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Research Article

Effect of Low and High-Dose Dexamethasone on CYP3A and P-glycoprotein Expression and Function in Mice

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Abstract

Dexamethasone, which acts on nuclear receptors, is used together with P-gp and Cyp3a4 substrates at different doses in the treatment of diverse diseases. The aim of the study is to determine the effect of different doses of dexamethasone on the expressions and functions of P-gp and Cyp3a4 (mouse homologues of Cyp3a4 are Cyp3a11 and Cyp3a13). Animals (n=54) were divided into 9 equal groups. The control group was given only water. (The second group received fexofenadine, the third group received midazolam, the fourth group received low-dose dexamethasone, and the fifth group received high-dose dexamethasone. Low dose dexamethasone was administered to the sixth and seventh groups, and high dose dexamethasone was administered to the eighth and ninth groups.) 24 hours after these applications, fexofenadine was administered to the sixth and eighth groups, and midazolam was administered to the seventh and ninth groups. Synthesis of mdr1a, mdr1b, Cyp3a11 and Cyp3a13 in the small intestine and liver were determined using RT-qPCR. Plasma concentrations of fexofenadine and midazolam were determined using HPLC-UV. The effect of dexamethasone on Cyp3a11 expression was determined to be dose-related, while its effect on P-gp expression was not dose-related. Midazolam, a CYP3A substrate, did not cause P-gp induction in the small intestine, but did cause P-gp induction in the liver. Fexofenadine and midazolam reduced the effect of dexamethasone on Cyp3a11 expression in the small intestine. Dexamethasone dose-dependently decreased the plasma concentration of midazolam and increased that of its metabolite. Dexamethasone caused a dose-independent decrease in plasma concentrations of fexofenadine. The effect of dexamethasone on P-gp expression is not dose-dependent, but its effect on Cyp3a11 ve Cyp3a13 expression is dose-dependent. Fexofenadine and midazolam caused a reduction in the effect of dexamethasone on Cyp3a11 expression in the small intestine.

Keywords: Cyp3a4, Dexamethasone, Dose, Mice, P-glycoprotein

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Introduction

Dexamethasone, which has a strong binding affinity to corticosteroid receptors, is a glucocorticoid with immunosuppressant and anti-inflammatory effects (Ferguson and Hoenig 1995, Shimmer and Parker 1996). This drug, which is more widely preferred in the clinic compared to other glucocorticoids, is used in different doses for several indications (Riviere and Papich 2017). Glucocorticoid receptors belong to the class of nuclear receptors.

Nuclear receptors regulate the transcription of genes that control different physiological events such as homeostasis, metabolism, proliferation and development (Chawla et al 2001). These receptors also transmit the effects of various xenobiotics and endogenous substances (Negishi et al 2020, Parlak et al 2024). Nuclear receptors on which glucocorticoids act in eukaryotic organisms regulate various metabolic and developmental events (Young 2008).

Permeability glycoprotein (P-gp) is encoded by the Multidrug Resistance 1 (MDR1) gene, is expressed in various tissues and organs, including the kidney, intestine, liver, and brain capillary endothelium (Gül et al 2016). It alters the pharmacokinetics and

Table 1. Drugs applied to animal groups, their doses and routes of administrationa (n=6)

Group	Dexamethasone (mg/kg)	Fexofenadine (mg/kg)	Midazolam (mg/kg)
C[b]	-	-	-
F	-	40	-
M	-	-	20
LD	5	-	-
HD	50	-	-
LDF	5	40	-
LDM	5	-	20
HDF	50	40	-
HDM	50	-	20

C: Kontrol, F: Fexofenadine, M: Midazolam, LD: Low Dose Dexamethasone, HD: High Dose Dexamethasone, LDF: Low Dose Dexamethasone+Fexofenadine, LDM: Low Dose Dexamethasone+Midazolam, HDF: High Dose Dexamethasone+Fexofenadine, HDM: High Dose Dexamethasone+Midazolam. [a]Midazolam and dexamethasone were administered intraperitoneally and fexofenadine was administered orally. Fexofenadine and midazolam were administered 24 hours after dexamethasone administration. [b]Distilled water was administered orally to the control group.

