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Hibiscus sabdariffa Improves Intestinal Barrier Function to Antibiotics than Nano capsules MIL-100(Fe) Drug Delivery Systems

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Abstract

Several studies have reported that a home-made aqueous extract of *Hibiscus sabdariffa* (HS) modified the absorption of drugs administered orally.

The present study aims to compare the capability of HS and large-pore iron (III) carboxylate MIL-100(Fe) Nanocapsules (NPs) drug delivery systems to enhance antibiotics permeations across mice intestine.

For that purpose, Oxytetracycline (OTC) was Encapsulated (NPs-OTC) in microparticles of iron III Metal Organic Frameworks (MIL100-Fe) or combined with aqueous dry extract of HS (HS-OTC), whereas Flumequine (FLM) was combined with HS (HS-FLM). Intestinal mucosa from mice were mounted in Using chambers to measure transepithelial fluxes (J_{ms}) and changes in short-circuit current (I_{sc}) elicited by these formulations.

The absorptive fluxes through intestinal epithelium were significantly increased with HS-OTC ($J_{ms} = 420 \pm 70$ ng.hr^{-1}. cm^{-2}, $p<0.001$) compared to NPs-OTC ($J_{ms} = 72 \pm 16$ ng.hr^{-1}. cm^{-2}) or control OTC ($J_{ms} = 21 \pm 5$ ng.hr^{-1}. cm^{-2}). The absorptive fluxes was also increased with HS-FLM ($J_{ms} = 376 \pm 65$ ng.hr^{-1}. cm^{-2}, $p<0.001$) compared to NPs-FLM ($J_{ms} = 45 \pm 12$ ng.hr^{-1}. cm^{-2}) and the control ($J_{ms} = 11 \pm 5$ ng.hr^{-1}. cm^{-2}).

Total transepithelial electrical conductance (G_t), and index of ionic permeability increased with addition of HS extract and remained unchanged with NPs formation.

In conclusion, our data suggests that Intestinal bioavailability of Oxytetracycline and Flumequine increased *in vitro* when it was used concomitantly with HS suggesting promising formulations for enhancing oral delivery of antibiotics.

Keywords: Antibiotics; Drug Delivery; Flu equine; *Hibiscus sabdariffa*; Intestinal Bioavailability; Intestinal Barrier Function; Ionic Conductance; Mice; Nano capsules Mil-100(Fe); Oral Bioavailability; Oxytetracycline; Pharmacological Enhancer; Using Chamber

Abbreviations

CFTR : Cystic Fibrosis Transmembrane Regulator

FLM : Flumequine

HS : *Hibiscus sabdariffa*

I.P : Intraperitoneal

IC_{50} : Concentration Yielding 50% Inhibition

I_{sc} : Short-Circuit Current

JFD : Dual Voltage Clamps TBC

MIL100-Fe : Microparticles of Iron III Metal Organic Frameworks

NPS : Carboxylate MIL-100(Fe) Nanocapsules

OTC : Oxytetracycline

PD : Potential Difference

PHZ : Phloridzin

TBC : Laboratoires TBC.

ΔI_{sc} : Difference between the peak I_{sc} after glucose challenge and the basal I_{sc}

Introduction

Flumequine (FLM) and Oxytetracycline (OTC) are antimicrobial widely used in veterinary care and they were available for veterinary medicine for the last half-century [1]. Oxytetracycline (OTC) is a commonly used antibiotic in veterinary and human medicine [2]. In veterinary medicine, OTC as well as the other major tetracycline's, are used to treat respiratory, gastrointestinal, skin, locomotive organs, and genitourinary bacterial infections, as well as systemic infections and sepsis. OTC and FLM are the cheapest antibiotics available, and this is due to modern manufacturing processes. Such conditions make it particularly attractive for its use in developing countries [1].

Although OTC and FLM are the cheapest and attractive, there are poorly absorbed from the gastrointestinal tract. Oral bioavailability of OTC is close to 12 % in broiler chickens [3] whereas that of FLM is close to 57 % [4] or lower (32%) in blunt snout bream [5]. In fishes, the bioavailability of FLM was relatively low in sea fishes 29% [6], 44% in channel catfish and in the range of 31 to 59% in cold-water fishes [7-9]. The injection represents the major route for administration of OTC and FLM. To reduce the difficulties that daily OTC and FLM injections represent to provide greater effective plasma concentrations during several days, the development of oral formulations to achieve fast and high blood levels might represent the good alternative such as including OTC and FLM in nanocapsules drug delivery systems or formulations of those antibiotics with drugs enhancers.

