine collected from smokers and non-smokers helps monitor smokers and non-smokers' exposure to nicotine and tobacco smoke [31]. The resulting finding helps direct health protection plans for the general population or identify vulnerable populations, as well as determine the efficacy of tobacco product regulation [32].

The remaining free NNK and NNAL are hydroxylated by cytochrome P450 2A6 (CYP2A6) and 2A13 (CYP2A13) enzymes. The reaction produces electrophilic species that react with DNA to form DNA adducts [33]. This aligns with a study in which inhalation exposure to carcinogens from tobacco smoke generates bronchial adducts if the formation of DNA adducts formation is persistent and some do not undergo DNA repair. Miscoding will occur during DNA replication, resulting in permanent mutations and genomic instability, affecting normal cellular growth control mechanisms and increasing the risk of lung cancer [34]. CYP2A6 is expressed in the liver, and CYP2A13 is expressed predominantly in the respiratory

https://doi.org/10.24191/sl.v18i1.21163

tract, including the lung [35]. Although both enzymes are homologous, the enzymes exhibited significant differences in their substrate preference for NNK, where the levels of α-hydroxylated NNK metabolites by CYP2A13 were higher than those by CYP2A6 in lung cells A549 and H1437, respectively [9].

Studies on the structures of both enzymes have been carried out to understand better the factors controlling the binding and metabolism of nicotine and NNK, two substrates responsible for human tobacco addiction and lung cancer. For example, a hydrogen bond between pyridine rings of both nicotine and NNK pyridine rings with CYP2A13 is a key interaction for enzyme affinities and ligand orientations. However, it is not present for CYP2A6 [25]. Furthermore, the docking study suggests that amino acid residues Met 365 and Leu 366 of CYP2A13 compared to Val 365 and Ile 366 of CYP2A6 may contribute to differences in NNK metabolism. On the contrary, most of the remaining differences between CYP2A6 and CYP2A13 are located outside the active site, either in the N-terminal transmembrane region or on the protein's surface. [36].

Evidently, inhibition of the CYP2A6 enzyme has been significant for smoking cessation. For example, selective inhibition of CYP2A6 by methoxsalen impeded nicotine first-pass metabolism, and the combination reduced smoking rates among smokers [37]. Genomic variation in CYP2A6 has also been found to be the most substantial risk factor for the heaviness of smoking, where slower metabolizers present better quit rates. This has been associated with failure to quit smoking cessation [38]. Another study on the effect of the CYP2A6 genotype and smoking status showed that nicotine patches are efficacious for smoking cessation. However, people with CYP2A6*4/*4 might be more sensitive to adverse effects and find it more challenging to quit smoking once they start smoking habit [39]. Furthermore, the polymorphisms of CYP2A13 enzymes influence the hydroxylation of NNK and NNAL, thus affecting the formation of urinary metabolites and modifying the risk of urothelial carcinoma [40]. Therefore, it is plausible that inhibition of this pathway or polymorphism of the genes CYP2A6 and CYP2A23 would affect the formation of DNA adducts and tobacco-related carcinogenesis, thus altering the risk of tobacco use disorder and smoking-related diseases.

Intracellular Bilirubin Management by CYP2A6 Enzyme

The production of bilirubin and biliverdin from hemolysis degradation is part of the pathophysiology response to inflammation [41]. Bilirubin has been established as an endogenous antioxidant that circulates in human plasma, and both bilirubin and biliverdin protect against tissue injury [42]. Mechanisms of antioxidant activity include scavenging reactive epoxides before they bind to DNA and cause mutation [43], activating the pathway to repair bilirubin-induced DNA damage [44], protecting against smoke-induced emphysema by suppressing inflammatory cell recruitment and pro-inflammatory cytokine secretion, increasing anti-inflammatory cytokine levels and antioxidant superoxide dismutase activity [45].

