


Effects of BCRP and P-gp Modulators on the Penetration of Aflatoxin B₁ into the Mouse Brain ^[1]

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Abstract

This study was conducted to determine whether the plasma and brain concentrations of AFB₁ are affected by the modulation of P-gp and BCRP using zosuquidar (ZQR) and prazosin (PRZ), respectively. In this study, a total of 40 healthy adult male BALB/c mice (32±3.7 g) were used. The animals were randomly divided into 5 groups, with 8 animals per group. Group 1 was used for method validation. Group 2 (AF) received intraperitoneal AFB₁ at a dose of 20 mg/kg of body weight. Groups 3 (AF+PRZ), 4 (AF+ZQR), and 5 (AF+PRZ+ZQR) received 20 mg/kg of AFB₁ intraperitoneally 30 min after the intraperitoneal administration of prazosin (0.3 mg/kg), zosuquidar (25 mg/kg), and prazosin+zosuquidar (0.3 mg/kg prazosin + 25 mg/kg zosuquidar), respectively. Six hours after the administration of AFB₁, blood and brain samples were collected from the animals in Groups 2 to 5. AFB₁ concentrations were determined using an HPLC system with fluorescence detection. Individual and simultaneous administration of prazosin and zosuquidar significantly reduced the brain concentrations of AFB₁ in comparison to a single administration of AFB₁ (P<0.05). The brain/plasma ratio of the AF group was higher than that of the other groups (AF+PRZ, AF+ZQR, and AF+PRZ+ZQR) (P<0.05). Inducers of transmembrane proteins, especially BCRP, can be life saving during acute AFB₁ poisoning.

Keywords: Aflatoxin B₁, Brain, Drug transporter proteins, Modulation, Mice

Aflatoksin B₁'in Fare Beynine Geçiş Üzerine BCRP ve P-gp Modülatörlerinin Etkisi

Özet

Çalışma, zosuquidar ve prazosin tarafından sırasıyla P-gp ve BCRP modülasyonunun AFB₁'in plazma ve beyin konsantrasyonlarının etkileyip etkilemediğini belirlemek için gerçekleştirildi. Bu çalışmada, 40 adet sağlıklı, erkek BALB/c ırkı fare (32±3.7 g) kullanıldı. Hayvanlar her grupta 8 fare olacak şekilde rastgele 5 gruba ayrıldı. Grup 1, metod validasyon çalışmalarında kullanıldı. Grup 2 (AF)'ye AFB₁ 20 mg/kg dozda intraperitoneal yolla verildi. Grup 3 (AF+PRZ), 4 (AF+ZQR) ve 5 (AF+PRZ+ZQR)'e ise sırasıyla intraperitoneal yolla prazosin (0.3 mg/kg), zosuquidar (25 mg/kg) ve prazosin+zosuquidar (0.3 mg/kg prazosin + 25 mg/kg zosuquidar) uygulamalarından 30 dk. sonra AFB₁ 20 mg/kg dozda intraperitoneal yolla uygulandı. Grup 2-5'de bulunan hayvanlardan AFB₁ uygulamasından sonraki 6. saatte kan ve beyin örnekleri alındı. AFB₁ düzeyleri floresans dedektör içeren HPLC sisteminde tayin edildi. Prazosin ve zosuquidarın tek ve eşzamanlı uygulanması AFB₁'in beyin konsantrasyonlarında sadece AFB₁ uygulamasına göre önemli oranda azalmaya neden oldu (P<0.05). AFB₁ grubunda AFB₁'in beyin/plazma oranı diğer gruplardan (AF+PRZ, AF+ZQR, and AF+PRZ+ZQR) önemli oranda daha yüksekti (P<0.05). Akut AFB₁ zehirlenmelerinde özellikle BCRP gibi transmembran proteinlerin indüklenmesi hayatta kalma oranını artırabilir.

Anahtar sözcükler: Aflatoksin B₁, Beyin, İlaç taşıyıcı proteinler, Modülasyon, Fare

INTRODUCTION

Aflatoxin B₁ (AFB₁), which is an environmental dietary carcinogen, is one of the most toxic mycotoxins and causes significant losses of livestock ^[1-3]. While the clinical

signs of acute aflatoxicosis include epistaxis, blood stained faeces, and convulsions, sudden death is observed as the clinical sign of severe acute aflatoxicosis ^[4,5]. The elimination half-life of AFB₁ was determined to be 77 min after intraperitoneal administration in mice ^[6]. There is a



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high interaction potential to occur of AFB₁ with food/feed because AFB₁ is a common food/feed contaminant. Also, the ingredients found in the food/feed compositions can cause to change the efficiency and disposition of drug and toxic substances through enzyme-and transporter [7-9].