Several studies have reported that a homemade aqueous extract of *Hibiscus sabdariffa* (HS) modified the absorption [10] of drugs administered orally. *In vitro* studies have shown that an aqueous extract of HS can also increase the intestinal absorption of Cisplatin, Oxaliplatin and antiretroviral drugs such as Efavirenz, Ritonavir and Atanazavir [11]. In the light of these observations, we considered HS as a candidate to enhance the intestinal absorption of OTC and FLM.

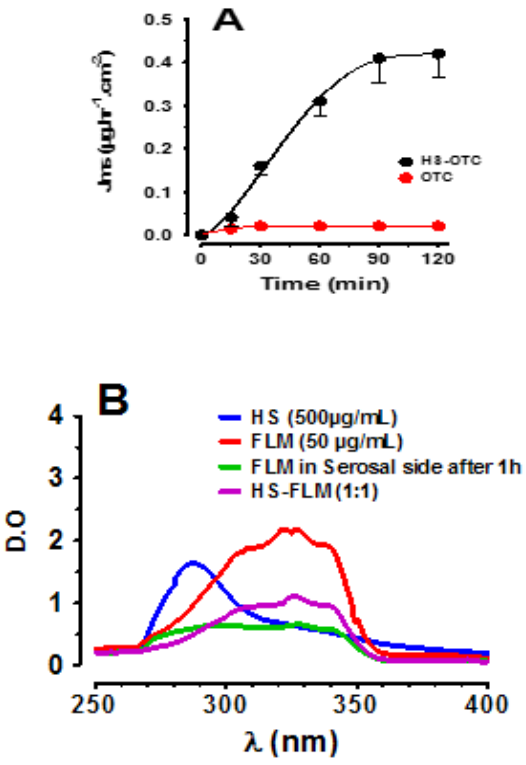
we conducted the present study in murine jejunum to compare the capability of HS and large-pore iron (III) Carboxylate MIL-100(Fe) Nanocapsules (NPs) drug delivery systems to enhance OTC and FLM permeations across the intestine using Using chambers.

Materials and Methods

Materials

Reagents and Standards

D-glucose, bumetanide hydrochloride, Benzene-1,3,5-tricarboxylic acid (Trimesic acid), Ferric Chloride Hexahydrate (Sigma, St. Quentin-Fallavier, France) are commercially available. Oxytetracycline, Flumequine (Sigma, Saint Louis, USA). Nanocapsules MIL-100(Fe), an iron(III) carboxylate with large pores were generous gifts by F. Ben ayed (Laboratory of professional metabolic biophysics and toxicology environment, faculty of medicine, Sousse, Tunisia). Aqueous dry extract of *Hibiscus sabdariffa* (HS) was supplied by TBC Laboratoires, France. Absorbance spectra of HS, and a combination HS Oxytetracycline (HS-OTC) or Flumequine (HS-FLM) were realized before the experiment (Figure 1).



Figures 1(A,B): Typical recordings of fingerprint of different UV spectra of *Hibiscus sabdariffa* (HS), Oxytetracycline (OTC), a combination HS and OTC (HS-OTC) before passage and in the serosal compartment 60 minutes after passage through murine jejunum (**A**). The same measures were repeated with Flumequine (**B**).

Preparation of combinations of antibiotics and *Hibiscus sabdariffa*

We prepared two combinations of antibiotics with *Hibiscus sabdariffa* (HS-OTC and HS-FLM). OTC or FLM in solution form was mixed with a standardized lyophilized aqueous extract of HS (Plantex, France, Reference PR 1765/LAB1388/FT940) in Ringer solution to obtain final concentrations of 1 mg antibiotics and 3 mg of HS in 1 mL.

