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Article

A study on enhanced intestinal permeability of clarithromycin nanoparticles

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The main objective of the present study was to determine the permeability of clarithromycin (CLA)-PLGA nanoparticles using single-pass intestinal perfusion technique in rats. Clarithromycin nanoparticles were prepared by nano-precipitation according to the modified quasi emulsion solvent diffusion technique and evaluated for their physicochemical characteristics. Permeability coefficients (P_{eff}) in anaesthetized rats were determined at 3 different concentrations. Drug solution or suspensions in PBS was perfused through a cannulated jejunal segment and samples were taken from outlet tubing at different time points up to 90 min. Microbiological assay of CLA and phenol red in the samples were analyzed using an agar well diffusion procedure and HPLC method respectively. The average particle size of prepared nanoparticles was 305 ± 134 nm. The mean P_{eff} of CLA solution in concentrations of 150, 250 and 400 µg/mL was found to be 1.20 (±0.32) $\times 10^{-3}$, 9.62 (±0.46) $\times 10^{-4}$, and 1.36 (±0.95) $\times 10^{-3}$ cm/sec, respectively. The corresponding values for the same concentration of nanoparticles were found to be 2.74 (±0.73) $\times 10^{-3}$, 2.45 (±0.88) $\times 10^{-3}$, and 3.68 (±0.46) $\times 10^{-3}$ cm/s, respectively. The two-tailed Student's t-test showed that the intestinal permeability of CLA nanoparticle suspensions in prepared concentrations were significantly increased in comparison with its solution.

Uniterms: Clarithromycin/nanoparticles/ permeability. Single-Pass Intestinal Perfusion. Intestinal permeability.

O objetivo principal do presente estudo foi determinar a permeabilidade de nanopartículas de claritromicina (CLA)-PLGA, utilizando a técnica de perfusão intestinal de passo único em ratos. As nanopartículas de claritromicina foram preparadas por nanoprecipitação, de acordo com a técnica modificada de difusão de solvente quase-emulsão, e suas características físico-químicas avaliadas. Os coeficientes de permeabilidade (P_{eff}) em ratos anestesiados foram determinados em três concentrações diferentes. A solução, ou suspensões, do fármaco em PBS foi perfundida através do segmento de jejuno canulado e as amostras foram tomadas do tubo externo em diferentes tempos até 90 minutos. Os ensaios microbiológico de CLA e de vermelho de fenol das amostras foram realizados, utilizando-se o procedimento de difusão em poço de ágar e de CLAE, respectivamente. O tamanho médio das partículas das nanopartículas preparadas foi de 305 ± 134 nm. O P_{eff} médio da solução de CLA em concentrações de 150, 250 and 400 µg/mL foi de 1.20(±0.32)$\times 10^{-3}$, 9.62(±0.46)]$\times 10^{-4}$ e de 1.36(±0.95)$\times 10^{-3}$ cm/s, respectivamente. O valor correspondente para a mesma concentração de nanopartículas foi de 2.74 (±0.73)$\times 10^{-3}$, 2.45(±0.88)$\times 10^{-3}$ e de 3.68 (±0.46)$\times 10^{-3}$ cm/s, respectivamente. O teste t de Student com duas variáveis mostrou que a permeabilidade intestinal das suspensões de nanopartículas de CLA nas concentrações preparadas foram significativamente aumentadas em comparação com sua solução.

Unitermos: Claritromicina/nanopartículas/permeabilidade. Perfusão Intestinal de Único Passo. Permeabilidade intestinal.

