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Design and development of microemulsion drug delivery system of atorvastatin and study its intestinal permeability in rats

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Abstract:

The objective of this study was to design and develop microemulsion drug delivery system of Atorvastatin and to investigate its intestinal transport behavior using the single-pass intestinal perfusion method in rat. Microemulsion drug delivery system of Atorvastatin was prepared by water titration method and optimized formulation was characterized. The permeability behavior of Atorvastatin over three different concentrations (10, 20 and 40 µg/mL) was studied in each isolated region of intestine (i.e. duodenum, jejunum, ileum and colon) of rat by single-pass intestinal perfusion method in rat method at a flow rate of 0.2 ml/min. The concentration of the sample was determined by HPLC and the effective permeability coefficients were calculated. Considering the high correlation of rat permeability coefficient values with those of human, the human intestinal permeability was predicted using the Lawrence compartment absorption and transit model. The intestinal permeability of Atorvastatin in microemulsion, plain drug suspension and marketed formulation was also compared. The particle size and zeta potential of Atorvastatin microemulsion were (18.2±0.3) nm and (–9.19±0.8) mV respectively. There was no significant difference in permeability coefficient in jejunum, duodenum and ileum with same concentration but higher in colon was observed. The permeability coefficient in jejunum at 10 µg/mL was significantly higher than that at 40 µg/mL ($p < 0.01$). The estimated human intestinal permeability of Atorvastatin for the microemulsion was relatively higher. Based on the above results, it could be concluded that microemulsion formulation could enhance the intestinal permeability of Atorvastatin and thus could be presented as a possible alternative to traditional oral formulations for improving the oral absorption of Atorvastatin.

Keywords: Microemulsion;, Zeta potential; Atorvastatin; Single-pass intestinal perfusion (SPIP) method; Compartment absorption and transit model (CAT)

Introduction

In recent years, much attention has been focused on using microemulsion drug delivery system for the purpose of improving the solubility and oral absorption of poorly water-soluble drugs [1-5]. Microemulsion

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system, an isotropic mixture of oil, surfactant and co-surfactant along with water is optically clear and thermodynamically stable system with a droplet size in a range of 10-100 nm. This system has been shown to improve absorption of drugs due to small droplet size and promotes intestinal lymphatic transport due to its specific components [6]. Atorvastatin, a semi synthetic hypolipidemic drug, selectively and competitively inhibits 3-hydroxy-3-methylglutaryl-coenzyme-A reductase (HMG-CoA reductase) catalyzes the conversion of HMG-CoA to mevalonate, which is required for cholesterol biosynthesis. Atorvastatin is a poor water-soluble drug and has a very low absolute bioavailability (about 5%) due to rapid metabolism in the gut and liver [7]. Since Intestinal permeability is necessary for oral administration so in this study, microemulsion drug delivery system was developed to improve the solubility and oral absorption of Atorvastatin. Labrafil M 1944CS, Cremophor RH 40 and ethanol were used as oil phase, surfactant and co-surfactant respectively. The intestinal permeability of Atorvastatin was determined using the single pass intestinal perfusion (SPIP) in rats since this method provides a unique advantages of experimental control (e.g. compound concentration and intestinal perfusion rate), ability to study regional differences and an intact intestinal blood supply and a functional intestinal barrier [8].

Materials and Methods

Materials

Atorvastatin was obtained from Torrent Research Center (Ahmedabad, India). Capmul MCM, Labrafac CC, Cremophor EL, Cremophor RH 40, Labrafil M 1944CS and Transcutol P were kindly provided by Gattefosse, france. Isopropyl Myristate (IPM), Tween 60, PEG 600, PEG 400, Ethanol, Glycerol, Ammonium acetate, Sodium citrate, Acetonitrile (HPLC grade) and Methanol (HPLC grade) were purchased from National Chemicals (Baroda, India). All other Chemicals were reagent grade.

Animals

Male Albino rats (250 ± 20 g) were used for the comparative *in vivo* studies. The animals were maintained at temperature (25±1°C), and humidity (60±5%) and were supplied with food and water ad libitum. Animal experiments were approved by Social Justice and Empowerment Committee, Ministry of Government of India, New Delhi, India, with the permission number of 404/01/a/CPCSEA. The protocol was approved by Local Animal Ethics Committee. Male albino rats were obtained from Zydus Health care, Ahmadabad, India.