pharmacodynamics of drugs by its effects on the absorption, distribution and excretion of drugs (Faber et al 2003, Ho and Kim 2005). Modifications of P-gp function are used to protect tissues and modulate the drug's effect. Foods and some drugs can cause drug-drug and drug-food interactions by inducing or inhibiting transport proteins (Fromm et al 1999, Dresser et al 2003, Tras et al 2017).

Cytochrome P450 3a4 (Cyp3a4), a sub-member of the cytochrome P450 3A (CYP3A) enzyme, is expressed mainly in the liver and small intestine and is responsible for the metabolism of approximately 50% of drugs (Wrighton et al 1996). Changes in the synthesis or function of this enzyme affect the clinical response of many drugs (Flockhart and Rae 2003). Cyp3a4 expression is regulated by nuclear receptors such as the glucocorticoid receptor (GR) and farnesoid X receptor. It is stated that the P-gp and Cyp3a4 substrates are overlapped and nuclear receptor ligands such as rifampicin increase CYP3A and P-gp expression (Tras et al 2017). It is stated that nuclear receptor ligands such as rifampicin increase CYP3A and P-gp expression (Zong and Pollack 2003). Cytochrome P450 3a11 (Cyp3a11), the murine homolog of human Cyp3a4, is synthesized in the liver of murine (Li et al 2009), while Cytochrome P450 3a13 (Cyp3a13) is synthesized in 95 tissues, mainly in the liver and jejunum of murine.

Glucocorticoid receptors act on Cyp3a4 and P-gp expression (Demeule et al 1999). Dexamethasone induces Pregnane X Receptor (PXR), retinoid X receptor (RXR) and constitutive androstane receptor (CAR) expression by enhancing GR signaling at low concentrations, while it directly activates PXR at high concentrations (Pascussi et al 2001, Shi et al 2010).

Fexofenadine, an H1-receptor antagonist and widely used in therapy, is a substrate of P-gp and Cyp3a4. It is largely excreted in feces (80%) and urine (12%) without being metabolized. Modulation of CYP3A and P-gp causes changes in the pharmacokinetics of fexofenadine (Simons and Simons 1999, Simpson and Jarvis 2000). Antihistamines such as fexofenadine are used together with glucocorticoids in the treatment of allergic rhinitis (Randall and Hawkins 2018).

Midazolam, a benzodiazepine derivative, is metabolized by Cyp3a4 and Cytochrome P450 3a5 (Cyp3a5) enzymes and converted to a pharmacologically active 1'-hydroxymidazolam metabolite (De Wildt et al 2002, Pacifici 2014).

It has been hypothesized that nuclear receptor agonists, such as dexamethasone, may alter plasma concentrations of drugs by affecting the function of enzymes and transporter proteins, which can alter the pharmacokinetics of drugs. The aim of the study is to determine whether the effect of dexamethasone on the expression and function of the enzyme (Cyp3a4) and transport proteins (P-gp) is dose-related. For this purpose, fexofenadine, a substrate of P-gp and Cyp3a4, and midazolam, which is metabolized by Cyp3a4 enzymes, were preferred. In addition, to determine whether fexofenadine and midazolam altered the effect of dexamethasone on Cyp3a4 and P-gp expression.

Material and Methods

Ethics committee approval for the use of mice was obtained at the meeting held on 21.02.2018 by the Selcuk University Experimental Medicine Application and Research Center Ethics Committee (SUDAM, 2018-5).

Chemicals and Solutions

Dexamethasone (Dekort, injectable solution, Deva Holding Inc., Türkiye), Fexofenadine (Fexadyne, 180 mg tablet, Ali Raif Pharmaceutical Industry Co., Turkey), and Midazolam (Demizolam, injectable solution, Solupharm, Germany). For animal applications, fexofenadine (1 mg/ml) dissolved in distilled water.

