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EX-VIVO PERMEABILITY STUDIES OF DIHYDROARTEMISININ-PIPERAQUINE ANTIMALARIAL IN THE PRESENCE OF FOOD COMPONENTS

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ABSTRACT

The work was aimed at assessing the interactions between dihydroartemisinin-piperaquine (DP) with food components. Food components [*i.e.*, albumin (ALB), starch (STC) and sunflower oil (SFO)] were co-loaded simultaneously with DP in excised intestinal tissues (*i.e.*, duodenum and ileum) from New Zealand white albino male rabbits. Loaded tissues were submerged in 100 mL Tyrode solution (TS) in organ bath. DP equivalent to (100 mg/mL) of PQ was similarly loaded in duodenum and ileum as controls (CRT1 and CRT2), respectively. Sampling (5mL) was performed from organ bath at timed interval post immersion. Analysis was done using Chemstation high pressure chromatographic reverse-phase (HPLC-RP) system with Zorbact XDB C8 column and mobile phase acetonitrile: 10 mM ammonium acetate (70: 30, %v/v). The wavelength of UV detection was at 220 nm and flow rate 0.7 mL/min. PQ permeation kinetics was zero order in the presence of food components. SFO treatment revealed significant reduction in C_{max} in duodenum (0.2951 versus 0.9800 µg/mL) and ileum (0.6150 versus 1.2170 µg/mL), $p<0.05$. ALB produced no significant difference in P_{eff} disappearance from lumen and P_{eff} appearance in the Tyrode solution for duodenum but higher values for ileum ($p<0.05$). There was higher rate for appearance (Ka) of PQ in organ bath for STC and SFO treatments in both intestinal segments, $p<0.05$. Similarly, the extents of permeation at 6 h (AUC_6) for the treatments were lower in duodenum than the control. ALB, SFO and STC significantly affected the rate and extent of permeation of PQ across the absorptive intestinal membrane.

KEYWORDS: Food components, Dihydroartemisinin-piperaquine, Intestinal membrane, Permeability, Antimalarial, Drug-drug interaction.

INTRODUCTION

The physiochemical properties of a drug substance, dosage form, physiological function of gastrointestinal tract along with the biochemical and physical properties of the epithetical barrier all influence the complex process of intestinal absorption.[1] The transport of molecules across intestinal membrane can be classified mechanistically into active transport, carrier-mediated or passive transport. Passive transport comprised of simple diffusion, facilitated diffusion and endocytosis or transcytosis.[2] The process of mass transfer across the partition wall is directed towards equilibrating any difference in chemical potential while internal or partial pressure gradient, constitute the driving forces.[3]

Co-administered agents may influence the mechanism and velocity of passage of drugs with respect to lag time and the time period for equilibrium to be attained.[4] Since drug administration is often linked with meal times, there may be variations in the pharmacokinetic disposition of orally administered drugs basically influenced by the quantity and type of meal ingested.[4]

Dihydroartemisinin-piperaquine (DP) is an antimalarial prescribed for multi-drug resistant *Plasmodium falciparum* malaria.[5,6] It has been reported to give variable oral absorption when taken with fatty meals.[7] Meal types, composition and volume vary widely in malaria endemic boundaries. Drug-food interactions in recent times have been of major concern because of poor clinical outcomes attendant to significant episodes.[8] It has also been noted as a source of inconvenience and non-adherence through disruptions in patients' daily schedule.[9]

The mechanisms of interactions and the consequences of different types of meals on the physiological nature of gastrointestinal medium have been reported.[10] In real life malaria conditions, meal types ingested with antimalarial may vary widely. Furthermore, the anorexic condition associated with the episode may prevent a sufferer from ingesting a particular prescribed meal. The array of possible predisposed food usually is not extensively researched into during drug development but the pharmacokinetic Food and Drug Administration

(FDA) experimental prescribed meal.[11] The molecular mechanisms of drug-food interaction have received some concern stemming from the observed grape fruit juice interactionwith warfarin.[12] It therefore becomes necessary to investigate the drug specific biopharmaceutical importance of food-drug interaction on drugs of interest at post-approval and drug marketing stages. Malaria drugs are key candidates for this investigation in the light of the widely reported therapeutic hitch and poor clinical outcomes.

