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Brain Targeted Delivery of Rizatriptan using Glutathione Conjugated Liposomes through Transmucosal Nasal Route

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ABSTRACT

Objectives: The objective of this work was to enhance the bioavailability of rizatriptan for brain targeted drug delivery through glutathione conjugated liposomes. **Methods:** Cholesterol glutathione conjugate was synthesized used as a rigidizing agent for liposomes. Liposomes with free cholesterol were also prepared for comparison. 9 batches each were prepared for glutathione conjugated liposomes and non-conjugated liposomes. All formulations were administered to rats. **Results:** For optimum non-conjugated liposomes batch particle size, drug release and entrapment efficiency were found to be 181nm, 90.2% and 88.1% respectively whereas the same values for glutathione conjugated batch were 194 nm, 84.9% and 86.4% respectively. Zeta potential was between 5 to 19. Polydispersity index was below 0.5. Scanning electron microscopy revealed slightly different shapes for both types of liposomes. These two types of rizatriptan liposomes and marketed oral tablet were administrated to rats to study plasma and brain levels. The t_{max} for liposomes was faster (1 hr) as compared to the oral tablet. C_{max} and AUC values for oral tablet, non-conjugated liposomes and conjugated liposomes were found to be 150.19 ng/ml and 223.99 ng.hr/ml; 320.55 ng/ml and 426.6 ng.hr/ml; 410.12 ng/ml and 543.49 respectively. Maximum brain levels were achieved by glutathione conjugated liposomes over other liposomes and oral delivery (C_{max} 310.46, 135.42 and 79.16 ng.ml respectively; AUC 786.94, 229.55 and 118.11 ng.hr/ml respectively). Drug targeting efficiency for conjugated liposomes was about 5 times higher. **Conclusion:** The study concluded that glutathione conjugated liposomes of rizatriptan administered by nasal transmucosal route can offer a promising approach to enhance targeted delivery to brain and bioavailability.

Key words: Glutathione, Liposomes, Migraine, Rizatriptan, Brain targeted drug delivery, *in-vivo* evaluation.

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INTRODUCTION

Rizatriptan is commonly used for the treatment of migraine headache. It acts through one or more of the following mechanisms: it stimulates the presynaptic 5-HT1D receptors and inhibits both dural vasodilation and inflammation; inhibition of trigeminal nuclei cell excitability in the brainstem and vasoconstriction of vessels. Although oral absorption of rizatriptan is about 90%, it has a bioavailability of only 45 to 47% due to extensive hepatic first pass metabolism.[1] The major enzyme that metabolizes rizatriptan in monoamino oxidase A. The metabolites produced are pharmacologically inactive.

Nasal transmucosal drug delivery offers a promising approach for systemic delivery due to numerous advantages like non-invasive nature, faster drug permeation across the mucus membrane etc.[2] The nasal route is also beneficial because of its preferential delivery to the brain via olfactory pathway without encountering the blood brain barrier.[3] The aforementioned challenges faced in the oral therapy of rizatriptan can be overcome with nasal administration.

Liposomes are vesicles found to be effective in crossing various body membranes.[4] The basic structure of liposomes is a bi-layer lipid vesicle with an inside aqueous compartment. High lipid content present in liposomes makes them suitable for targeting the drug across the highly lipophilic blood brain barrier.[5] BBB contains tight junctions of highly specialized brain endothelial cells and epithelial structure of the fully differentiated neurovascular system.

Cholesterol is an integral part of liposomes as it gives rigidity to liposomal walls. It provides thermal and physical stability to liposomes. Cholesterol is also a potential candidate for conjugation with peptides which are intended for targeting the drug specifically to certain organs in the body. Glutathione is proposed to be an excellent agent for selective targeting to the brain. Conjugation of cholesterol with glutathione would assert that liposomal surface would bear the glutathione moiety. As cholesterol is present in the membrane of liposomes, the conjugated moieties would protrude outwards and insides of the liposomes. The mere addition of glutathione during the preparation of liposomes would not be sufficient for successful targeting of liposomes as these targeting ligands should be present on the surface of liposomes.