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INTRODUCTION

Oral administration remains the most convenient and useful route for delivering most pharmaceutical agents. However, the major problem of many orally administered drugs is to overcome several barriers before reaching their target site (Cook, Shenoy, 2003; Rao *et al.*, 2008). Several approaches have been applied in order to improve the oral bioavailability of poorly permeable and soluble compounds intended for oral administration. Using nanoparticulate drug delivery system is considered as one of these strategies (Cai *et al.*, 2010; Saha *et al.*, 2010). These formulations have been shown to be efficient approaches to enhance the transport of a large number of drugs including nucleic acids and genes across many biological membranes as well as to improve the stability of these materials. This could result in enhanced oral bioavailability of poorly bioavailable drugs due to their specialized uptake mechanisms such as circumventing the P-gp efflux and protecting incorporated drug molecules from the gastro-intestinal tract (GIT) degradation as well as gut wall and first pass metabolism (Arayne, Sultana, 2006; Florence, 2004; Moinard-Checot *et al.*, 2006). Since the intestinal epithelium is one of the main obstacles the drugs should pass through, it is both a great interest and a medical need for improving the intestinal permeability of various poorly bioavailable drugs. Intestinal permeability or the ability of a compound to move across the intestinal epithelial barrier is an important and critical determinant of its rate and extent of absorption (Zakeri-Milani *et al.*, 2009a; Zakeri-Milani *et al.*, 2007). A number of different *in vivo, in situ* and *in vitro* methods have emerged to determine the intestinal permeability of drugs and their mechanism of absorption. These include diffusion studies with intestinal segments from various species (e.g. rat and rabbit) or with cultured cell monolayers (e.g. Caco-2 cells); uptake studies in brush-border membrane vesicles prepared from intestinal segments of various species; and the single-pass intestinal perfusion (SPIP) in small mammals, most commonly rats (Artursson, Karlsson, 1991; Hillgren *et al.*, 1995; Salphati *et al.*, 2001). Among these, single-pass intestinal perfusion (SPIP) provides conditions closer to what is faced following oral administration (Cook, Shenoy, 2003; Jeong *et al.*, 2004). This technique has low sensitivity to pH variations due to the preserved microclimate above the epithelial cells, and it maintains an intact blood supply to the intestine as well as providing the unique advantages of experimental control (e.g. compound concentration and intestinal perfusion rate), and the ability to study regional differences (Song *et al.*, 2006; Wu *et al.*, 2004; Zakeri-Milani *et al.*, 2009b). Clarithromycin (CLA) 6-*O*-methylerythromycin, is a semi-synthetic macrolide antibiotic with a broad antibacterial spectrum which is used in many infectious conditions like upper and lower respiratory tracts infections, skin, ear and other soft tissues infections, caused by different bacterial groups (Alkhalidi *et al.*, 2008; Gomez-Burgaz *et al.*, 2009; Lu *et al.*, 2008). Clarithromycin is also the drug of choice to treat peptic ulcer as *H. pylori* resistance rate is much lower for clarithromycin as compared to other antibiotics like amoxicillin and tetracycline (Jain *et al.*, 2006; Zakeri-Milani *et al.*, 2005). However, like many other macrolide antibiotics, clarithromycin exhibits poor absorption and low bioavailability when administered orally (Inoue *et al.*, 2007). It is extensively metabolized by CYP3A (which is highly expressed in the gastrointestinal tract) to 14-hydroxyclarithromycin and *N*-desmethylclarithromycin and exhibits nonlinear pharmacokinetics, demonstrated by reduced clearance with increasing doses. Although these metabolites may also inactivate CYP3A, they occur at low systemic concentrations in vivo such that circulating metabolites will probably have an insignificant effect on CYP3A (Quinney *et al.*, 2010). Therefore it is desirable to design CLA-loaded biodegradable nanoparticles with improved physicochemical properties and antibacterial activity against intracellular bacteria as well as an enhanced intestinal permeability and thereafter an increased bioavailability. The objective of the present study was to determine the permeability of clarithromycin previously (Mohammadi *et al.*, 2011) prepared as PLGA nanoparticles in comparison with pure drug solution using single-pass intestinal perfusion technique in rats and to investigate whether a nanoparticulate formulation could improve intestinal permeability of the compound.

MATHERIAL AND METHODS

Material

Clarithromycin powder was obtained from Dr Reddy's Pharmaceutical Company, India. Poly (d,l-lactide-co-glycolide) (PLGA) (50:50 d,l-lactide:glycolide) with average molecular weight of 12,000 g/mol (Resomer RG 502), was purchased from Boehringer Ingelheim, Germany. Micrococcus luteus ATCC 9341 was purchased from Pasteur Institute, Iran. Poly vinyl alcohol, PVA, with molecular weight of 95000 (Acros Organics, Geel, Belgium) were used. Phenol red was purchased from Sigma Chemical Co. (St. Louis, MO). KH_2PO_4, NaH_2PO_4,

Na_2HPO_4, Orthophosphoric acid, NaOH, and NaCI were purchased from Merck (Darmstadt, Germany). All other materials were of analytical or HPLC grade and obtained from Merck (Darmstadt, Germany).