Tri Reagent (Sigma-Aldrich, USA), iScript™ cDNA Synthesis Kit (Bio Rad, USA, Cat. No: 170-8891), and SYBR Green Master Mix (SYBR Green Supermix Biorad, USA).

Diethyl ether (Sigma-Aldrich, USA), sodium dihydrogen phosphate (Sigma-Aldrich, USA), acetonitrile (Sigma-Aldrich, USA), methanol (Sigma-Aldrich, USA), sodium hydroxide (NaOH) (Sigma-Aldrich, USA), and sodium acetate buffer (10 mmol, pH: 4.7) (Sigma-Aldrich, USA).

Animals and study design

In the study, 54 healthy male mice (Swiss albino, 8-10 weeks, 32 ± 1.92 g) were used and divided into 9 equal groups. The groups and the drugs administered to the animals (dexamethasone, fexofenadine, midazolam) are presented in Table 1. The groups were created as control (C), Fexofenadine [(F), 40 mg/kg, PO], Midazolam [(M), 20 mg/kg, IP], low-dose dexamethasone [(LD), 5 mg/kg, IP], high-dose dexamethasone [(HD), 50 mg/kg, IP], low-dose dexamethasone + Fexofenadine [(LDF), 5 mg/kg, IP + 40 mg/kg, PO], low-dose dexamethasone + Midazolam [(LDM), 5 mg/kg, IP + 20 mg/kg, IP], high-dose dexamethasone + Fexofenadine [(HDF), 50 mg/kg, IP + 40 mg/kg, PO] and high-dose dexamethasone + Midazolam [(HDM), 50 mg/kg, IP + 20 mg/kg, IP], respectively.

During the experiment, animals were housed in polysulfone cages in SUDAM under controlled conditions (24±1°C room temperature, 12 h light/dark cycle and 60% atmospheric humidity) and were provided with water and food ad libitum. In the study, fexofenadine and midazolam, chosen as probe drugs of P-gp and Cyp3a4, respectively, were administered 24 hours after dexamethasone administration.

In the study, 25 hours after dexamethasone (first) administration, blood was taken from the hearts of all animals under anesthesia with ketamine at a dose of 90 mg/kg (IP) and xylazine at a dose of 10 mg/kg (IP) and they were sacrificed by cervical dislocation. The time of blood collection was determined taking into account a previous study with midazolam in mice (Pieri 1983). The blood taken into heparin tubes was centrifuged at 3000 g, 4°C for 10 minutes. The plasma samples, liver and small intestine tissues obtained were stored at -80°C until measurement. The tissues were first frozen in liquid nitrogen.

Real-Time PCR (RT-qPCR)

Total RNAs were isolated from tissue samples according to the Tri Reagent protocol. RNAs quality was checked spectrophotometrically. The integrity of RNAs was examined by agarose gel electrophoresis. iScript™ cDNA Synthesis Kit was used for cDNA synthesis of all samples. RT-qPCR was performed on a LightCycler®Nano platform (Roche, Switzerland) using 4 µl of cDNA in a system containing 0.1 µl of forward and reverse primers (100 µM) and 10 µl of SYBR Green Master Mix (Bozkaya et al 2016). Cycling conditions were as follows: preincubation at 98°C for 3 min, followed by 40 cycles of 15 s at 95°C, 30 s at the annealing temperature (Table 2), and finally 30 s at 72°C. The temperature was increased from 60°C to 95°C to create the melting curve. A negative control was run with every assay (Mutlu et al 2023).

