

Formulation and *in vivo* Evaluation of Niosomes containing Oxcarbazepine

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Abstract

The purpose of this research work was to prepare Oxcarbazepine niosomes to improve the anticonvulsant activity. The nonionic surfactant vesicles were prepared by the thin film hydration method. The lipid mixture consists of drug, cholesterol, and surfactant in the molar ratio of 1:1:3 respectively to achieve prolonged circulation time and sustained release. The *in vivo* study revealed that the prepared niosomal dispersion shows improved anticonvulsant activity of Oxcarbazepine in albino wistar rats. After a single dose of the niosomal characteristics.

In conclusion the niosomal formulation could be a promising delivery system for Oxcarbazepine with improved anticonvulsant activity,

Key words: Oxcarbazepine, Niosomes, Anticonvulsant Activity, *In vivo* studies.

INTRODUCTION

Epilepsy is among the most prevalent of the serious neurological disorders, affecting, from 0.5 to 1% of the world population¹. Interestingly, the prevalence of epilepsy in developing countries is generally higher than in developed countries². Epileptic seizures are paroxysmal clinic events arising from neuronal hyper-excitability and hyper-synchrony of the cerebral cortex, either locally initiated neuronal activity that occurs during a seizure is caused by a sudden imbalance between the inhibitory and excitatory signals in the brain with gamma amino butyric acid, noradrenaline respectively being the most important neurotransmitters involved³.

Oxcarbazepine is one of the most effective drug in the treatment of epilepsy⁴. Niosomes lipid bilayered vesicles in effects. A vehicle for drug formulation, they may reduce the systemic toxicity of clinically important anticonvulsant agents. Niosomes may improve therapeutic index by restricting drug effects to target cells⁵. The applications of the conventional drug delivery system have been limited by their short circulation half-life. In this content the niosomal formulation has a relatively long half-life than the conventional dosage forms in blood circulation and expected to an altered bio distribution *in vivo*. Niosomes are nonionic surfactant vesicles that one well recognized as drug delivery vehicles. Niosomes can carry hydrophilic drugs by encapsulation are quite stable, and require no

In the present study Oxcarbazepine loaded niosomes were formulated and evaluated for *in vivo* release and pharmacokinetic parameters.

MATERIALS AND METHODS

Materials

Oxcarbazepine was obtained as a gift sample from Micro Labs, Hosur. Cholesterol and Spans were purchased from Qualigens fine chemicals Ltd, Mumbai. All other materials used in the study were of Analytical grade.

Formulation of Oxcarbazepine Niosomes

Oxcarbazepine niosomes were prepared by Thin Film Hydration Technique using Rotary Flash Evaporator⁶. Weighed quantity of cholesterol and surfactant were weighed into a round bottom flask and dissolved in a thermostatically controlled water bath at 60°C ± 2°C. The solvent was then removed by rotary evaporation. The residue was then dissolved in a small amount of chloroform. The chloroform was then removed by rotary evaporation and a slimy layer was deposited on the flask. To the thin dry lipid formed, Oxcarbazepine solution was added previously dissolved in 10ml of phosphate buffer saline pH 7.4 and the mixture was then subjected to rotary evaporation for removal and dispersion^{9,10}.

The niosomal suspension so formed was then transferred to a suitable glass container and sonicated for 30 minutes using bath sonicator in an ice bath for heat dissipation. The sonicated dispersion was then allowed to stand for about 2 hours at room temperature to form niosomes. The formulation was sterilized by passing into 0.2 µm membrane filter and stored in a refrigerator¹¹.

Table number -1: Formulation of Oxcarbazepine niosomes

Formulation Batch Code	Oxcarbazepine	Cholesterol	Span 40	Span 60	Span 80	Chloroform	Methanol	Phosphate Buffer Saline pH 7.4	Drug+ Cholesterol + Surfactant Ratio
F - I	10mg	10mg	30 mg	-	-	10 ml	10 ml	10ml	1:1:3
F- II	10mg	10mg	-	30mg	-	10 ml	10 ml	10ml	1:1:3
F- III	10mg	10mg	-	-	30mg	10 ml	10 ml	10ml	1:1:3

IN VIVO STUDIES

Animals used

Albino wistar rats weighing (150-200g) of male rat were obtained from the animal house in Periyar College of Pharmaceutical Sciences, Trichy. The animals were maintained in a well ventilated room with 25°C to 30°C, 12 hours light / dark cycle in poly propylene cages¹². The animals were fed with standard pellet feed and water ad libitum. All experiments performed in this study were reviewed and accepted by the Institutional Animal Ethics Committee (IAEC) PCP /IAEC/011/2012.

