Research Article

Transmembrane passage of Sulfamethoxazole/Trimethoprim in combination with leaves extracts of *Pseudocedrela kotschyi* and *Crossopteryx febrifuga*

Thierry Lenoir Ayoman Djadji*, Eric Joseph Balayssac, Armelle Sandrine Aka-Any Grah, Nounaferitien Awa Silue, Gbongue Eric Tia, Sylvain Landry Kouakou, Gisèle N'doua Kouakou-Siransy

Pharmaceutical and Biological Sciences, Felix Houphouet Boigny University, 01 BPV34 Abidjan

Received: 11 July 2022 Revised: 213 August 2022 Accepted: 23 August 2022

Abstract

Background: The use of herbal medicines in combination is common throughout the world and particularly in Africa. It can be the cause of many adverse effects that can lead to death. **Objective**: The aim of this study is to evaluate the absorption of the transmembrane passage of *Crossopteryx febrifuga* and *Pseudocedrela kotschyi* in association with Sulfamethoxazole. **Material and Methods**: We used the Ussing Chamber (TBC-Biomécatronics SAS, France) and HPLC DAD methods to analyse transepithelial passage of *Crossopteryx febrifuga* and *Pseudocedrela kotschyi* in association with Sulfamethoxazole in order to evaluate the herbal and drugs pharmacokinetic absorption. **Results**: Combination of the aqueous extract *Pseudocedrela kotschyi* with *Sulfamethoxazol*e in the donor chamber of the Ussing Chamber causes an increase in Sulfamethoxazole concentrations in the recipient chamber ($p<0.0001$). This concentration is 5 to 8 times higher than the concentration of Sulfamethoxazole alone. The association Sulfamethoxazole + the aqueous extract *Crossopteryx febrifuga* causes a decrease in Sulfamethoxazole concentrations. This decrease is observed from the 30th minute to the 120th minute compared to Sulfamethoxazole alone. **Conclusion**: There are interaction pharmacokinetic between *Crossopteryx febrifuga* and *Pseudocedrela kotschyi* in association with Sulfamethoxazole. These plants should be used with caution in association with Sulfamethoxazole.

Keywords*:* Ussing Chamber, Drugs-Herbal interactions, Pharmacokinetic, *Crossopteryx febrifuga, Pseudocedrela kotschyi,* Sulfamethoxazole/Trimethoprim

Introduction

The Using chamber provides a physiologically relevant system for measuring the transport of ions, nutrients, and drugs across various epithelial tissues (Clarke, 2009). It is also a system to determine the short-circuit current as an indicator of net ion transport taking place across tissue or membrane of animals (Sjöberg et al., 2013). These dispositifs are increasingly being used to measure ion transport in native tissue, like gut mucosa, and in a monolayer of cells grown on permeable supports. However, the Ussing chamber system is, to date, not often applied for the investigation of the impact of food bioactives (proteins, sugars, lipids) on health (Clarke, 2009; Lennernäs, 2007).

Address for Corresponding Author:
Thierry Lenoir Ayoman Djadji
Pharmaceutical and Biological Sciences, Felix Houphouet Boigny University, 01 BPV34 Abidjan
Email**:** djadji_thierry@yahoo.fr

The use of herbal medicines in combination is common throughout the world and particularly in Africa. It can be the cause of many adverse effects that can lead to death. For example, Warfarin (Anticoagulant) was the most common cardiovascular drug involved with herbal to interact with many others plants such as boldo, curbicin, fenugreek, garlic, danshen, devil's claw, don quai, ginkgo, papaya, lycium, mango and cause heamorrhagies (Izzo & Ernst, 2009, Izzo et al., 2005). Gum guar, St. John's wort, Siberian ginseng and wheat bran were found to decrease plasma digoxin concentration; aspirin interactions include spontaneous hyphema when associated with ginkgo and increased bioavailability if combined with tamarind. There are two main types of interactions from a pharmacological point of view: pharmacokinetic interactions and pharmacodynamic interactions.the pharmacokinetic mechanism involved in altering drug efficacy and the potential occurrence of adverse events is the mechanism involving absorption.

