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Investigation of Expression of CYP3A4 in Diabetic Rats in Xenobiotic Metabolism

Short Title in English: Investigation of Expression of CYP3A4

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ABSTRACT
Objectives: This study aimed to investigate the impact of high-fat diet and STZ induced diabetes as well as dapagliflozin treatment on hepatic protein expression of CYP3A4.
Materials and Methods: In our study, 34 male Sprague-Dawley rats were randomly divided into four groups: control, high-fat diet and streptozotocin induced diabetes, dapagliflozin treated control and dapagliflozin treated diabetes. In the microsomes obtained from livers of these rats, the protein expression levels of CYP3A4 were determined by Western blot.
Results: Hepatic CYP3A4 protein expression levels in control group treated with dapagliflozin were statistically significantly decreased compared to control group. Besides, hepatic CYP3A4 protein expression levels were decreased in dapagliflozin treated diabetic Sprague-Dawley rats compared to both control and diabetic group rats, but the difference between the groups was not statistically significant.
Conclusion: According to these two results, the use of dapagliflozin resulted in inhibition of hepatic CYP3A4 protein expression.
Key words: CYP3A4, dapagliflozin, diabetes mellitus, microsome, protein expression

INTRODUCTION

Cytochromes P450 monooxygenases (CYP450), a superfamily of heme-containing proteins, is responsible for the biotransformation of a vast majority both endobiotics and xenobiotics by converting these lipophilic compounds into their hidrophilic forms.[1,2] More than 95% of available pharmaceuticals used clinically are metabolized by CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. Different CYP450s, which have varying degrees of abundance in smooth endoplasmic reticulum of the human hepatocyte have been determined to be 13% CYP1A2, 4% CYP2A6, 1% CYP2B6, 20% CYP2C, 2% CYP2D6, 7% CYP2E1 and 30% CYP3A4.[3] CYP3A4 is a major CYP450 enzyme that particularly mediates biotransformation of approximately 50% of marketed drugs, including benzodiazepines (alprazolam, diazepam, midazolam), calcium channel blockers (amlodipine, diltiazem, verapamil), immunosuppressives (cyclosporine, tacrolimus, sirolimus), macrolide antibiotics (clarithromycin, erythromycin), statins (atorvastatin, simvastatin). Along with many medications, CYP3A4 also catalyzes the metabolism of a wide range of endogenous molecules such as steroids (estradiol, progesterone, testosterone), and vitamin D.[4,5] CYP3A4 is also responsible for bioactivating aflatoxin B1, which is an environmental carcinogen.[6] A high level of CYP3A4 gene expression is found in the liver of a human. In addition, extrahepatic tissues expressing CYP3A4 include prostate, breast, intestine, colon, small intestine, and brain.[7]
Diabetes Mellitus (DM) is a heterogeneous group of diseases characterized by hyperglycemia due to an absolute or relative deficiency of insulin secretion and/or insulin action. The hyperglycemia associated with DM, which is a chronic disease, damages the heart, blood vessels, eyes, kidneys, and nerves.[8] There are two main types of DM, type 1 and type 2. Type 1 diabetes mellitus (T1DM), known as an autoimmune disease, results from a lack of insulin production caused by damages to the pancreatic beta cells. Type 2 diabetes mellitus (T2DM) has three characteristic

features: insulin resistance, beta cell secretory dysfunction, increased production of hepatic glucose.[9,10] The consequences of DM, which is a treatable disease, are prevented or delayed by diet, physical activity, medications, regular screening and treatments for complications.[11] Dapagliflozin, chosen as an antidiabetic agent in our study, is a medicine used in the management of T2DM by selectively inhibiting sodium-glucose co-transporter 2 (SGLT2), thus preventing the reabsorption of glucose from the urine. In January 2014, the U.S. Food and Drug Administration (FDA) approved its use in combination with diet and exercise to treat adult T2DM patients by providing glycemic control. According to the results of some in vitro studies examining the relationship between dapagliflozin metabolism and CYP450 enzymes, it has been shown that dapagliflozin metabolism can be catalyzed by CYP1A1, CYP1A2, CYP2A6, CYP2C9, CYP2D6 and CYP3A4.[12,13] Sprague Dawley rats with T2DM induced by combination of high-fat diet and STZ were used in our study. High- fat diet feeding leads to insülin resistance in rats. Besides, treatment with STZ contributes to beta cell dysfunction. Namely, co-administration of a high-fat diet and STZ creates alike metabolic profile observed in humans who suffer from T2DM.[14]