Awofisayo *et al.* reported on the *in vitro* drug-food interaction with DP and concluded that there was no change in the peaks and troughs due to PQ and DHA from Fourier transform infra-red spectra characteristics of dihydroartemisinin (DHA) or PQ in DP.[14] Consequently, this study was aimed at assessing the effect of the basic food components on intestinal permeability of the PQ component in DP following oral administration *post cibos*.

EXPERIMENTAL

Materials and Chemicals

PQ and tinidazole reference standard powder were kind donations from Central Research Laboratory, University of Lagos, Nigeria. High pressure liquid chromatographic (HPLC) grade acetonitrile, ammonium acetate and methanol were products of Sigma Aldrich, Germany. Magnesium chloride, calcium chloride, sodium chloride, sodium bicarbonate, potassium chloride were products of Sigma Aldrich, Germany. Glucose (Evans PLC) and P-ALAXIN® (BVS, India) were bought from a registered pharmacy, Lagos Nigeria. Albumin, sunflower oil and soluble starch powder were purchased from a local distributor in Lagos, Nigeria.

Preparation of Standard Solution/ Working Solutions/ Physiologic solutions

Stock solution of PQ was prepared by dissolving 50 mg accurately weighed reference standard powder in 10 mL volumetric flask. Serial dilutions were made to produce graded concentrations of working solutions in the range 1-100 mg/mL. Tyrode solution (TS) was prepared according to the method of Awofisayo and co-workers.[13]

Preparation of admixture of drugs with food components

The admixture of DP suspension with the respective food components were prepared from the volume of DP suspension required for intestinal loading equivalent to PQ at 10 mg PQ content/kg body weight of rabbit and the food components at 40 mg/kg body weight.

Handling of Animals

New Zealand albino male rabbits were employed for the experiment. Animals were fed with standard pellets, free access to water and allowed to acclimatize to the environment for one week, prior to the experiment. The protocol of this study was approved by Faculty of Pharmacy, University of Uyo Ethics Committee on the Use of Laboratory Animals (UFP012). Good Laboratory Practice Guideline was observed.

Loading of Excised Tissue Segments

The excised tissues were tied at one end with silk suture and loaded with DP (100 mg/mL) at 10 mg of PQ/kg body weight and food components (STC, ALB and SFO) at 40 mg, simultaneously.

Sampling and Analysis of Samples

Sampling (5 mL) was taken from the organ bath at 0, 1, 2, 4 and 6 h post immersion of loaded tissues in organ bath. Chromatographic analyses of samples were performed by injecting 1 µL of filtered sample into the system. The HPLC system comprised of C8 Zorbact XDB (150 x 4.6 mm, 4.6 µm) column and mobile phase was acetonitrile: 10 mM ammonium acetate (70:30 %, v/v). UV detection of analyte at 220 nm and flow rate at 0.7 mL/min.

Statistical Analyses

Statistical evaluation of data was performed using statistical package for social scientists (SPSS) version 20 (IBM, USA). Paired T-test was employed to analyze for difference between treatments and control set-ups while statistical difference was taken at confidence interval of 5%.

RESULTS

The representative chromatogram for PQ analysis in HPLC is presented in Figure 1. Peaks due to PQ were shown at elution time of 4.25 min while that of the IS at 2.10 min. Peaks There were no interference with the peaks for the analyte and the IS. The concentrations of PQ versus time profile for the experimental conditions in the duodenum and ileum segments are presented in Figures 2a and 2b, respectively. The AUC measurements in Table 1 revealed information on the rate and extent of permeation under the experimental conditions. AUC_2 values revealed significantly higher permeation wit ALB in duodenum than other treatments (p=0.001, 0.003 and 0.001 for ALB, STC and SFO, respectively). SFO treatment had no significant difference compared with the control but higher than STC and ALB in the ileum (p=0.004 and 0.001, respectively). The control had higher overall (AUC_6) permeation for the analyte than the food-based treatments in ileum but ALB revealed significantly higher value in duodenum (Table 1).