DOI:https://doi.org/10.31024/ajpp.2022.8.4.1

The pharmacokinetic mechanism involved in the modification of drug efficacy and the potential occurrence of adverse events is the mechanism involving absorption. One of the methods for evaluating drug penetration through the limb is an in vitro method using the Ussing Chamber. It allows to assess the amount of substance that passes through a biological membrane (Animal, Rat) (Verhoeckx et al., 2015).

The aim of this study is to evaluate the absorption of the transmembrane passage of *Crossopteryx febrifuga* and *Pseudocedrela kotschyi*

Material and methods

Reagents

Some chemicals products has been used to study the transepithelial study: Histamine, 400µg/mL; d- glucose, 20mM, Mannitol, 500µg/mL and Chlorure de potassium (KCl) saturated 3M from Sigma Aldrich, Altrim ® (Sulfamethoxazole (SMX)/ trimethoprim (TMP) suspension buvable from OLEA Pharma Cote d'Ivoire ; Ringer lactate from PharmIvoire Nouvelle (Côte d'Ivoire) , ultrapure water, acetonitrile and triethylamine HPLC grade, glacial acetic acid and methanol

Preparation of extracts and solutions

Isotonic Ringer's solution (mM) (15 NaCl, 25 $NaHCO_3$, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 2.4 K_2HPO_4 and 0.4 KH_2PO_4) containing aqueous extracts of *Pseudocedrela kotschyi*, *Crossopteryx febrifuga* and cotrimoxazole was used for assay. An intestinal preparation is mounted between two plexiglass half-chambers with a mucosal compartment and a serous compartment. These half-chambers, whose interior is applied to the exposed surface of the intestinal tissue, the Ringer's liquid buffered at pH = 7.6 maintains at 37°C and which remains permanently with the carbogen and carbon dioxide in the proportions 95% O_2 and 5% CO_2). Bubbling also ensures permanent mixing of the incubation medium in both compartments. The transepithelial electrical potential, which is the asymmetry of the electrical charges between the mucosa and the serosa of the membrane, is measured by the calomel electrodes (TBC-Biomecatronics SAS, France) by agarose bridges in a KCl (3 M). These bridges are visible on both sides of the fabric and are adapted to each half-chamber. Everything is connected to a high impedance voltmeter (TBC-Biomécatronics SAS, France).

The potential difference passes through the calomel electrodes is short-circuited by a short-circuit current (Icc) via agarose bridges containing 3M KCl and in each tank. The whole being connected to a tension-clamp system (TBC-Biomécatronics SAS, France). BiodacqSoft (TBC-Biomecatronics SAS, France). Icc reresents the sum of the ion flux transported through the epithelium in the absence of an electrochemical gradient (mainly Na^+ Cl^{-2} and HCO^-_3). Measurement of bidirectional flow through the rat gut (**Figure** 2).

Preparation of the solutions

Quantity of substances to be administered into the receiving chamber of the Ussing Chamber should not exceed 1500 µg / ml. Therefore, the cotrimoxazole concentration was prepared as follows (**Figure** 1).

Tissue preparation

The rats from which the samples were taken were fed by the FACI company's of animal food until the studies were carried out. The food was removed 18 hours before the experiments, but the animals have free access to drinking water. For studies of transmembrane passage, the animals were killed by cervical dislocation after anesthesia (Ether) according to ethical rules and good laboratory practices in force.

Fasting animal gut segments are removed and rinsed from the intestinal ashes with Ringer Lactate solution. The intestinal fragment were cut at 10 cm after the pylorus of the stomach and 10 cm before the colon. The pieces of tissue are then opened along the mesenteric border and are mounted flattened between the two half-chambers of Ussing (TBC-Biomecatronics SAS, ZI of Ruitz, France).

Drug- Herbal interaction

The different products used to measure the flow (passage through the rat intestine) are introduced at the concentration of 1 mg / ml in the donor compartment.