Furthermore, the distribution of drugs and transport across BBB can be enhanced by targeting the drug selectively to the brain. For targeting the drug to brain glutathione was proposed as a targeting ligand in the present work. GSH is present in high amounts in the body. GSH is a natural antioxidant, found at high levels in the brain, with an excellent safety profile; only the BBB possesses GSH transporters that actively carry GSH to the brain against a gradient concentration.[6,7] It does not require the modification of the active components, thereby avoiding the need for extensive preclinical tests and clinical trials before regulatory agencies approval, can carry various classes of molecules, has low costs and straightforward manufacturing.

Attributing to all these advantages, the liposomal drug delivery system containing GSH as the ligand was used to target delivery of rizatriptan to brain.

MATERIALS AND METHODS

Materials

Rizatriptan was obtained as a gift sample from Cadila Pharmaceuticals. Soya phosphatidylcholine Lipoid S 100 was provided by Lipoid, Germany. Cholesterol was purchased from Analab Fine Chemicals, Mumbai and polyethylene glycol 400 from Loba Chemie Private Limited, Mumbai. Glutathione was purchased from Oxford Laboratory, Thane, Mumbai. Male Wistar rats weighing were obtained from Agarkar Institute, Pune, India.

Methods

Synthesis of cholesterol acrylate from cholesterol

Cholesterol-acrylate was synthesized by a procedure described by Patil *et al*. and Qiong *et al*.[8,9] Reagents were used in the following proportion. Cholesterol (4gm) and triethanolamine (4.3 ml) were initially dissolved in 50 ml of anhydrous tetrahydrofuran (THF). Separately, acroloyl chloride was dissolved in 5ml of THF and added to cholesterol solution dropwise. Temperature was maintained at 0°C. Nitrogen environment was maintained in the mixing flask. After 10 min, the flask was removed from ice bath and kept stirring for 24 hr.

The solvent was removed by rotary vacuum evaporator. The crude solid was dissolved in 50 ml of dichloromethane and washed with the following solutions: deionized water, hydrochloric acid (0.5 M), de-ionized water, sodium bicarbonate (1 M) and deionized water. The product was dried and purified by silica gel column chromatography (dichloromethane/hexane 1/1 v/v) after the removal of the solvent by rotary evaporator. The formation of cholesterol acrylate was confirmed by carrying out FTIR and ^{1}HNMR studies.

Synthesis of cholesterol-glutathione conjugates from cholesterol acrylate

The process of Conjugating glutathione to cholesterol acrylate was carried out in a mixture of dimethyl sulfoxide and tetrahydrofuran at the presence of triethylamine. The mixture was kept on magnetic stirrer for 48 hr at 37°C while maintaining nitrogen environment. Dialysis tubes were used to obtain the synthesized product which was further dryed using a lyophilizer. The formation of conjugation was confirmed by ^{1}HNMR and FTIR studies. The reactions involved are Figure 1.

Liposome preparation

The thin-film hydration method was used for the preparation of liposomes.[10] The required amount of Lipoid S100, cholesterol and rizatriptan (10mg) were weighed and dissolved in chloroform in a round bottom flask. The flask was handshake till the solvent got evaporated; as the solvent evaporated a thin film was formed along the inner wall of the flask. The flask was kept overnight for the complete removal of the organic solvent. After keeping for overnight sufficient amount of phosphate buffer of pH 6.8 was poured into the flask along with the surfactant PEG 400 to hydrate the film. Benzalkonium chloride was added to this dispersion as preservative. The liposomal dispersion thus formed contained large lamellar vesicles. To convert them into unilamellar vesicles, the dispersion was ultrasonicated for 30 min. The same method was used to prepare GSH-CHL conjugated liposomes by replacing cholesterol by cholesterol acrylate glutathione conjugate. Total 9 formulations (Table 1) were prepared for each type of formulations with varying levels of cholesterol/cholesterol-GSH conjugate and Lipoid S 100.