UGT1A1 in the liver primarily regulates bilirubin metabolism. Therefore, impaired glucuronidation of bilirubin affects elimination. It can result in high blood bilirubin levels, which have been linked to neurotoxicity and movement disorders [46]. However, slower elimination of bilirubin is beneficial under oxidative stress while restoring the balance of free radicals and antioxidants. For example, cadmium-induced oxidative stress activates Nrf2, a transcription factor that controls the expression of many cytoprotective genes in response to oxidative stress. This activation activates Nrf2-dependent hemooxygenase-1 (HO-1) and causes an increase in bilirubin concentration, resulting in high bilirubin oxidant scavenging activity. The study also found that mouse cytochrome P450 CYP 2A5 can be a bilirubin oxidase to metabolize bilirubin to biliverdin. Therefore, it was postulated that Cyp2a5's metabolism of bilirubin to biliverdin by Cyp2a5 does not aim to eliminate bilirubin but to prevent the accumulation of bilirubin at pro-oxidant levels, indicating the role of Cyp2a5 in the management of intracellular bilirubin [47].

The mouse Cyp2a5 and human CYP2A6 genes are considered orthologous due to their similar function and regulation [48]. CYP2A plays a significant role in the metabolic activation and detoxification of many exogenous and endogenous compounds [49]. CYP2A6 has been established to involve the activation of free radicals derived from inhalation of smoke from tobacco products. Thus, the regulation of CYP2A6 in response to cellular perturbations and the activity of aspects that govern the CYP2A6 is intriguing. A study

https://doi.org/10.24191/sl.v18i1.21163

reported that unspecific cellular stress, such as DNA damage and transcriptional arrest, activates p53 and increases the expression of CYP2A6. However, the interaction of p53 and Nrf2 at the distal promoter inhibits CYP2A6 activation [50]. As members of the CYP2A family, the substrates for CYP2A6 and CYP2A13 vary from drugs to toxins. Therefore, it was reasonable to learn that bilirubin is an endogenous substrate of CYP2A6, and a high bilirubin concentration can regulate CYP2A6 gene expression. Furthermore, bilirubin treatment was also found to delay the degradation of the CYP2A6 protein in a dose-dependent manner. However, it did not alter the level of CYP2A6 mRNA, suggesting the ability of bilirubin to stabilize the CYP2A6 protein in its active conformation [51].

It can be deduced that induction of CYP2A6 could be due to specific cellular events associated with exposure to inducers, such as the NNK. Oxidative stress increases bilirubin production, but some of the bilirubin would be oxidized by free radicals and eliminated as waste products, which is relevant to previous epidemiological observations that associate bilirubin level with cancer risk. As an endogenous substrate for CYP2A6, bilirubin prevents the development of tobacco-induced lung cancer by directly acting on the free radical, leading to its oxidation to biliverdin, where biliverdin can be reduced back to bilirubin by biliverdin reductase. The remaining bilirubin would bind to CYP2A6 to stabilize the proteins, causing its oxidation to biliverdin. It is plausible that the CYP2A6 gene is part of the defense machinery that controls intracellular bilirubin levels during and after transient attack by free radicals [48], but which mechanism is dominant would depend on the levels of intracellular free radicals and bilirubin [52].

The high affinity of bilirubin as an endogenous substrate of the CYP2A6 enzyme suggests the potential of bilirubin to inhibit NNK metabolism mediated by CYP2A6, which will be demonstrated in the near future. To date, no research on substrate preference between CYP2A13 and bilirubin that could impact the CYP2A13 activity has been conducted. However, the enzymes CYP2A6 and CYP2A13 are homologous despite some differences. Thus, it is postulated that bilirubin could also influence NNK metabolism by CYP2A13. However, this remains to be confirmed.

The in silico approach has been advantageous in characterizing and elucidating protein-ligand binding sites. It also allows exploration of the protein-ligand interaction at active and allosteric sites. The approach can be adopted to evaluate the binding of bile pigments, bilirubin, and biliverdin to the active and allosteric sites of the enzymes CYP2A6 and CYP2A13. Furthermore, the relation between bilirubin level and urinary NNAL concentration has yet to be established, which may be an exciting point to approach for future studies.

CONCLUSION

The relationship between serum bilirubin levels and the probability of developing cancer among smokers has received significant interest. Prolonged exposure to procarcinogens could increase the serum level. Bilirubin is an endogenous antioxidant that is cytotoxic at high concentrations. However, the discovery of bilirubin as a high-affinity endogenous substrate for CYP2A6 suggests a cytoprotective role of CYP2A6 against bilirubin toxicity by lowering cellular levels of bilirubin. This review discusses the mechanisms that naturally regulate the bilirubin concentration within the optimal therapeutic range for its antioxidant activity.

