Preparation of Idebenone as a Thermosetting Nasal Gel for Better Bioavailability and Histopathological Effect

Abstract:

Background Idebenone is an extensively metabolized drug with poor water solubility that is used to treat Leiber's hereditary optical neuropathy.

Objective This study aims to prepare idebenone nanoemulsion as a poloxamer-based nasal gel to overcome the extensive rate of hepatic metabolism for better bioavailability and lower histopathological effect on the nasal mucosa.

Methods The formulation strategy was based on eliciting mutual concentration reduction between the nanoemulsion and the carrier gel by setting their gelation temperature between 30-32°C to overcome the mucociliary dose washout. The o/w nanoemulsions rely on cremophor EL and transcutol as an emulsifying system to stabilize idebenone-loaded lemongrass oil. The spontaneous emulsification method was used to prepare nanoemulsions that were characterized by zeta sizer while the thermosensitive hydrogels were prepared using the cold method. In-vitro dissolution test and ex-vivo permeation study through excised sheep nasal mucosa were performed to evaluate the enhanced permeation ratio, rate of permeation, and permeation coefficient. The histopathological effect of direct application on sheep nasal mucosa was studied using optical microscopy to evaluate cellular toxicity.

Results The formula prepared from NE1 with poloxamer 407: poloxamer188 in concentrations 10:3% w/w respectively showed almost complete drug release in 120 minutes due to complete polymers blend erosion. Furthermore, thermosensitive nano-emulgel at a temperature of gelation 31.8°C was obtained at much lower concentrations of poloxamer 407 (10%) compared to previous studies. Nanoemulsions retained their globular size below 100nm due to further gel entrapment stabilization.

Conclusions Drug permeation through excised sheep nasal mucosa elicited an increase in enhanced permeation ratio to 20.3 times and other flux kinetics parameters compared to those of IDB oil dispersion. Direct cellular toxicity showed a minor inflammatory response characterized by serous infiltration of inflammatory cells and edema. In contrast, most of the epithelial cells retained their histological characteristics compared to control slides.

Keywords: Bioavailability, Idebenone, Nanoemulsion, Permeation.

Introduction

Nasal drug delivery offers an optimal chance for certain drugs of low oral bioavailability to be absorbed effectively via the nasal route. The highly vascularized mucosa is characterized by appreciable blood flow rate, adequate surface area, and low enzymatic activity; therefore, good plasma concentration can be attained (1). However, nasal drug administration faces several challenges, of which mucociliary clearance is the most important. This process takes between 12 and 15 minutes (2) and can be a major reason for the dose washout of poorly formulated nasal preparations. Poloxamers are a group of thermosensitive polymers that are described as having lower critical solution temperature, i.e., they form a gel on heating. They are composed basically of polyethylene oxide.

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the most predominant polymorph. It is practically insoluble in water but freely soluble in methanol, oils (log P=3.865). Idebenone was assigned by the European medical agency (EMA) as a treatment for Leiber’s hereditary optic neuropathy (LHON) (5,6), an autosomal recessive neurodegenerative disease of mitochondrial origin that is characterized by gradual loss of visual acuity ending with complete blindness and this disease has no approved treatment yet other than IDB(7). Although IDB is almost completely absorbed from GIT, it has low aqueous solubility as it belongs to the class II of the biopharmaceutical classification system (BCS). Still, its extensive rate of metabolism reduces the drug’s oral bioavailability to less than 1% (8).

This study aims to utilize several well-characterized IDB nanoemulsions (NEs) from previous work to formulate the drug as a thermosensitive nasal gel (NG). Those NGs were supposed to prolong dose residency time and decrease surfactant toxicity through overall dilution, circumventing the extensive hepatic metabolism and increasing the transmucosal permeation of the drug. Consequently, an increase in drug bioavailability with decreased cellular toxicity would be anticipated.

Materials and methods

Materials

Idebenone was purchased from (XI’An Geekee Biotech Co., Ltd, China), lemongrass oil (LGO), was purchased from (Shaanxi Guangie Technology Co., Ltd, China). Cremophor EL® (CrEL) was purchased from (Shanghai Taijie Chemical Co., Ltd, China). Transcutol P® (TC) was purchased from (Dayhang Chemicals Co., Ltd Hangzou, China). Poloxamer 407 (P407), poloxamer 188 (P188), NaCl, KCl, CaCl2, NaHPO4, and KH2PO4 were purchased from (Green land chemical comp.U.K), and de-ionized water (DIW) was purchased from a local manufacturer.

2.2 Methods

2.2.1 Preparation IDB-loaded nanoemulsion

Three well-characterized NE formulas were used to prepare a set of drug-loaded NEs as shown in table (1), using a self-emulsification method at which an oily phase, made of the drug in the oil, was added gradually under continuous stirring at 300rpm to the already prepared aqueous phase which is formed by dissolving the stated amount of CrEl and TC in the DIW.