Solubility Studies

The solubility of atorvastatin in various oils (Sunflower oil, Isopropyl Myristate, soya bean oil, Labrafil M 1944CS, Capmul CMC), surfactant (Labrafac CC, Tween 60, Cremophor EL, Cremophor RH 40), and cosurfactants (PEG 400, PEG 600, glycerol, ethanol) was determined. A total of 2 mL of each of the selected vehicle were added to each cap vial containing 100mg of Atorvastatin. After sealing, the mixture was heated at 40°C in a water bath to facilitate the solubilization and then mixed using a vortex mixer. Mixtures were then shaken with mechanical shaker at 25°C for 48 hrs. Then each vial was centrifuged at 8000 rpm for 15 min, and insoluble as well as soluble Atorvastatin was quantified by UV spectroscopy [9]. Table 1 shows the results of the solubility studies.

Table 1: Solubility studies data of Atorvastatin in different vehicles

S. No.	Vehicles	Solubility (mg/ml±SD)
1	Sunflower oil	24.9 ± 2.2
2	Isopropyl Myristate	34.9 ± 1.8
3	Soya bean oil,	20.1 ± 1.1
4	Labrafil M 1944CS	57.7 ± 1.3
5	Capmul CMC	8.4 ± 1.2
6	Labrafac CC	26.5 ± 2.7
7	Tween 60	5.56 ± 0.9
8	Cremophor EL	11.4 ± 1.2
9	Cremophor RH 40	28.2 ± 1.1

Pseudo-ternary Phase Diagram Study

Pseudo-ternary phase diagrams of microemulsion were to obtain the optimum concentration of oil, surfactant and co-surfactant. After performing the solubility study and phase diagram study, components were selected for microemulsion formulation. The effect of Atorvastatin in the phase diagrams was also investigated [10].

Preparation of Atorvastatin microemulsion system

Atorvastatin containing microemulsion was prepared by water titration method [11]. Atorvastatin was first

dissolved in the pre-measured volume of oil by stirring on a magnetic stirrer. A mixture of the surfactant and co-surfactant at a fixed ratio (v/v) was added to the above resulting mixture. Finally this mixture was titrated with distilled water. The optimized microemulsion drug delivery system was also subjected for accelerated stability study.

Particle size and zeta potential measurement

Particle size and zeta potential of Atorvastatin containing microemulsion drug delivery system was carried out by dynamic light scattering through with Zetasizer 3000HS, Malvern Instruments Corporation, U.K. Drug loaded microemulsion was also assayed for % drug content.

Single-pass intestinal perfusion (SPIP) of the rat segments

Before the SPIP experiment, sufficient Krebs-Ringer's buffer solution was added to adjust the formulations to a designed concentration. The intestinal permeability experiments of the microemulsion formulation with different concentrations of the Atorvastatin were investigated and compared to both PDS and MFA of equivalent drug concentration. Preliminary studies are necessary before commencing the SPIP studies to ensure that the loss of drug from perfusion is due to absorption and not by other losses (e.g. the binding of the intestinal wall or degradation) [12]. Approximately 10 cm from the rat intestine was clipped, intestinal sac were turned over and put into 50 mL Krebs-Ringer's buffer solution, in which Atorvastatin (20µg/mL) was incubated at 37±2°C for 3 Hrs. Samples of 40µl were withdrawn at 3 Hrs interval, diluted with acetonitrile, methanol and water (mobile phase-4.5:4.5:1) and assayed by HPLC. SPIP studies were performed according to previously described methods [13, 15]. After an overnight fast, male albino rats (weighing approximately 250 ± 20 g) were anesthetized with an i.p. injection of 20% (w/v) urethane solution (urethane: 1g/kg). A surgical lamp was kept under the rat's cage to maintain the body temperature. The abdomen was opened with a midline incision, an intestinal segment of approximately 10 cm was chosen and separated. Intestinal segment was then rinsed with isotonic saline (37°C) until the outlet solution was clear. Using an infusion pump the intestinal segments were perfused at a flow rate of 0.2 ml/min for 30 min with Krebs-Ringer's buffer solution. Then the pump was connected to the reservoir containing Atorvastatin loaded microemulsion system with the Phenol Red (Marker). Setting t=0 when the buffer solution has been completely pushed out. Each perfusion experiment lasted for 180 min. and the perfusate was quantitatively collected in 60, 75, 90, 105, 120, 135, 150, 165 and 180 min. Samples were stored in a refrigerator at 4°C until analysis by HPLC.