Table 2. Sequence of primers, amplicon sizes and their annealing temperatures

Target	Primers	Annealing Temperatures	Lenght (bp)*	Accession Number
CYP3A11-F	5'ACAAACAAGCAGGGATGGACCTGG3'	59.7 °C	269	NM_007818.3
CYP3A11-R	5'TGTGACAGCAAGGAGAGGCGTTT3'			
CYP3A13-F	5'AGCAGTGCTCTTTCCTTTGC3'	57.2 °C	243	NM_007819.4
CYP3A13-R	5'CCCTTTGGGAATGAATAGCC3'			
MDR1A-F	5'GGATGAAATTGATAATTTAGACATG3'	54.3 °C	232	NM_011076.3
MDR1A-R	5'TCCATTTATTATGGCACAGAATATA3'			
MDR1B-F	5'AACACAGCCAACCTTGGAAC3'	54.9 °C	180	NM_008830.2
MDR1B-R	5'TGTTGCAATCTTTCCAGCAG3'			
ACTB-F	5'GGCTGGCCGGGACCTGACAGACTAC3'	57.8 °C	150	NM_007393.5
ACTB-R	5'GCAGTGGCCATCTCCTGCTCGAAGTC3'			
*PCR product size (base pair).				

Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). ACTB (beta-actin) was used as the housekeeping gene, and the control group served as the calibrator. Each experimental group consisted of six biological replicates (n=6), and all RT-qPCR reactions were performed in technical triplicates. Primer specificity and efficiency were validated prior to analysis. Standard curves generated from serial dilutions of pooled cDNA were used to assess amplification efficiency. All primer sets had efficiency values ranging between 1.9 and 2.2. Melt curve analysis confirmed the specificity of all reactions by showing single, sharp peaks.

Drugs Analysis

Plasma concentrations of fexofenadine, midazolam, and its metabolite, 1'-hydroxymidazolam, were determined using the HPLC system (Shimadzu, Tokyo, Japan).

Fexofenadine Analysis

The plasma fexofenadine level was determined according to the method previously reported (Helmy and El Bedaiwy 2016). A total of 1.5 ml of diethyl ether was added to the plasma sample (100 µL) and this mixture was vortexed for 1 min and then centrifuged at 3000 g for 10 min. The ether phase was transferred to tubes with 1.5 ml, evaporated in a water bath at 60 °C and dissolved with 100 µl mobile phase. The solution obtained was transferred to autosampler vials and 25 µl of its was injected into the HPLC-UV system. The mobile phase containing sodium dihydrogen phosphate (pH: 3.0 adjusted with 20 mmol orthophosphoric acid) and acetonitrile (70:30, v/v) was sent to HPLC at a flow rate of 1.0 ml/min and detection was performed with SPD-10A UV-VIS detector (225 nm).

The analytical standard of fexofenadine hydrochloride was prepared in methanol (100 µg/ml). Calibration standards (20-2000 ng/ml) and control samples at concentrations of 40, 400 and 2000 ng/ml were prepared by diluting the stock solution. Calibration standards of fexofenadine prepared from fexofenadine-free mouse plasma were linear (r2;> 0.9994) in 20-2000 ng/ml the concentration range. The lower limit of quantification (LLOQ) of fexofenadine was 20 ng/ml. The recovery of fexofenadine from plasma samples was >91%. The variation coefficient of the precision within the day and between days was <8%. The intraday and inter-day bias of the accuracy were ±7%.

Midazolam and 1'-hydroxymidazolam Analysis

Plasma levels of midazolam and its metabolite (1'-hydroxymidazolam) were analyzed using the method previously reported (Juřica et al 2007). After 0.1 M sodium hydroxide (NaOH) was added to 100 µL plasma sample, 1 ml of ether was added, mixed by vortexing at 2000 rpm for 10 minutes, and centrifuged at 3,000 g for 5 minutes. The samples were kept at -40 °C for 35 minutes and then the ether phase was transferred to a 1.5 ml tube and evaporated under nitrogen (40°C). The residue obtained was dissolved with 100 µl mobile phase, transferred to autosampler vials, and 30 µl of its was injected into the HPLC-UV system. The sodium acetate buffer (10 mmol, pH: 4.7) and acetonitrile (60:40, v/v) were used as mobile phases. The detection was performed at 1.0 ml/min flow rate of the mobile phase and using an SPD-10A UV-VIS detector set at 220 nm.

