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DEVELOPMENT OF CARBOPLATIN DRUG DELIVERY SYSTEM FOR ITS OCULAR USE

Pharmacy

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ABSTRACT

Carboplatin is platinum based anti-carcinogenic agent useful in the treatment of Retinoblastoma. Experiments have been performed to formulate ocular patch of carboplatin in order to enhance residual time and prolong drug release. Using gelatin, ocular inserts were prepared, mixed with water. After properly autoclaving the mixture, drug at different concentration were incorporated into the gelatin-water mixture, DMSO (Di-Methyl Sulfoxide) was used as a solubility enhancer. The cross linking was done by pouring glutaraldehyde (GTA) on to the formulation mixture and allow for drying overnight. The patches were then evaluated for uniformity of thickness, folding endurance, loss on drying, tensile strength and in vitro and ex vivo drug release study. Finally, LC-MS/MS (Liquid Chromatography- Mass Spectroscopy/Mass Spectroscopy) study was carried out to see the drug release kinetics (in vitro and ex vivo). By this study the researcher took an attempt to formulate a superior ocular drug delivery system with pronounced therapeutic efficacy, and to improve the penetration of Carboplatin in eye to give localized targeted effect.

KEYWORDS

Retinoblastoma, gelatin patch, in vitro study, ex vivo study

INTRODUCTION [1-8]

Eye is often used for drug delivery. Topical application of drugs in the form of driblet or ointments to the eye is the well- established route for curing number of diseases like dryness, pink eye, eye flu etc. Due to activity of eye like tear flow, flickering, lacrimal nasal drainage etc., the preparations which are instilled in to the eye, rapidly drains away from the ocular surface. As the availability of the drug is less for its therapeutic effect, there is frequent dosing of drug. In order to solve these issues, new formulations of ophthalmic such as in-situ gel, nanoparticles, nanosuspension, microemulsion, intophoresis and ocular inserts have been made to enhance the bioavailability of the drug in controlled and sustained manner. The objective of the improvements is to maintain the drug in the bio phase at different time intervals. Ocular inserts are characterized as single, sterile, thin and multi-layered drug infused, solid or semisolid consistency devices, whose shape as well as size is particularly designed for application in eye. Ocular inserts which may or may not consist of drug, a polymer support is must. Later on, the drug is snared or disseminate or the drug can be subsume as a solution in the polymeric supports so that residence time of drug in the eye increases and a sustained release dosage form can be formulated. There are different types of inserts based on solubility as insoluble, soluble and bioerodible inserts. There are three procedures by which drug release from the inserts takes place, namely: 1) diffusion, 2) osmosis, and 3) bioerosion. The drug is subsumed as a solution or dispersion in insoluble inserts which are polymeric systems. Soluble inserts contains all monolytic polymeric devices will dissolve or erode at the end of their release. Bioerodible inserts polymers eliminate the need for removing the implant after complete drug release. The development of polymeric inserts and discs have been taken place to avoid the difficulties faced by conventional dosage form. From decreased frequency of administration and reduced incidence of systemic side effects, polymeric inserts gives accurate dosing, decreases systemic absorption and enhances the patient compliance. Also, nasolacrimal drainage and tear flow have least effect on the insert, thus providing suitable drug release and longer residence in cul-de-sac region.

Retinoblastoma is a cancer that originates in retina, where images are formed. It is mostly occur in children. The eye cells namely, retinoblasts splits into daughter cells and fill up the retina. At a particular point in time, the disintegration of cells ceases to exist, developing into mature retinal cells. It is very rare that something goes wrong in this process. Some retinoblasts continues to expand rapidly and out-of-control, instead of developing themselves into special cells that detects light and form a cancer called **retinoblastoma.** If these tumours are not put into medical attention, then they will rapidly break off from the retinal tumour and they will float through vitreous to reach other parts of the eye. The channels that distribute fluid throughout the eye are blocked by the tumour resulting in pressure increase within the eye. This causes **glaucoma**, one of the severe complications of retinoblastoma, which results in pain and loss of vision in the affected eye. The process that leads to retinoblastoma is rather complex, but it is always initiated by a mutation in a gene called the retinoblastoma (Rb or RB1) gene. The normal RB1 gene prevents cells from growing out of control. The majorily involved gene in retinoblastoma is the **RB1 tumor suppressor gene**. This gene forms a protein (pRb) that unable cells from growing rapidly. Each cell generally consists of 2 RB1 genes. Until retinal cell has at least one RB1 gene which works as it was suppose to, it will not form a retinoblastoma. If both of the RB1 genes are mutated or missing, a cell can grow uncontrollably. This can lead to further changes in the gene, as a result causing cells to become cancerous. Conventional system to administer carboplatin for the treatment of retinoblastoma is I.V route administration. This system is easy to administer but suffer from the inherent drawback of poor ocular absorption. In case of ocular drop, drug is diluted immediately in the tear film as soon as the eye drop solution is instilled into the cul-de-sac and is rapidly drained away from the precorneal cavity by constant tear flow and nasolacrimal drainage. Therefore, a very minute fraction of instilled dose is absorbed by the target tissue and hence frequent dosing is required to achieve an adequate level of therapeutic effect. Here we took an attempt to design a novel cross linked gelatin patch for management of retinoblastoma achieving a site specific delivery and minimising associated side effects.