The metabolism of NNK and NNAL by CYP2A13 and CYP2A6 results in a cellular disturbance that disturbs the free radical-antioxidant balance, leading to the development of lung cancer. As a high-affinity substrate for CYP2A6, the efficacy or possibility of bilirubin interacting with CYP2A6 and/or CYP2A13 in the presence of NNK is still unknown. This review highlighted the potential of bilirubin as a CYP2A6 substrate to limit the production of NNK-derived free radicals. The interaction of bilirubin with CYP2A13 has yet to be explored.

ACKNOWLEDGEMENTS

https://doi.org/10.24191/sl.v18i1.21163

This review is part of a study funded by the Ministry of Higher Education Malaysia (FRGS/1/2017/STG04/UITM/02/3). Special thanks to Dr A'edah Abu Bakar, Specialist (Toxicology) at PETRONAS, Kuala Lumpur and Professor Dr Ong Chin Eng, School of Pharmacy, International Medical University (IMU) for research opportunity and valuable suggestions.

AUTHOR'S CONTRIBUTION

The article was part of Nurnadia Majid MSc. thesis. The manuscript was revised by Hasseri Halim. Salfarina Ramli conceptualized the main idea for the review, oversaw its progress, anchored the review, approved corrections, and approved the publication submission.

CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted without any self-benefits, commercial, or financial conflicts and declare the absence of conflicting interests with the funders.

REFERENCES

[1] P. L. M. Jansen and E. E. Bittar, "Bilirubin Metabolism," *Principles of Medical Biology*, vol. 15, no. C. 2004, doi: 10.1016/S1569-2582(04)15010-1.

[2] A. Nakeeb and H. A. Pitt. (2007). Chapter 7 - Biliary Tract Pathophysiology. In L. H. Blumgart, J. Belghiti, W. R. Jarnagin, R. P. DeMatteo, W. C. Chapman, M. W. Büchler, L. E. Hann, & M. D'Angelica (Eds.), *Surgery of the Liver, Biliary Tract and Pancreas (Fourth Edition)* (pp. 79-97). W.B. Saunders. https://doi.org/https://doi.org/10.1016/B978-1-4160-3256-4.50015-6

[3] A. Kalakonda, B. Jenkins, and S. John, "Physiology, Bilirubin," *StatPearls*, 2021.

[4] X. Wang, J. R. Chowdhury, and N. R. Chowdhury, "Bilirubin metabolism: Applied physiology, *Curr. Paediatr.*, vol. 16, no. 1, 2006, doi: 10.1016/j.cupe.2005.10.002.

[5] S. Gazzin, L. Vitek, J. Watchko, S. M. Shapiro, and C. Tiribelli, "A Novel Perspective on the Biology of Bilirubin in Health and Disease," *Trends in Molecular Medicine*, vol. 22, no. 9. 2016, doi: 10.1016/j.molmed.2016.07.004.

[6] L. Ngashangva, V. Bachu, and P. Goswami, "Development of new methods for determination of bilirubin," *J. Pharm. Biomed. Anal.*, vol. 162, pp. 272–285, 2019, doi: 10.1016/j.jpba.2018.09.034.

[7] T. Hemalatha, D. Ahino Mary, and A. Saravana Ganthi, "Acute and sub-acute toxicity study of Trema orientalis (L.) Bl. methanol extract in rats," *J. Drug Deliv. Ther.*, vol. 9, no. 1-s, 2019, doi: 10.22270/jddt.v9i1-s.2353.

[8] S. Suh, Y. R. Cho, M. K. Park, D. K. Kim, N. H. Cho, and M. K. Lee, "Relationship between serum bilirubin levels and cardiovascular disease," *PLoS One*, vol. 13, no. 2, 2018, doi: 10.1371/journal.pone.0193041.

[9] H. chih Chiang, C. Y. Wang, H. L. Lee, and T. C. Tsou, "Metabolic effects of CYP2A6 and CYP2A13 on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced gene mutation-A mammalian cell-based mutagenesis approach," *Toxicol. Appl. Pharmacol.*, vol. 253, no. 2, pp. 145–152, 2011, doi: 10.1016/j.taap.2011.03.022.