Method validations of midazolam and its metabolite were performed similarly to fexofenadine. Calibration standards of midazolam and its metabolite (1'-hydroxymidazolam) were linear (r2;>0.9993). The LLOQ of midazolam and its metabolite was 20 ng/ml. The recovery of midazolam and its metabolite 1'-hydroxymidazolam from plasma samples was over 93% and 89%, respectively. The variation coefficient of the precision within a day and between days for midazolam and 1'-hydroxymidazolam were <%5 and <%8, respectively. The intraday and inter-day bias of the accuracy for both the theme were ±8 %.

Statistical analyses

Statistical analysis of gene expression was performed using the ΔCt values. One-way ANOVA test was performed with normalized values and then Duncan's multiple range test was used to compare means between groups. Results were considered statistically significant at $P<0.05$. Relative gene expression levels were visualised using $2^{-\Delta\Delta Ct}$ values.

The plasma concentration values of fexofenadine, midazolam, and its metabolite (1'-hydroxymidazolam)

Table 3. Plasma concentrations of 1-hydroxyimidazolam (1-HMZM) and midazolam in mice treated with different doses of dexamethasone (n=6, mean±SD)

Group	1-HMZM (ng/ml)	Midazolam (ng/ml)	1-HMZM/Midazolam
M	1141.67±397.43^{c}	371.17±80.33^{a}	3.33±1.21^{c}
LDM	1960.17±277.63^{b}	265.00±38.62^{b}	7.33±1.03^{b}
HDM	2701.67±302. 44^{a}	180.33±31.66^{c}	15.67±4.27^{a}

^{a,b,c}Different letters in the same column are statistically significant ($p<0.05$). 1-HMZM; 1-hydroxyimidazolam, M: Midazolam, LDM; Low Dose Dexamethasone+Midazolam, HDM; High Dose Dexamethasone+Midazolam

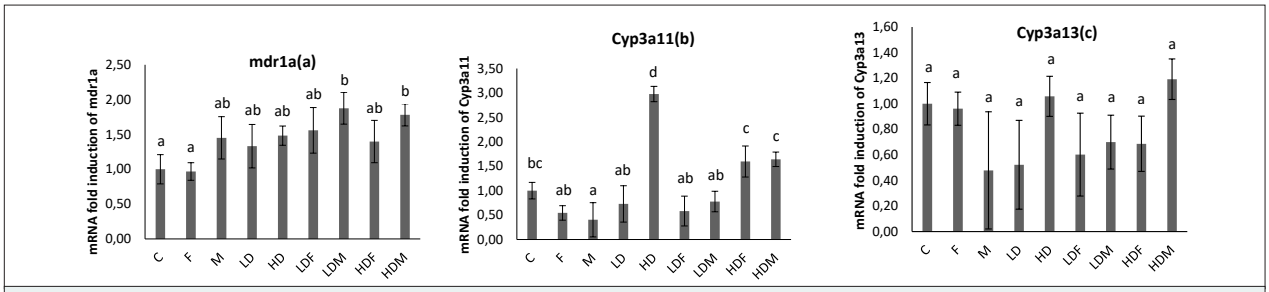

Figure 1. mRNA levels of mdr1a (a), CYP3A11 (b) and CYP3A13 (c) in small intestines of mice treated with different doses of dexamethasone (n=6, mean±SEM). [a,b,c] Different letters among groups are statistically significant (p <0.05). C: Control, F: Fexofenadine, M: Midazolam, LD: Low Dose examethasone, HD: High Dose Dexamethasone, LDF: Low Dose Dexamethasone+Fexofenadine, LDM: Low Dose Dexamethasone+Midazolam, HDF: High Dose Dexamethasone+Fexofenadine, HDM: High Dose Dexamethasone+Midazolam

were normalized and presented as mean±SD, and differences between groups were determined by the Mann-Whitney U test; statistical significance was set at P<0.05. All statistical analyses were performed using SPSS v22.0 (IBM Corp.).