Liposome characterization

Malvern Zetasizer 90 was used to measure the particle size, zeta potential and polydispersity index of liposomes formulations. For entrapment efficiency calculation, liposome suspension was centrifuged at 10000 rpm to separate un-entrapped drug. The free drug present in the supernatant was determined using a UV spectrophotometer at 225nm. EE (%) was calculated by the following equation:

$$EE\ (\%) = [(C_{Total} - C_{Free})/C_{Total}] \times 100$$

Where, C_{Total} = total drug added, C_{Free}= unentrapped drug[11]

In vitro drug release

In vitro drug release of liposomes was studied using a dialysis bag (molecular weight cut off 12000) soaked in distilled water for 24 hr and then washed with water thoroughly before use to open the pores. Liposomal dispersion (2 ml) was placed in the dialysis bag. The dialysis bag was sealed from both the sides with cotton thread. USP Type II dissolution apparatus was used for the *in-vitro* dissolution study. Dialysis bag was tied to the stainless steel part of the paddle and then immersed in a dissolution tester beaker containing 100 ml of bile salt solution as the release medium. The temperature was set at 37 ± 0.2°C and the rotation speed of the paddles was set at 100 rpm.[12]

Scanning electron microscopy

The morphology and surface topography of the liposomes were examined by SEM (Nova nanosem 450). The liposomes suspension was mounted on silicon wafers and carefully air-dried. The samples were coated with chromium (200 A0) under reduced pressure (0.001 torr) for 2 min using an ion sputtering device. The chromium-coated samples were observed under the SEM and photomicrographs of suitable magnifications were obtained.[13]

Animals used and approval of the protocol

Male Wistar rats were used for the study. The study groups each containing 6 animals were control, marketed oral tablet, non-conjugated liposomes and GSH-CHL conjugated liposomes of rizatriptan. The Institutional Animal Ethical Committee (IAEC) reviewed and approved experimental procedures and protocols. Animals were maintained in standard condition with adequate supply of water and feed.

Administration of formulations to animals and sample collection

GSH conjugated and non-conjugated liposomes formulations at a rizatriptan concentration of 1 mg/ml were used for intranasal

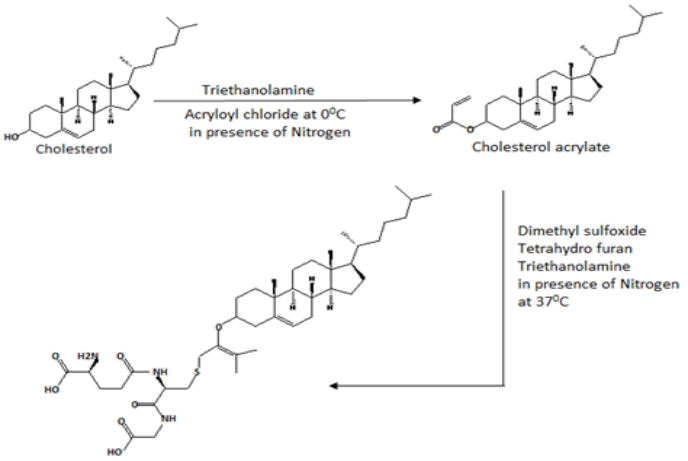

Figure 1: Synthesis of cholesterol-glutathione conjugate.

administration. Rizatriptan in the form of a tablet (Rizact™) powder equivalent to 1mg was dissolved in distilled water and was administered orally through a forced feeding tube. The animals were anesthetized with the use of anaesthetic ether for IN administration. Rats were placed in a supine position. Rizatriptan suspensions were administered into nostrils using a microsyringe and polyethylene tube. Dose given was 1mg per each kilogram of body weight.[14] Blood samples (0.5 ml) were collected at time intervals of 0, 30, 60, 90, 120 and 150 min. Blood samples collected were centrifuged and serum was collected. At the specified intervals mentioned above, after the withdrawal of blood samples from the animals; one animal from each group was sacrificed by cervical dislocation method, perfused with saline its and brain was removed for detection of levels of rizatriptan in the brain.[15] 20% aqueous brain homogenates from rat was prepared in potassium chloride solution. A centrifuge with cooling system was used to process the homogenized tissue. The operating speed was 15000 rpm and temperature was maintained at 4°C. To the clear supernatant liquid, methanol was added as deproteinizing agent in 1:2 proportion. The dispersion was again centrifuged at the same conditions. Equal volume of water was mixed with the supernatant liquid. Samples so prepared were used for HPLC analysis.

Determination of pharmacokinetic parameters: Peak Plasma Concentration (C_{max}), Time of Peak Plasma Concentration (t_{max}) and Area under Curve (AUC)

The peak plasma concentration, time of peak and extent of absorption expressed as area under curve were used as pharmacokinetic parameters for comparison among the formulations.