There are a variety of components that influence the expression of each CYP450, including genetic polymorphisms, xenobiotics, cytokines, hormones, disease states, and sex, age, and others.[15] Factors that induce CYP3A4 include wide variety of medicines such as antiandrogens, antibiotics, antiemetics, antiepileptics, antineoplastic agents, antipyretic analgesic, antiretrovirals, barbiturates, cystic fibrosis medications, glucocorticoids, retinoid receptor modulators, steroidogenesis inhibitors, kinase inhibitors and different types of herbal compounds such as ginkgolide A and B, hyperforin, quercetin. Moreover, dichlorodiphenyltrichloroethane and endrin, which are organochlorine pesticides as well as ethanol have been associated with CYP3A4 induction.[16] The induction of CYP3A4 by different exogenous substances, which upregulate gene transcription by binding to pregnan X receptor or constitutive androstane receptor, is an important factor regulating its expression.[17] The activity and expression level of CYP3A4 enzyme has also been associated with environmental factors such as diet and xenobiotic exposure. Since CYP3A4 widely distributed throughout intestinal mucosa, CYP3A4 enzyme levels are affected by fasting symptoms and are increased in starvation. There is a gender-specific difference in induction of CYP3A4 in humans. Studies have shown that women are more likely to have a higher CYP3A4 metabolism rate.[18]

Among these factors mentioned above, DM can modulate CYP450 enzyme levels, drug metabolism, and drug response. Moreover, patients with DM often require pharmaceutical therapy more frequently than healthy ones. For these two reasons, it is important to understand how DM affects biotransformation of endogenous and exogenous compounds.[19,20] Effects of both types of diabetes on CYP450 enzyme expression and activity have been shown in different human samples, experimental animal models, and cell lines. A number of xenobiotic-metabolizing enzymes are impacted by DM, including CYP1A1, CYP1B1, CYP2B1, CYP2B4, CYP2C6, CYP2C11, CYP2C23, CYP2E1, CYP3A1, CYP3A4, CYP3A11, CYP3A5, CYP7A1.[21-28] However, the number of studies examining the effects of DM on CYP3A4 protein expression is limited. Researchers found that the levels of CYP3A4 proteins and catalytic activity were significantly reduced by DM.[28] T2DM is acknowledged as a chronic condition characterized by low inflammation. In patients with T2DM, certain cytokines have been associated with changes in CYP450 enzyme expression levels and/or activity. There is a correlation between T2DM and increased inflammatory markers, especially interleukin-6 and tumor necrosis factor alpha. Many drug metabolizing enzymes, particularly CYP450 enzymes in the CYP3A subfamily, are downregulated in response to higher levels of interleukin-6 and tumor necrosis factor alpha.[29] In contrary to these findings, another study showed that both T1DM and T2DM significantly increased hepatic CYP3A expression.[30] CYP3A4 enzyme inhibition and/or induction increases risk of undesirable drug- drug interactions and subsequent drug toxicity. Since CYP3A4 is main and most important enzyme involved in metabolism of more than half of drugs prescribed and administered, it is thought that this may be principal cause for clinical failures and withdrawal of marketed drugs.[31]

Our study was aimed to understand the alteration of CYP3A4 expression under diabetic conditions. With this purpose, CYP3A4 hepatic expression was investigated in liver microsomes obtained from control, high fat diet and STZ induced diabetes, dapagliflozin- treated control and dapagliflozin treated diabetes Sprague-Dawley rats using by Western blot.

