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Influence of Poloxamer188 on the Intestinal Transport of Diltiazem Hydrochloride

Hafsa Rana[1], Sunitha B[2], Shravan Kumar Y[2*],Avinash M[2]

Assistant Professor at M.A.K college of Pharmacy, Moinabad, Hyderabad[1].

Vaagdevi college of Pharmacy, Kakathiyauniversity, Warangal[2].

ABSTRACT:

Background: Many Excipients are known to interact with P-gp and CYP enzyme system thereby limiting the oral bioavailability. The present study was aimed to investigate the influence of poloxamer 188(with different concentrations) pretreatment on the intestinal transport of Diltiazem Hydrochloride.

Methods: The transport of Diltiazem across different parts of rat intestine was studied by everted and non-everted sac methods. The control and poloxamer 188 (0.25%, 0.5% and 1% for 7 days) pre-treated rats were sacrificed and isolated the intestine. The sacs of intestine were prepared, filled with Diltiazem solution and then placed in dulbeccos buffer. Samples were collected periodically and the drug content was estimated using HPLC.

Results and conclusion: The results show that there was a significant ($p<0.05$) difference in the transport of Diltiazem from the intestinal sacs of pretreated with poloxamer 188 and control. Pretreatment with Poloxamer decreased drug exsorption in everted studies and increased transport in non everted studies compared to control indicating the inhibition of P-gp transporter and CYP3A enzyme respectively.

KEYWORDS: Diltiazem, Poloxamer 188, Everted, Non Everted, Exsorption and High Performance Liquid Chromatography method (HPLC).

I. INTRODUCTION

Layer transporters can influence the pharmacokinetic, security and viability profiles of substrate medications. P-gp, very communicated in gastrointestinal tract and tumor cells, is an ABC transporter with a vital part in shielding tissue from xenobiotics, in this manner restricting oral bioavailability and prompting

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multidrug resistance. In this way, hindrance of P-gp-intervened efflux is considered as normal methodology to enhance the oral bioavailability and to overcome multidrug resistance [1]. It has been accounted for that some P-glycoprotein inhibitors, for example, verapamil and cyclosporin A could improve the bioavailability of P-gp substrate drugs. Yet, it is no doubt understood that these medications themselves have pharmacological exercises. Subsequently, it is critical to discover a P-gp inhibitor which does not have pharmacological exercises. It has been shown that various excipients could repress the capacity of P-gp, consequently expanding the retention of P-gp substrate drugs [2].

Along these lines, hindrance of P-gp-interceded efflux is considered as normal method to enhance the oral bioavailability and to overcome multidrug resistance [1]. It has been accounted for that some P-glycoprotein inhibitors, for example, verapamil and cyclosporin A could improve the bioavailability of P-gp substrate drugs. Anyhow, it is surely understood that these medications themselves have pharmacological exercises. Hence, it is critical to discover a P-gp inhibitor which does not have pharmacological exercises. It has been exhibited that various excipients could restrain the capacity of P-gp, in this way expanding the retention of P-gp substrate drugs[2].

Excipients are crucial for the meaning of pharmaceuticals to support their course of action and patient ampleness. Starting late, it has been represented that couple of ordinary excipients can alter the development of the efflux transporter P-glycoprotein (P-gp/MDR1)[3]. Surfactants are generally used as a piece of pharmaceutical definitions as wetting authorities to upgrade crumbling and maintenance of inadequately dissolvable solutions. A couple of nonionic surfactants have been demonstrated to limit transporters. Most reports of surfactant-influenced obstacle of film transporters have focused on P-glycoprotein (P-gp). Tests of nonionic surfactant with development fuse Tweens, Spans, Cremophors (EL and RH40), Pluronic square copolymers, and vitamin E TPGS[4]. Pluronic square copolymers are recorded in the US and British Pharmacopeia under the name "poloxamers', and are broadly utilized as a part of a mixture of pharmaceutical applications. A normal for Pluronic atoms is the capacity to self-gather into micelles in watery arrangements. Hence, polymer micelles have been utilized as transporters for medication conveyance .The noncovalent consolidation of medications into the hydrophobic PO (propylene oxide) center of the Pluronic micelle brings about expanded solvency, expanded metabolic dependability, and expanded flow time. Pluronic applies its belongings through synchronous intracellular ATP consumption and layer

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fluidization, bringing on modifications of the liking of the efflux drug transporter for the medication and ATP atoms [5].