Preparation of solutions

The perfusion buffer consisted of 5.77 g/L Na_2HPO_4 (anhydrous), 4.085 g/L $NaH_2PO_4.2H_2O$, and 7 g/L NaCl. The pH of prepared buffer was adjusted to 7.2. Phenol red (0.7 mM) and metoprolol (0.07 mM) were added to the solution in all experiments as non-absorbable marker and internal standard respectively. Phenol red (0.7 mM) was added to the solution as a non-absorbable marker in all experiments. Control drug solutions were prepared in perfusion buffer (PBS) to obtain clarithromycin concentrations of 150, 250 and 400 µg/mL.

Preparation of nanoparticles

Nanoparticles were prepared by nano-precipitation according to the modified quasi emulsion solvent diffusion technique. CLA and PLGA powders (with 1:3 ratio) were co-dissolved in internal phase containing acetone, 2.5 mL, at room temperature (25 °C). The resulting organic solution was injected at the constant rate of 0.5 mL/min in aqueous phase (40 mL) containing PVA 95000 (2% w/v), as a stabilizing agent (Salem, Duzgunes, 2003). The process was carried out under homogenization for 5 min using the Silent Crusher M (Heidolph, Germany). The agitation speed was 13000 rpm in an ice-water bath. Organic phase was eliminated at room temperature under stirring for 12 hours. The final nano-suspension was centrifuged (Beckman Centrifuge, AvantiTMJ-25, USA) at 14000 rpm for 30 min and the precipitated nanoparticles were washed twice with water, using the previously described centrifugation approach, and then lyophilized using a lyophilizer (Christ Alpha 1-4; Germany). Final dry powder was taken out for further investigations. The nanoparticle suspensions were prepared to obtain concentrations of 150, 250 and 400 µg/mL (Mohammadi *et al.*, 2011).

Physicochemical evaluation of nanoparticles

Evaluation of the physicochemical properties of the prepared nanoparticles was performed using encapsulation efficiency and dissolution studies, particle size analysis, zeta potential determination, differential scanning calorimetry and Fourier-transform infrared spectroscopy. The nanoparticle production yield was determined from the mass ratio of the prepared nanoparticle to the initially added PLGA and drug (Zakeri-Milani *et al.*, 2013). The experiment was carried out using 3 samples equivalent to 5 mg drug. The mean particle-size values were measured using a laser diffraction particle-size analyzer (Sald 2101, Shimadzu, Japan) equipped with Wing software (version 1.20). Scanning electron microscopy (SEM) (LEO 440i, Leo Electron Microscopy Ltd., Cambridge, UK) at an accelerating voltage of 20 kV was used to examine the morphology of the nanoparticles. The particle surface charge was quantified as zeta potential using a Zetasizer 4 (Malvern Instr., UK). Thermograms of CLA, PLGA, nanoparticles and corresponding physical mixture of the drug with the polymer were recorded on a DSC-60 (Shimadzu, Kyoto, Japan). For drug release study, the prepared nanoparticles were placed in the vessels containing 250 mL phosphate buffer (pH 6). The vessels were incubated at 37°C with continuous orbital mixing (50 rpm). At specified time intervals, 3 mL of medium was removed using a glass syringe fitted to cellulose acetate membrane, 25 mm diameter and 20 nm pore diameter (Whatman, UK). After each sampling, an equal volume of fresh dissolution media was passed through the same filtration assembly to replace the withdrawn aliquots. The cumulative amount of the released CLA was calculated considering the replaced volume of the dissolution medium and the cumulative percentage of the released drug was plotted versus time. The mean calculated values were obtained from 3 replicates (Mohammadi *et al.*, 2011).