MATERIALS AND METHODS

Animals and study design

4-5 week old male Sprague-Dawley rats (100–150 g) were obtained by Bilkent University Genetics and Biotechnology Research Center (Ankara, Turkey). The rats were housed with 2 or 3 rats in each cage and maintained on a 12 h light/dark cycle at constant room temperature (22±1 ºC) with tap water and standard rat chow (Purina) *ad libitum*. One week after quarantine, rats were given either standard chow or high-fat diet (35% fat; Arden Research & Experiment) during the rest of the experiment. Rats of the control group received only citrate buffer (pH: 4.5) intraperitoneally. After another 4–5 weeks, diabetes was induced followed by a single-dose injection of STZ (25 mg/kg; i.p.) dissolved in citrate buffer (0.1 N; pH 4.5) in rats of feeding with high-fat diet. After 72 hours of STZ injection, blood glucose level was evaluated for each rat from the tail. A second or a third STZ injection was given to animals whose blood glucose levels were <200 and <140 mg/dl. Rats were accepted as diabetic when blood glucose level was than higher than 140 mg/dl. After diabetes had been established, half of control and diabetic group rats were treated orally 1 mg/kg/day of dapagliflozin for 12 weeks. Dapagliflozin suspension was prepared by pulverizing Forziga ® tablets (10 mg as 12.3 mg dapagliflozin propanediol monohydrate) and then dissolving them in distilled

water. Among the pharmaceutically inactive ingredients were microcrystalline cellulose, anhydrous lactose, crospovidone, silicon dioxide, and magnesium stearate in tablets. They are coated with polyvinyl alcohol, titanium dioxide, polyethylene glycol, talc, and yellow iron oxide.
Thus, the animals were divided into four groups: control (n = 10), diabetic rats (n = 6), control rats treated with dapagliflozin (n = 10) and diabetic rats treated with dapagliflozin (n = 8). All animal procedures were performed in accordance with the Ankara University Animal Care and Use Committee (permit 2018-6-45).

Preparation of microsomes

12 weeks after treatments, rats were sacrificed under anaesthesia. Liver tissues were rapidly excised, weighed, and preserved at -80 until to use. Liver tissues about 1.5 g, were homogenized in Potter Elvehjem homogenizer using 1.15% potassium chloride (w/v) (Sigma- Aldrich) at 3000 rpm in an ice-cold bath. The homogenate was then centrifuged at 11 000 x g for 25 min. The supernatant fractions were centrifuged again at 108 000 x g for 60 min. After ultracentrifugation microsomal pellets were resuspended in 20 % glycerol (Sigma-Aldrich); microsomal fractions were stored at -80°C.

Western blotting

Protein level for CYP3A4 were assessed by western blotting. Firstly, total protein content was measured using the BCA Protein Assay Kit (Pierce). Samples were heated with sample buffer (Sigma-Aldrich) at 70 °C for 5 min to denaturate the protein. 30 µg of samples were loaded onto a 10% SDS-PAGE gel. Proteins were separated by using electrophoresis conducted at 100 V for approximately 2 hours. Following gel electrophoresis, seperated proteins were transferred onto nitrocellulose membrane (Biorad) via wet transfer method at 100 V for 2 hours. After transfer, the membranes were blocked with 5% BSA (Sigma- Aldrich) in Tris-buffered saline that contained Tween 20 (Sigma-Aldrich) at room temperature for 1 hour. Membranes were then incubated with primary antibody (Abcam; 1:5000 dilution) at 4 ℃ overnight. Horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (Advansta; 1:5000 dilution) was used as secondary antibody. Detection of specific bands was performed by chemiluminescence using ECL reagent (Advansta). Imaging was conducted via Li-Cor Odyssey imaging system. Beta-actin (Biolegend; 1/2000 dilution) was used as a loading control, for normalize the density of each band.

Statistical analysis

All statistical analysis were performed using Statistical Package for the Social Sciences (SPSS, version 25). The Shapiro-wilk test was used to check the normal distribution for all variables. Statistically significant differences between groups were analyzed with one-way analysis of variance, followed by a post hoc least significant difference test. The data were expressed as mean, standard error and standard deviation. P-value < 0.05 was considered statistically significant.