[10] L. A. Peterson, "Context matters: Contribution of specific DNA adducts to the genotoxic properties of the tobacco-specific nitrosamine NNK," *Chem. Res. Toxicol.*, vol. 30, no. 1, pp. 420–433, 2017, doi: 10.1021/acs.chemrestox.6b00386.

[11] S. S. O'Malley, R. Wu, S. T. Mayne, and P. I. Jatlow, "Smoking cessation is followed by increases in serum bilirubin, an endogenous antioxidant associated with lower risk of lung cancer and cardiovascular disease," *Nicotine Tob. Res.*, vol. 16, no. 8, 2014, doi: 10.1093/ntr/ntu067.

[12] L. J. Horsfall, S. Burgess, I. Hall, and I. Nazareth, "Genetically raised serum bilirubin levels and lung cancer: A cohort study and Mendelian randomization using UK Biobank," *Thorax*, vol. 75, no. 11, 2020, doi: 10.1136/thoraxjnl-2020-214756.

[13] N. L. Ngu and M. McEvoy, "Environmental tobacco smoke and peripheral arterial disease: A review," *Atherosclerosis*, vol. 266, pp. 113–120, 2017, doi:10.1016/j.atherosclerosis.2017.09.024.
[14] B. Dellinger, L. Khachatryan, S. Masko, and S. Lomnicki, "Free Radicals in Tobacco Smoke," *Mini. Rev. Org. Chem.*, vol. 8, no. 4, 2012, doi: 10.2174/157019311797440281.
[15] M. Shein and G. Jeschke, "Comparison of Free Radical Levels in the Aerosol from Conventional Cigarettes, Electronic Cigarettes, and Heat-Not-Burn Tobacco Products," *Chem. Res. Toxicol.*, vol. 32, no. 6, 2019, doi: 10.1021/acs.chemrestox.9b00085.
[16] P. Laxmi and S. P, "Assessment of Biochemical Profile in Smokers," *Int. J. Med. Heal. Res.*, vol. 3, no. 3, 2017.
[17] S. S. O. Malley, R. Gueorguieva, R. Wu, and P. I. Jatlow, "Acute alcohol consumption elevates serum bilirubin : An endogenous antioxidant," vol. 149, pp. 87–92, 2015.
[18] C. P. Wen *et al.*, "The ability of bilirubin in identifying smokers with higher risk of lung cancer: A large cohort study in conjunction with global metabolomic profiling," *Clin. Cancer Res.*, vol. 21, no. 1, 2015, doi: 10.1158/1078-0432.CCR-14-0748.
[19] R. Zhang *et al.*, "Elevated serum bilirubin may significantly reduce coronary heart disease risk in females : A prospective cohort study," pp. 648–657, 2022.
[20] T. Inoguchi, Y. Nohara, C. Nojiri, and N. Nakashima, "Association of serum bilirubin levels with risk of cancer development and total death," *Sci. Rep.*, vol. 11, no. 1, pp. 1–12, 2021, doi: 10.1038/s41598-021-92442-2.
[21] L. J. Horsfall *et al.*, "Serum Bilirubin and Risk of Respiratory Disease and Death," *JAMA*, vol. 305, no. 7, p. 691, Feb. 2011, doi: 10.1001/jama.2011.124.
[22] A. C. Bulmer, K. Ried, J. T. Blanchfield, and K.-H. Wagner, "The anti-mutagenic properties of bile pigments," *Mutat. Res. Mutat. Res.*, vol. 658, no. 1–2, pp. 28–41, Jan. 2008, doi: 10.1016/j.mrrev.2007.05.001.
[23] J. E. Lim, H. Kimm, and S. H. Jee, "Combined effects of smoking and bilirubin levels on the risk of lung cancer in Korea: The Severance cohort study," *PLoS One*, vol. 9, no. 8, 2014, doi: 10.1371/journal.pone.0103972.
[24] Q. L. Da Jiang, Jian Shi , Meng Yuan, Xiaoyang Duan , Lihua Li, "Levels of serum bilirubin in small cell lung cancer and non-small cell lung cancer patients," *Cell. Mol. Biol.*, vol. 64, no. 6, pp. 71–76, 2018.
[25] J. B. Kodal, Y. Çolak, C. J. Kobylecki, S. Vedel-Krogh, B. G. Nordestgaard, and S. Afzal, "Smoking reduces plasma bilirubin: Observational and genetic analyses in the copenhagen general population study," *Nicotine Tob. Res.*, vol. 22, no. 1, 2020, doi: 10.1093/ntr/nty188.
[26] M. Shong Tang *et al.*, "DNA damage, DNA repair and carcinogenicity: Tobacco smoke versus electronic cigarette aerosol," *Mutat. Res. - Rev. Mutat. Res.*, vol. 789, no. December 2021, 2022, doi: 10.1016/j.mrrev.2021.108409.
[27] A. K. Gupta, S. Tulsyan, M. Bharadwaj, and R. Mehrotra, "Grassroots approach to control levels of carcinogenic nitrosamines, NNN and NNK in smokeless tobacco products," *Food Chem. Toxicol.*, vol. 124, no. September 2018, pp. 359–366, 2019, doi: 10.1016/j.fct.2018.12.011.
[28] E. Konstantinou *et al.*, "Tobacco-specific nitrosamines: A literature review," *Food Chem. Toxicol.*, vol. 118, no. May, pp. 198–203, 2018, doi: 10.1016/j.fct.2018.05.008.
[29] Y. Wang, L. Shi, J. Li, H. Wang, and H. Yang, "Involvement of twist in NNK exposure-promoted lung cancer cell migration and invasion," *Toxicol. Vitr.*, vol. 63, no. 100, p. 104740, 2020, doi: 10.1016/j.tiv.2019.104740.
[30] G. Chen, S. Luo, S. Kozlovich, and P. Lazarus, "Association between Glucuronidation Genotypes and Urinary NNAL Metabolic Phenotypes in Smokers," doi: 10.1158/1055-9965.EPI-15-1245.
[31] X. Luo *et al.*, "A new method for the simultaneous determination of urinary NNAL and cotinine concentrations using HILIC-MS/MS coupled with PRiME HLB SPE," *Int. J. Mass Spectrom.*, vol. 465, pp. 1–9, 2021, doi: 10.1016/j.ijms.2021.116579.
[32] Wei B, Blount BC, Xia B, Wang L. Assessing exposure to tobacco-specific carcinogen NNK using its urinary metabolite NNAL measured in US population: 2011–2012. Journal of exposure science & environmental epidemiology. 2016 May;26(3):249-56, doi:10.1038/jes.2014.88.