Aflatoxin B₁ is a substrate of BCRP, and BCRP function may affect systemic exposure to this mycotoxin [10,11]. Additionally, AFB₁ and its metabolite aflatoxin B₁-epoxsit-glutathione are substrates of multidrug resistance protein 1 (MRP1), although the affinity of MRP1 for AFB₁ is low [12-14]. BCRP substrates tend to overlap with P-gp substrates [13,15], but no information is available concerning whether AFB₁ is a substrate of P-gp. Van Herwaarden et al. [10] noted that BCRP plays an important role in the renal excretion of AFB₁. If AFB₁ is a substrate of both BCRP and P-gp, the tissue penetration of AFB₁ may be changed by the modulation of these transmembrane proteins.

The blood-brain barrier (BBB) protects the brain from a variety of endogenous and exogenous substances. The BBB not only limits substance flow from the blood to the brain tissue via the paracellular and transcellular routes but also permits the efflux of substances via several transmembrane proteins, such as P-glycoprotein (P-gp) and the breast cancer resistance protein (BCRP). P-gp (ABCB1) and BCRP (ABCG2) are members of the ATP-binding cassette transporter superfamily. Both P-gp and BCRP are expressed in mammalian capillary endothelial cells at BBB sites, and these transporters work in tandem to limit the accumulation of substances in tissues [11,16-19]. P-gp and BCRP are inducible and inhibitable *in vivo* and *in vitro* by various substances. The efflux activity of these transmembrane proteins has been described as saturable [19]. Because P-gp and BCRP have a broad substrate specificity and tissue distribution, the modulation of these transmembrane proteins may result in significant alterations in the pharmacokinetics, pharmacodynamics, and toxicity of their substrates. For example, Van Herwaarden et al. [10] found that the brain concentration of [¹⁴C] 2-amino-3-methylimidazo[4,5-f]quinoline, which is a BCRP substrate, was higher in bcrp-/- mice than wild-type mice.

The aim of this study was 1) to determine the passage of AFB₁ into the brain and 2) to evaluate whether the plasma and brain concentrations of AFB₁ are affected by the modulation of P-gp and BCRP using prazosin and zosuquidar, respectively. This study is the first *in vivo* experimental study intended to evaluate the modulation of AFB₁ passage into the brain.

MATERIAL and METHODS

Chemicals and Reagents

All reagents were of recognised analytical grade. AFB₁ was obtained from Biopure Chemical Co. (Romer Labs

Inc., 1301 Stylemaster Drive Union) and dissolved in corn oil for injection. Zosuquidar hydrochloride (99%) was obtained from MedKoo Biosciences (Canada, USA) and dissolved in a solution that included glycine (15 mg) and mannitol (200 mg) in 1 mL of distilled water for injection. Prazosin hydrochloride (≥99%) was purchased from Sigma Chemical Co. (Saint Louis, MO, USA) and dissolved in 1 mL of distilled water for injection. Immunoaffinity columns (Aflatest® WB) were purchased from Vicam (Watertown, MA, USA). Monobasic potassium phosphate (KH₂PO₄), sodium phosphate dibasic (Na₂HPO₄), sodium chloride (NaCl), potassium bromide (KBr), nitric acid (4 mol/L), and HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). All water used in this study was deionised and distilled. For the high performance liquid chromatography (HPLC), water was passed through a water purification system (aqua Max-Ultra System, Younglin Instrument Co. Ltd., South Korea). Phosphate buffer solution (PBS) was prepared by dissolving 0.2 g of KCl, 0.2 g of KH₂PO₄, 1.16 g of anhydrous Na₂HPO₄, and 8 g of NaCl in 1,000 mL of water, and the pH of the solution was adjusted to 7.4 with 0.1 N NaOH. A Tween-20 (Amresco, USA) solution was prepared by adding 15 mL of Tween to 85 mL of PBS and mixing.

Experimental Design

In this study, a total of 40 healthy adult male BALB/c mice (32±3.7 g) were used. Mice were supplied from Experimental Animal Production and Research Laboratory, Faculty of Veterinary Medicine, University of Mehmet Akif Ersoy, Burdur, Turkey. All experimental administrations on animals were carried out in here. The animals were housed individually in plastic cages with grated stainless steel floors in a controlled environment (temperature 25±1°C, relative humidity 60±10%, and artificial lighting sequenced to generate a 12-h light/dark cycle). The animals had *ad libitum* access to water and a commercial diet (Optima Feeds, Kirklareli, Turkey) that included the following: 89% dry matter, 21% crude protein, 2850 kcal/kg metabolic energy, 5% crude fibre, 0.75% methionine and cysteine, 1.0-2.0% calcium, 0.5-1.0% phosphorus, and 0.5% sodium.

All animal protocols in this study were approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Mehmet Akif Ersoy (No: 2014-71).