Single-pass intestinal perfusion experiment

Single-pass intestinal perfusion (SPIP) studies were performed according to the previously established methods (Jeong *et al.*, 2004; Salphati *et al.*, 2001; Zakeri-Milani *et al.*, 2007, 2008, 2009a). Briefly, male Wistar rats (250-300 g) were maintained on a 12 h light-dark cycle and fasted 12-18 h overnight before the experiment. Drinking water was readily accessible. After anesthesia via intra-peritoneal administration of pentobarbital (60 mg/kg), rats were placed on a heating pad to maintain body temperature at 37 °C. Upon verification of the loss of pain reflex the abdomen was opened by a 3-4 cm midline longitudinal abdominal incision and a 10 cm section of the proximal rat jejunum was located, isolated and cannulated at both ends with plastic tubing. Then the intestinal segment was gently rinsed with saline (37 °C) and attached to the perfusion assembly. Blank perfusion buffer was infused for 10 min by a syringe pump (Palmer, UK), followed by perfusion of

the clarithromycin solution or nanoparticle suspension in 3 different concentrations at a flow rate of 0.2 mL/ min for 90 min. The outlet samples were collected every 10 minutes, frozen immediately and stored at -20 °C until analysis. The length of the perfused segment was measured at the end of the experiment and the animal was euthanized with a cardiac injection of a saturated solution of KCl. In all animal studies, The Guide to the care and use of experimental animals by the Canadian council on animal care was followed.

Microbial analysis of clarithromycin in the samples

Microorganism and inoculums standardization

Micrococcus luteus ATCC 9341 was purchased in lyophilized form (Pasteur Institute, Iran) and activated in trypticase soy broth medium. Fifty microliters of the growth medium was transferred into antibiotic agar medium I (24 h before assay) and incubated at 35 °C for one day. The bacterial growth culture was diluted with a 0.9% w/v saline solution, in order to reach 30% turbidity at 580 nm, and the resultant bacterial suspension was then used as culturing inoculums.

Well diffusion assay

Microbiological assay of clarithromycin in the samples was performed using an agar well diffusion procedure (USP, 2008). Briefly, the assay plate contained 25 mL of antibiotic agar I inoculated with the bacterial inoculums. Wells of 6mm diameter were punched and filled with 100 µL of calibration samples or test samples. After 24 h of incubation at 35 °C, the diameter of the inhibition zone was measured. The method was validated by determination of the following operational characteristics: linearity, precision and accuracy (Mohammadi *et al.*, 2011). The linearity was evaluated using the linear regression analysis which was calculated by the least squares regression method. Clarithromycin calibration standards were prepared at concentrations of 500, 450, 350, 250, 150 and 50 µg/mL. Each level was made in triplicate and employed on the well diffusion assay method described above. The precision of the assay method was determined by evaluating repeatability (intra-assay) and intermediate precision (inter-assay), and expressed as the relative standard deviation (RSD) of four quality control samples. The accuracy was determined by adding known amounts of clarithromycin reference substance (quality control samples) at the beginning of the process, followed by calculation of the value: measured value/nominal value × 100.

Liquid chromatographic conditions for analysis of phenol red

For analysis of phenol red in the samples, Shimadzu HPLC system (Shimadzu, Kyoto, Japan) comprising an LC-10ADvp pump and a variable wavelength ultraviolet spectrophotometric detector (SPD-10Avp) set at 430 nm was used.

The phenol red concentrations in the perfusion buffer were determined using a previously developed HPLC method in which the mobile phase consisting of a mixture of 55% methanol and 45% of 0.05 mol/L KH_2P0_4 aqueous solution (adjusted to pH 2.6) was pumped into a Shimpack VP-ODS 5 µm 4.6 × 250 mm with a Shimpack VP-ODS 5 µm 4.6 ×50 mm guard column at a flow rate of 1 mL/min. Under these conditions the phenol red retention time was found to be 3 min (Swenson *et al.*, 1994; Valizadeh *et al.*, 2006; Zakeri-Milani *et al.*, 2005). Class VP software was used for data acquisition and processing.