Results

The mdr1a expression level in the small intestine was presented in Figure 1a. It was determined that mdr1a expression increased statistically in the small intestine in the experimental groups (LDM and HDM) compared to the control group (p<0.05). The Cyp3a11 expression was statistically increased in the small intestine only in the HD group, while it was decreased in the M group (compared to the control group) (p<0.05). In addition, fexofenadine and midazolam significantly decreased the Cyp3a11 expression enhancing effect of HD in the intestine (Figure 1b) (p<0.05). No statistically significant difference was found in the Cyp3a13 expression between groups in the small intestine (Figure 1c) (p<0.05).

In the liver, a statistically significant increase in mdr1b expression was found in all experimental groups compared to the control group (Figure 2a)(p<0.05). Compared to the control group, in the LD, HD, LDF, LDM, HDF, and HDM groups, a statistically significant increase in the Cyp3a11 expression in the liver was detected (Figure 2b) (p<0.05).In the liver, a statistically significant decrease in the Cyp3a13 expression was observed in the groups of F, LD, and HDF compared to the control group (Figure 2c) (p<0.05).

Plasma concentrations of fexofenadine in mice treated with low and high doses of dexamethasone are presented in Figure 3. The combined administration of fexofenadine with low and high doses of dexamethasone significantly decreased the plasma concentration of fexofenadine compared to alone administration of fexofenadine (p<0.05). The plasma fexofenadine concentrations in mice in the groups administered high and low doses of dexamethasone were similar.

The plasma concentrations of midazolam and its metabolite 1'-hydroxymidazolam in mice treated with low and high doses of dexamethasone are presented in Table 3. Depending on the dose, LDM and HDM administration decreased the plasma concentration of midazolam and increased the plasma concentration of 1'-hydroxymidazolam (p<0.05). Plasma 1- HMZM/MZM ratio increased significantly depending on the dexamethasone dose (p<0.05).

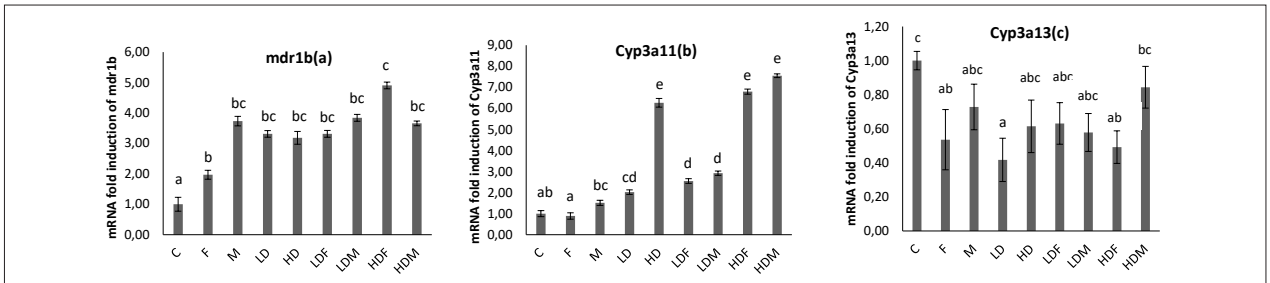

Figure 2. mRNA levels of mdr1b (a), CYP3A11 (b) and CYP3A13 (c) in liver of mice treated with different doses of dexamethasone (n=6, mean±SEM). [a,b,c]Different letters among groups are statistically significant (p <0.05). C: Control, F: Fexofenadine, M: Midazolam, LD: Low Dose Dexamethasone, HD: High Dose Dexamethasone, LDF: Low Dose Dexamethasone+Fexofenadine, LDM: Low Dose Dexamethasone+Midazolam, HDF: High Dose Dexamethasone+Fexofenadine, HDM: High Dose Dexamethasone+Midazolam

