Nanoparticulate Drug Delivery: A Newer Drug Delivery Concept

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Abstract

Aim: To compare change in intravitreal drug concentration with time for pristine (plain) and nanoparticulate drug.

Method: Pristine drug solution & liposome nano-particulate prepared. Pristine & Nano particulate administered by topical, subtenon and intravitreally. Vitreous sample were collected at different interval & drug concentration was measured by High Performance Liquid Chromatography (HPLC).

Results: The concentration of drug in vitreous was more in intravitreal group as compared to subtenon group at different time interval. Nano particulate drug was present for long duration in vitreous as compared to pristine.

Conclusion: Nano particulate drug prolong the drug action and can reduce number of intravitreal injections as compared to pristine.

Introduction

Eyeball is divided as anterior and posterior segments. Drug delivery to the eye can vary in case from the simple topical eye drop, which rapidly penetrates to the anterior chamber, to the complicated engineering skills required to develop intravitreal implants. The anterior segment and the posterior segment are two entirely different ocular regions and the challenges faced in delivering therapeutic drugs to each of these areas are unique.

Drug delivery to eye can be by topical, subconjunctival, subtenon, periocular, intravitreal and systemic routes. To reach the drug in posterior segment in maximum concentration we have to inject drug by intravitreal injections. Many times intravitreal injections have to be repeated like in age related macular degenerations, diabetic macular edema...etc.

To prevent multiple intravitreal injection related complications either we may decrease the frequency of intravitreal injections or we may change the route of administering the drug.

Nanoparticle are very small size particle the formulations which can incorporate drug inside it or over its surface. Nano particulate drug provide the slow drug release so it prolong the drug action and that may decrease the frequency of intravitreal injections.

Aim of study

To compare change in intravitreal drug concentration with time for pristine(plain) and nanoparticulate drug after administering dexametha-sone and it's liposomal formulation by following routes:

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- Topical
- Subtenon
- Intravitreal

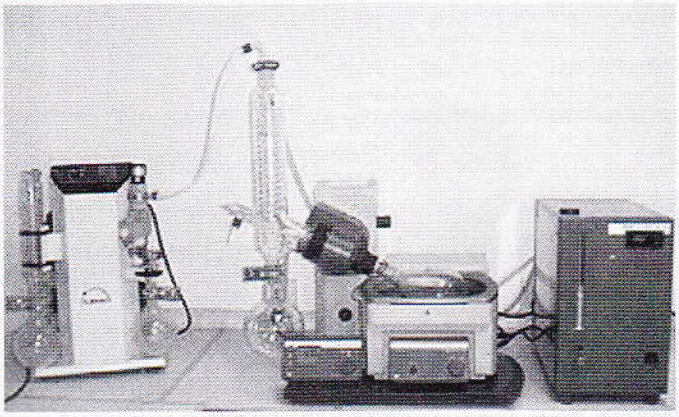
Material and method:

- Material:
  - Dexamethasone sodium phosphate.
  - Drugs for liposomal nano formulations.
  - HPLC machine.
  - New Zealander Rabbits
- **Nanoparticle preparation :**

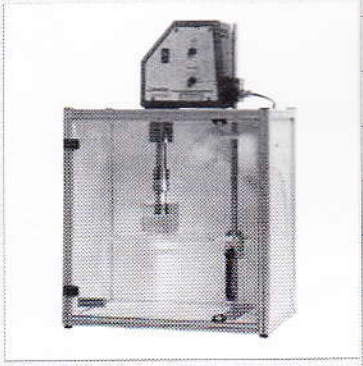
After weighting following contents were mixed in a round bottle flask.

Content name	Amount
Phosphatidylcholine	70mg
Cholesterol	18mg
Tocopherol polyethylene glycol (Vitamin E TPGS)	14mg
Chloroform	2ml
Methanol	4ml

Organic solvents were removed completely by a rotary flash evaporator (IKA® RV 10) above the lipid transition temperature (51 °C) at 75 rpm for 3 h to obtain a uniform thin lipid film on the wall of the flask. 10 ml distilled water was added to make it 1 mg/ml sample and mixed by the same.



Small unilamellar vesicles (SUV) were obtained by subjecting the dispersion to probe sonication (Ultrasonic Processor, UP200S, Hielscher Ultrasound Technology) for 1, 3, 5 min using 6 mm ultrasonic probe at 60 % amplitude and 0.5 cycles per second.



Sample	Dexamethasone (in mg)	Probe sonification time (minutes)
A1	10	1
A2	10	3
A3	10	5
B1	20	1
B2	20	3
B3	20	5
C1	30	1

- **Characterization of nanoparticles:**

Nano formulation was subjected to **particle size and polydispersity index analysis** using Photon Correlation Spectroscopy (PCS) Delsa Nano C (Beckman Coulter Counter, USA) particle Size analyzer.

Zeta potential of nano suspension was measured using a Delsa Nano C (Beckman Coulter, conter, USA).

The **per cent encapsulation** of DEX in DEX-Lipo was determined by direct method using Ultra Violet Spectroscopy.

- **In vitro release:**

In – vitro drug release study of selected batches were determined in distilled water as a dissolution medium using dialysis bag diffusion method.

Sample was analyzed using Ultra violet spectroscopy at 260 nm.