The animals were randomly divided into 5 groups of 8 animals each. Group 1 was used for method validation without any treatment of the animals. AFB₁ was administered intraperitoneally at a dose of 20 mg/kg of body weight to mice in Group 2-5. In the present study, the dose of AFB₁ was determined by taking into consideration concerns about AFB₁ analysis, the natural poisoning state and the dose previously reported Bastaki et al. [6] because no experimental research is available concerning the passage of AFB₁ into the brain. Group 2 (AF) received intraperitoneal (IP) AFB₁ at a dose of 20 mg/

kg of body weight 30 min after the IP administration of 1 mL of the glycine/mannitol solution. Groups 3 (AF+PRZ), 4 (AF+ZQR), and 5 (AF+PRZ+ZQR) were administered IP AFB₁ at a dose of 20 mg/kg of body weight 30 min after the IP administration of prazosin (0.3 mg/kg of body weight) plus the glycine/mannitol solution (1 mL), zosuquidar (25 mg/kg of body weight), and prazosin (0.3 mg/kg of body weight) plus zosuquidar (25 mg/kg of body weight), respectively. The animals were observed for general behavioural changes, signs of toxicity, and mortality continuously for 6 h following the administration of AFB₁. Six hours after the administration of AFB₁ in Groups 2 to 5, blood samples from the hearts of animals under ether anaesthesia were collected into tubes containing heparin. All animals were euthanized using the cervical dislocation method. Plasma and brain tissues were collected from the animals and carried to Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Selcuk, Konya, Turkey for HPLC analysis. All samples were stored at -70°C until the time of analysis.

HPLC and Chromatographic Conditions

HPLC analyses were performed using an Agilent 1100 series HPLC system, which consisted of a G1379A degasser, a G1310A isocratic pump, a G1313A autosampler, a G1316A column oven, and a fluorescence detector (model G1321A, Agilent Technologies, Palo Alto, California, USA). Data acquisition was performed using the Chemstation 3D software (Agilent Technologies, Palo Alto, California, USA). For HPLC analysis, a reverse-phase ACE C18 analytical column (5 mm particle size, 4.6 x 250 mm) was employed. The column temperature was maintained at 30°C. Postcolumn derivatisation resulting in enhanced fluorescence was achieved with electrochemically-generated bromine in a Cobra cell (Coring System Diagnostics GmbH, Gernsheim, Germany), using a reaction tube that consisted of a 0.5 mm id x 34 cm length of polyether ketone tubing. The LC mobile phase solvent used with electrochemically-generated bromine was water/methanol/acetonitrile (60:20:20, v/v/v). To each litre of mobile phase, 120 mg of potassium bromide and 350 mL of 4 M nitric acid were added. The flow rate was 1 mL/min. The fluorescence detector was set to excitation and emission wavelengths of 360 and 430 nm, respectively. The injection volume for the standards and samples was 100 µL.

Standard Preparation

A stock solution was prepared by dissolving 5 mg of AFB₁ in 5 mL of methanol and stored at -70°C. The stock solution was further diluted with water/methanol/acetonitrile (60:20:20, v/v/v) to generate a series of working standard solutions. Calibration standards of AFB₁ (0.5, 1, 5, 10, 25, 50, 100, 200 ng/mL) were prepared by adding 20 µL of the working standard solution to 180 µL of plasma or 180 mg of brain tissue. Quality control samples (QC) at low (5 ng/mL), medium (25 ng/mL) and high (200 ng/

mL) concentrations were used for the method validation program. These calibration standards and QC samples were processed according to the described method of sample preparation prior to HPLC analysis.

Sample Preparation

Plasma and brain tissue samples were extracted and cleaned up according to the procedures described by Association of Official Analytical Chemist^[20] and Chiavaro et al.^[21], with some modifications. Briefly, 200 µL of plasma or 200 mg of tissue sample were blended with 200 mg of NaCl in 1.2 mL of methanol-water (80:20) using a tissue homogeniser (Heidolph Silent Crusher M, Germany) at 9.000 rpm for 1 min. The extract was filtered through Whatman No. 4 filter paper. A 1-mL filtrate sample was diluted to 7.2 mL with PBS containing 0.1% Tween-20. The final filtrate was passed through an immunoaffinity column at a flow rate of 1 drop/s. The column was then washed twice with 4 mL of ultrapure water. AFB₁ was eluted with 0.5 mL of methanol followed by 0.5 mL of water in a glass vial. Finally, 100 µL of the sample was injected into the HPLC system.