Preparation of Nano capsules (NPs*)*

Nanocapsules MIL-100(Fe), an iron(III) carboxylate with large pores was prepared as previously described by Horcajada et al. [12]. Flumequine and oxytetracycline encapsulation was evaluated by a simple impregnation by suspending the solid beforehand dehydrated (100° while 10 hours) into methanol antibiotic solution at room temperature under bidirectional stirring for 6h (material: antibiotic ratio of 2:1, methanol antibiotic concentration: 1mg/ml) . The nanocapsules were then recovered by simple filtration and the antibiotics encapsulation has been confirmed by X-ray powder and IR spectroscopy, the amount of active ingredient adsorbed was directly determined by thermo gravimetric analysis (TGA) and by indirect dosage method by HPLC.

Permeation Study in Using Chambers

Animals

Mature male mice (C57BL/6JRj), weighing 20-25 g, were obtained from Janvier SAS (Route des Chênes, Le Genest-st-Isle, St Berthevin, France), housed in individual cages with free access to standard laboratory chow (UAR, Villemoisson s/Orge, France) and water.

The study was conducted in accordance with the principles outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health, and all efforts were made to minimize animal suffering and the number of animals used. Ethical approval was obtained from Paris Diderot University - Paris 7.

The studies were conducted in fasted animals. Food was withdrawn 18 hours before the experiments, although they had access to drinking water. The animals were killed by CO_2 inhalation, their jejunum were removed and rinsed free of intestinal content by flushing with ice-cold Ringer's solution. We checked that the animals' stomachs were empty. The jejunum were opened along the mesenteric border, and mounted as flat sheets between the two halves of acrylic Using chambers, as previously described [10,13].

Trans epithelial Electrical Conductance

The isotonic Ringer's solution used throughout the experiments contained (in mM) 115 NaCl, 25 $NaHCO_3$, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 2.4 K_2HPO_4, and 0.4 KH_2PO_4. The pH was 7.40 at 37°C when bubbled with the 95% O_2 - 5% CO_2 mixture circulating the chambers. Different solutions were used in the two bathing reservoirs on each side of the ileum defining the two compartments: mucosal (or luminal) and serosal (or blood) compartments, separated by the iliac mucosa.

The spontaneous transmural electrical Potential Difference (PD) reflecting the asymmetry of electrical charges between the luminal and serosal jejuna mucosa was measured via agar bridges containing 3 M KCl solution in 4% agar (w/v). These bridges were placed on both sides of the tissue and connected to calomel half-cells, linked to a high-impedance voltmeter. The PD was short-circuited and maintained at 0 mV throughout the experiment by a short-circuit current (I_{sc}) via two stainless steel 316L working electrodes directly placed in each reservoir as previously described [14,16], connected to a voltage-clamp system (JFD-1V, Laboratoires TBC, Paris, France). Delivered I_{sc}, corrected for fluid resistance, was recorded continuously using Biodaqsoft software (Laboratoires TBC, Paris, France). The I_{sc} (in µA/cm^2) represents the sum of the net ion fluxes transported across the epithelium

in the absence of an electrochemical gradient (mainly Na^+, Cl^-, and HCO_3^-). The transepithelial electrical conductance (G_t) was calculated according to Ohm's law. G_t, the inverse of resistance and a permeability parameter, was expressed in mS/cm^2.

Oxytetracycline and Flumequine Transepithelial Fluxes

At the steady state of electrical parameters, tissues were paired according to their conductance value (± 20%). After a stable I_{sc} had been obtained, different formulations of Oxytetracycline or Flumequine were introduced into the mucosal compartment. A 1 mL sample from the opposite compartment was withdrawn at 0, 60, 120 and 150 min and replaced by 1 mL of Ringer's solution at 37°C, for UV measurements at 327 for FLM and 374 nm for OTC using spectrophotometer UV (UVIKON 941, KONTRON Instruments). Unidirectional fluxes Hdrx permeability (J_{ms}) were determined during the steady state of transport (at 60-180 min).

Materials and Methods

In the *in vitro* study, the data are expressed as mean ± standard error (SE), n = number of tissues from at least 3 mice. The statistical analyses were performed using one-way analysis of variance (ANOVA), followed by the Dennett's multiple comparison test (GraphPad software for Windows version 5. GraphPad, San Diego, CA, USA). P<0.05 was considered as significant.