- **In vivo administration and vitreous sampling**

After taking institutional animal ethical clearance the procedure was performed on rabbit eyes.

- Groups: Total 24 rabbits were included in this study.
  - Species/Common name :New Zealander Rabbits
  - Weight : 2-2.5 KG
  - Gender : Male
  - Total Number of rabbits : 24
  - Number of day rabbit housed : 30 days.

Total number of groups were 4 and in each group 6 rabbits were included.

Group A : Rabbits in this group were applied with 100µl of topical pristine dexamethasone.

Group B : Rabbits in this group were applied with 100µl of subtenon liposomal nano formulation of dexamethasone.

Group C : Rabbits in this group were applied with 100µl of subtenon pristine dexamethasone.

Group D : Rabbits in this group were applied with 100µl of subtenon liposomal nano formulation of dexamethasone.

Group E : Rabbits in this group were applied with 100µl of intra vitreal pristine dexamethasone.

Group F : Rabbits in this group were applied with 100µl of intra vitreal liposomal nano formulation of dexamethasone.

100µl of vitreous humor was aspirated from all rabbits at day1st, day3rd, day7th, day14th and day 21st from each study included eye.

B. Anaesthesia

C. Drug administration

- We instilled few drops of standard povidine iodine solution in eye and washed with balanced salt solution.
- After this in each group according to the study we administered the drug in eyes by insulin syringe.

D. Vitreous sample taking:

- Anaesthesia given first as mentioned earlier .
- With proper technique, aspiration of vitreous done.
- Vitreous samples were collected through pars plana approach at predetermined time on day 1st, 3rd,7th,14th and 21st after dose under proper anaesthesia.

Sample analysis by HPLC

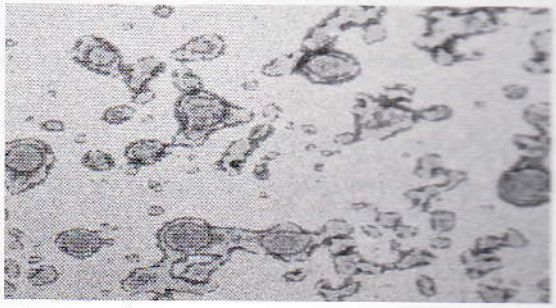
The collected Vitreous samples were stored at −20 ºC until the analysis. The Vitreous samples were with 100 of mobile phase and was injected in to HPLC for analysis

Observation and Results:

- **Characterization of nanoparticles:**

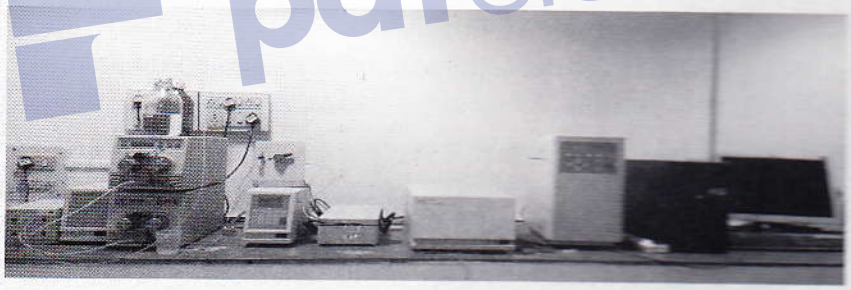
Formulation	Composition Dexamethasone (In mg)	Ultra Sonification time(in minutes)	Size(in nm)	Zeta potential	% Entrapment Efficiency
1	10	1	83.8	-9.74	74.04941
2	10	3	76.6	-5.91	65.74901
3	10	5	58.9	-6.95	62.7253
4	20	1	128.4	-11.11	50.45257
5	20	3	75.6	-10.84	44.64229
6	20	5	71	-6.83	17.48814

Liposomal nano formulation TEM (Transmission Electron Microscopy) image:



Vitreous sample analysis: (done by HPLC)

After the injection as mentioned earlier, vitreous sample was withdrawn on day 1st, 3rd, 7th, 14th and 21st and analyzed by the help of HPLC.



Results of Vitreous Sample Analysis (µl/ml)

Time in days	DEX-Lipo intravitreal	Pristine DEX intravitreal	DEX-Lipo subtenon	Pristine DEX subtenon	DEX-Lipo Topical	Pristine DEX Topical
1	30	27	13	9	9	5
3	23	13	10	4	7	3
7	15	5	7	0	3	0
14	4	0	0	0	0	0
21	0	0	0	0	0	0

- Overall the drug concentration was maximum for intravitreal injections at the time of injections on day 1.

- Subtenon administered drug also reached to the posterior segment but its concentration was less as compared to intravitreal injections.
- With time the pristine drug clearance was faster than the liposomal nano formulations.

Discussion:

- In our study we found that the drug concentration was more for intravitreal injections as compared to subtenon injections.
- The eyes where we injected nano particulate dexamethasone drug, concentration was more as compared to pristine drug and for longer duration
- Our study show that we can inject the drug by the subtenon route also for posterior segment diseases but the bioavailability is less in vitreous so we have to inject the more concentrated drug solution.
- These days endophthalmitis is most highlighted issue these days realated to intravitreal injections so this mode of drug delivery will decrease the number of injections and it may replace completely the requirement of intravitreal injections.

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