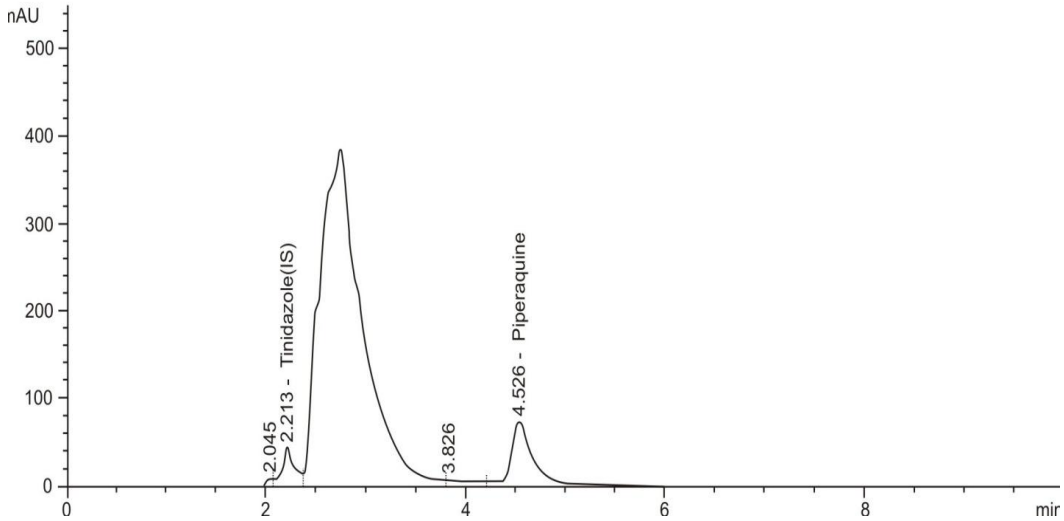

Figure 1: Representative chromatograph of sample from intestinal perfusate containing piperaquine.