Drug targeting

The degree of drug targeting to the brain through nasal transmucosal administration can be expressed as drug targeting efficiency (DTE) and nose-to-brain direct transport (DTP). DTE% in comparison with oral formulations is calculated as:

$$DTE\ \% = \frac{(AUC^{brain}/AUC^{blood})in}{(AUC^{brain}/AUC^{blood})oral} \times 100$$

The drug targeting efficiency (DTE) was calculated using above equation where AUC_{brain} is the area under curve of brain concentration of rizatriptan and AUC_{blood} is the area under curve of blood concentration of rizatriptan concentration over the study period.

The nose-to-brain direct transport (DTP) percentages of rizatriptan formulations were calculated according to following equation where $B_{i.n.}$ is the total brain AUC following intranasal administration and F is a fraction of the brain AUC contributed by the systemic circulation through the BBB following the intranasal administration and was calculated according equation.

Where, B_{oral} is the brain AUC following oral administration, P_{oral} is the blood AUC following oral administration and $P_{in.}$ is the blood AUC following intranasal administration.[16]

$$DTE\ \% = \frac{(B_{in}\text{-}F)}{B_{in}} \times 100$$

RESULTS

Characterization of cholesterol-glutathione conjugate

The synthesis scheme of cholesterol- glutathione conjugate is described in Figure 1. Formation of cholesterol acrylate glutathione conjugate was confirmed using ^{1}HNMR as shown in Figure 2. The yield of the product was about 37%.

Evaluation of liposomes

The outcomes of the evaluation of liposomes formulation are shown in Table 2. For formulations F1 to F9 (non-conjugated liposomes group) the particle size was found to range between 181 to 540 nm and zeta potential between 10.34 to 19.04 mV. Entrapment efficiency and *in vitro* drug release ranged between 70.7 to 92.1% and 59.3 to 90.2 % respectively. For formulations G1 to G9 (GSH conjugated liposomes group) the particle

Table 1: Composition of liposomes formulations for cholesterol-GSH conjugated liposomes and non-conjugated liposomes.

Formulation code	Rizatriptan (mg)	Cholesterol (mg)	CHL-GSH conjugate (mg)	Lipoid S 100 (mg)	PEG 400 (ml)	Benzalkonium chloride (ml)	Phosphate buffer q.s. (ml)
F1	10	150	-	75	2	0.1	10
F2	10	150	-	100	2	0.1	10
F3	10	150	-	125	2	0.1	10
F4	10	300	-	75	2	0.1	10
F5	10	300	-	100	2	0.1	10
F6	10	300	-	125	2	0.1	10
F7	10	450	-	75	2	0.1	10
F8	10	450	-	100	2	0.1	10
F9	10	450	-	125	2	0.1	10
G1	10	-	75	75	2	0.1	10
G2	10	-	100	100	2	0.1	10
G3	10	-	125	125	2	0.1	10
G4	10	-	75	75	2	0.1	10
G5	10	-	100	100	2	0.1	10
G6	10	-	125	125	2	0.1	10
G7	10	-	75	75	2	0.1	10
G8	10	-	100	100	2	0.1	10
G9	10	-	125	125	2	0.1	10

size was found to range between 194 to 590 nm and zeta potential between 5.09 to 9.54 mV. Entrapment efficiency and *in vitro* drug release ranged between 66 to 88% and 56.2 to 86.4 % respectively.

It was observed that the batch F3 and G3 showed the maximum amount of drug release in 8 hr, along with good amounts of % entrapment efficiency and least particle size in each group of formulations respectively. These batches were used for *in-vivo* studies.

Polydispersity index (PDI) is used to express droplet size uniformity and it varies from 0.0 to 1.0. PDI is the ratio between the standard deviation to mean droplet size. The closer to zero the polydispersity value the more homogenous are the droplets.[17] The values of the polydispersity index were observed to be 0.3 to 0.5 indicating that, considerable homogenous nature of particles.