HPLC analysis of samples

All samples were diluted to 5 ml with the mobile phase and measured by HPLC (Shimadzu, Japan) system consisting of membrane degasser, binary solvent delivery system, a pump, a rheodyne injector equipped with a 20 µl sample loop and UV detector (Shimadzu, Japan) [16]. Aliquots of 20 µl were injected onto the HPLC system, was used to analyze Atorvastatin at 332 nm. Chromatographic separations were achieved using an Hypersil ODS C18 column (250×4.6 i.d.×5 µm). The mobile phase used for the sample analysis was Acetonitrile, methanol and water of HPLC grade (45:45:10 V/V) with the flow rate of 1.0 ml/min.

Table 2. Data of optimized microemulsion

Characterization	Particle size (nm)	Zeta Potential (mV)	Poly dispersibility Index (PDI)	% Drug Content
Atorvastatin ME	18.2±0.3	–9.19±0.8	0.281	98.72±1.87

Water absorption or secretion (flux) was measured by gravimetric method [17-18]. The net water flux (NWF) per cm of each segment was calculated using the following equation and results were tabulated in Table 3:

$$NWF\ (\mu l/min/cm) = [(1-CPR_{out} / CPR_{in})\times Q_{in} /L]\times 1000.$$

Where, CPR_{in} and CPR_{out} were the inlet and outlet concentration of phenol red, respectively. Qin was the flow rate of the perfusion solution entering the intestinal segment and L was the length of the intestinal segment (cm). If Intestinal lumen absorbs water NWF will be negative and if intestinal lumen secretes water NWF is positive value, however under normal condition, intestinal lumen absorbs water except when

the high osmotic solution is administered [19]. The effective permeability (P_{eff}) was calculated based on the inlet and outlet concentration of Atorvastatin (C_{in} and C_{out}, respectively) and result was shown in Figure 1.

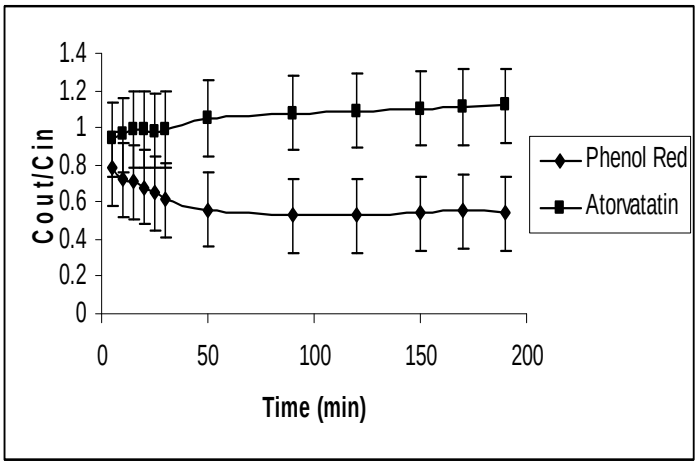

Figure 1. Plot of the concentration ratio of Cout/Cin V/s Time.

Steady state was considered to have been achieved when the concentration of phenol red and Atorvastatin were constant. The permeability values are calculated after steady state was achieved using the following equation [20]:

$$P_{eff} = [Q \ln (C_{in} / C_{out})']/ 2\pi rL$$

Where Q is the flow rate in mL/min, r is radius of the intestine (0.18 cm in rat and 1.75 cm in man), L is the length of the perfused intestinal segment (cm), and $(Cin/Cout)'$ is the concentration ratio corrected for fluid flux. Using ANOVA, the statistical significance of the differences among group means were assessed with the least significant difference (LSD) test and a value of $p<0.05$ was considered statistically significance.