Data Analysis

Effective permeability coefficients (P_{eff}) were calculated after correcting the steady-state outlet concentrations for water flux based on the ratio of inlet and outlet concentrations of an un-absorbable marker, phenol red. The SPIP technique performed in the present study for estimating intestinal permeability coefficient uses a mass balance approach. Two models namely Well-stirred model (i.e. mixing tank model) and Parallel tube model (i.e. complete radial mixing model) are used to explain this approach. Steady state was reached about 40 min after the beginning of the perfusion and was confirmed by plotting the ratio of the outlet to inlet concentrations versus time. The representative results for CLA 250 µg/mL in perfusion solution are plotted in Figure 1. Assuming the parallel tube model, the P_{eff} values were calculated using the following equation:

$$P_{eff} = -Q_{in} [C_{out}/C_{in}]/2\pi rl$$

where Q is the perfusion buffer flow rate (0.2 mL/ min), C_{out}/C_{in} is the ratio of the outlet concentration and inlet concentration of clarithromycin that has been adjusted for water transport during the perfusion, r is the radius of the intestinal segment (0.18 cm) and l is the length of the intestinal segment. Values were indicated as mean ± SD for permeability in four independent rats. Statistical difference between the permeabilities of different concentrations of clarithromycin in the form of solution and nanoparticle

TABLE I - Loading efficiency of different CLA concentrations loaded in PLGA nanoparticles

Initially applied drug concentration (µg/mL)	diameter of the inhibition zone (mm)	Measured drug concentration (µg/mL)	Loading efficiency (%)
400	24.76	287.1	71.78%
250	23.85	247.3	98.92%
150	21.58	148.2	98.80%

suspension was evaluated by two-tailed Student's t-test. P-values<0.05 were considered significant.

RESULTS AND DISCUSSION

Micrococcus luteus was selected for performing the antibacterial assay as it is a non-pathogenic bacterium and has good sensitivity to clarithromycin (Mohammadi *et al.*, 2011). The six point linearity curves for clarithromycin were constructed in the range of 50-500 µg/mL. These concentration ranges were selected based on the drug concentrations used in the permeability studies. The regression analysis of the obtained responses generated a linear curve with a correlation coefficient of 0.992. Nanoparticles with efficient loading of clarithromycin and submicron size range were obtained. Loading efficiencies of the obtained particles were calculated using the equation shown below:

Loading efficiency (%) = (actual drug content in nanoparticles/theoretical drug content) × 100.

Loading efficiencies for three different concentrations of drug (400, 250 and 150 µg/mL) were between 71.78% - 98.8% (Table I). Poor entrapment of drug in the applied concentration of 400 µg/mL could be due to the low aqueous solubility of both polymer and CLA (practically insoluble) in water. Higher loading efficiency was obtained with decreasing the initially applied amount of the drug.

According to the results of particle size analysis and SEM studies (Figure 1), the obtained nanoparticles were between 200 to 500 nm in size with narrow size distribution, and spherical shape. The mean particle size was found to be 305 ± 134 nm.

Zeta potential of the nanoparticles, intact polymer and CLA were -20.32 ± 2.84 and 1.47 ± 1.61, and -14.26 ± 1.92 mV, respectively. Intact CLA powder had an endothermic peak corresponding to its melting point at 231.34 °C, there was no distinct CLA melting endotherm in the nanoparticle, suggesting a complete amorphization of the drug in the prepared nanoparticles (Figure 2).

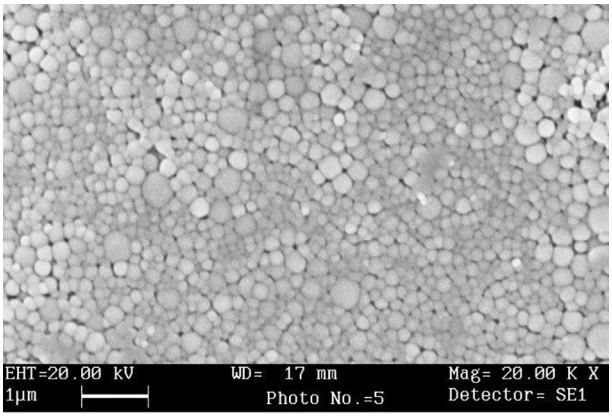

FIGURE 1 - SEM image of clarithromycin loaded nanoparticles of 1:3 drug to polymer ratio.

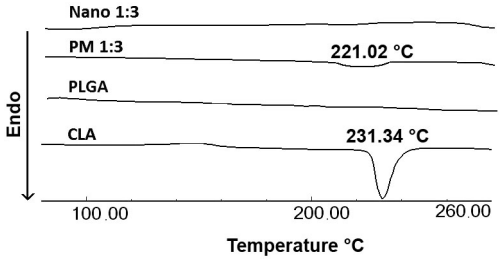

FIGURE 2 - DSC curves of the intact clarithromycin (CLA), PLGA, their physical mixture (PM) and clarithromycin loaded nanoparticles (NANO) of 1:3 drug to polymer ratio.