Results

Antibiotics Transepithelial Fluxes

Figure 1A shows the spectra of OTC, HS and a combination of HS and OTC (HS-OTC) in mucosal medium, before passage through the intestinal mucosa and after 120 min in serosal medium (green colour). The profile of the absorbance spectrum of OTC was changed after passage through the jejunum epithelium. In the order hand Figure 1B, shows the spectra of FLM, HS and a combination of HS and FLM (HS-FLM) in the same condition of experimentation without modification of the profile of the absorbance spectrum of FLM in the serosal medium after 60 min of passage through the jejunum epithelium. When HS-OTC or HS-FLM was introduced into the mucosal compartment, the unidirectional fluxes of OTC and FLM increased compared to the control (Figures 2,3).

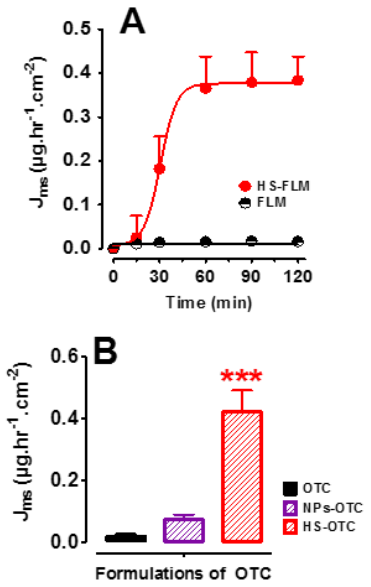


Figure 2: Time course of mucosal to serosal unidirectional fluxes **(A)** of Oxytetracycline control (OTC) and formulated with *Hibiscus sabdariffa* (HS-OTC). Different fluxes were determined after 120 min of permeation **(B)** with OTC formulated into Nanocapsules (NPs-OTC) or with HS (HS-OTC) through murine jejunum (mean ± SEM, n = 7 tissues), ***p < 0.01.

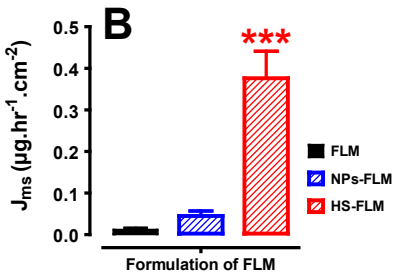


Figure 3: Time course of mucosal to serosal unidirectional fluxes **(A)** of Flumequine Control (FLM) and formulated with *Hibiscus sabdariffa* (HS-FLM). Fluxes were determined after 120 min of permeation **(B)** with FLM formulated with HS (HS-FLM) through murine jejunum (mean ± SEM, n = 7 tissues), ***p < 0.01.

The values of absorptive fluxes of OTC were HS-OTC: J_{ms} = 420 ±70 ng.hr^{-1}. cm^{-2}, p<0.001, NPs-OTC: J_{ms} = 72 ± 16 ng.hr^{-1}. cm^{-2} and control OTC: J_{ms} = 21 ± 5 ng.hr^{-1}. cm^{-2}, whereas that of FLM were HS-FLM: J_{ms} = 376 ± 65 ng.hr^{-1}. cm^{-2}, p<0.001, NPs-FLM, J_{ms} = 45 ± 12 ng.hr^{-1}. cm^{-2} and the control J_{ms} = 11 ± 5 ng.hr^{-1}. cm^{-2}.

Trans Epithelial Electrical Conductance *(Gt)*

The transepithelial electrical conductance (an index of ionic permeability) increases after addition in the preparation of HS-antibiotics combination (Figure 4).

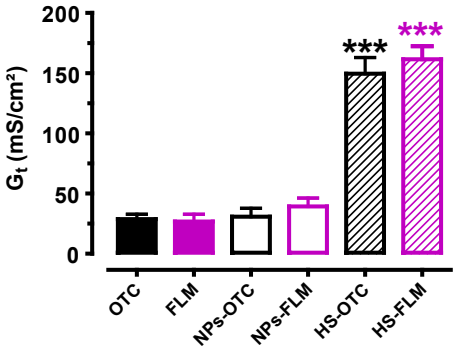


Figure 4: Transepithelial Electrical Conductance (Gt) in the presence of different formulations. This figure shows that the effect of the drug enhancer (HS) on G_t was observed is only with the formulations which contain HS such as HS-OTC and HS-FLM (mean ± SEM, n = 9 tissues), ***p < 0.01.