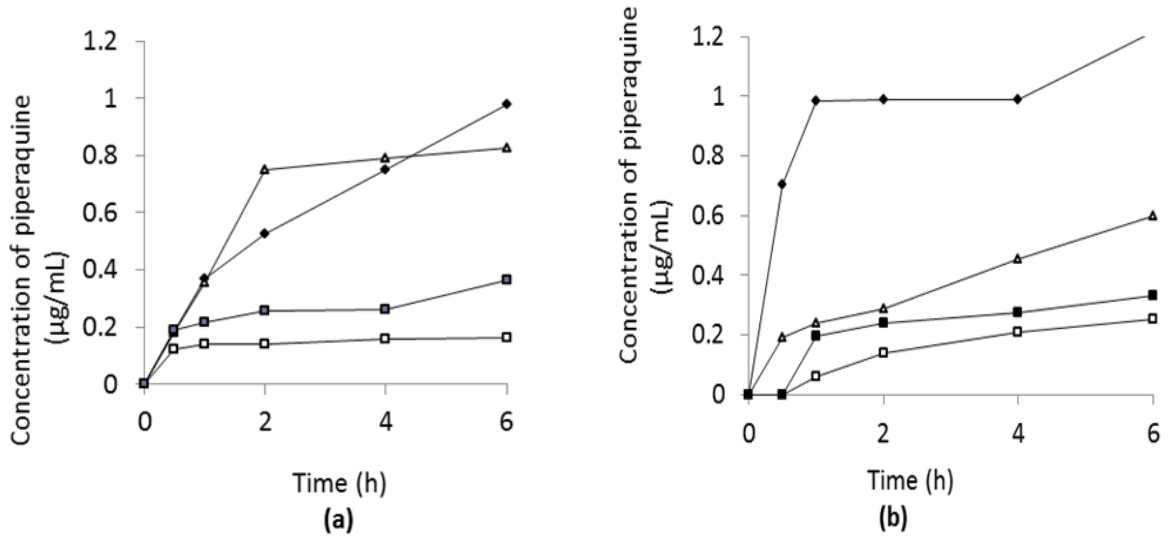

Figure 2: Piperaquine permeation across intestinal epithelia from (a) duodenum and (b) ileum, in the presence of □ sunflower oil, Δ albumin, ■ starch and ♦DP alone.

Table 1: AUC measurements for the treatments and intestinal regions.

Media condition	AUC_{0-2} ($\mu g.hmL^{-1}$)		AUC_{0-6} ($\mu g.hmL^{-1}$)	
	Duodenum	Ileum	Duodenum	Ileum
DP + ALB	0.7457±0.0153	0.4357±0.0154	4.2580±0.4843	2.3657±0.1554
DP + STC	0.2728±0.0373	0.2798±0.0113	0.7738±0.0828	1.4495±0.0630
DP +SFO	0.3877±0.0385	1.2325±0.0078	1.6710±0.1442	1.0133±0.0878
Control	0.6198±0.0083	1.5408±0.4275	3.5228±0.3638	5.6603±0.1073

NB: AUC_{0-2} and AUC_{0-6} represent areas under the curve for PQ permeation from 0-2 h and 0-6 h, respectively.

There was no significant difference in the parameter P_{eff} appearance in organ bath and P_{eff} disappearance from intestinal lumen for ALB treatment and its control values in the duodenal set up (Figure 3). However, treatments with STC and SFO revealed significantly higher P_{eff} appearance in organ bath and P_{eff} disappearance from intestinal lumen, $p<0.05$.

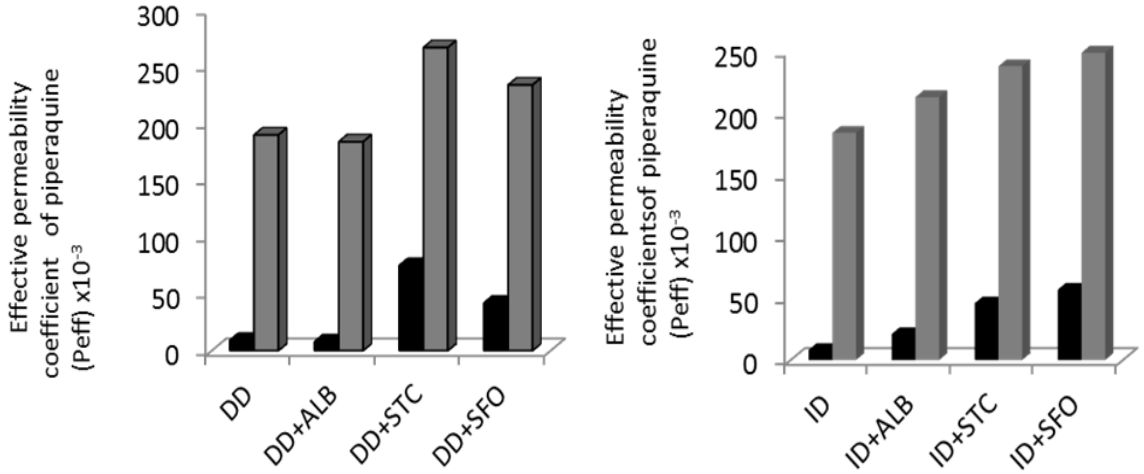

Figure 3: Effective permeability coefficients of piperaquine from ■appearance in organ bath and □disappearance from lumen in (a) duodenum and (b) ileum, in the presence of albumin (DD+ALB), starch (DD+STC) and sunflower oil (DD+SFO).