MATERIALS AND METHODS

Carboplatin (Sigma Aldrich, India), Gelatin (Qualigens Fine Chemicals), Formic acid (Qualigens Fine Chemicals), Glutaraldehyde (Sigma Aldrich, India), acetic acid, Acetonitrile HPLC grade methanol, DMSO (Sigma Aldrich, India).

Preparation of drug loaded ocular patch

Ocular patches were prepared by solvent casting method. Gelatin was used as a polymer. Glutaraldehyde was used as cross linking agent and DMSO was used as solubility enhancer. Required quantities of gelatin and water as shown in table 1 were weighed accurately and transferred to beaker and mixed thoroughly with the help of magnetic stirrer and the formulation without drug was autoclaved. Autoclaved formulation was taken to a sterilized room and the sealed beaker was opened under laminar air hood. DMSO was added with continuous stirring. Drug (Carboplatin) was added to the solution and mixed thoroughly. Finally GTA (Glutaraldehyde), a cross linking agent was added. The formulation was immediately poured into petri dish to form patch of uniform thickness. Then the patch was allowed to dry under laminar flow for 4 hours. Then the patch was peeled using blade, and cut into 3mm×3mm, and finally stored at room temperature for further studies.

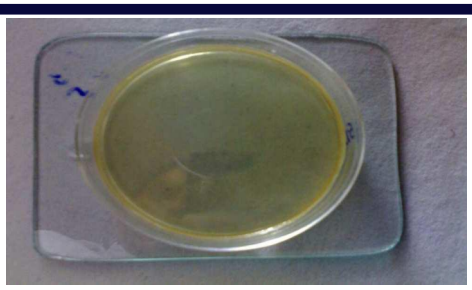

Fig 1: Formulation GP1 with 5mg drug

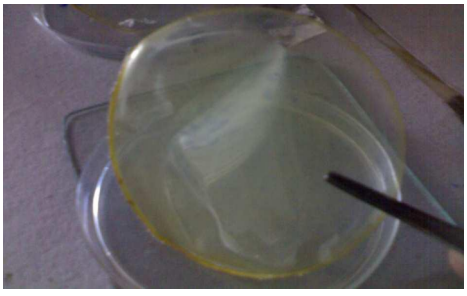

Fig 2: Formulation GP5 with 12.5mg drug

TABLE 1 FORMULATION TABLE FOR DRUG LOADED OCULAR PATCH

S.No	Formulation	Drug (mg)	Gelatin (mg)	Distilled water (ml)	Glutaraldehyde 10% (µl)	DMSO (µl)
1	GP1	5.0	400	5.0	100	400
2	GP4	12.5	400	5.0	100	400

EVALUATION OF CROSS-LINKED GELATIN -PATCH [12-19]

Patch thickness: Thickness was assessed by using digital micrometer (Mitutoyo Co., Kanagawa, Japan). Four patches from each batch were taken and mean and standard deviation were calculated.

Folding endurance: The film was repeatedly been folded from the same place till it broke in order to calculate folding endurance. It describes the ability of the film been folded number of times at the same location without breakdown

Loss on drying: The patches were cut into a dimension of 3mm×3mm and placed into MCT (Micro Centrifuge Tube) and weighed. Then the MCT along with the patch was kept in incubator in open conditions at a temperature of 60°C. At the time interval of 6hrs, 24hrs, 36hrs and 48hrs, the MCT along with patch were taken out and reweighed. This procedure was followed till the concordant reading came.