The therapeutic effectiveness of the developed delivery system is evaluated by the *in vivo* experiments. *In vivo* evaluation study was carried out for the best FII formulation which has shown better performance than other formulations during *in vitro* study.

In vivo evaluation of Oxcarbazepine niosomes

The *in vivo* evaluation study design of formulated niosomes was carried out in albino wistar rat which was already given electroshock through a pair of ear electrodes. Rat of male sex weighing 150-200gm were selected for the study and they were fed with a standard pellet diet and water ad libitum¹³. The animals were divided into 3 groups each group containing 6 animals. The groups under treatment were designed as follows.

- Group I : Treatment control
- Group II : Pure Oxcarbazepine drug solution
- Group III: Niosomes FII formulation

The drug treatment of 10mg/kg was given the day of electroshock previously the rats were kept at 12 hours fasting. A catheter was implanted in the femoral artery with the outlet exiting cut the neck to facilitate blood sampling. Blood samples were collected from the animals at the intervals of 0.5hours, 2hours, 4hours, 8hours, 12hours and 16 hours from the catheter were continuously flushed with physiological saline. The collected blood samples were centrifuged in a cooling centrifuge at 12000 rpm for 3minutes and blood plasma is separated. The separated blood plasma was stored in a freezer at -4°C until use.

HPLC Analysis of Oxcarbazepine

The concentration of Oxcarbazepine was measured by HPLC after addition of trichloroacetic acid (2mm, 5µl) Plasma (100µl) to precipitate plasma proteins. It is filtered and the filtrate was analyzed by HPLC¹⁴. This treatment of the sample did not affect Oxcarbazepine levels. The retention time was 2.8 ± 0.4 min.

Analysis of plasma levels of Oxcarbazepine

A simple and rapid HPLC assay was developed for determination of Oxcarbazepine. The HPLC system consisted of a Shimadzu LC-6AD using ODS column (25cmx0.46mm) and a model SPD-6A UV detector

(Shimadzu, Japan). The wavelength was set at 255 nm¹⁵. The stationary phase was 5mm Budpen (C18 (0.39x30cm) column the mobile phase was aqueous 0.02m potassium dihydrogen phosphate acetonitrile- methanol. The flow rate was set at 1.2 ml /min⁻¹.

Estimation of Oxcarbazepine in plasma samples

The plasma samples stored in the freezer was brought to room temperature and 24 µl of internal standard (2.0 µg/ml) was added to each of the plasma sample. The amount of drug present is determined by HPLC and the chromatogram was recorded. The concentration of Oxcarbazepine was determined from the ratio of area under the curve of Oxcarbazepine for each plasma sample using the standard curve¹⁶.

Pharmacokinetic Analysis

Pharmacokinetics parameters were calculated from the individual plasma concentration time curves for Oxcarbazepine after the administration of its free solution (or) niosomal dispersion. The values of peak height (C_{max}) and peak time (T_{max}) were obtained directly from the individual plasma drug concentration time curves. The areas under the serum concentration time curves (AUC) were estimated by linear trapezoidal rule. The terminal elimination rate constants (K_e) were calculated by applying linear regression on the log concentrations time curve. Half life ($t_{1/2}$) was calculated as 0.693 divided by K_e . The absorption rate constants (K_a) determined from the plasma concentration time data. The mean residence time (MRT) corresponding to transit time of drug in the body was calculated from equation $MRT = 1/K_a + 1/K_e$.