https://doi.org/10.24191/sl.v18i1.21163

[33] E. S. Carlson, P. Upadhyaya, and S. S. Hecht, "Evaluation of Nitrosamide Formation in the Cytochrome P450-Mediated Metabolism of Tobacco-Specific Nitrosamines," *Chem. Res. Toxicol.*, vol. 29, no. 12, pp. 2194–2205, Dec. 2016, doi: 10.1021/acs.chemrestox.6b00384.

[34] M. Ceppi, A. Munnia, F. Cellai, M. Bruzzone, and M. E. M. Peluso, "Linking the generation of DNA adducts to lung cancer," *Toxicology*, vol. 390, 2017, doi: 10.1016/j.tox.2017.09.011.

[35] L.-R. Zhu *et al.*, "CYP2A13 in Human Respiratory Tissues and Lung Cancers: An Immunohistochemical Study with A New Peptide-Specific Antibody," doi: 10.1124/dmd.106.011049.

[36] B. D. Smith, J. L. Sanders, P. R. Porubsky, G. H. Lushington, C. D. Stout, and E. E. Scott, "Structure of the human lung cytochrome P450 2A13," *J. Biol. Chem.*, vol. 282, no. 23, pp. 17306–17313, 2007, doi: 10.1074/jbc.M702361200.

[37] D. Bagdas, P. P. Muldoon, A. Z. X. Zhu, R. F. Tyndale, and M. I. Damaj, "Effects of methoxsalen, a CYP2A5/6 inhibitor, on nicotine dependence behaviors in mice," *Neuropharmacology*, vol. 85, pp. 67–72, 2014, doi: 10.1016/j.neuropharm.2014.05.006.

[38] J. A. Tanner and R. F. Tyndale, "Variation in CYP2A6 activity and personalized medicine," *J. Pers. Med.*, vol. 7, no. 4, Dec. 2017, doi: 10.3390/JPM7040018.

[39] S. Ozaki *et al.*, "Smoking cessation program and CYP2A6 polymorphism," *Front. Biosci.*, vol. 11, pp. 2590–2597, 2006, Accessed: Sep. 04, 2022. [Online]. Available: http://www.imm.ki.se/CYPalleles/.