Tensile strength: It is used to know the modulus of elasticity, elastic limit, elongation, proportional limit, yield strength etc. A patch of dimension 2cm×1cm and two clamps were taken. One clamp was tied to the stand holding one end of the patch and the other clamp was tied to a conical flask (to which load would be added) holding other end of the patch. Water was added to the conical flask till the patch between the two clamps would break. Then the weight of the beaker along with the water was measured and tensile strength was calculated using the formula kg/cm^2.

In-vitro drug release studies: 25ml of 5% DMSO solution with deionised water was taken into a bottle with closer. Then a patch containing drug with dimensions 3mm×3mm was inserted into the bottle. The bottle along with patch was kept on water bath maintained at 37°C with occasional shaking. At the time intervals of 30min, 60min, 2hrs, 4hrs, 6hrs, 8hrs, 12hrs, 24hrs and 36hrs, 200µl of sample was taken and volume was replaced with 200µl of fresh 5% DMSO solution. The samples were stored at -20°C for further analysis by LC-MS/MS to determine the release kinetics of formulation.

LC-MS/MS METHOD FOR CARBOPLATIN[20,21]

Materials and reagents: Carboplatin was procured from Sigma Aldrich. Homatropine (Merck, Germany), water (18.2MΩ) was purified using a Milli-Q purificatioin system (Millipore Corp., Bedford, MA, USA).

Calibration standard and quality control samples

Carboplatin stock solution was prepared in Milli-Q water at 1mg/ml. Then the solution was diluted accordingly with 50% methanol that contains 0.1% formic acid.

Stock solution Homatropine 1mg/ml of internal standard (IS) was prepared by dissolving in Milli-Q water. It was further diluted and added to extracting solvent containing 90% acetonitrile and 0.1% formic acid to reach 500ng/ml.

LC-MS/M: Chromatography separation was achieved using Thermo Surveyor System (Thermo Electron Corp, Waltham MA, USA) with quaternary pump connected to an online degasser and photodiode array detector (PDA). Chromquest software version 4.1 was used to control all parameters of HPLC. For the analytical separation Zic-Hilic (50 X 4.6 mm, 5m, 22A, Merck, Darmstadt, Germany HILIC-Hydrophilic Interaction Liquid Chromatography, SeQuant ZICHILIC HPLC column, Merck, Germany) column was used. The isocratic mobile phase consists of Acetonitrile which contains 0.1% formic acid (C) and MilliQ water (A) in the ratio of 75:25, followed by pumping at the rate of 0.5 mL/min. The column and autosampler tray was kept at ambient temperature. In HPLC, 20µl sample was injected into the with 5 min run time. Internal standard and tandem mass spectrometric detection of analyte were carried out using Applied Bio Systems 4000 triple quadrapole instrument (ABS Biosystems, Foster City, CA, USA) equipped with a Turbo IonSpray (ESI) source that operated in the positive ion mode. Based on molecular adduct ion and fragment ion for Carboplatin m/z 371.1→293 (transition 1), m/z 72.1→294.1 (transition 2), m/z 373.1→295.1 (transition 3) respectively, quantification was performed using multiple reaction monitoring (MRM) mode. The transition for Homatropine was m/z 276.1→142.2. Data acquisition and integration was performed by Analyst 1.5.2 software (ABS Biosystems, Foster City, CA, USA).

Ex-vivo drug release study

Extraocular tissues were dissected from the goat eye (procured from slaughter house). Balanced salt solution was injected through the optic nerve to increase the intraocular pressure of the eye for the proper implantation of carboplatin patch (3mm×3mm). Under the surgical microscope, pocket has been made and patch was implanted into the scleral portion of the eye. After the implantation of the patch, the anterior segment of eye (intracameral region) was cannulated using tubing connected with 26 G needle for input and output of the solvent (balanced salt solution). The cannulated eye was placed above the custom made eye chamber and the whole chamber was maintained at 37°C throughout the experiment. The flow of the solvent was maintained at 5µl/min. The effluent was collected in the pre-weighed tubes at different time intervals (30min, 1hr, 2hrs, 4rs, 8hrs, 12hrs and 24hrs). The effluents were weighed after the collection and subjected for analysis using LC/MS/MS.

RESULTS AND DISCUSSIONS

Optimizing the required concentration of gelatin:

To optimize the appropriate concentration of gelatin, different formulations were prepared, starting from lowest concentration i.e.100mg to highest concentration i.e. 350mg and evaluated in terms of clarity, thickness, flexibility and stickiness. The results were noted in below

Table 2: Selection Of Appropriate Concentration Of Gelatin

Formulation	Concentration of gelatin (mg)	Volume of solvent (ml)	Appearance
F1	100	5.0	Stick and difficult to peeled off.
F2	350	5.0	Easily peeled off and break down.