Results

Microemulsion drug delivery system formulations

Labrafil M 1944CS was selected as the oil phase for formulation development because it provided higher solubility than other oils and oral compatibility. Cremophor RH 40 as surfactant and ethanol as co-surfactant were used on the basis of their drug solubilising capacity and might influence the tight junctions of the epithelial cells. From the results of the pseudo-ternary phase diagram, 3:1 ratio of Cremophor RH 40 and ethanol was selected for microemulsion preparation. The optimal formulation of microemulsion drug delivery system was 3% oil, 27.75% surfactant, 9.25% co-surfactant and 60% as aqueous phase. Moreover Atorvastatin microemulsion drug delivery systems were stable for 6 months at ambient temperature.

Particle sizes and zeta potential measurement

Particle size, zeta potential and % drug content of Atorvastatin microemulsion were tabulated in Table 2.

Validation of HPLC assay

The regression equation for the concentration of Atorvastatin (ng/ml) V/s response in the perfusion fluid ranging from 50 ng/ml to 500 ng/ml was $A = 93.21C+620.3$ ($R^2 = 0.9998$). The mean recovery of Atorvastatin was 100.8±1.31%. The intra-day and inter-day RSDs were less than 2%.

Table 3: The net water flux (NWF) in each intestinal segment (n=4)

Time (min)	Duodenum	Jejunum	Ileum	Colon
70	-0.584±0.121	-0.144±0.175	-0.102±0.056	-0.129±0.018
90	-0.555±0.212	-0.117±0.128	-0.238±0.078	-0.197±0.056
110	-0.789±0.374	-0.209±0.124	-0.237±0.032	-0.426±0.244
130	-0.672±0.221	-0.206±0.226	-0.345±0.057	-0.298±0.057

Water absorption or secretion of intestinal lumens

The NWF for all intestinal segments were negative value as listed in Table 3, which suggested that the water was mainly absorbed in the intestine tract. The NWF for the duodenum was significantly higher than that for the jejunum, ileum and colon ($P<0.05$) and NWF for the jejunum, ileum and colon were not significantly varied ($P>0.05$).

SPIP studies

The amount of Atorvastatin remained after incubated in the Krebs-Ringer's buffer with the intestinal sac for 3 hrs was 99.32 ± 1.23%, which was not significantly decreased which suggested that neither Atorvastatin markedly bind to the intestinal wall nor the potential metabolites were formed. The intestinal permeability of

Atorvastatin was studied as a function of concentration in each segment of the intestine and 40 µg/ml concentration was chosen because of its limited aqueous solubility. Steady state is confirmed by plotting the concentration of Atorvastatin and phenol red versus time in Figure 1. Steady state, which was assessed by the constant concentrations of both phenol red and Atorvastatin, was reached about 50 min after the beginning of the perfusion. The permeability values were calculated only after steady state was achieved in the experiments. The permeability values of microemulsion drug delivery system for each intestinal segment and concentrations are listed in Table 4. P_{eff} in the jejunum at 10 µg/mL (0.388±0.021 cm/s) was significantly higher than P_{eff} at 40 µg/mL (0.229±0.037 cm/s, $p<0.01$). There was no statistical difference in P_{eff} at three investigated concentrations in other segments (including duodenum, ileum and colon). Comparisons across each segment revealed that P_{eff} at each concentration in colon was higher than all other segments ($p<0.01$). Moreover, the permeability values in duodenum, jejunum and ileum were not statistically different ($p>0.05$).

Table-4: Comparison of effective permeation coefficients (Peff $\times 10^{-4}$) of different intestinal segments with three concentrations. Results are given as mean ± S.D (n=4).

Conc. of Atorvastatin (µg /mL)	Peff (cm/s) in Duodenum	Peff (cm/s) in Jejunum	Peff (cm/s) in Ileum	Peff (cm/s) in Colon
10	0.435±0.049	0.445±0.026	0.475±0.052	0.625±0.096
20	0.402±0.045	0.414±0.042	0.456±0.067	0.598±0.085
40	0.401±0.068	0.377±0.057	0.437±0.116	0.532±0.068

Permeability values of Atorvastatin in the formulation of microemulsion drug delivery system, PDS and MFA for each segment at 10 µg/mL were shown in Figure-2. P_{eff} of microemulsion drug delivery system was significantly higher than PDS and MFA in each segment ($p<0.01$). Except the duodenum ($p<0.05$), P_{eff} of microemulsion drug delivery system and MFA in the jejunum, ileum and colon did not all reach statistical difference ($p>0.05$).