[40] C. Chung *et al.*, "4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) metabolism-related enzymes gene polymorphisms, NNK metabolites levels and urothelial carcinoma," vol. 216, pp. 16–22, 2013.

[41] D. D. Haines and A. Tosaki, "Heme degradation in pathophysiology of and countermeasures to inflammation-associated disease," *International Journal of Molecular Sciences*, vol. 21, no. 24. 2020, doi: 10.3390/ijms21249698.

[42] S. W. Ryter, "Bile pigments in pulmonary and vascular disease," vol. 3, no. March, pp. 1–8, 2012, doi: 10.3389/fphar.2012.00039.

[43] C. Mölzer *et al.*, "Bilirubin and Related Tetrapyrroles Inhibit Food-Borne Mutagenesis: A Mechanism for Antigenotoxic Action against a Model Epoxide," *J. Nat. Prod.*, vol. 76, no. 10, pp. 1958–1965, Oct. 2013, doi: 10.1021/np4005807.

[44] V. Rawat, G. Bortolussi, S. Gazzin, C. Tiribelli, and A. F. Muro, "Bilirubin-induced oxidative stress leads to DNA damage in the cerebellum of hyperbilirubinemic neonatal mice and activates DNA double-strand break repair pathways in human cells," *Oxid. Med. Cell. Longev.*, vol. 2018, 2018, doi: 10.1155/2018/1801243.

[45] J. Wei, H. Zhao, G. Fan, and J. Li, "Bilirubin treatment suppresses pulmonary inflammation in a rat model of smoke-induced emphysema," *Biochem. Biophys. Res. Commun.*, vol. 465, no. 2, 2015, doi: 10.1016/j.bbrc.2015.07.133.

[46] J. Rose and R. Vassar, "Movement disorders due to bilirubin toxicity," *Seminars in Fetal and Neonatal Medicine*, vol. 20, no. 1. 2015, doi: 10.1016/j.siny.2014.11.002.

[47] A. Abu-Bakar, V. Lämsä, S. Arpiainen, M. R. Moore, M. A. Lang, and J. Hakkola, "Regulation of CYP2A5 gene by the transcription factor nuclear factor (erythroid-derived 2)-like 2," *Drug Metab. Dispos.*, vol. 35, no. 5, pp. 787–794, 2007, doi: 10.1124/dmd.106.014423.

[48] A. Abu-Bakar, J. Hakkola, R. Juvonen, M. Rahnasto-Rilla, H. Raunio, and M. A. Lang, "Function and Regulation of the Cyp2a5/CYP2A6 Genes in Response to Toxic Insults in the Liver," *Curr. Drug Metab.*, vol. 14, no. 1, pp. 137–150, 2012, doi: 10.2174/138920013804545223.

[49] Y. Xu, Z. Shen, J. Shen, G. Liu, W. Li, and Y. Tang, "Computational insights into the different catalytic activities of CYP2A13 and CYP2A6 on NNK," *J. Mol. Graph. Model.*, vol. 30, pp. 1–9, 2011, doi: 10.1016/j.jmgm.2011.05.002.

[50] H. Hu, T. Yu, S. Arpiainen, M. A. Lang, J. Hakkola, and A. Abu-Bakar, "Tumour suppressor protein p53 regulates the stress activated bilirubin oxidase cytochrome P450 2A6," *Toxicol. Appl. Pharmacol.*, vol. 289, no. 1, pp. 30–39, 2015, doi: 10.1016/j.taap.2015.08.021.

[51] A. Abu-Bakar *et al.*, "Metabolism of bilirubin by human cytochrome P450 2A6," *Toxicol. Appl.*

Pharmacol., vol. 261, no. 1, pp. 50–58, 2012, doi: 10.1016/j.taap.2012.03.010.

[52] S. N. F. Muhsain, M. A. Lang, and A. Abu-Bakar, "Mitochondrial targeting of bilirubin regulatory enzymes: An adaptive response to oxidative stress," *Toxicol. Appl. Pharmacol.*, vol. 282, no. 1, pp. 77–89, 2015, doi: 10.1016/j.taap.2014.11.010.

https://doi.org/10.24191/sl.v18i1.21163