- A donor chamber has received 400 µL of cotrimoxazole alone

- A donor chamber has received 400 µL of cotrimoxazole and 400 µL of *Pseudocedrela .kotschyi* extract

- A donor chamber has received 400 µL of cotrimoxazole and 400 µL of *Crossoptéryx. febrifuga* extract

The samples with replacement with lactate ringer (400 µL) in the recipient chambers are then carried out at 30, 60, 90, 120 minutes and then frozen until the HPLC assay of cotrimoxazole and Trimethoprim. The interpretation of electrical parameters was not been done in this work. The operations were repeated three times and the samples obtained made it possible to average the concentrations.

Cotrimoxazole (Sulfamethoxazole et Trimethoprim) assay

Cotrimoxazole assay was done using a HPLC chain coupled to a diode array spectrophotometer (Water alliance e2695 XE). This unit is equipped with a stainless steel R6 column (25 cm x 4.6 mm) packed with base-deactivated silica gel particles, the surface of which has been modified

with chemically bonded octadecylsilyl groups (5 µm). The mobile phase used is a mixture of 1400 ml of ultrapure water, 400 ml of HPLC grade acetonitrile and 2 ml of HPLC grade triethylamine in 2000 ml volumetric flask. The mixture was then equilibrated at room temperature and adjusted with dilute glacial acetic acid (10 g / L) TS at pH 5.9, finally the mixture volumes were filtered by volume with ultrapure water. The samples were pretreated by adding 50 µl of methanol and sonicating, stirring intermittently for 5 minutes. Then allow to cool at room temperature, to make up to the volume with HPLC grade methanol, then mix and filter.

Statistical Analysis

Results are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by multiple Tukey's comparison test. A value of $p < 0.05$ was considered statistically significant.

Ethical Considerations

The experimental protocols and procedures used in the study were approved by the Institutional Animal Ethics Committee of the GMC and conformed to the Guidelines for Care and Use of Animals in Scientific Research (Indian National Science Academy, 1998, Revised 2000).

Table 1. Mean of concentration (µg/L) in SMX and TMP in the received chamber

Concentration (µg/ml)	Times (min)			
	30	60	90	120
SMX	0.14	0.35	0.60	0.90
TMP	0.04	0.06	0.09	0.15
SMX+PK	1.23	2.65	3.39	5.03
TMP+PK	0.12	0.09	0.14	0.16
SMX+CR	0.04	0.17	0.41	0.73
TMP+CR	0.09	0.07	0.12	0.12

SMX : Sulfamethoxazole ; TMP : Trimethoprim

Results

Combination of the aqueous extract *Pseudocedrela kotschyi* with Sulfamethoxazole in the donor chamber of the Ussing Chamber causes an increase in Sulfamethoxazole (SMX) concentrations in the recipient chamber ($p<0.0001$) (Table 2). The concentration of SMX in the recipient chamber increase as following at T0=0 ; T30=1.23 ; T60=2.65, T90=3.39 ; T120=5.03 (µg/L) (Table 1 and Figure 2).

The concentrations of the TMP when combined to *Pseudocedrela kotschyi* have evolved as follows at T0=0; T30=0.12; T60=0.09, T90=0.14; T120=0.14 (µg/L) (Table 1).

Combination of the aqueous extract *Crossopteryx febrifuga* with Sulfamethoxazole in the donor chamber of the Ussing Chamber causes an increase in Sulfamethoxazole (SMX) concentrations in the recipient chamber without any significative ($p>0.05$) (Table 2). The concentration of SMX in the recipient chamber stay stable as following at T0=0; T30=0.04 ; T60=0.17, T90=0.41 ; T120=0.73 (µg/L) (Table 1 and Figure 2).

The concentrations of the TMP when combined to *Crossopteryx febrifuga* have evolved as follows at T0=0; T30=0.09; T60=0.07, T90=0.12 ; T120=0.12 (µg/L) (Table 1 and Figure 2).

Discussions

This study evaluated a possible pharmacokinetic interaction at the absorption level between the plant extract (*Pseudocedrela kotschyi* and *Crossopteryx febrifuga*) of and Cotrimoxazole widely used in bacterial infections.