Preparation of cross linked gelatin patch:

Cross linked gelatin patches were prepared by using solvent casting method and their appearance was noted and shown in below table3.

Evaluation of cross linked gelatin patch: The formulated patches were evaluated for thickness, folding endurance, tensile strength (table4), loss on drying (table5), in-vitro & ex-vivo release studies (table6).

TABLE 3: FORMULATION DESIGN AND APPEARANCE FOR CROSS LINKED GELATIN PATCHES

Formulation	Drug(mg)	Gelatin (mg)	Distilled water (ml)	Glutaraldehyde 10% (µl)	DMSO (µl)	Appearance
GP1	5.0	400	5.0	100	400	Clear and transparent
GP2	12.5	400	5.0	100	400	Clear and transparent

TABLE 4: EVALUATION DATA FOR PATCHES

Formulation	Thickness (mm)	Folding endurance	Tensile strength (kg/cm2)
Gp1	0.04	130	0.1895
GP2	0.043	131	0.191

TABLE 5: ASSESSMENT OF LOSS ON DRYING (FOR EACH FORMULATION THREE MCT WERE TAKEN)

S. No	Weight of empty MCT	Weight of MCT+ 9mm2 patch	Weight of MCT+ 9mm2 patch after 6hrs.	Weight of MCT+ 9mm2 patch after 12hrs	Weight of MCT+ 9mm2 patch after 24hrs	Weight of MCT+ 9mm2 patch after 36hrs
1	1.0520	1.0590	1.0587	1.0584	1.0580	1.0577
2	1.0523	1.0589	1.0584	1.0581	1.0577	1.0572
3	1.0535	1.0596	1.0589	1.0585	1.0582	1.0577
4	1.0510	1.0582	1.0573	1.0567	1.0561	1.0554
5	1.0512	1.0584	1.0575	1.0569	1.0563	1.0555
6	1.0511	1.0582	1.0574	1.0567	1.0562	1.0554

TABLE 6: IN- VITRO DRUG STUDY AT DIFFERENT TIME INTERVALS

Concentration of drug(mg)	Time (hours)	Cumulative percentage of drug release
5.0	0.5	1.10±9.70
	1	8.41±6.22
	2	25.00± 9.16
	4	49.70± 9.16
	8	73.13 ±2.79
	12	101.59 ±1.90
	24	107.028 ±5.87
12.5	0.5	5.16 ±8.12
	1	11.36 ±9.22
	2	22.76 ±6.40
	4	52.83 ±6.44
	8	82.26 ±8.10
	24	103.48 ±1.59

TABLE 7: EX- VIVO DRUG RELEASE STUDY AT DIFFERENT TIME INTERVALS

Concentration of drug (mg)	Time (hours)	Cumulative percentage of drug release
5.0	0.5	1.11±2.69
	1	2.75± 8.50
	2	6.39± 2.19
	4	10.38± 4.42
	8	21.27± 4.29
	12	29.97± 1.55
	24	33.24± 4.05
12.5	0.5	0.27±4.01
	1	0.65±3.00
	2	1.41±7.75
	4	3.47±9.49
	8	10.9±5.82
	12	22.41±2.62
	24	66.2±2.26

DISCUSSION:

The objective of this research was to design and evaluate cross- linked gelatin patch for intraocular delivery of Carboplatin for the management of retinoblastoma. The preparation of intraocular using gelatin as the polymer of choice to produce different formulation. During the preformulation stage, the drug compatibility with excipient was evaluated by Fourier Transformed Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetery (DSC).

During optimization stage, it was observed that there was lower concentration of gelatin to yield sticky solution and so, the patch was difficult to cast. When the concentration of gelatin increases at uniform rate to about 350mg, a smooth clear transparent patch was casted with appreciable aesthetic appeal with uniform distribution of the ingredients. The patch was later evaluated for thickness parameter. No significant change in the thickness and folding endurance was found between the two patches. The tensile strength also somewhat affected and some difference was observed between the patches.

Likewise the loss on drying value of the formulations was not significantly affected.

In the *in vitro* performance study of gelatin patches showed a peak drug release at 4th hours with steady release up to 20th hour, but the formulation six shows slower drug release compared to other two formulations.

In the *ex vivo* study of the gelatin patches showed a peak drug release at 4th hour with steady release up to 20hrs, but for formulation one drug release rate falls at 12hrs and here formulation two shows good release compared to other two formulations

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