Method Validation

The specificity, linearity, sensitivity, recovery, precision, and accuracy of the employed method were determined to evaluate the performance of the analytical method. The specificity of the method was evaluated to identify interference from plasma and brain tissue. The calibration curve of AFB₁ was constructed using seven calibration standards over a calibration range of 0.5-200 ng/mL. The limits of detection (LOD) and quantification (LOQ) were determined via signal-to-noise ratio evaluations of samples spiked from 0.1-10 ng/mL. The LOD was defined at a signal-to-noise (S/N) ratio of 3:1. The LOQ was defined as the lowest quantifiable concentration of analyte with an accuracy within 20% and a precision <20%. The recovery of AFB₁ was evaluated at concentrations corresponding to the three QC values (5, 25, and 200 ng/mL), with six replicates. This parameter was determined by comparing the peak areas of the extracted QC samples with those of the working standards. The intra- and inter-day precision and accuracy were assessed by extracting and analysing five replicates of each QC sample. The intra- and inter-day precision and accuracy of the assay were determined based on the percent coefficient of variation (CV) and percent relative error (RE) values, respectively.

Statistical Analysis

All data were expressed as the mean ± SD. Data obtained from plasma and brain concentrations were analysed using one-way ANOVA, followed by the *Duncan* test. Statistical significance was assigned at P<0.05. The SPSS (Version 16.0 for Windows, Chicago, USA) statistical software was used for the statistical analyses.

RESULTS

No interfering peaks were observed in the blank plasma and brain tissue samples after the extraction. The assay was linear from 0.5 to 200 ng/mL, and the concentrations of the calibration standards were back-calculated using the peak area ratios of AFB₁. The data were analysed using linear regression analysis, and the correlation coefficients for the calibration curves prepared from plasma and brain tissue were ≥ 0.9996 and ≥ 0.9994 , respectively. The LOD values were determined to be 0.2 and 0.5 ng/mL in plasma and brain tissue, respectively. The LOQ values were determined to be 0.5 and 1 ng/mL in plasma and brain tissue, respectively. The values obtained for the recovery of AFB₁ ranged from 89.3 to 97.9% for the plasma and brain samples and from 86.4 to 94.3% for the QC samples. In the plasma and brain samples, the intra-day precision that was calculated from three QC samples that were injected six times on the same day was low, with a precision ranging from 3.94% to 10.27% for the CV and an accuracy ranging from 94.3% to 107.6%. The inter-day precision and accuracy varied from 1.54% to 12.48% and from 92.1% to 109.71%, respectively.

Table 1. The plasma and brain concentrations of aflatoxin B₁ at six hours following the intraperitoneal administration of aflatoxin B₁ (20 mg/kg) in mice (n=8)

Tablo 1. Farelerde aflatoksin B₁'in (20 mg/kg) intraperitoneal yolla uygulanmasını takiben 6. saatte alınan plazma ve beyin örneklerindeki AFB₁ konsantrasyonları (n=8)

Group	Plasma (ng/mL)	Brain (ng/g)
AF	23.45±13.84 ^{ab}	181.23±89.40 ^a
AF+PRZ	15.53±10.10 ^b	6.69±2.81 ^b
AF+ZQR	31.96±14.28 ^a	24.07±19.64 ^b
AF+PRZ+ZQR	13.37±9.50 ^b	24.85±11.66 ^b

^{ab} Different letters in the same column indicate statistically significant differences (P<0.05); AF: aflatoxin B₁; PRZ: prazosin; ZQR: zosuquidar

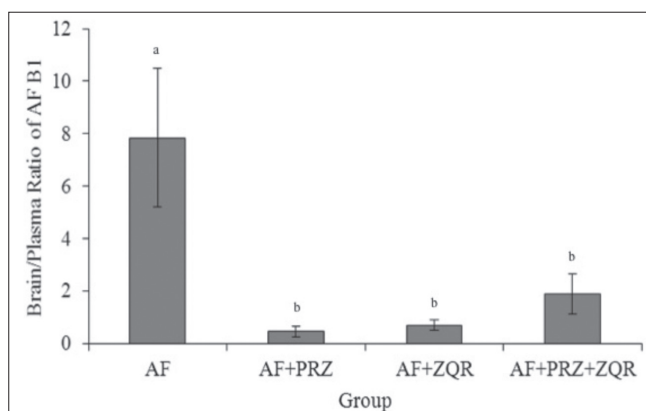


Fig 1. Ratios of brain concentrations to plasma concentrations of aflatoxin B₁. AF; aflatoxin B₁, PRZ; prazosin, ZQR; zosuquidar. ^{ab} Different letters are statistically significant (P<0.05)

Şekil 1. AFB₁'in beyin/plazma konsantrasyon oranları. AF; aflatoksin B₁, PRZ; prazosin, ZQR; zosuquidar. ^{ab} Farklı harfler istatistiksel açıdan önemlidir (P<0.05)

The effects of prazosin and zosuquidar on murine plasma and brain concentrations of AFB₁ after the IP administration of AFB₁ at a dose of 20 mg/kg of body weight are presented in Table 1. Single and simultaneous administrations of prazosin and zosuquidar significantly reduced brain concentrations of AFB₁ in comparison to a single administration of AFB₁ (P<0.05). The ratios of the brain concentrations of AFB₁ to the plasma concentrations of AFB₁ in the mice are shown in Fig 1. The brain/plasma ratio in the AF group was higher than those of the groups (AF+PRZ, AF+ZQR and AF+PRZ+ZQR) that received prazosin and zosuquidar (Fig. 1, P<0.05).