Pseudocedrela kotschyi

Combination of the aqueous extract of *Pseudocedrela kotschyi* with Sulfamethoxazole in the donor chamber of the using Chamber causes an increase in Sulfamethoxazole

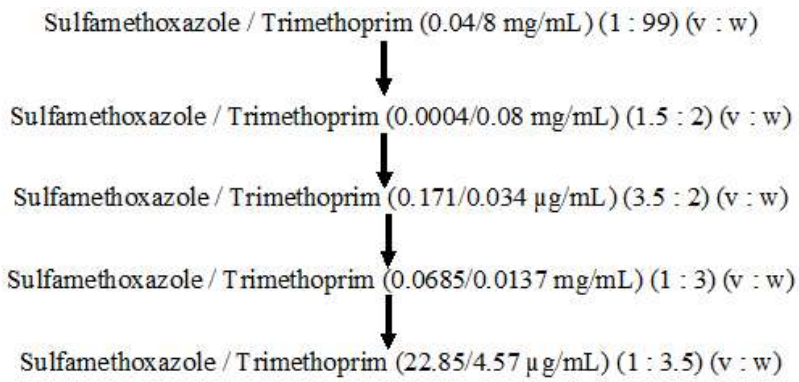

Figure 1. Diagram of the preparation of Cotrimoxazole (Sulfamethoxazole/Trimethoprim) solution to be injected into the donor chamber

Table 2. *p*- value Sulfamethoxazole/Trimethoprim transmembrane passage

Concentration (µg/mL)	Times (min)			
	30	60	90	120
SMX vs. SMX+PK	0.0001[d]	0.0001[d]	0.0001[d]	0.0001[d]
SMX vs. SMX+CR	0.21[ns]	0.0097[c]	0.38[ns]	0.97[ns]
TMP vs. TMP+PK	0.42[ns]	0.80[ns]	0.98[ns]	0.99[ns]
TMP vs. TMP+CR	0.84[ns]	0.99[ns]	0.99[ns]	0.99[ns]

*a*p<0,05; b**p<0,01; c***p<0,001; d ****p< 0, 0001; ns: no significative p: >0,05; SMX : Sulfamethoxazole ; TMP : Trimethoprim*