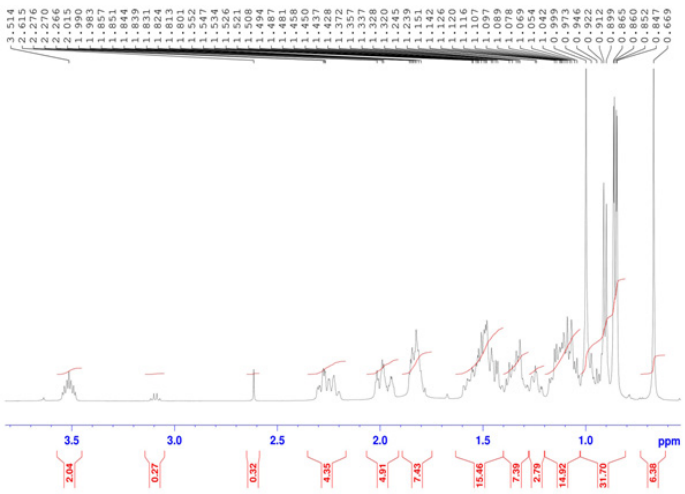

Figure 2: ^{1}HNMR spectrum of cholesterol glutathione conjugate.

Entrapment efficiency

Percent entrapment efficiency of the liposomal dispersion was found to be in the range of 72.4 to 92.1%. While preparing liposomes, drug mixed with lipid gives more entrapment efficiency as compared to drug mixed with aqueous phase and then encapsulated into liposomes. The percent entrapment efficiency of the liposomal dispersion was found to be in the range of 72.4 to 92.1%. While preparing liposomes, drug mixed with lipid gives more entrapment efficiency as compared to drug mixed with the aqueous phase and then encapsulated into liposomes.

In-vitro drug release

The drug release of the non-conjugated batch was ranged between 59.2 to 90.2% whereas the HSG-CHL conjugated batch showed drug release of between 56.2 to 86.4%. This decrease in the levels of drug release in the case of conjugated liposomes could be because of the greater particle size of conjugated liposomes, as the conjugated GSH would take a longer time to get across the dialysis membrane into the receiving compartment containing dissolution fluid.

Scanning electron microscopy

Conjugated liposomes exhibited a higher range of particle size and non-spherical shape. Both the observations could be attributed to the attachment of the GSH to the liposomes. The images are shown in Figure 3.

In-vivo plasma and brain level studies

The mean pharmacokinetic parameters calculated from the plasma levels of rizatriptan in rats after administration of marketed oral tablet, non-conjugated liposomes and GSH-CHL conjugated liposomes showed great variations. The values are summarized in Table 3 and 4. Peak plasma concentration of 150.19, 320.55 and 410.12 ng/ml respectively for these formulations showed a remarkable increase over oral delivery.

Table 2: Evaluation of liposomes formulations.

Formulation	Zeta Potential Mv	Particle size nm	PDI	Entrapment efficiency %	*In-vitro* drug release %
F1	10.34±0.5	290±7.2	0.31	81.5±1.5	75.3±1.3
F2	14.54±0.2	380± 10.3	0.36	85.2± 1.6	66.1±1.7
F3	12.90±0.1	181± 12.6	0.45	88.1± 1.4	90.2±0.9
F4	11.51±0.1	245± 5.4	0.42	76.3± 1.7	87.3±1.5
F5	16.53±0.1	355± 14.9	0.40	82.4± 1.5	69±1.8
F6	14.60±0.1	470± 12.7	0.34	92.1± 1.7	61.1±0.8
F7	19.04±0.1	195± 16.1	0.37	70.7± 1.8	80.2±1.5
F8	17.09±0.5	280± 16.5	0.50	78± 1.6	70±1.3
F9	12.54±0.1	540± 25.2	0.40	80.6± 1.9	59.3±0.9
G1	8.34±0.2	305± 12.3	0.43	78.2±1.2	72±2.1
G2	9.54±0.1	402±18.5	0.36	82.3±1.5	62±1.1
G3	5.90±0.1	194± 22.7	0.50	84.9±1.7	86.4±1
G4	7.51±0.2	272± 8.8	0.35	72.4±1.6	83.4±1.9
G5	6.53±0.1	370± 17.4	0.30	79.2±1.8	66.2±1.2
G6	7.60±0.2	490± 15.9	0.38	88±1.4	57.1±0.5
G7	6.04±0.1	220± 10.2	0.49	66±0.9	78.1±1.2
G8	5.09±0.3	291± 19.6	0.46	75.2±1.1	66.4±1.3
G9	9.54±0.2	590± 32.2	0.32	77.5±1.4	56.2±0.7

n=3. Values mentioned above are mean of three observation with standard deviation.