RESULTS

Blood glucose levels and body weight of animals

Diabetic rats exhibited statistically significant higher blood glucose levels, as compared with rats of control and treatment groups ($p<0.05$). Body weight of dapagliflozin treated control group were found to be statistically significantly lower than control group ($p<0.05$). Body weight of the dapagliflozin treated control group were lower than those of diabetes and dapagliflozin-treated diabetes groups. However, no statistically significant difference on body weight was found difference between dapagliflozin treated control group and each of diabetes and dapagliflozin treated diabetes groups ($p>0.05$). Data about blood glucose levels and body weights at the time of death was indicated in Table 1.

Table 1. The blood glucose levels and the body weights at the time of death for each group of animals

	Control	**Diabetes**	**Dapagliflozin treated control**	**Dapagliflozin treated diabetes**
Body weight (g)	430.2 ± 7.374	410.67 ± 18.204	303.3 ± 55.059*	418.75 ± 7.343
Blood glucose levels (mg/dl)	100.2 ± 2.149	233.67 ± 54.769Δ	98.7 ± 2.135	129 ± 12.186

Significant changes are expressed as * ($p < 0.05$; One-way ANOVA, Post Hoc-LSD) in comparison with control group; Δ ($p < 0.05$; One-way ANOVA, Post Hoc-LSD) in comparison with control and treatment groups.

CYP3A4 protein levels in the liver of animals

Protein expression level of CYP3A4 in dapagliflozin treated rats was statistically significantly decreased as compared to the control rats ($p<0.05$). In addition, hepatic CYP3A4 protein expression level of high-fat diet and STZ induced diabetes group was lower than rats of control group, but difference between these two groups was not statistically significant ($p>0.05$). It was also reported that CYP3A4 protein expression level of rats placed in dapagliflozin treated diabetes were lower than those of control and diabetes groups, while higher than those of dapagliflozin treated control group, but difference between groups was not statistically significant ($p>0.05$). Protein expression bar graphs and representive protein bands for each group in Figure 1 and Figure 2.

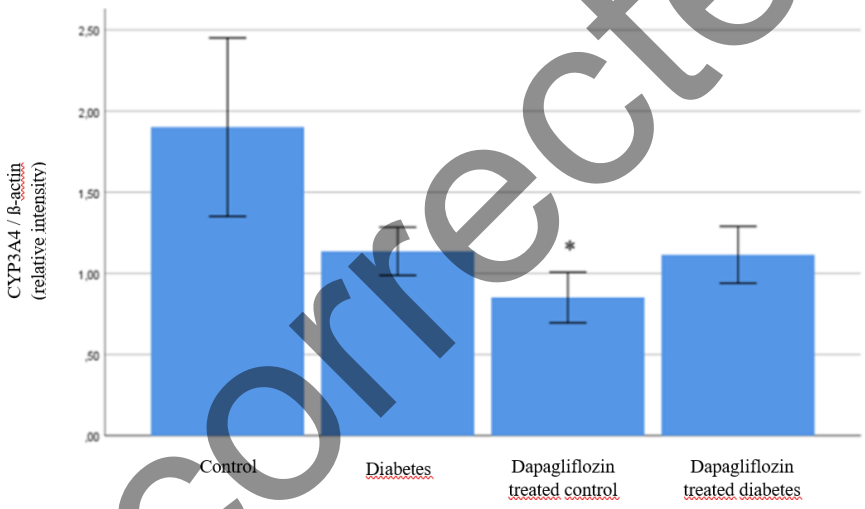

Figure 1. Hepatic CYP3A4 protein expression levels of for each animal group. Significant changes are expressed as * ($p < 0.05$; One-way ANOVA, Post Hoc-LSD) in comparison with control group.