Similarly, SFO treatment produced significantly higher P_{eff} appearance and P_{eff} disappearance compared with other treatments, $p<0.05$. The rate for PQ appearance (Ka) in organ bath for treatments with STC and SFO were higher compared with the control in duodenum ($p=0.019$ and 0.012, respectively) while the same parameter had values with no significant difference for PQ disappearance. Similarly in ileum, Ka for PQ appearance values for STC and SFO were significantly higher than the control $p=0.021$ and 0.004, respectively (Table 2). In the same vein, Ka for PQ disappearance were higher than the control, with $p=0.035$ and 0.033, respectively.

Table 2: Kinetics of piperaquine permeation.

Parameters	Media conditions							
	Duodenum				Ileum			
	DP alone	DP+ALB	DP+STC	DP+ SFO	DP alone	DP+ALB	DP+STC	DP+SFO
Ka* (h^{-1})	0.0367±0.0006	0.0317±0.0032	0.3013±0.0076	0.1679±0.0031	0.0327±0.0011	0.0854±0.0021	0.0182±0.0024	0.2297±0.0011
Ka **(h^{-1})	0.7600±0.0190	0.7360±0.0165	1.0676±0.0105	0.9345±0.0539	0.0327±0.0011	0.0854±0.0021	0.1842±0.0039	0.2297±0.0024
C_{max}± SEM (µg/mL)	0.9800±0.0025	0.8270±0.0060	0.1640±0.0020	0.2951±0.0030	1.2170±0.0070	0.6000±0.5970	0.3310±0.0040	0.6150±0.0060
R^2	0.9438	0.7463	0.4438	0.6160	0.8652	0.9270	0.7434	0.8652

NB: R^2 represents the regression coefficient for PQ permeation across the absorptive membrane; Values presented are the highest R^2 values denoting the kinetics of permeation; Ka* is the rate of appearance of PQ in Tyrode solution while Ka** is rate of disappearance from intestinal lumen; C_{max} is the maximum concentration achieved in the TS.

DISCUSSION

In the recent years effect of food on the bioavailability of drugs has received particular importance necessitating food and medication time counseling for optimum therapeutic implications.[15] Since a vast number of medications are absorbed in the intestine, this work has followed other researcher's interests in this regard and considerations are highlighted on the quantitative permeation of drug molecules through the intestinal barrier.[16] This flux or permeability study therefore was designed to assess the effects of food components on the intestinal permeation of PQ from DP antimalarial drug. The major effect of diets on drugs is alteration of absorption by fatty, high protein and fiber-rich diets. The consequent bioavailability variations due to food composition have been correlated with clinical outcomes of drugs.[17] This preliminary study was designed to showcase the salient biopharmaceutical implications of DP antimalarial drug administration's effective absorption in the presence of concomitantly administered agents.

Mass transport of molecules in a solution or molecular transport across biomembrane barriers is measured in fluxes, in this experiment, net movement of PQ molecules are considered in the presence of concentration gradient as the major diffusion factor. Permeability data were therefore obtained and used to predict drug bioavailability and its consequent clinical outcomes in-vivo. Permeability coefficient, related to flux, is the transport flux of molecules through biomembranes per unit driving force per unit membrane thickness.[18]

Previous studies on transepithelial transport of drugs by Gershkovich *et al* and other researchers have concluded that the absorption of PQ from DP was enhanced when taken with fatty meal.[19,21] This study however believes that malaria drugs may be taken with varying meal types with differing proportions of food components. Therefore, the study design was to assess the different components of meals as to project at the probable outcome on bioavailability on different food component with varying proportions. The drug components of DP (*i.e*., DHA and PQ) being lipophilic in nature are expected to dissolve effectively in lipid medium and by inference the outcome in any mix of food components with which the drug may possibly be taken. This becomes very important as real life circumstances in malaria presents with anorexia in patients and differing preferences for food types.