Diltiazem, a calcium channel blocker of the benzodiazepine family, has been generally utilized for the treatment of angina pectoris and hypertension. Diltiazem is widely metabolized by the liver and discharged by the kidney. Also, it is assimilated portion up to around 80%. Then again, because of a broad first-impact, Diltiazem is liable to an outright bioavailability of around 40%. The plasma end half-life taking after single or numerous organization is give or take 3-5 h. Incessant organization of quick discharge arrangements is regularly prescribed to keep up compelling blood plasma levels of Diltiazem [6]..

II. MATERIALS AND METHODS

Materials:

Diltiazem Hydrochloride was gifted from Divi's Labs (Hyderabad, India).Verapamil pure drug and Poloxamer 188 were gifted from Dr. Reddy's Lab Ltd.(Hyderabad, India), Dulbeccos phosphate buffer pH of 7.4 (Hi Media Ltd Mumbai, India), Methanol, Acetonitrile (E. Merck Ltd Mumbai, India) and all chemicals used in this study are AR grade.

Experimental animals: Male Wistar rats weighing about 200±25 g were selected and the study was conducted according to the protocol approved by animal ethics committee, Kakatiya University, India.

Methods:

*In vitro transport study:*The vehicle of diltiazem crosswise over rodent digestive tract (duodenum, jejunum and ileum) was concentrated on by utilizing *in vitro* everted and non-everted sac systems. The rats were dealt with independently with poloxamer 188 (0.25%, 0.5%, 1%) and verapamil (1%) in gatherings of 3 to 7 days. The intestinal sections were separated and after that the sacs were arranged. The medication arrangement was set in sac and kept in 40 ml dulbeccos buffer. Test samples were gathered at preset time points for 120 min by supplanting with crisp support and their medication substance was assessed utilizing approved HPLC strategy. Control examinations were additionally performed[7].

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Inhibition Studies: In Inhibition study, inhibitor verapamil 250µm was added to mucosal medium. Utilizing these media the vehicle of diltiazem in nonappearance (control) or vicinity of inhibitor (250µm verapamil) and under affected conditions after pretreatment with poloxamer 188 in distinctive proportions (0.25%, 0.5% and 1%) for 7 days was examined [8].

*Precipitation method:*Methanol (100 µl) was added to intestinal sac tests (200 µl) vortexed on cyclomixer for 2 min, centrifuged at 2500 rpm for 15 min utilizing Biofuge Fresco Centrifuge (Heraeus, Germany) and supernatant was isolated. Twenty microlitres of the supernatant was taken into Hamilton syringe and infused into HPLC [7].

*HPLCAnalysis:*Shimadzu HPLC framework outfitted with a LC-20AD pump and SPD 20 An UV unmistakable finder and RP C18 section (Kromosil, 250 mm x 4.6 mm ID, molecule measure 5 µm) was utilized for the examination of tests. The portable stage utilized was a blend of acetonitrile and double distilled water with potassium phosphate buffer (10 mM, pH 4.6) 35:65 v/v, the pH was acclimated to 4.6 with orthophosphoric acid. The elution was monitored at 238 nm, at a flow rate of 1 mL/ min.
The calibration curve was plotted in the range of 0.25 to 10 µg.ml-1[9]. A linear relationship was observed between the concentration and the peak height of diltiazem with a correlation coefficient (R2 = 0.997).

Statistical analysis: The *in vitro* results were compared by student t-test using GraphPad PrismSoftware. A value of $P<0.05$ was considered to be statistically significant.

III. RESULTS AND DISCUSSION

In the present study, the mean cumulative transport of diltiazem from non-everted sac (mucosal to serosal surface) and everted sac (serosal to mucosal surface) were determined in different regions of the rat intestine of the control, verapamil and different concentrations of Poloxamer (1%, 0.5%, 0.25%) treated groups.

The rate of the adjustment in medication transport was high in all parts of digestive tract treated with poloxamer 188 unique fixations and low on account of verapamil regarded gathering as in instances of non everted sac studies. Medication transport if there should arise an occurrence of everted studies was all the more in control gathering contrasted with the Poloxamer 188 treated gathering. The time course of diltiazem

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transport at distinctive focuses crosswise over rodent small digestive tract of duodenum, jejunum and ileum was demonstrated in Table 1, 2 and Figure 1, 2. P_{app} was likewise computed utilizing the equation

$$P_{app} = \frac{dQ}{dt} \times \frac{1}{A.C_o}$$

Table 1: Cumulative amount (µg) of Diltiazem (Mean±S.D) transported in Non Everted sacs in albino wistar rat (n=3).