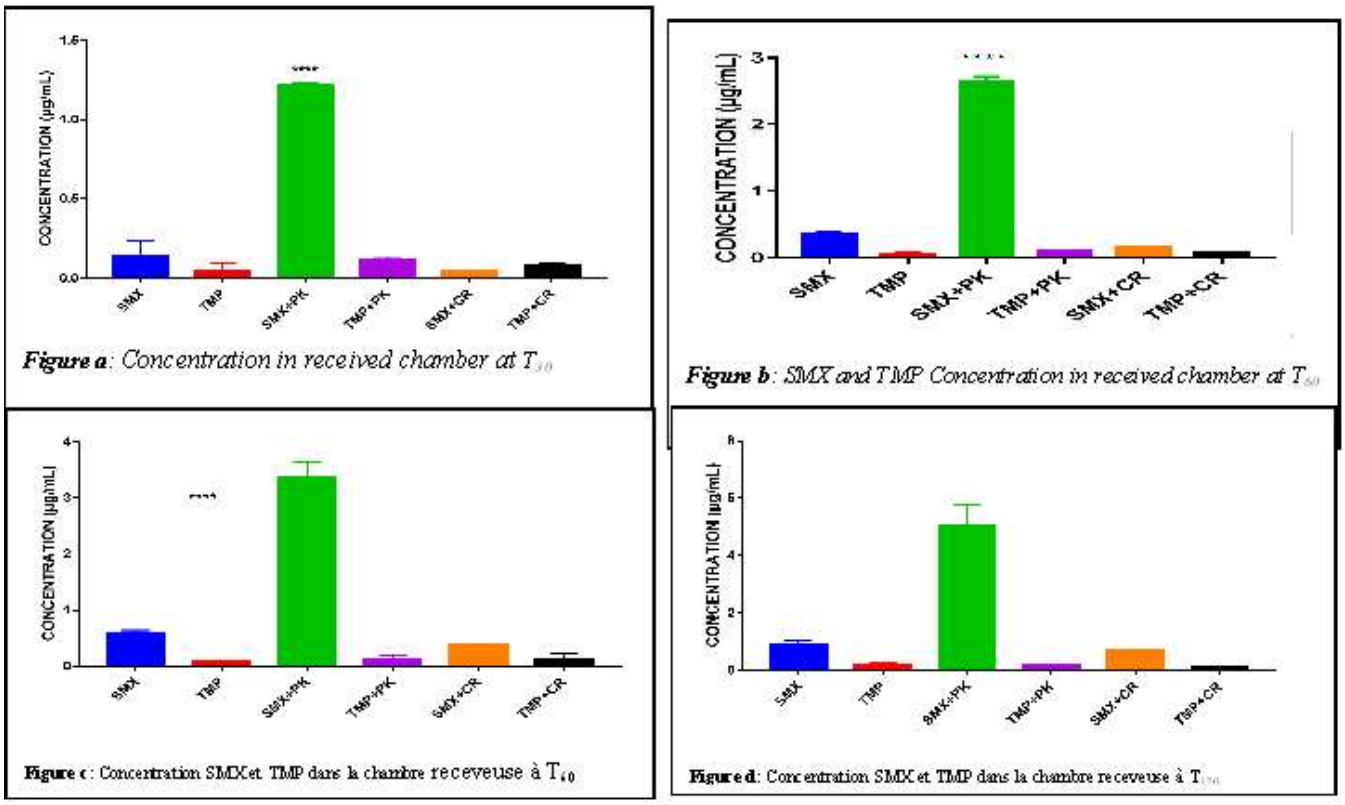

Figure 2. SMX et TMP concentration after transmembrane from T_{30} to T_{120}. SMX: Sulfamethoxazole; TMP: Trimethoprim; SMX+PK: Sulfamethoxazole+ *Pseudocedrela kotschyi;* TMP+PK: Trimethoprim+ *Pseudocedrela kotschyi;* SMX+CR: Sulfamethoxazole+ *Crossopteryx febrifuga,* TMP+CR: Trimethoprim+ *Crossopteryx febrifuga*

concentrations in the recipient chamber (p<0.0001). This concentration is 5 to 8 times higher than the concentration of Sulfamethoxazole alone i.e. not associated with the aqueous extract *Pseudocedrela kotschyi*. This increase in the Sulfamethoxazole concentration in the presence of the aqueous extract *Pseudocedrela kotschyi* is observed throughout the duration of the study, i.e. from the 30th to the 120th minute. Thus the aqueous extract *Pseudocedrela kotschyi* leads to an exaltation of the concentrations of Sulfamethoxazole. There could thus be in the aqueous extract *Pseudocedrela kotschyi* bioactive constituents capable of increasing the absorption of Sulfamethoxazole. Thus, this study suggests that one or more constituents of the extract would act by inhibition of the efflux protein (PgP) which would promote the absorption of SMX and thus increase bioavailability. Indeed, the literature reports that plants can influence Pgp. Products based on Allium sativum inhibit Pgp activity. The catechics contained in green tea and the polyphenols in grapefruit juice are powerful inhibitors of Pgp (Zhou et al., 2004).

Sulfamethoxazole is a highly metabolized molecule (85%). The literature reports that the metabolized form amounts to about 80%. Assuming that part of Sulfamethoxazole is metabolized in the intestine, it could be hypothesized that the plant extract inhibits SMX-degrading enzymes (Zhou et al., 2007).

Furthermore, since SMX diffusion results in 5 to 8 times higher concentrations than the control lot (SMX alone). There is a potential risk of reaching toxic doses when SMX is

administered concomitantly with this plant extract (Ali et al., 2011).

The use of medicinal plants is common practice in Africa (WHO), including *Pseudocedrela kotschyi*, and there is a real risk of toxicity in patients when combined treatments with traditional remedies and conventional drugs. The toxicity of SMX is manifested by hematological and lymphatic system damage; hepatobiliary and renal disorders, metabolic disorders (Hypoglycaemia); central nervous system disorders (ANSM, 2019).

Some compounds of *Pseudocedrela kotschyi* totum may be structurally similar to sulfamethoxazole and give false impressions of high serum concentrations. The literature reports cases of structural similarity between phytochemical compounds and conventional drugs. This is the case of Kyushin which is a traditional Chinese remedy containing bufalin and cinobufaginale which are cardiotonic steroids derived from toad venom (Bufo bufo gargarizans) whose chemical structures are similar to digoxin and which can react with antibodies in immunoassay reactions (Kwan et al., 1992).