SFO treatment in the two regions of the intestine studied agreed with the findings of the earlier researchers. The physicochemical basis for solubility includes higher degree of association of solute molecules with triglyceride-rich lipoproteins (TRL) leading to enhanced solubilizing power.[22] Since meals taken with drugs, will be composed of different food components in varying proportions, we expect an inter- and intra-individual variability with a specified dosage of medication at defined physiological and physicochemical conditions. It therefore will be necessary to determine the effects of the different meal types, so as to predict the nature of interaction of a mix of food components, obtainable in different geographical settings. Therefore, ALB, STC and SFO were adopted as basic treatments representing the classes of food (*i.e*, protein, carbohydrate and fat/oil).

This present study also reflects on the hypotheses of co-existing passive transcellular diffusion and carrier mediated mechanisms of PQ molecules in the presence of food components.[20] ALB did not cause any significant difference in PQ diffusion in duodenum in the study. ALB is a protein with high molecular weight. It does not transit biomembranes because of its inherent physicochemical limitations. However, a significant reduction in PQ permeation was observed due to ALB in the ileum. The ileum is a distal segment of the intestine with absorptive functions similar to the duodenum. Previous workers have reported on the effect of protein-rich food on drug absorption from intestinal regions.[23] Protein-rich foods cause reduced gastric emptying and will increase the absorption of acidic drugs in the stomach. The reduced gastric emptying may however lead to reduced rate and bioavailability of drugs that are susceptible to acid hydrolysis and enzymatic degradation.

STC is a polymer of glucose units that possess significant gumming or adhesive capacities with molecules in solution with which it is in contact, hence this treatment caused a significant reduction in PQ permeation across duodenal epithelium. Previous work by Awofisayo *et al.* revealed similar reduction in drug absorption of artemether-lumefantrine, a similar antimalarial of the fixed-dose combination regimen, due to co-administration with STC.[24] Similarly co-administration of STC resulted in significant reduction in PQ permeation across ileal epithelium and the same argument is proffered for the mechanism. SFO treatment caused significant increase in PQ permeation across the duodenal and intestinal epithelium, respectively considering its output with the other food components. This is in agreement with the manufacturer's recommendation that the drug be taken with fatty meal.

Where the effect of co-loading with DP in both regions of the intestine considered caused reduction sequentially, it is believed that food components will pose an interaction likely to cause treatment failure, in the additive effects.[25] The rate of drug permeation observed with food components in this work showed wide variations among the treatments. Similarly, the AUC values at 2 and 6 h revealed that food components caused significant reduction in the overall amount of drug that transited the intestinal membranes. Antimalarial drugs like other anti-infectives are meant for sufficient systemic exposure for therapeutic success.

AUC values for SFO treatment revealed higher values than STC and ALB but lower value than the control. This is as a result of the solubilisation of the poorly water-soluble PQ by a fatty milieu. This study therefore supports DP drug manufacturer's recommendation for DP's optimum absorption. Furthermore, as all the treatments presented lower AUC values than the respective control at 2 and 6 h post-loading, it could therefore be suggested that DP be taken before meals for improved absorption. The foregoing recommendation ensues as it appears from this study that food components significantly reduced the intestinal permeability of PQ following oral absorption as compared with the drug alone. The lower values from SFO treatment compared with the control were due to the sequestrating effect of lipid medium on lipophilic compounds when in excess of the solubilisation properties. [26] This study therefore recommends that DP is taken on empty stomach for predictable absorption.

CONCLUSION

This study revealed a reduction in intestinal permeability of PQ due to the presence of food components (*i.e*., ALB, STC and SFO) in the absorptive lumen of the duodenal and ileal intestinal region. This is suggestive of better bioavailability of PQ when DP was administered before food. The study also confirmed the significant increase in PQ permeation due to SFO than other food components.

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Conflicts of Interest

Authors have no financial and non-financial conflicts to declare.

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