Region of Intestine	Control group (µg)	Poloxamer treated group			Verapamil treated group (µg)
		1%	0.50%	0.25%	
Duodenum	1.53 ± 0.14	15.67± 2.11*	21.84± 5.27*	4.78±2.4	1.73±0.99
Jejunum	1.81 ± 1.3	18.15±3.55*	31.96±4.02*	5.31±2.41	2.08±1
Ileum	2.74 ± 1.45	18.33±3.51*	40.5±5.06*	10.17±4.15*	8.47±4.25

(Note: * values indicate statistical significant)

Table 2: Cumulative amount (µg) of Diltiazem (Mean±S.D) transported in Everted sacs in albino wistar rat (n=3).

Region of Intestine	Control group (µg)	Poloxamer treated group			Verapamil treated group (µg)
		1%	0.50%	0.25%	
Duodenum	31.87±2.65	13.18±4.18*	2.25±1.33*	7.17±2.28*	26.42±7.98
Jejunum	33.2±7.76	13.95±4.53*	10.14±3.89*	12.53±4.52*	32.14±3.78
Ileum	35.65±2.28	14.1±5.17*	10.79±3.76*	23.61±5.20*	33.98±6.39

(Note: * values indicate statistical significant)

Where dQ/dt is the steady-state appearance rate on the acceptor solution, A is the surface area of the intestinal sacs. C_o is the initial concentration inside the sacs. The results of Papp are tabulated in table 3 and4.

Table 3: Apparent Permeability (P_{app}) of Diltiazem transported in Non Everted sacs in albino wistar rat (n=3).

Region of Intestine	Control group (cm/s)	Poloxamer treated group			Verapamil treated group (cm/s)
		1%	0.50%	0.25%	
Duodenum	0.06×10^{-6}	0.55×10^{-6}	0.75×10^{-6}	0.12×10^{-6}	0.06×10^{-6}
Jejunum	0.06×10^{-6}	0.59×10^{-6}	0.73×10^{-6}	0.15×10^{-6}	0.10×10^{-6}
Ileum	0.06×10^{-6}	0.81×10^{-6}	1.59×10^{-6}	0.30×10^{-6}	0.14×10^{-6}

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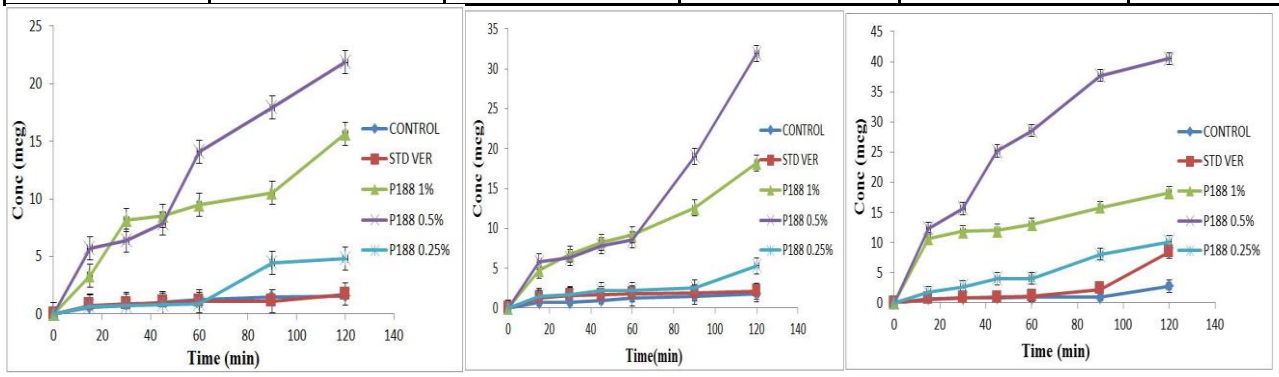
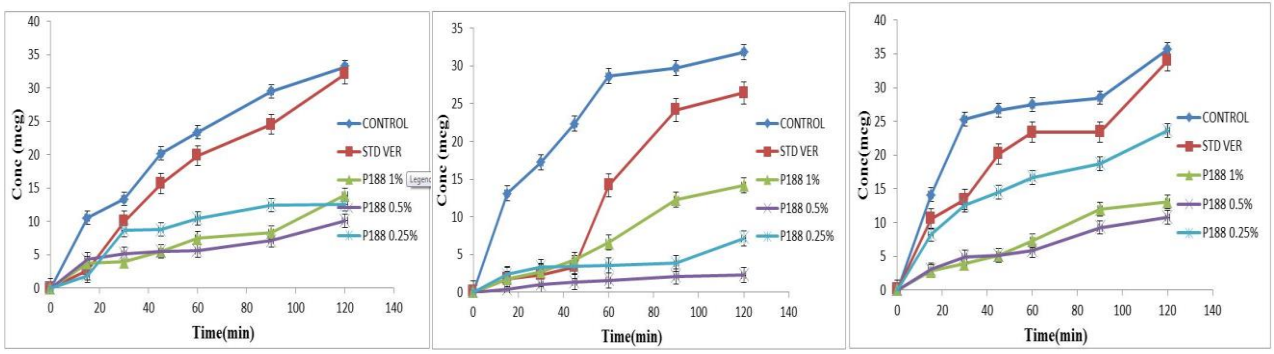
Table 4: Apparent Permeability (P_{app}) of Diltiazem transported in Everted sacs in albino wistar rat (n=3).