No mortality was observed in the AFB₁-treated groups during the study; however, signs of toxicity, including oedema of the lower extremities, lethargy, muscular weakness, and convulsions, were observed in the AF group.

DISCUSSION

Although AFB₁ was found at levels of 1-5 ppb in human brain biopsy samples, as reported by Hooper et al.^[22] no experimental study investigated the ability of AFB₁ to cross the BBB. However, AFB₁ was reported to be unable to pass into the rat brain^[23]. In this study, AFB₁ crossed the BBB after the administration of 20 mg/kg AFB₁, and the brain/plasma ratio of AFB₁ was 7.85 six hours after administration. Our results suggest that AFB₁ passed more intense to the brain and cannot be removed immediately. We concluded that AFB₁ accumulates in tissues, such as the brain, and cannot be removed immediately due to the greater concentration of AFB₁ in the brain than that in the plasma at the 6-hour blood collection time.

Contrary to expectation in this study, prazosin administration caused a statistically significant decline in the brain concentration of AFB₁ (P<0.05); however, the decline in the plasma concentration of AFB₁ was not statistically significant in the AF group (P>0.05). The brain/plasma ratio of AFB₁ in prazosin-treated mice was 0.45 (Fig. 1). Our results indicate that prazosin is an inducer of BCRP, although some authors reported that prazosin is a BCRP inhibitor and substrate^[24-27]. The reason for this contradiction may be differences in the materials used in the studies and in the employed doses of BCRP inhibitors and substrates. The drug affinity and the expression rate of BCRP differ among species, breeds, and organs^[28,29]. Studies of inhibitors and inducers are typically conducted *in vitro*. The majority of *in vivo* studies concerning the passage of BCRP and P-gp substrates into the brain were conducted using transgenic experimental animals (bcrp/p-gp -/-), such as mice and rats. Our study was conducted *in vivo* using wild-type mice. The type of modulation (i.e., induction and/or inhibition) and the effectiveness of a substance on transporter proteins are reported to depend on the dose, the type of tissue, the substrate examined, and the time of administration^[30,31]. Additionally, the

difficulties of predicting *in vivo* relationships based on *in vitro* transport assays were discussed by Enokizono et al.^[32]. Alternatively, the causes of the decreased AFB₁ concentration in the brain that is caused by prazosin may be associated with the roles of AFB₁ as a P-gp substrate and prazosin as a P-gp inducer. The inhibition of BCRP by prazosin may cause the activation of P-gp in the brain capillaries of mice. A compensatory relationship has been reported to exist between P-gp and BCRP, and P-gp has also been reported to be more prominent and inducible than BCRP at the BBB^[33,34]. Prazosin has been indicated to be only an inducer of P-gp^[23-27]. In addition, many BCRP and P-gp substrates and modulators overlap^[13,27,35].

In the present study similar to the results of prazosin, zosuquidar, which is a potent and selective P-gp inhibitor *in vitro*, caused a non-statistically significant increase in the plasma concentration of AFB₁ but a statistically significant decrease in the brain concentration of AFB₁ (P<0.05). Cripe et al.^[34] reported that the *in vivo* inhibition of P-gp by zosuquidar might lead to the activation of non-P-gp transmembrane proteins, such as BCRP. In addition, BCRP and P-gp presumably work in synergy or in a compensatory manner to restrict the passage of their concerted substrates into the CNS in mice, and P-gp can compensate for the deletion of BCRP, as suggested by Zhou et al.^[33]. In addition, zosuquidar can be considered to be an inducer of P-gp or a dual P-gp/BCRP inducer in brain capillaries. Zosuquidar is described as a specific P-gp inhibitor^[13,19,35], and a number of BCRP and P-gp substrates and modulators overlap^[3,27,35].

Although the co-administration of prazosin and zosuquidar reduced the level of AFB₁ in the brain, this reduction was not statistically significant in comparison to the other two treatment groups. Thus, prazosin and zosuquidar do not show the combined effect to prevent the passage of AFB₁ into the brain. This may be associated with that prazosin or zosuquidar in combined use alter the response to the modulation of other transmembrane protein. Also, it has been reported that BCRP and P-gp work in synergy or in a compensatory manner for the efflux of their substrates and the modulation of a transmembrane protein by the inducer/inhibitor alters the efflux effect of other transmembrane protein^[33,34].