Table 3: Plasma and brain levels of rizatriptan in rats after administration of oral and liposomes formulations.

Time (min)	Marketed oral tablets		Non Conjugated liposomes		GSH-CHL Conjugated Liposomes	
	Plasma level (ng/ml)	Brain level (ng/ml)	Plasma level (ng/ml)	Brain level (ng/ml)	Plasma level (ng/ml)	Brain level (ng/ml)
0	0	0	0	0	0	0
30	57.12±4.1	24.12±0.5	80.43±16.2	59.15±0.6	138.54±21.1	82.45±4.2
60	89.41±16.2	36.23±0.3	320.55±23.5	102.68±1.8	410.12±32.8	161.72±11.2
90	132.22±21.4	68.54±0.2	240.64±31.3	135.42±1.9	270.24±28.4	310.46±21.3
120	150.19±24.5	79.16±0.6	170.36±19.7	120.56±2.1	195.28±21.3	170.24±19.7
150	38.11±3.1	56.37±0.7	82.44±9.1	82.61± 0.8	145.61±19.5	124.14±11.4

n=3, Values obtained as mean of three readings with standard deviation.

Table 4: Mean pharmacokinetic parameters after administration of rizatriptan as oral marketed oral tablet and liposomes formulations.

Parameter	Marketed oral tablet	Non Conjugated liposomes	GSH-CHL Conjugated Liposomes
C_{max} (ng/ml)	150.19	320.55	410.12
t_{max} (min)	120	60	60
AUC (ng.hr/ml)	223.99	426.6	543.49

Table 5: Drug targeting efficiency % and direct nose to brain transport % for targeted and non-targeted liposomes.

Formulation	DTE%	DTP%
Non-conjugated liposomes	370.13	20.05
GSH-CHL conjugated liposomes	1616.50	63.58

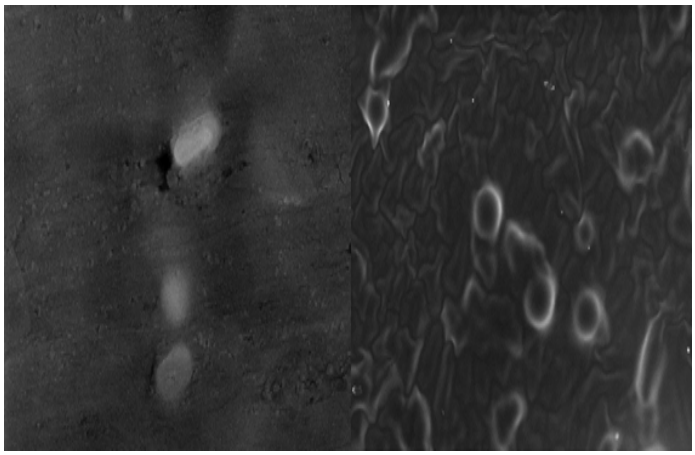

Figure 3: Scanning electron microscopic image of (a) non-conjugated liposomes and (b) CHL-GSH conjugated liposomes.

Drug targeting efficiency (%DTE) and nose to brain transport (% DTP)

Drug targeting efficiency and direct nose to brain transport were evaluated and mentioned in Table 5. Both types of liposomes differed significantly in the magnitude of brain targeting. A considerable direct nose to brain transport was also detected. These values demonstrate the efficacy of GSH-CHL conjugated liposomes for brain targeting of rizatriptan.