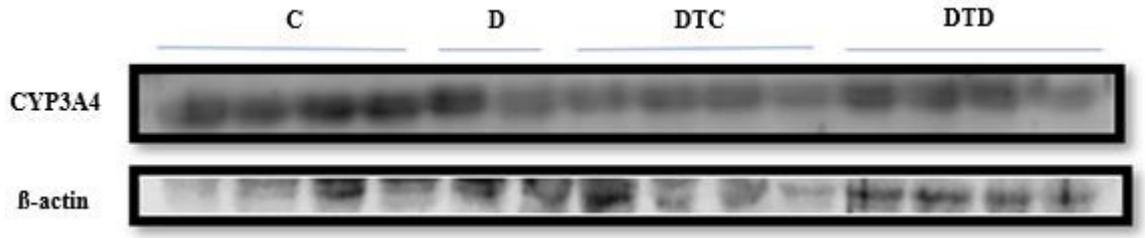

Figure 2. Western blot images of protein/loading control for each group. C, control, D, diabetes, DTC, dapagliflozin treated control, DTD, dapagliflozin treated diabetes

DISCUSSION

DM is a metabolic disease characterized by insufficient benefit of organism from carbohydrates, lipids and proteins and hyperglycemia which caused by defects in insulin secretion or insulin effect or both. DM is demonstrated to regulate protein expression of CYP450 enzyme. Alterations in expression of CYP450 enzymes are associated with changes in metabolism caused by diabetes (increased ketone bodies, lipids and carbohydrates) and regulation of some hormones such as insulin, glucagon. Researches reported that protein expression of CYP3A4, which is one of the most important enzymes in proses of biotransformation, is effected by DM. Considering this information, our study makes several implications. First, it shows that diabetes downregulates CYP3A4 protein expression in the rat liver microsome. This result was consistent with previous study, which revealed that CYP3A4 hepatic expression level was significantly lower in diabetic human liver microsomes. The same research study also showed that there has been no significant difference in CYP3A5 protein level in diabetic and non-diabetic individuals.[28] However, little is known about how to effect CYP3A5 protein expression in rat liver microsomes under diabetic conditions. Changes caused by DM in metabolic process, such as elevations in ketone bodies, lipids, and carbohydrates, as well as hormonal regulation, such as insulin, glucagon, leptin, and growth hormone, might influence hepatic CYP450 expression.[32] Different opinions have been suggested regarding the mechanism of decreased CYP3A4 hepatic expression in individuals with DM. These mechanisms comprise the effects of pre- inflammatory cytokines, non-cytokinin components, oxidative stress, and obesity. It has been reported that elevated levels of cytokines (interleukin-1ß, interleukin-6 and tumor necrosis factor alpha) contribute to decrease in CYP3A4 enzyme expression in individuals with DM.[28] Also, another data suggested, that protein level and activity of CYP3A4 reduced in liver microsomes of DM and non-alcoholic fatty liver disease patients. Contrary to these results it was demonstrated that induction activity and up-regulated protein level of CYP3A4 were found in HepG2 cells incubated with serum from rats developing diabetes with STZ. Based on results of the study, AMP-activated protein kinase, protein kinase C, and nuclear factor kappa B pathways were most likely involved in oleic acid-induced CYP3A4 activity, while PKC might be involved in palmitic acid-induced activity.[33] Similar to results of the previous study, markedly increased hepatic CYP3A protein level were determined in both STZ- induced T1DM mice and db/db T2DM mice. Although there are differences in their pathophysiology, these two diseases seem to have the same modulating effect on CYP3A expression.[30] When all these results are considered together, it seems that CYP3A4 protein expression and activity is modulated differently by DM in human samples, diabetic animal models, and cell culture. A disruption of CYP3A4 protein expression and activity associated with DM may alter xenobiotic elimination half- life, and bioavailability, efficacy, and safety of CYP3A4 substrates. A second important implication of our study is that hepatic CYP3A4 protein expression level of dapagliflozin- treated control rats was found to be significantly lower than control group. Based on this result, the use of dapagliflozin is the most likely to inhibit hepatic CYP3A4 protein expression. Therefore, drugs that are CYP3A4 substrates such as acetaminophen, lovastatin, diltiazem, vardenafil should be used with caution in patients with DM using dapagliflozin.[34] According to rat, dog, monkey, and human liver microsomal studies, dapagliflozin undergoes oxidative metabolism. Various human CYP450 enzymes metabolized dapagliflozin in vitro, and the highest metabolism was attributed to the enzymes CYP2D6, CYP1A2, CYP3A4, CYP2C9, CYP1A2, CYP3A5 and CYP2E1 in order of highest to lowest.[35] According to this information and the result obtained from our study, inhibition of hepatic expression of CYP3A4, which is involved in the metabolism of dapagliflozin, by dapagliflozin may also cause a decrease in the biotransformation of the aforementioned drug. In a study in which STZ-induced T1DM rats, it was found that combining dapagliflozin with a low dose of insulin stabilizes CYP1A, CYP2D, CYP2E and CYP3A activities.[36] In addition to these, blood glucose levels and body weights at time of death were measured for each group of animals. Blood glucose levels in diabetic rats were significantly higher than those of control and dapagliflozin-treated control rats. Accordingly, it has been demonstrated that the STZ-induced DM model was confirmed. Body weights were found to be statistically significantly lower in dapagliflozin treated control group compared to control group. The mechanism of action of dapagliflozin is through glucose excretion, suggesting that it may have caused a decrease in body weights in the treated groups.