The administration of prazosin, zosuquidar and prazosin+zosuquidar did not cause a statistically significant reduction of the plasma concentrations of AFB₁ in comparison to a single administration of AFB₁ (AF group). Similar to our findings, some previous studies reported that transmembrane protein modulators caused no changes in the plasma concentrations or pharmacokinetics of the substrates of transmembrane proteins but did cause important changes in tissue concentrations^[34,36,37]. Based on the results of studies in this field, we believe that this difference may occur because the transmembrane proteins

in each tissue respond differently to inducers and because drugs/substances are transported by more than one transmembrane protein. Demeule et al.^[38] found that dexamethasone, which is a P-gp inducer, increased P-gp expression in the liver and the lung but reduced the expression of this molecule in the kidney. Transmembrane proteins in each tissue respond differently to inducers, as stated by Drescher et al.^[39].

AFB₁ is activated via the conversion into AFB₁ 8-9 epoxide by cytochrome 450 enzymes, especially CYP1A2 and CYP3A4, and glutathione S-transferases are the most important enzymes for detoxifying AFB₁ in all species, including mice^[40-42]. The plasma concentration of AFB₁ in the ZQR-AF group was found to be significantly higher than those of the PRZ-AF and PRZ+ZQR-AF groups. The causes of this difference may be the inhibition of AFB₁ metabolism by zosuquidar and/or the induction of BCRP in excretion organs by prazosin. Van Herwaarden et al.^[10] posited that BCRP plays an important role in the renal excretion of AFB₁. P-gp inhibitors have also been reported to inhibit CYP3A activity, which plays an important role in AFB₁ metabolism.

In summary, both prazosin and zosuquidar significantly reduced the brain concentration of AFB₁ but not the plasma concentration of this molecule. Thus, prazosin is a better inducer than zosuquidar for both BCRP and P-gp in brain capillaries. In addition, AFB₁ may be a substrate of both BCRP and P-gp. Inducers of transmembrane proteins, such as prazosin, can be life-saving during acute poisoning with AFB₁, based on the overall health status and brain concentrations of AFB₁ in mice. The results of *in vitro* studies in this issue should be confirmed with *in vivo* studies of wild-type animals.

REFERENCES

- Dalvi RR:** An overview of aflatoxicosis of poultry its characteristics, prevention and reduction. *Vet Res Commun*, 10, 429-443, 1986. DOI: 10.1007/BF02214006
- Oguz H:** A review from experimental trials on detoxification of aflatoxin in poultry feed. *Eurasian J Vet Sci*, 27, 1-12, 2011.
- Zain ME:** Impact of mycotoxins on humans and animals. *J Saudi Chem Soc*, 15, 129-144, 2011. DOI: 10.1016/j.jscs.2010.06.006
- Bortell R, Asquith RL, Edds GT, Simpson GF:** Acute experimentally induced aflatoxicosis in the weanling pony. *Am J Vet Res*, 44, 2110-2114, 1983.
- Chao TC, Maxwell SM, Wong SY:** An outbreak of aflatoxicosis and boric acid poisoning in Malaysia: A clinicopathological study. *J Pathol*, 164, 225-233, 1991. DOI: 10.1002/path.1711640307
- Bastaki SA, Osman N, Kochiyil J, Shafiullah M, Padmanabhan R, Abdulrazzaq YM:** Toxicokinetics of aflatoxin in pregnant mice. *Int J Toxicol*, 29, 425-431, 2010. DOI: 10.1177/1091581810369565
- Bailey DG:** Fruit juice inhibition of uptake transport: A new type of food-drug interaction. *Brit J Clin Pharmacol*, 70, 645-655, 2010. DOI: 10.1111/j.1365-2125.2010.03722.x
- Abdallah HM, Al-Abd AM, El-Dine RS, El-Halawany AM:** P-glycoprotein inhibitors of natural origin as potential tumor chemosensitizers: A review. *J Adv Res*, 6, 45-62, 2015. DOI: 10.1016/j.jare.2014.11.008
- An G, Mukker JK, Derendorf H, Frye RF:** Enzyme- and transporter-mediated beverage-drug interactions: An update on fruit juices and

green tea. *J Clin Pharmacol*, 55, 1313-1331, 2015. DOI: 10.1002/jcph.563

10. Van Herwaarden AE, Wagenaar E, Karnekamp B, Merino G, Jonker JW, Schinkel AH: Breast cancer resistance protein (Bcrp/Abcg2) reduces systemic exposure of the dietary carcinogens aflatoxin B1, IQ and Trp-P-1 but also mediates their secretion into breast milk. *Carcinogenesis*, 27, 123-130, 2006. DOI: 10.1093/carcin/bgi176

11. Robey RW, To KKK, Polgar O, Dohse M, Fetsch P, Dean M, Bates SE: ABCG2: A perspective. *Adv Drug Deliver Rev*, 61, 3-13, 2009. DOI: 10.1016/j.addr.2008.11.003

12. Loe DW, Stewart RK, Massey TE, Deeley RG, Cole SP: ATP-dependent transport of aflatoxin B1 and its glutathione conjugates by the product of the multidrug resistance protein (MRP) gene. *Mol Pharmacol*, 51, 1034-1041, 1997.