DISCUSSION

The objective of the work was to improve bioavailability of rizatriptan over oral delivery and to achieve brain targeting using GSH. Blood brain barrier is more accessible to liposomes owing to their lipid nature. Liposomes which have principle constituent as lipid often need a rigidizing agent. Cholesterol is frequently for this purpose. In the present work cholesterol was labelled with GSH through the synthesis of an intermediate cholesterol acrylate. Simultaneously liposomes with free cholesterol were also prepared to compare and explore the efficacy of GSH in brain targeting. In order to select between natural and synthetic lipids, stability of them was a prime consideration. Synthetic lipids are more stable as compared to those obtained from natural sources which are more susceptible to oxidation due to higher levels of polyunsaturated fatty acids. Lipoid S100 (synthetic soya phosphatidylcholine) is hydrogenated soya phosphatidylcholine, mainly a mixture of C16 and C18 fatty acid and soya phosphatidylcholine. Liposomes were prepared and characterized to select the optimum batch from each group i.e. non conjugated and GSH-CHL conjugated liposomes.

Increase in the levels of cholesterol was found to increase the particle size and decrease the drug release. Cholesterol makes the liposomes rigid and does not allow the drug to freely permeate across the liposomal layers; also during ultra-sonication, the formation of small uni-lamellar vesicles from multi-lamellar vesicles becomes difficult because of the rigidizing effect shown by higher values of cholesterol. On the other hand, Lipoid S 100 had a positive effect on entrapment efficiency as an increase in levels of lipid markedly increased levels of % entrapment efficiency.[18,19] Particle size was observed to be more for conjugated liposomes. GSH conjugated with cholesterol is responsible for forming liposomes of larger size. The difference in particle size of two types of liposomes was significant. Low values of the polydispersity index indicated the homogenous size of the liposomes.

Zeta potential plays an important role to keep the liposomes separated or aggregated. It is generated by the electrical double layer formed around the liposomes which also moves along with the particle when the particle is in motion in the solution. Generally, an increase of electrostatic repulsive forces between liposomes prevents the coalescence of particles. Whereas phase separation can be caused by decrease in electrostatic repulsive forces.[20] All the excipients used were non-ionic in nature; the liposome thus formed carried a positive charge. Lipoid S 100 contains C=O, phosphate and N function in its structure. It exists in zwitterion form. As the pH of the aqueous system was adjusted to the slightly acidic side of pH 6.4, the free H atoms are drawn by phosphate group making

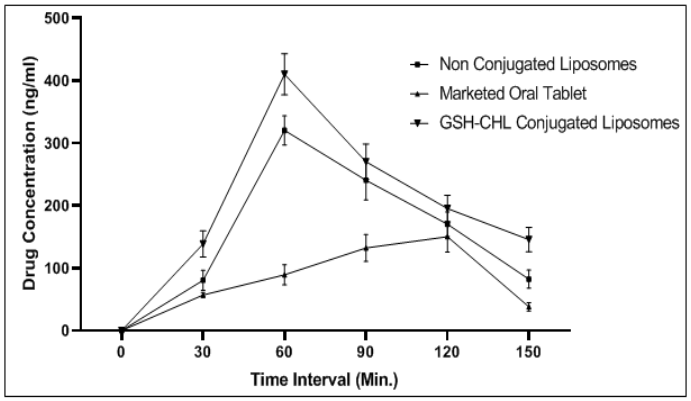

Figure 4: Plasma levels of rizatriptan in rats after administration of marketed oral tablet and liposomes formulations. Study was carried out in three replicates (*n*=3). The values of mean ± SD are mentioned in Table 3.

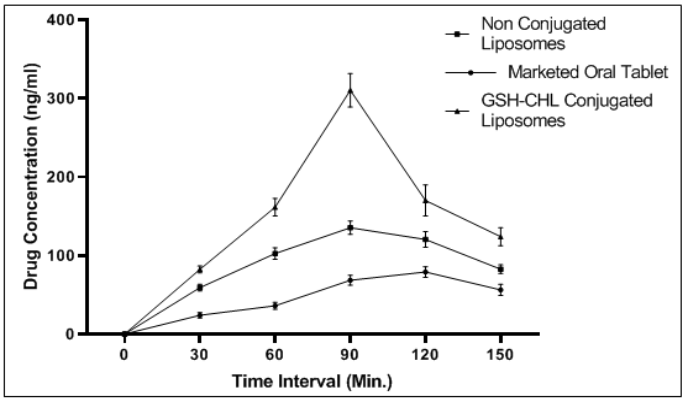

Figure 5: Brain levels of rizatriptan in rats after administration of marketed oral tablet and liposomes formulations. Study was carried out in three replicates (*n*=3). The values of mean ± SD are mentioned in Table 3.

the N function imparting a positive charge to the structure. Hence the liposomes prepared carried a positive charge on them. The slight positive charge is beneficial for nasal administration as it would increase mucoadhesion with negatively charged mucosal lining. Sialic groups present on mucin are responsible for carrying negative charge which is why the liposomes are likely to interact with these groups.[21] In the case of conjugated liposomes, the presence of GSH which contains N function; reduces the positive charge slightly as compared to non-conjugated liposomes. Hence, the conjugated liposomes showed slightly lesser positive values of zeta potential than the non-conjugated liposomes.