The association Trimethoprim + the aqueous extract *Pseudocedrela kotschyi* discreetly increases the concentrations from the 30th to the 120th minute but this increase is not significant ($p>0.05$).

The intestinal mucous membrane constitutes a "natural barrier" limiting the entry in the general circulation. Membrane permeability, active transport, first pass effect, food interactions or solubility are all factors that influence the amount of active ingredient reaching the portal vein (Ayme-Dietrich and Verstuyft, 2011).

P-gp is an efflux transporter expressed throughout the body within "barriers" where it has a protective and excretion role. It plays an important role in the intestinal absorption of many drugs because it limits their bioavailability. Its action consists of exporting different substrates to the lumen of the intestine, using energy by ATP hydrolysis. The amount of P-gp varies by increasing from the duodenum to the ileum, in contrast to CYP3A4 (Bruyère et al., 2010). It possesses a large number of substrates, including drugs that are very different in structure (mainly hydrophobic and amphiphilic molecules, uncharged or basic). Some substrates are also inhibitors of P-gp and can therefore block its activity (Marzolini et al., 2004). The first agents discovered as inhibitors are verapamil and cyclosporin. Interactions between drugs are dependent on substrates, concentrations (Litman et al., 1997) and tissues (Choo et al., 2006).

Crossopteryx febrifuga

The association Sulfamethoxazole + the aqueous extract *Crossopteryx febrifuga* causes a decrease in Sulfamethoxazole concentrations. This decrease is observed from the 30th minute to the 120th minute compared to Sulfamethoxazole alone. However, this decrease is not significant until the 60th minute ($p<0.0001$).The combination TMP + *Crossopteryx febrifuga* aqueous extract does not influence TMP absorption ($P<0.005$).

However in term of toxicity and contrary to *Pseudocedrela kotschyi*, the antipyretic properties of *Crossopteryx febrifuga* could be used without major risks in febrile states during a bacterial infection with germs sensitive to Cotrimoxaole. However, it would be necessary to ensure through activity tests that the antipyretic effects of *Crossopteryx febrifuga* are maintained in the presence of Cotrimoxazole.

In terms of activity, the observed decrease in activity could be the cause of therapeutic failure and Cotrimoxazole resistance phenomena. It would be preferable to use *Crossopteryx febrifuga* at a distance from Cotrimoxazole. This decrease could be explained by an activation of efflux proteins such as the glycoprotein -P (Pgp) or else an activation of biotransformation enzymes at the intestinal level (Zhou et al., 2004).

Intestinal absorption of certain drugs is regulated in enterocytes by the presence of an enzyme, CYP3A4, coupled with an efflux transporter, P-glycoprotein (P-gp) (Zhou et al., 2004; Yang et al., 2014; Pal & Mitra, 2006) CYP3A4 directly metabolizes these drugs in the enterocyte, while P-gp promotes their rejection in the intestinal lumen. Because of their potent CYP3A4 inhibitory effect, certain substances (bergamottine and 6,7 dihydroxybergamottine), present in grapefruit, compete with this system, thus increasing intestinal absorption of CYP3A4 substrate drugs (Zhou et al., 2004).

Also, naringin is a potent and selective inhibitor of the enteric transporter OATP1A2, in vitro, compared to P-gp (Zhou et al., 2004; Pal and Mitra, 2006; Yang et al., 2014). Similarly, orange juice and hesperidin, its main flavonoid, inhibit in vitro the influx of fexofenadine by OATP1A2 and could be safely used clinically (Zhou et al., 2004).

Conclusion

In conclusion, there are interactions pharmacokinetic between *Crossopteryx febrifuga* and *Pseudocedrela kotschyi* in association with Sulfamethoxazole. The aqueous extracts of *Pseudocedrela kotschyi has* increased transmembranaire passage of sulfamethoxazole. The aqueous extracts of *Crossopteryx febrifuga has* decreased transmembranaire passage of sulfamethoxazole Therfore, theses plants should be used with caution in association with Sulfamethoxazole.