13. Löscher W, Potscka H: Role of drug efflux transporters in the brain for drug disposition and treatment of brain diseases. *Prog Neurobiol*, 76, 22-76, 2005. DOI: 10.1016/j.pneurobio.2005.04.006

14. Sharom FJ: 2008: ABC multidrug transporters: Structure, function and role in chemoresistance. *Pharmacogenomics*, 9, 105-127, 2008. DOI: 10.2217/14622416.9.1.105

15. Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, Chu X, Dahlin A, Evers R, Fischer V, Hillgren KM: International transporter consortium. Membrane transporters in drug development. *Nat Rev Drug Discov*, 9, 215-236, 2010. DOI: 10.1038/nrd3028

16. Eisenblatter T, Galla HJ: A new multidrug resistance protein at the blood-brain barrier. *Biochem Biophys Res Commun*, 293, 1273-1278, 2002. DOI: 10.1016/S0006-291X(02)00376-5

17. Aszalos A: Role of ATP-binding cassette (ABC) transporters in interactions between natural products and drugs. *Curr Drug Metab*, 9, 1010-1018, 2008. DOI: 10.2174/138920008786927776

18. Zhou SF: Structure, function and regulation of P-glycoprotein and its clinical relevance in drug disposition. *Xenobiotica*, 38, 802-832, 2008. DOI: 10.1080/00498250701867889

19. Durmus S, Xu N, Sparidans IRW, Wagenaar E, Beijnenb JH, Schinkel AH: P-glycoprotein (MDR1/ABCB1) and breast cancer resistance protein (BCRP/ABCG2) restrict brain accumulation of the JAK1/2 inhibitor, CYT387. *Pharmacol Res*, 76, 9-16, 2013. DOI: 10.1016/j.phrs.2013.06.009

20. AOAC (Association of Official Analytical Chemist): Official method of analysis. Natural Toxins. Vol. 2, 17th edn., 20-22, Association of Official Analytical Chemist, Gaithersburg, MD, USA, 2000.

21. Chiavaro E, Cacchioli C, Berni E, Spotti E: Immunoaffinity clean-up and direct fluorescence measurement of aflatoxins B1 and M1 in pig liver: Comparison with high-performance liquid chromatography determination. *Food Addit Contam*, 22, 1154-1161, 2005. DOI: 10.1080/02652030500307115

22. Hooper DG, Bolton VE, Guilford FT, Straus DC: Mycotoxin detection in human samples from patients exposed to environmental molds. *Int J Mol Sci*, 10, 1465-1475, 2009. DOI: 10.3390/ijms10041465

23. Gangolli S: The dictionary of substances and their effects. In, Gangolli S (Ed): The Royal Society of Chemistry. 2nd edn., Cambridge, England, 1999.

24. Takano M, Yumoto R, Murakami T: Expression and function of efflux drug transporters in the intestine. *Pharmacol Ther*, 109, 137-161, 2006. DOI: 10.1016/j.pharmthera.2005.06.005

25. Liu Y, Liu H, Yang H, Wen T, Shang Y, Liu X, Xie L, Wang G: Impaired expression and function of breast cancer resistance protein (Bcrp) in brain cortex of streptozocin-induced diabetic rats. *Biochem Pharmacol*, 74, 1766-1772, 2007. DOI: 10.1016/j.bcp.2007.08.021

26. Juvalé K, Wiese M: 4-Substituted-2-phenylquinazolines as inhibitors of BCRP. *Bioorg Med Chem Lett*, 22, 6766-6769, 2012. DOI: 10.1016/j.bmcl.2012.08.024

27. Takara K, Yamamoto K, Matsubara M, Minegaki T, Takahashi M, Yokoyama T, Okumura K: Effects of α -adrenoceptor antagonists on ABCG2/BCRP-mediated resistance and transport. *PLoS One* 7 (2): e30697. 2012. DOI: 10.1371/journal.pone.0030697

28. Tanaka Y, Slitt AL, Leazer TM, Maher JM, Klaassen CD: Tissue distribution and hormonal regulation of the breast cancer resistance protein (Bcrp/Abcg2) in rats and mice. *Biochem Biophys Res Commun*, 326, 181-187, 2004. DOI: 10.1016/j.bbrc.2004.11.012