Preparation of standard Curve

A series of standard solution of Oxcarbazepine was prepared by diluting a stock solution (1mg/ml of Oxcarbazepine) in water so as to give solution containing 10 µg/ml, 15 µg/ml and 20 µg/ml of Oxcarbazepine. Plasma samples were prepared by mixing each of the above solution with equal volume of Oxcarbazepine free plasma. An aliquot of 20 ml of Oxcarbazepine solution in water (2.0 µg/ml) was added as internal standard to each of the above plasma samples. The amount of drug present is determined by HPLC method and the chromatograms were recorded. A standard curve was plotted by taking the concentration of Oxcarbazepine in plasma in X-axis and corresponding ratio of area under the curve of Oxcarbazepine in Y-axis

Statistical analysis

All the values were expressed as mean ± SEM. The data was statistically analyzed by one-way ANOVA followed by Dunnett test. P values < 0.01 were considered significant.

RESULT AND DISCUSSION

In vivo evaluation of Oxcarbazepine niosomes

Based on *in vitro* evaluation the FII formulation was selected to carry out the pharmacokinetic study.

The niosomes prepared with span 60 markedly altered the pharmacokinetic profile of Oxcarbazepine. The serum levels of Oxcarbazepine were significantly higher in Oxcarbazepine entrapped niosomes and free Oxcarbazepine in the form of solution.

As niosomes FII formulation showed prolonged release of drug *in vitro* studies and got increased entrapment efficiency and stability they were selected to carry out the *in vivo* studies. Standard curve for Oxcarbazepine drug was plotted and the data was given in Table number 2 and Figure number 1.

The parameter evaluated were absorption rate constant, elimination rate constant, absorption half life and elimination half life, T_{max} , C_{max} , AUC, AUMC and MRT.

The elimination half life of niosomes FII formulation were found to be 21.65 hours when compared to free drug having 1.080 hours as $t_{1/2}$. This shows that niosomes FII formulation will have prolonged circulation when compared to free Oxcarbazepine.

The elimination rate constant was found to be 0.032 and 0.641 hours for FII niosomes formulation and free Oxcarbazepine respectively.

The pharmacokinetic parameter T_{max} was found to be 4 and 0.5 hours for Niosomes FII formulation and free Oxcarbazepine respectively.

C_{max} was found to be 49.54 and 26.39 mg.hr/ml for Niosomes FII formulation and free Oxcarbazepine.

These results show that the time taken to reach the peak serum concentration was higher for Niosomes FII formulation. When compared to free Oxcarbazepine the serum concentration at the particular time was found to be lower Niosomes formulation. This shows that niosomes FII formulation were found to be prolonged circulation time.

The area under the peak serum concentration curve was found to be 731.56mg hr/ml for FII formulation for free Oxcarbazepine it was found to be 62.07mg hr/ml. The elimination of Oxcarbazepine from the rate was slower when given as niosomes. A notable increase in the area

under the Oxcarbazepine concentration time curve and MRT of Oxcarbazepine could be noticed after injection of Oxcarbazepine entrapped niosomes containing drug as compared to free Oxcarbazepine. The MRT was calculated as 9.678, 1.719 hours for niosomes FII formulation and free Oxcarbazepine respectively. The data were shown in Table number 6.

The niosomes did not give a sharp peak in the serum concentration curve, but produced a sustained plateau of the drug. This may be due to the slow rate of release and a longer residence time.

The niosomes were superior to free Oxcarbazepine in terms of reducing the peak and maintenance of drug concentration in serum. AUC was also higher for niosomes FII formulation when compared to free Oxcarbazepine. Niosomes FII formulation showed a substantial increase in blood circulation.

Thus it was evident that niosomes made with span 60 entrapped with Oxcarbazepine was more effective compared to free Oxcarbazepine.

Table number - 2: Standard curve for Oxcarbazepine (HPLC)

Concentration ($\mu\text{g/ml}$)	Area
10	13962
15	22312
20	27849

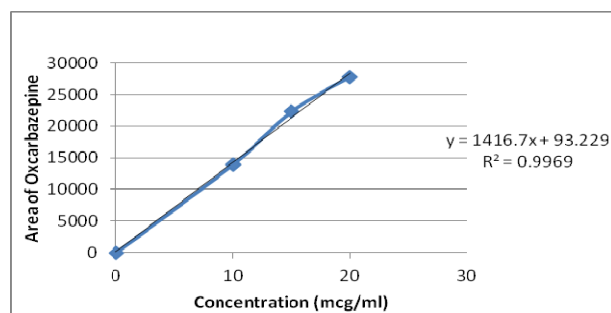


Figure 1: Standard curve for estimation of Oxcarbazepine through HPLC in plasma

Table number - 3 Plasma concentration of pure Oxcarbazepine after intraperitoneal injection in rat (HPLC determination).