The time required for 50% of drug dissolved ($t_{50\%}$) which is inversely related to the dissolution rate are 30 min for CLA and 180 min for nanoparticles. The presence of insoluble polymer in the nanoparticles matrix body reduces the water penetration, hence dissolution and diffusion. A very slow release pattern for nanoparticles was seen after an initial burst within 4 h. 59% of drug was released during the first 4 hours from nanoparticles. This may be attributed to the dissolution of the drug that is poorly entrapped in the polymer matrix, while the slower and continuous release may be ascribed to the diffusion of the drug localized in the PLGA core of the nanoparticles. In SPIP experiments steady state was reached about 40 min after the beginning of the perfusion and was confirmed

by plotting the ratio of the outlet to inlet concentrations versus time. The representative results for CLA 250 µg/mL in perfusion solution are plotted in Figure 3.

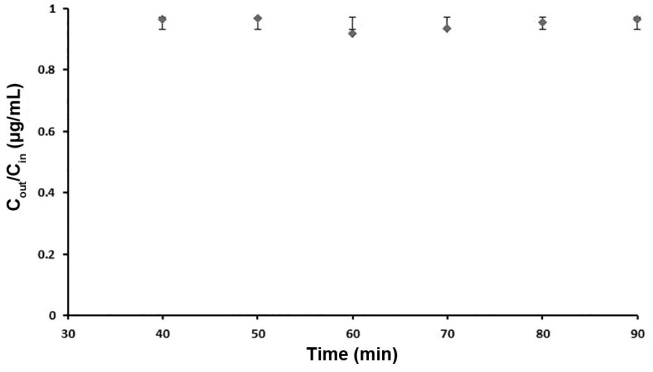

FIGURE 3- Plot of concentration ratio of the inlet and outlet tubing (C_{in}/C_{out}) vs. time for CLA (250 µg/mL) perfusion study (n=4, error bars represent SD).

The intestinal permeability of clarithromycin in three different concentrations was determined in rat jejunum using the *in situ* single-pass perfusion technique. The samples were analyzed by the above mentioned method. Prior to the *in situ* study, stability assessment was performed to evaluate the stability of the test drug in blank perfusion samples by simulating the same conditions which occurred during the study. The results showed that drug was sufficiently stable throughout the duration of experiment (120 min) in all investigated solutions. The mean effective permeability coefficients of clarithromycin solution in concentrations of 150, 250 and 400 µg/mL in perfusion solution were found to be $1.20(\pm0.32)\times10^{-3}$ cm/s, $9.62(\pm0.46)\times10^{-4}$ cm/s, and $1.36(\pm0.946)\times10^{-3}$ cm/s respectively. The corresponding values at the same concentration of nanoparticles were found to be $2.74(\pm073)\times10^{-3}$ cm/s, $2.45(\pm0.877)\times10^{-3}$ cm/s, and $3.68(\pm0.46)\times10^{-3}$ cm/s respectively. The effective permeability of clarithromycin from nanoparticle suspension was 2.28 fold enhanced in comparison to the solution form at the concentration of 150 µg/mL whereas these enhancement ratios were 2.54 and 2.70 in the case of 250 and 400 µg/mL samples, respectively. The obtained data suggests a slight increase in the enhancement ratio by increasing the concentration of drug within nanoparticles. As it is shown in Figure 4, the two-tailed Student's t-test showed that the intestinal permeability of clarithromycin was significantly increased in the nanoparticle suspension form in all four clarithromycin concentrations used ($P < 0.05$).