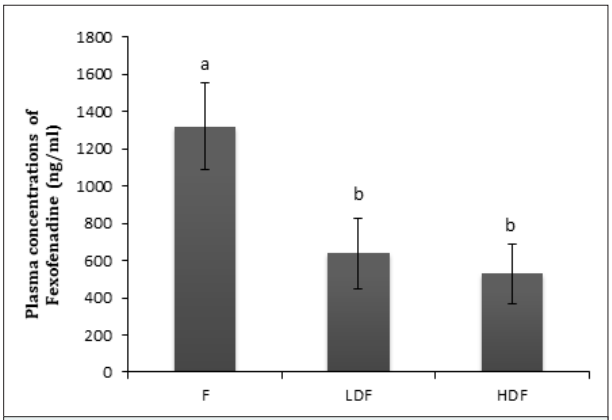

Figure 3. Plasma fexofenadine concentrations in mice treated with different doses of dexamethasone (n=6, mean±SD). a,b: Different letters are statistically significant (P <0.05). F: Fexofenadine, LDF: Low Dose Dexamethasone+Fexofenadine, HDF: High Dose Dexamethasone+Fexofenadine.

Discussion

The expressions of the Cyp3a4 enzyme and P-gp were determined by the RT-PCR method, while their functions were determined by HPLC measurement of plasma concentrations of substrate and metabolite.

We found that both doses of dexamethasone did not cause a statistically significant increase in P-gp expression in the intestine compared to the control, but in the liver. Midazolam also showed a similar effect to dexamethasone on P-gp expression in tissues. In addition, the co-administration of midazolam with both doses of dexamethasone caused a significant increase in the expression of P-gp in the intestines compared to the control. This result suggests that the concurrent use of dexamethasone with benzodiazepine drugs such as midazolam may increase the importance of pharmacokinetic-based drug interactions in multiple drug use.

CYP3A and mdr1a expressions were determined in liver and intestinal (duodenum, jejunum, and ileum) tissues in a study conducted on mice by administered cyclosporine A and different doses (1-75 mg/kg) of dexamethasone. In conclusion, similar to the results of our study, they revealed that dexamethasone in the liver induced mdr1a in a dose-dependent manner, but contrary to our results, it also induced in the small intestine. The intestines-related difference may be related to our use of only the duodenal part of the intestine. Because it is reported that dexamethasone causes the highest induction in the ileum and the least in the duodenum (Jin et al 2006).

Studies have been conducted on the effects of dexamethasone on P-gp expression in different tissues in other species besides mice. In the study conducted with rats, similar results were found in P-gp synthesis in the liver and intestine at different doses (1-73 mg/kg) to ours (Wrighton et al 1996). However, no changes in mdr1a/b expression were detected in the liver at a dose of 1-25 mg/kg. This difference may be due to the animal species used (Micuda et al 2007). It is reported that mdr1a/b expression varies according to the species, tissue difference and dosage regimen (Zong and Pollack 2003, Tanaka et al 2005, González-Lobato et al 2010, Tras et al 2017).

We found that dexamethasone increased Cyp3a11 expression in the small intestine only at high dose, while it increased it in the liver at both low and high doses. This suggests that the use of low and high doses of dexamethasone may alter the plasma levels of other concomitantly used drugs. Similar results were found with our results regarding the effect of dexamethasone on CYP3A expression in the liver and small intestine in mice (Jin et al 2006). On the other hand, it is stated that 10 mg/kg of dexamethasone does not cause any change in liver CYP3A expression in mice (Rudek et al 2014). These discrepancies (oppositeness) may be due to the dose difference used. The inducer effect of dexamethasone (50 mg/kg) on Cyp3a11/13/25 expression in the liver in mice of different ages was investigated and it was reported that it caused different degrees of expression increase in all three enzymes, regardless of age and gender (Down et al 2007). The mentioned study findings are similar to the results of our study for Cyp3a11 but differ for Cyp3a13. The differences may be due to dexamethasone's dosage regimen.