Control of the Intestinal Functional Viability at the end of Permeation Experiments

Physical and functional viability of tissues were usually controlled at the end of fluxes studies by measuring electro genic ions transport due to response of the tissue after stimulation. Glucose (30 mM) was added to mucosal side to induce electro genic absorption following with bumetanide (5x10^{-5} M) in the serosal side of the preparation to provoke electro genic ionic secretion inhibition (Figure 5). The variation of I_{sc} indicate physical and functional viability of the tissue at the end of permeation studies.

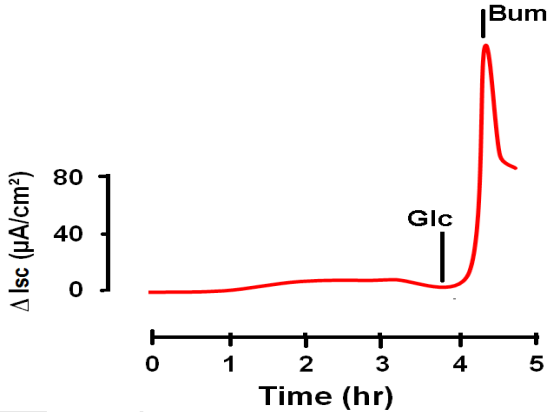


Figure 5: Typical recording of the effect of D-glucose (30 mM) and bumetanide (Bum) 5x10^{-5} M on short-circuit current at the end of the permeation studies (120 min). Glucose was added to mucosal side whereas bumetanide was added in the serosal side of the preparation. The variation of I_{sc} indicate physical and functional viability of the tissue at the end of permeation studies.

Discussion

This is the first study conducted to compare the capability of HS and large-pore iron (III) Carboxylate MIL-100(Fe) nanocapsules (NPs) drug delivery systems to enhance antibiotics permeations across the murine intestine. In this study we voluntary choose two antibiotics which are cheapest, attractive but poorly absorbed from the gastrointestinal tract [3,5]. The data of the study shows that the absorptive fluxes through intestinal epithelium were significantly increased with combination of HS-and antibiotics. In the addition the augmentation of intestinal bioavailability with utilization of HS was more important to that observed with NPs. We noted that total transepithelial electrical conductance (G_t), and index of ionic permeability increased with addition of HS extract and remained unchanged with NPs formation.

Our results are similar to that obtained with hydroxocobalamin [10], anticancer and antiretroviral drugs [11]. In all that studies augmentation in permeability was associated with an increase in transepithelial conductance, a non-specific measure of small water-soluble molecule permeability. Iinterestingly, the relationship between transepithelial electrical conductance (G_t) and antibiotics

with combination with HS. It could be postulated that in us *in vitro* experimental set-up OTC and FLM might passively be transported through the murine jejunum. Variability in small molecule passive diffusion is frequently explored by measurements of changes in transepithelial electrical conductance. This method is based on the observation of a linear relationship between conductance and the transepithelial flux of small water-soluble molecules, including sodium chloride (MW 58), xylose (MW 150), mannitol (MW 182), or polyethylene glycol (MW 2000) [17,18]. The MW of OTC (MW 460.43) and FLM (160.25) could be considered as small water-soluble molecule when compared to polyethylene glycol.

Total transepithelial electrical conductance (G_t), and index of ionic permeability increased with addition of HS extract and remained unchanged with NPs formation. In conclusion, our data suggests that Intestinal bioavailability of Oxytetracycline and Flumequine increased *in vitro* when it was used concomitantly with HS suggesting promising formulations for enhancing oral delivery of antibiotics.

A caution about the potential risk of increasing the conductance in a non-selective manner. In other word the clinical safety study may not cover all the potential risk of increasing the non-selective gut permeability in particularly case which OTC or FLM was concomitantly with others drugs. We suggest that OTC and FLM with combination with HS must be taken alone or delayed the time with others drugs consumption.

Conclusion

Our *in vitro* study suggests that the action mechanism of adding Hibiscus Subdariffa involves an increase in intestinal epithelial permeability.

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Conflict of Interest

No conflict of interest.

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