Region of Intestine	Control group (cm/s)	Poloxamer treated group			Verapamil treated group (cm/s)
		1%	0.50%	0.25%	
Duodenum	1.33×10^{-6}	0.40×10^{-6}	0.08×10^{-6}	0.24×10^{-6}	0.72×10^{-6}
Jejunum	1.26×10^{-6}	0.42×10^{-6}	0.37×10^{-6}	0.54×10^{-6}	1.04×10^{-6}
Ileum	1.57×10^{-6}	0.44×10^{-6}	0.35×10^{-6}	0.94×10^{-6}	1.16×10^{-6}



(a) (b) (c)

Figure 1 a, b, c : Cumulative transport pattern of Diltiazem in (a) Duodenum, (b) Jejunum and (c) Ileum Non Everted sac in Wistar rats.



(a) (b) (c)

Figure. 2 a, b, c : Cumulative transport pattern of Diltiazem in (a) Duodenum, (b) Jejunum and (c) IleumEverted sac in Wistar rats.

In recent years a number of studies have suggested that several common pharmaceutical surfactants/excipients can modulate the activity of the efflux transporter P-gp, and possibly other transporters. Thus, the concept of all excipients being "inactive" has been challenged, and the idea that they

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were largely inert molecules used to control the stability and solubility of drugs has had to be reassessed[10]. Pluronic block copolymers are listed in the US and British Pharmacopoeia under the name ‘’poloxamers’exerts its effects through simultaneous intracellular ATP depletion and membrane fluidization, causing alterations of the affinity of the efflux drug transporter for the drug and ATP molecules. The synergy between these two effects results in a potent inhibition of P-gp efflux system, as well as some other ATP-dependent drug efflux transporters, such as MRP [5].

The non-everted sac model was initially used to assess medication transport instruments. Genty et al., looked at the penetrability estimations of some effectively transported drug molecules through everted and non-everted sacs and found that the porousness was higher for effectively transported particles when the sacs were everted. The permeability of passive absorption drug diazepam remained the same whether the sacs were everted or not. These results suggested that the passive permeability of actively transported molecules can be determined through non-everted rat gut sacs [11].

Huang J et al., considered the Effect of pluronic F68 piece copolymer on P-glycoprotein transport and CYP3A4 digestion system and found that pluronic F68 expanded apical-to-basolateral penetrability (AP-BL) and diminished basolateral-to-apical transport (BL-AP) of the P-gp substrate Celiprolol in Caco-2 cell monolayer [12].

Diltiazem hydrochloride delivers its antihypertensive impacts principally by unwinding of vascular smooth muscle and the resultant abatement in fringe vascular resistance. It has poor bioavailability because of broad first-pass digestion system and yields desacetyl diltiazem. Poloxamer pretreatment seem to have a huge impact on CYP3A4 intervened intestinal digestion system of Diltiazem.

IV. CONCLUSION

The effect of excipient (Poloxamer 188) on the vehicle of Diltiazem was resolved. Pretreatment with Poloxamer 188 diminished medication exsorption in everted studies and expanded transport in non everted studies contrasted with control demonstrating the hindrance of P-gp transporter and CYP3A compound individually may contribute to increment in bioavailability. Among the distinctive amassings of Poloxamers, 0.5% was found to restrain the transporter and chemical adequately. This presumes that excipients can be

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utilized to increase the bioavailability of medications by repressing transporters however precautionary measures ought to be taken.

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