Our study has reported the effect of DM on CYP3A4 expression in rat liver, but the impact of DM on CYP3A4 enzymatic activity and/or mRNA levels were not studied. Also, the mechanism behind this decrease in CYP3A4 expression has not been clarified in our study.

Further research is needed to shed light on these issues. To the best of our knowledge, this paper is the first to study investigate the effect of dapagliflozin on CYP3A4 expression.

Therefore, it is very important to support our study by designing studies in which the number of animals is increased and relationship between dapagliflozin and CYP3A4 expression in different species of experimental animals is

evaluated. Our study had been done on exclusively male rats. However, the impact of the gender factor should also be assessed in other studies involving female rats. Moreover, it can be evaluated that effect of STZ administration on CYP3A4 expression in early and late applications. When the studies are examined, it is seen that the antidiabetic agent whose effect on CYP450 expression and activity is investigated is generally insulin. Therefore, it is important to design studies that examine the effect of antidiabetic agents other than insulin on CYP450 enzyme expression. Research on expression of other CYP450 enzymes involved in dapagliflozin metabolism in addition to CYP3A4 in diabetic conditions will also be complementary to our study.

CONCLUSION

The findings of our study showed that hepatic CYP3A4 protein expression levels in control group treated with dapagliflozin were statistically significantly decreased compared to control group. Besides, we reported that hepatic CYP3A4 protein expression levels were decreased in dapagliflozin treated diabetic Sprague-Dawley rats compared to both control and diabetic group rats, but difference between groups was not statistically significant. According to these two results, the use of dapagliflozin resulted in inhibition of hepatic CYP3A4 protein expression. This result was consistent with data of two previous studies. Another important conclusions of our study was regarding physical and biochemical characteristics of rats. It was observed that blood glucose levels of both control and dapagliflozin-treated control groups were lower than diabetes and dapagliglozin-treated diabetes groups, but it was not statistically significant. On the other hand, blood glucose levels of diabetic rats was found to be statistically significantly higher than the control and administration groups. Accordingly, the control of DM model induced by STZ and high fat diet provided. Body weights of dapagliflozin-treated control group at time of death were lower than diabetes and dapagliglozin-treated diabetes groups, but it was not statistically significant. Moreover, body weights at time of death were found to be statistically significantly lower in rats of dapagliflozin-treated control group compared to rats of control group. According to this data, significant decrease in body weight of dapagliflozin-treated control rats was associated with mechanism of action of dapagliflozin, leading excretion of urinary glucose. As a final point, we showed for the first time that impact of dapagliflozin treatment on hepatic CYP3A4 protein expression levels. Many clinically used drugs, including dapagliflozin, and endogenous substances are metabolically processed by CYP3A4. Diabetic condition, complications related to diabetes and antidiabetic agents are among the factors that play a role in regulating CYP3A4 protein expression. Therefore, it is necessary to design studies examining relationship between these factors and CYP3A4 protein expression.