Acknowledgement

Thanks to the Laboratory of Galenic and Pharmacotechnical Pharmacy of the training and research unit Pharmaceutical and Biological Sciences of the Felix houphouet Boigny University for allowing us to have access to the USSING chamber

References

Ali NH, Faizi S, Kazmi SU. 2011. Antibacterial activity in spices and local medicinal plants against clinical isolates of Karachi, Pakistan. Pharmaceutical Biology 49(8): 833-839.

ANSM. (2019). base-donnees-publique.medicaments.gouv.fr/affichageDoc.php?specid=67891013&typedoc=R.

Bruyère, Declèves, Bouzom. 2010. Effect of varia-tions in the amounts of P-glycoprotein (ABCB1), BCRP (ABCG2) and CYP3A4 along the human small intestine on PBPK models for predicting intestinal first pass. Molecular Pharmaceutics, 4;7(5):1596–60.

Choo, Kurnik, Muszkat. 2006. Differential in vivo sensitivity to inhibition of P-glycoprotein located in lympho-cytes, testes, and the blood-brain barrier. Journal of Pharmacology and Experimental Therapeutics, 317:1012-8.7.

Clarke LL. 2009. A guide to Ussing chamber studies of mouse intestine. In American Journal of Physiology - Gastrointestinal and Liver Physiology 296(6):G1151-G1166.

Ayme-Dietrich E, Verstuyft C. 2011. Les transporteurs membranaires et leur rôle en thérapeutique Transporteurs impliqués dans l'absorption digestive des médicaments et interactions médicamenteuses. La Lettre Du Pharmacologue, 25(4):1–6.

Izzo AA, Ernst E. 2009. Interactions between herbal medicines and prescribed drugs: An updated systematic review. Drugs, 69(13):1777–1798.

IzzoAA, Di Carlo G, Borrelli F, ErnstE. 2005. Cardiovascular pharmacotherapy and herbal medicines: The risk of drug interaction. International Journal of Cardiology. 98(1):1-14.

Kwan, Paiusco, Kohl. 1992. Digitalis toxicity caused by toad venom. Chest, 102, 949–950.

Lennernäs H. 2007. Animal data: The contributions of the Ussing Chamber and perfusion systems to predicting human oral drug delivery in vivo. Advanced Drug Delivery Reviews. 59(11):1103-1120

Litman, Zeuthen, Skovsgaard, Stein. 1997. Competitive, non-competitive and cooperative interactions between substrates of P-glycoprotein as measured by its ATPase activity. Biochim Biophys Acta, 1361(6):169–176.

Marzolini, Paus, Buclin, Kim RB. 2004. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. Clinical Pharmacology and Therapeutic, 75(5):13–33.

Pal D, Mitra AK. 2006. MDR- and CYP3A4-mediated drug-herbal interactions. Life Sciences, 78(18):2131-2145

Sjöberg Å, Lutz M, TannergrenC, Wingolf C, Borde A, Ungell AL. 2013. Comprehensive study on regional human intestinal permeability and prediction of fraction absorbed of drugs using the Ussing chamber technique. European Journal of Pharmaceutical Sciences. 48(1-2):166-180

Verhoeckx K, Cotter P, López-Expósito I, KleivelandC, Lea T, MackieA, Requena T, Swiatecka D, Wichers H. 2015. The impact of food bioactives on health: In vitro and Ex Vivo models. In The Impact of Food Bioactives on Health: In Vitro and Ex Vivo Models. https://doi.org/10.1007/978-3-319-16104-4

Yang Y, Zhang Z, Li S, Ye X, Li X, He K. 2014. Synergy effects of herb extracts: Pharmacokinetics and pharmacodynamic basis. In Fitoterapia. 92:133-147

Zhou S, Lim LY, Chowbay B. 2004. Herbal Modulation of P-Glycoprotein. Drug Metabolism Reviews, 36(1):57–104.

Zhou, Xue Y, C L, G W. 2007. Clinically important drug interactions potentially involving mechanism-based inhibition of cytochrome P450 3A4 and the role of therapeutic drug monitoring. In Therapeutic Drug Monitoring 29:6.