Entrapment efficiency was little higher in the non-conjugated liposomes than the GSH-CHL conjugate group. There has been a change in the characteristics of cholesterol after the formation of a conjugate with GSH. The effect of cholesterol on liposomal lamella was pronounced as the conjugated batch showed a marked reduction in encapsulation efficiency. As cholesterol is a major factor in influencing the liposome's efficiency to enclose the drug, clearly change in its characteristics has been observed in terms of entrapment efficiency values.

The rigidity provided by cholesterol decreases the chances of solutes leaching out of the liposomes and also increases the drug permeation time through the lipid bilayer. Relating to this effect the drug release values decreased with an increase in levels of cholesterol.[22] Both types of liposomes (transmucosal nasal route) and oral formulation of rizatriptan were administered in rats and plasma and brain levels were studied. For liposomes, the plasma peak was achieved in 1 hr, much faster as compared to 2 hr for oral delivery. Similarly, rizatriptan achieved higher levels in the brain as compared to oral delivery when administered through liposomes via nasal route. Both plasma level and brain level profiles of rizatriptan as shown in Figures 4 and 5 demonstrate the superiority of the nasal transmucosal route. AUC values were found to enhanced by at least twice over oral delivery. The nasal route of administration bypasses the first-pass metabolism in the liver delivering higher concentrations in systemic circulation also at a faster rate.[23,24]The nasal transmucosal route was found to increase plasma levels of rizatriptan which contributed to its increased concentration in target organ i.e brain. Secondly, the nasal route can take the drug towards the brain via the olfactory pathway. Lipophilic nature of liposomes that act as drug carrier enhances the permeation across the BBB as compared to the free drug.[25] Enclosed inside GSH-CHL conjugated liposomes, rizatriptan gets selectively targeted towards brain as glutathione receptors are exclusively present in brain. GSH is an antioxidant which handles reactive oxidative species and protects tissues from oxidative damages. GSH receptors are present in abundant numbers in the brain. Transport across BBB requires special mechanisms. Active transport of certain species across BBB involves proteins located in the brain that can move across the membrane. GSH interacts with them. This GSH transport mechanisms can be utilized in brain targeting across the BBB.[26] GSH conjugated species enjoy easy passage through the BBB. Drug delivery systems can be conjugated with GSH and used for brain targeting. The increased levels of rizatriptan in brain could be due to a combined effect of GSH receptor binding and olfactory pathway i.e. nose to brain transport. Thus GSH was found to be very effective for brain targeting of liposomes of rizatriptan.

CONCLUSION

The present work concludes that bioavailability and brain targeting of a drug like rizatriptan can be effectively enhanced through nasal transmucosal route. Conjugate of glutathione with cholesterol can act as driving species for the liposomes across the blood brain barrier. This approach overcomes major drawbacks of oral delivery of rizatriptan. This enhancement in bioavailability may help in reducing dose and side effects of rizatriptan as well as may offer a promising non-invasive approach for its systemic delivery.

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CONFLICT OF INTEREST

The author declare no conflict of interest.

ABBREVIATIONS

BBB: blood brain barrier; **GSH:** glutathione; **CHL:** cholesterol; **PEG:** polyethylene glycol; **FTIR:** Fourier transform infra-red spectrophotometry; **^{1}HNMR:** nuclear magnetic resonance; **PDI:** Poly dispersity index; **EE:** entrapment efficiency; **DTE:** the drug targeting efficiency; **DTP:** nose-to-brain direct transport; **C_{max}:** Peak Plasma Concentration; **t_{max}:** Time of Peak Plasma Concentration; **AUC:** Area under Curve.

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