The effect of dexamethasone on the CYP3A enzyme has also been studied in other species besides mice. In a study conducted, CYP3A expression was determined in liver and intestinal tissue at different times (6, 12, 24, 36, and 48 hours) by giving dexamethasone at different doses (5, 16.6, 50, and 150 mg) to mice (Iwanaga et al 2013). In conclusion, similar to the findings of our study, the researchers reported that dexamethasone increased CYP3A induction in the liver depending on the dose, contrary to our findings, it did not increase CYP3A induction in the intestine. The intestine-related difference may arise from the difference in species and the section of the intestine studied. We used the only duodenum, while the entire intestinal tissue was used in the relevant study. Enzyme activity differs in the sections of the intestine (Li et al 2016). Additionally, no significant increase in Cyp3a13 synthesis was detected.

When the results (plasma levels of MZM and 1-HMZM in M, LDM, and HDM groups and 1-HMZM/MZM ratio) of our study regarding the function of CYP3A are evaluated, we determined that dexamethasone causes dose-dependent expression in Cyp3a11. It is reported that the administration of 1.5 mg dexamethasone daily for 4

days to humans does not alter the plasma concentration of triazolam. It is stated that this situation is due to low dose dexamethasone and may change when administered at high doses or for a long time (Villikka et al 1998).

When the results (the plasma level of fexofenadine in F, LDF, and HDF groups) of our study related-the function of P-gp are evaluated, it confirms the effect of dexamethasone on P-gp expression. The decrease in the plasma level of fexofenadine may be caused by increased P-gp expression in the liver and intestines because fexofenadine is minimally metabolized. Although we do not find an increase in the duodenum, it can be stated that there is more increase in other parts of the intestine than the duodenum. Besides, the effect of dexamethasone on the Cyp3a4 enzyme may contribute to the decrease in plasma fexofenadine level because fexofenadine is minimally metabolized by the Cyp3a4 enzyme. Similar to the results of our study, it has been stated that rifampicin, a nuclear receptor agonist, causes induction in P-gp and decreases the plasma concentration of fexofenadine by increasing the excretion of its from the intestines (Hamman et al 2001).

We determined that midazolam caused a statistically significant increase in mdr1b expression in the liver but not in the intestine. The absence of an increase in the intestine may be related to our using only the duodenum or to the concentration of the drug in the intestinal tissue because the tissue concentrations of midazolam and its metabolites can be different. In addition, benzodiazepines such as bromazepam, diazepam, and chlordiazepoxide were also reported to cause an increase in P-gp activity (Lima et al 2008).

We found that the effect of dexamethasone on Cyp3a11 expression in the intestines was reduced by fexofenadine and midazolam. The stated effect may be related to the fact that fexofenadine and midazolam reduce the entry of dexamethasone into the cell or its binding to the target point. Because there is a competitive type of relationship between P-gp substrates (Wessler et al 2013). In addition, our results revealed that fexofenadine and the combination of the low dose of dexamethasone plus midazolam caused a decrease in Cyp3a13 expression in the liver.

Conclusion

In conclusion, it can be indicated that i) the effect of dexamethasone on Cyp3a11 expression is dose-dependent, whereas its effect on P-gp is not dose-dependent, ii) The effect of dexamethasone on CYP3A changes according to its isoenzyme, iii) midazolam, a CYP3A substrate, does not cause P-gp induction in the small intestine but does cause P-gp induction in the liver, iv) fexofenadine and midazolam significantly decreased the Cyp3a11 expression enhancing effect of HD in the intestine.

Declarations

Competing Interests

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author.

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Ethical Statement

Selcuk University Experimental Medicine Application and Research Center, Animal Experiments Ethics Committee 21.02.2018, 2018-5 Number Ethics Committee Decision.

Author Contributions

Motivation/Concept: HEF, BT; Design: HEF, BT; Control/Supervision: KU, BT; Data Collection and Processing: HEF, GS, TMP; Analysis and Interpretation: HEF, KU, BT; Literature Review: TMP, ZOK, HA

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