REFERENCES

1. Kiani YS, Jabeen I. Lipophilic metabolic efficiency (LipMetE) and drug efficiency indices to explore the metabolic properties of the substrates of selected cytochrome p450 isoforms. ACS Omega. 2019;5(1):179-188.
2. Rourke JL, Sinal CJ. Biotransformation/Metabolism. In: Wexler P. Encyclopedia of Toxicology (3rd ed). Cambridge; Academic Press; 2014: 490-502.
3. Jin SE, Ha H, Seo CS, Shin HK, Jeong SJ. Expression of cytochrome p450s in the liver of rats administered with socheongryong-tang, a traditional herbal formula. Pharmacogn Mag. 2016;12(47):211-8.
4. Bauters T. Clinically Relevant Drug Interactions in HSCT. In: Carreras E, Dufour C, Mohty M, Kröger N, eds. The EBMT Handbook: Hematopoietic Stem Cell Transplantation and Cellular Therapies [Internet]. (7th ed). Cham (CH); Springer; 2019: Chapter 31.
5. Thummel KE. Gut instincts: CYP3A4 and intestinal drug metabolism. J Clin Invest. 2007;117(11):3173-6.
6. Martínez-Jiménez CP, Jover R, Donato MT, Castell JV, Gómez-Lechón MJ. Transcriptional regulation and expression of CYP3A4 in hepatocytes. Curr Drug Metab. 2007;8(2):185-94.
7. Wei Z, Jiang S, Zhang Y, Wang X, Peng X, Meng C, Liu Y, Wang H, Guo L, Qin S, He L, Shao F, Zhang L, Xing Q. The effect of microRNAs in the regulation of human CYP3A4: a systematic study using a mathematical model. Sci Rep. 2014;4:4283.