This finding is in agreement with the previously published literature about the intestinal uptake of nano

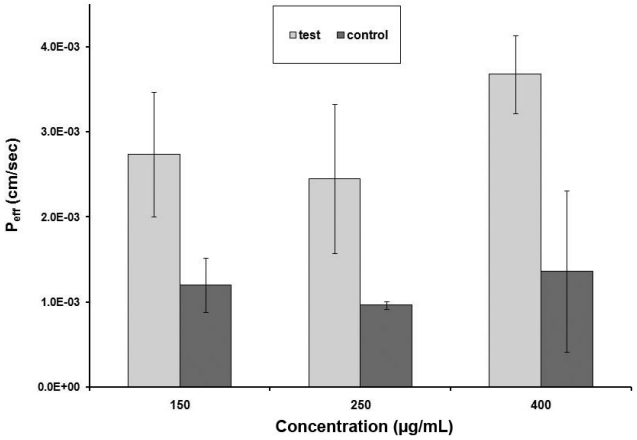

FIGURE 4 - Intestinal permeability of clarithromycin in control and test samples at three concentration levels. Values are the mean ± SD of four determinations.

sized particles. For instance in one study the results of in vitro permeation experiments by Ussing chambers has shown that the permeability of tacrolimus was enhanced significantly in the form of nanoparticles in comparison with its solution in both healthy and inflamed tissues (Lamprecht *et al.*, 2005). In another study, PLGA nanoparticles loaded with curcumin was designed and prepared and the comparison of *in situ* intestinal permeability and also the bioavailability of these nanoparticles with that of native curcumin showed that encapsulating the drug in PLGA polymers could enhance its intestinal permeability as well as its in vivo bioavailability (Xie *et al.*, 2011). In a similar study, the cyclosporine nanoparticles showed significantly higher intestinal uptake of around 90% as compared to Sandimmune Neoral®, which showed around 55% and Cyclosporine sodium CMC suspension with an uptake of only 10% (Italia *et al.*, 2007).

It has been demonstrated that the effect of delivering the drug in the nanoparticle form on bioavailability are primarily based on the fundamentals that nanonization increases the contact surface area of drugs. Drug nanonization would result in an increase in adhesion surface area between intestinal epithelial cells and nanoparticles as well as an increased dissolution rate and also an increase in saturation solubility that favors increased concentration gradient between intestinal epithelial cells and the mesenteric circulation beneath (Mohanraj, Chen, 2006). The results of such studies showed that PLGA may have bioadhesive properties and bind with the mucosa of the gastrointestinal tract. This may increase the residency time and enhance drug absorption due to intimate contact with epithelium cells. It was also reported that PLGA as a drug carrier moderates the P-gp

effect and MDR reversal activity, however, the P-gp inhibition mechanism of PLGA, which mainly involves changing the fluidity of the cellular membrane, inhibiting P-gp ATPase, and reducing P-gp expression, remains unclear (Xie *et al.*, 2011). In general, the gastrointestinal absorption of macromolecules and particulate materials involves either paracellular route or endocytotic pathway. Using polymers such as chitosan, starch or poly (acrylate) can increase the paracellular permeability of macromolecules. Endocytotic pathway for absorption of nanoparticles is either by receptor-mediated endocytosis, that is, active targeting, or adsorptive endocytosis which does not need any ligands. This process is initiated by an unspecific physical adsorption of material to the cell surface by electrostatic forces such as hydrogen bonding or hydrophobic interactions. Adsorptive endocytosis depends primarily on the size and surface properties of the material. This shows that a combination of size, surface charge and hydrophilicity play a major role in affinity (Damge *et al.*, 1996; Kotze *et al.*, 1998; Win, Feng, 2005).

Although clarithromycin exhibits undesirable physicochemical and biopharmaceutical properties (e.g., large molecular weight, extensive potential for hydrogen bonding, high polar surface area, substrates for efflux transporter P-glycoprotein), based on the obtained results it showed high intestinal permeability in rats. The drug was previously reported to have moderate to excellent oral bioavailability in preclinical species and humans (Lan *et al.*, 2009). A potential explanation for this paradox is that intestinal transporters may facilitate CLA absorption. Previous studies in rats suggested that oral absorption of azithromycin and clarithromycin is mediated by an Oatp and/or other rifamycin SV-sensitive intestinal transporter (Garver *et al.*, 2008). This indicates that the drug may be involved in active transport mechanisms which causes the drug fell outside the lipiniski 'rule of 5' (Doppenschmitt *et al.*, 1999).

CONCLUSION

The results of the present study suggest that formulating clarithromycin as nanoparticles, could improve its intestinal permeability in comparison with the pure drug solution. This could be at least partly, due to the contribution of PLGA in alteration of the intestinal permeability.

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CONFLICTS OF INTEREST

The authors indicate that they have no conflicts of interest in this report.

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