S. No	Time (hr)	Concentration($\mu\text{g/ml}$)	CT ($\mu\text{g hr/ ml}$)	AUC ($\mu\text{ghr/ml}$)	AUMC ($\mu\text{ghr}^2/\text{ml}$)
1	0.5	26.39	13.197	37.038	44.382
2	2	22.99	45.98	25.034	54.179
3	4	5.620	8.1796	-	-

$[\text{AUC}]_0^4 = 62.072 \mu\text{ghr/ml}$

$[\text{AUMC}]_0^4 = 98.5616 (\mu\text{ghr}^2/\text{ml})$

Table number- 4: Plasma concentration of (F_{II}) formulation after intraperitoneal injection in rat (HPLC determination)

S. No	Time (hr)	Concentration ($\mu\text{g/ml}$)	CT ($\mu\text{ghr/ml}$)	AUC ($\mu\text{ghr/ml}$)	AUMC ($\mu\text{ghr}^2/\text{ml}$)
1	0.5	26.13	13.06	50.54	71.68
2	2	41.26	82.52	90.80	280.68
3	4	49.54	198.16	191.88	1138.72
4	8	46.40	371.2	144.60	1364.00
5	12	25.90	310.8	253.74	4224.96
6	16	16.39	393.36	-	-

$[\text{AUC}]_0^4 = 731.56 \mu\text{ghr/ml}$

$[\text{AUMC}]_0^4 = 9680.04 (\mu\text{ghr}^2/\text{ml})$

Table number -5: Estimation of Oxcarbazepine in plasma

S. No	Formulation	Dose Recovered ($\mu\text{g} / \text{ml}$)					
		0.5 hr	2 hr	4 hr	8hr	12 hr	16 hr
1	Oxcarbazepine free drug Concentration	26.39	22.99	5.620	-	-	-
2	Niosome (F_{II}) formulation	26.13	41.26	49.54	46.40	25.90	16.39

Table Number- 6: Pharmacokinetic parameters

Parameter	For pure Drug Oxcarbazepine	Oxcarbazepine FII formulation.
C_{\max}	26.39 $\mu\text{g} \cdot \text{hr} / \text{ml}$	49.54 $\mu\text{g} \cdot \text{hr} / \text{ml}$
t_{\max}	0.5hrs	4 hrs
AUC	62.07 $\mu\text{g} \cdot \text{hr} / \text{ml}$	731.56 $\mu\text{g} \cdot \text{hr} / \text{ml}$
AUMC	98.56 $\mu\text{g} \cdot \text{hr}^2 / \text{ml}$	9680.04 $\mu\text{g} \cdot \text{hr}^2 / \text{ml}$
MRT	1.7195 hrs	9.678 hr
Elimination rate constant	0.6411 hr^{-1}	0.032 hr^{-1}
Elimination $t_{1/2}$	1.080 hrs	21.65 hrs
Absorption Rate Constant	0.544 hr^{-1}	0.133 hr^{-1}
Absorption $t_{1/2}$	1.272 hrs	5.198 hrs

CONCLUSION

Niosomes are novel form of drug delivery. They offer a great way of delivering drugs at a higher efficacy and lower toxicity. The prepared Oxcarbazepine niosomes have unilamellar spherical vesicles shape.

In this study the niosomal formulation were formulated and evaluated to explore the advantages of Oxcarbazepine niosomes over the conventional dosage forms. Pharmacokinetic studies have shown that Oxcarbazepine niosomes have increased elimination half life and the area under the curve also higher when compared with pure Oxcarbazepine and justify their potential in strengthening the efficacy and safety profile of the drug. Thus the prepared niosomes could be promising delivery system for Oxcarbazepine with sustained drug release profiles.

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