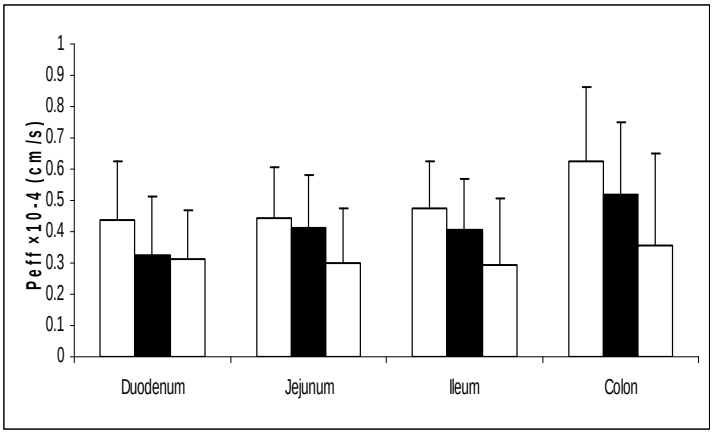

Figure 2. Comparison of *P*eff of the ME, PDS and MFA in different intestinal segments (n=4) at concentration of the Atorvastatin as 10 µg/mL.

Discussions

P_{eff} in the colon at each concentration was significantly higher than those in other segments of rat intestine as shown in Table 3. There are various reports showing the regional difference in the expression and activity of P-glycoprotein throughout the gastric intestinal tract [21]. The order of the expression and activity of P-glycoprotein is ileum> duodenum and jejunum> proximal and distal colon [22]. As a multi-drug resistance-reversing agent, the intestinal absorption of Atorvastatin was limited due to the presence of the P-glycoprotein throughout the intestinal tract [23]. The lower expression activity of P-glycoprotein conduced to be increasing the permeability of Atorvastatin in colon as compared to other sections (Table 3). It has been shown that there is a selectivity of permeation depending of the gut sections. Moreover, small intestine has more cationic selectivity, whereas, the colon has more anionic selectivity [24]. Another possible mechanism for higher P_{eff} values in colon was due to the higher crypt surface area in the colonic mucosa compared with the small intestine which could account for its high paracellular permeability [25]. The extent of drug absorption in human can be predicted from rat intestinal permeability experiments. Human intestinal permeability values may be estimated using the Lawrence compartmental absorption and transit (CAT) model [26]:

Fa (fraction of dose absorbed) $=1-[1+0.54\ P_{eff\,(man)}]^{-7}$

$P_{eff\,(man)} = 3.6 \times P_{eff\,(rat)} + 0.03 \times 10^{-4}$

The estimated Fa for Atorvastatin in the microemulsion system, MFA and PDS were 85.5±1.9% 59.3±3.1% and 81.2±2.2%, respectively. It suggested that the estimated oral absorption in human for microemulsion drug delivery systems would be higher than that for PDS and MFA ($p<0.01$). Microemulsion drug delivery system is known to improve the oral absorption of lipophilic drugs. The main mechanism reported include increasing membrane fluidity, opening tight junction, inhibiting P-gp and/or CYP450 by surfactants and stimulating lipoprotein/chylomicron production by lipid.

Conclusions

According to these results, several conclusions can be drawn. The Atorvastatin microemulsion drug delivery system had a small particle size (28.6±0.3 nm) and a native charge. Atorvastatin microemulsion drug delivery system and its dilutions were stable. The P_{eff} in jejunum at 10 μg/mL were significantly higher than that at 40 μg/mL ($p< 0.01$). Compared to the jejunum, duodenum and ileum, the higher P_{eff} in colon were observed at the same concentration. The estimated absorption of Atorvastatin in human for the microemulsion drug delivery system was higher than that for PDS and MFA ($p<0.01$).

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