8. Uazman A, Omar A, Shazlı A, Rayaz MA. General Aspects of Diabetes Mellitus. In: Zochodne DW, Malik RA. Handbook of Clinical Neurology. 2014;126:211-222.
9. Steck AK, Rewers MJ. Genetics of type 1 diabetes. Clin Chem. 2011;57(2): 176–185.
10. Shi Z, He Z, Wang DW. CYP450 epoxygenase metabolites, epoxyeicosatrienoic acids, as novel anti-inflammatory mediators. Molecules. 2022;27(12):3873.
11. World Health Organization (2019). Available from: https://www.who.int/news-room/fact-sheets/detail/diabetes
12. Balakumar P, Sundram K, Dhanaraj SA. Dapagliflozin: glucuretic action and beyond. Pharmacol Res. 2014;82:34-9.
13. Kasichayanula S, Liu X, Lacreta F, Griffen SC, Boulton DW. Clinical pharmacokinetics and pharmacodynamics of dapagliflozin, a selective inhibitor of sodium-glucose co-transporter type 2. Clin Pharmacokinet. 2014;53(1):17-27.
14. Gheibi S, Kashfi K, Ghasemi A. A practical guide for induction of type-2 diabetes in rat: incorporating a high-fat diet and streptozotocin. Biomed Pharmacother. 2017;95:605-613.
15. Zanger UM, Schwab M. Cytochrome p450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther. 2013;138(1):103-41.
16. Hakkola J, Hukkanen J, Turpeinen M, Pelkonen O. Inhibition and induction of CYP enzymes in humans: an update. Arch Toxicol. 2020;94(11):3671-3722.
17. Wolbold R, Klein K, Burk O, Nüssler AK, Neuhaus P, Eichelbaum M, Schwab M, Zanger UM. Sex is a major determinant of CYP3A4 expression in human liver. Hepatology. 2003;38(4):978-88.
18. Crago J, Klaper RD. Influence of gender, feeding regimen, and exposure duration on gene expression associated with xenobiotic metabolism in fathead minnows (Pimephales promelas). Comp Biochem Physiol C Toxicol Pharmacol. 2011;154(3):208-12.
19. Yoshinari K, Takagi S, Sugatani J, Miwa M. Changes in the expression of cytochromes P450 and nuclear receptors in the liver of genetically diabetic db/db mice. Biol Pharm Bull. 2006;29(8):1634-8.
20. Gravel S, Chiasson JL, Dallaire S, Turgeon J, Michaud V. Evaluating the impact of type 2 diabetes mellitus on CYP450 metabolic activities: protocol for a case-control pharmacokinetic study. BMJ Open. 2018;8(2):e020922.
21. Oh SJ, Choi JM, Yun KU, Oh JM, Kwak HC, Oh JG, Lee KS, Kim BH, Heo TH, Kim SK. Hepatic expression of cytochrome P450 in type 2 diabetic Goto-Kakizaki rats. Chem Biol Interact. 2012;195(3):173-9
22. Arinç E, Arslan S, Bozcaarmutlu A, Adali O. Effects of diabetes on rabbit kidney and lung CYP2E1 and CYP2B4 expression and drug metabolism and potentiation of carcinogenic activity of N-nitrosodimethylamine in kidney and lung. Food Chem Toxicol. 2007;45(1):107-18.
23. Takatori A, Akahori M, Kawamura S, Itagaki S, Yoshikawa Y. The effects of diabetes with hyperlipidemia on P450 expression in APA hamster livers. J Biochem Mol Toxicol. 2002;16(4):174-81.
24. Dey A, Williams RS, Pollock DM, Stepp DW, Newman JW, Hammock BD, Imig JD. Altered kidney CYP2C and cyclooxygenase-2 levels are associated with obesity- related albuminuria. Obes Res. 2004;12(8):1278-89.
25. Cheng Q, Aleksunes LM, Manautou JE, Cherrington NJ, Scheffer GL, Yamasaki H, Slitt AL. Drug-metabolizing enzyme and transporter expression in a mouse model of diabetes and obesity. Mol Pharm. 2008;5(1):77-91.
26. L.D. Bao, C.Q. Li, R. Peng, X.H. Ren, R.L. Ma, Y. Wang, H.J. Lv. Correlation between the decrease of cholesterol efflux from macrophages in patients with type II diabetes mellitus and down-regulated CYP7A1 expression. Genet. Mol. Res. 2015;14(3): 8716-8724.
27. Raza H, Prabu SK, Robin MA, Avadhani NG. Elevated mitochondrial cytochrome P450 2E1 and glutathione S-transferase A4-4 in streptozotocin-induced diabetic rats: tissue-specific variations and roles in oxidative stress. Diabetes. 2004;53(1):185-94.
28. Dostalek M, Court MH, Yan B, Akhlaghi F. Significantly reduced cytochrome p450 3A4 expression and activity in liver from humans with diabetes mellitus. Br J Pharmacol. 2011;163(5):937-47.
29. Darakjian L, Deodhar M, Turgeon J, Michaud V. Chronic inflammatory status observed in patients with type 2 diabetes induces modulation of cytochrome p450 expression and activity. Int J Mol Sci. 2021;22(9):4967.
30. Patoine D, Petit M, Pilote S, Picard F, Drolet B, Simard C. Modulation of CYP3a expression and activity in mice models of type 1 and type 2 diabetes. Pharmacol Res Perspect. 2014;2(6):e00082.
31. Sevrioukova IF, Poulos TL. Current approaches for investigating and predicting cytochrome p450 3A4-ligand interactions. Adv Exp Med Biol. 2015;851:83-105.
32. Park SY, Kim CH, Lee JY, Jeon JS, Kim MJ, Chae SH, Kim HC, Oh SJ, Kim SK. Hepatic expression of cytochrome p450 in Zucker diabetic fatty rats. Food Chem Toxicol. 2016;96:244-53.
33. Hu N, Hu M, Duan R, Liu C, Guo H, Zhang M, Yu Y, Wang X, Liu L, Liu X. Increased levels of fatty acids contributed to induction of hepatic CYP3A4 activity induced by diabetes - in vitro evidence from HepG2 cell and Fa2N-4 cell lines. J Pharmacol Sci. 2014;124(4):433-44.

34. DRUGBANK, Cytochrome P-450 CYP3A4 Substrates. Available from: https://go.drugbank.com/categories/DBCAT002646
35. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2014/202293Orig1s000PharmR.pdf
36. Sayed N, Murata I, Abdalla O, Kilany O, Dessouki A, Sasaki K. Effects of dapagliflozin in combination with insulin on cytochrome P450 activities in a diabetes type 1 rat model. J Vet Med Sci. 2021;83(10):1597-1603.