29. González-Lobato L, Real R, Prieto JG, Alvarez AI, Merino G: Differential inhibition of murine Bcrp1/Abcg2 and human BCRP/ABCG2 by the mycotoxin fumitremorgin C. *Eur J Pharmacol*, 644, 41-48, 2010. DOI: 10.1016/j.ejphar.2010.07.016

30. Zong J, Pollack GM: Modulation of P-glycoprotein transport activity in the mouse blood-brain barrier by rifampin. *J Pharmacol Exp Ther*, 306, 556-562, 2003. DOI: 10.1124/jpet.103.049452

31. Cetin G, Tras B, Uney K: Effects of dexamethasone and fexofenadine on the penetration into brain and testes tissues of levofloxacin, a P-gp substrate. *12th International Congress of the European Association for Veterinary Pharmacology and Toxicology (EAVPT 2012)*, July 8-12, Noordwijkerhout-Netherlands, 2012.

32. Enokizono J, Kusahara H, Ose A, Schinkel AH, Sugiyama Y: Quantitative investigation of the role of breast cancer resistance protein (Bcrp/Abcg2) in limiting brain and testis penetration of xenobiotic compounds. *Drug Metab Dispos*, 36, 995-1002, 2008. DOI: 10.1124/dmd.107.019257

33. Zhou L, Schmidt K, Nelson FR, Zelesky V, Troutman MD: The effect of breast cancer resistance protein and P-glycoprotein on the brain penetration of flavopiridol, imatinib mesylate (Gleevec), prazosin, and 2-methoxy-3-(4-(2-(5-methyl-2-phenyloxazol-4-yl)ethoxy)phenyl) propanoic acid (PF-407288) in mice. *Drug Metab Dispos*, 37, 946-955, 2009. DOI: 10.1124/dmd.108.024489

34. Cripe LD, Uno H, Paietta EM, Litzow MR, Ketterling RP, Bennett JM, Rowe JM, Lazarus HM, Luger S, Tallman MS: Zosuquidar, a novel modulator of P-glycoprotein, does not improve the outcome of older patients with newly diagnosed acute myeloid leukemia: A randomized, placebo-controlled trial of the Eastern Cooperative Oncology Group 3999. *Blood*, 116, 4077-4085, 2010. DOI: 10.1182/blood-2010-04-277269

35. Mittapalli RK, Vaidhyanathan S, Sane R, Elmquist WF: Impact of P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) on the brain distribution of a novel BRAF inhibitor: Vemurafenib (PLX4032). *J Pharmacol Exp Ther*, 342, 33-40, 2012. DOI: 10.1124/jpet.112.192195

36. De Lange EC, Marchand S, van den Berg D, van der Sandt IC, de Boer AG, Delon A, Bouquet S, Couet W: *In vitro* and *in vivo* investigations of fluoroquinolones: Effects of the P-glycoprotein efflux transporter on brain distribution of sparfloxacin. *Eur J Pharm Sci*, 12, 85-93, 2000. DOI: 10.1016/S0928-0987(00)00149-4

37. Tamai, I, Yamashita J, Kido Y, Ohnari A, Sai Y, Shima Y, Naruhashi K, Koizumi S, Tsuji A: Limited distribution of new quinolone antibacterial agents into brain caused by multiple efflux transporters at the blood-brain barrier. *J Pharmacol Exp Ther*, 295, 146-152, 2000.

38. Demeule M, Jodoin J, Beaulieu E, Brossard M, Beliveau R: Dexamethasone modulation of multidrug transporters in normal tissues. *Febs Letters*, 442, 208-214, 1999. DOI: 10.1016/S0014-5793(98)01663-9

39. Drescher S, Glaeser H, Mürdter T, Hitzl M, Eichelbaum M, Fromm MF: P-glycoprotein-mediated intestinal and biliary digoxin transport in humans. *Clin Pharmacol Ther*, 73, 223-231, 2003. DOI: 10.1067/mcp.2003.27

40. Kirby GM, Chemin I, Montesano R, Chisari FV, Lang MA, Wild VP: Induction of specific cytochrome P450s involved in aflatoxin B1 metabolism in hepatitis B virus transgenic mice. *Mol Carcinogen*, 11, 74-80, 1994. DOI: 10.1002/mc.2940110204

41. Van Vleet TR, Klein PJ, Coulombe RA: Metabolism of aflatoxin B1 by normal human bronchial epithelial cells. *J Toxicol Environ Health Part A*, 63, 525-540, 2001. DOI: 10.1080/15287390152410156

42. Yang X, Lu H, Li Z, Bian Q, Qiu L, Li Z, Liu Q, Li J, Wang X, Wang S: Cytochrome P450 2A13 mediates aflatoxin B1-induced cytotoxicity and apoptosis in human bronchial epithelial cells. *Toxicology*, 300, 138-148, 2012. DOI: 10.1016/j.tox.2012.06.010