Table (1): Formulas of Drug-Loaded Nanoemulsions

<table>
<thead>
<tr>
<th>Code</th>
<th>CrEL</th>
<th>TC</th>
<th>IDB: LGO</th>
<th>DIW</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE1</td>
<td>48.9</td>
<td>6.1</td>
<td>10 10 25</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>NE2</td>
<td>52.0</td>
<td>13</td>
<td>10 10 15</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>NE2</td>
<td>57.7</td>
<td>7.3</td>
<td>10 10 15</td>
<td>100</td>
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</tr>
</tbody>
</table>

Preparation of thermosensitive nasal gels

Poloxamer 407 in three concentrations, 10, 11, and 12 w/w% was used as gel backbone. Poloxamer 188 can raise the temperature of gelation (Tgel) of P407. Therefore, P188 at three ratios 1, 2, and 3% w/w with each P470 percentage ratio was used to prepare nine thermosensitive polymer blend of P407:P188 hydrogels (HG) (10:3), (10:2), (10:1), (11:3), (10:2), (10:1), (12:3), (12:2), and (12:1). A quantity equal to 0.1g of IDB-loaded NE1, NE2, and NE3 were cross-mixed with 0.4g of (P407:P188) polymer blends percent ratios (10:3, 10:2, 10:1, 11:3, 11:2, 11:1, 12:3, 12:2, 12:1 w/w) using a magnetic stirrer to prepare 9 drug-loaded thermosensitive nasal gel (NG) formulas as shown in table (2).

Characterization of the IDB-loaded nasal gels

Determination of temperature of gelation

The Tgel values were determined by using the tube inversion method. This method involves the loading of 1g of each NG into (2ml) sealable Eppendorf tubes and mounting them into a thermo-regulated water bath G.F.L, Karl Kolb, Germany. The temperature increased from 10°C to 34°C at a rate of 0.5 degrees/min. The Tgel can be determined by tilting the tube 90° every minute, and the temperature at which the meniscus of the gel stands still without movement is recorded as the Tgel(8).

Determination of average globule size and polydispersity index (PDI)

<table>
<thead>
<tr>
<th>ME code</th>
<th>Gel code</th>
<th>Nasal Gel code</th>
<th>CrEL</th>
<th>TC</th>
<th>LGO</th>
<th>IDB</th>
<th>P407</th>
<th>P188</th>
<th>DIW</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE1</td>
<td>10:3</td>
<td>NG1(10:3)</td>
<td>9.78</td>
<td>1.22</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>2.4</td>
<td>74.6</td>
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<td>NG1(10:2)</td>
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<td>2</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>1.6</td>
<td>75.4</td>
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<tr>
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<td>NG1(10:1)</td>
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<td>1.22</td>
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<td>2</td>
<td>2</td>
<td>8</td>
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<td>76.2</td>
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<td>NG2(10:3)</td>
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<td>2.6</td>
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<td>2</td>
<td>2</td>
<td>8</td>
<td>2.4</td>
<td>72.6</td>
</tr>
<tr>
<td>NE2</td>
<td>10:2</td>
<td>NG2(10:2)</td>
<td>10.4</td>
<td>2.6</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>1.6</td>
<td>73.4</td>
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<tr>
<td>NE2</td>
<td>10:1</td>
<td>NG2(10:1)</td>
<td>10.4</td>
<td>2.6</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>0.8</td>
<td>74.2</td>
</tr>
<tr>
<td>NE3</td>
<td>10:3</td>
<td>NG3(10:3)</td>
<td>11.54</td>
<td>1.46</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>2.4</td>
<td>72.6</td>
</tr>
<tr>
<td>NE3</td>
<td>10:2</td>
<td>NG3(10:2)</td>
<td>11.54</td>
<td>1.46</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>1.6</td>
<td>73.4</td>
</tr>
<tr>
<td>NE3</td>
<td>10:1</td>
<td>NG3(10:1)</td>
<td>11.54</td>
<td>1.46</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>0.8</td>
<td>74.2</td>
</tr>
</tbody>
</table>
The mean globule diameter of the prepared drug-loaded NE1, NE2 and NE3 as well as the prepared NGs were determined using Zetasizer (Nano ZS red label, Malvern, UK) operated at 25°C, the angle of incident light was 173° and equipped with an optical filter (9). Samples of NGs were diluted 100 times by using ultra-purified cold water to allow free Brownian’s motion and to avoid multiple diffractions. They were introduced to Zetasizer by disposable polystyrene cells (10).

Determination of Zeta potential: Using DTS 1070 zeta-cell, samples prepared as in the previous section were introduced into the Zetasizer to measure the potential of the diffused layer (zeta potential) that reflects NE electrostatic stability (11)

Morphological characterization by Transmission electron microscope (TEM): A drop of well-characterized NG was diluted and left to dry onto carbon-coated copper grids and negatively stained for 10 s with 2% filtered aqueous sodium phosphotungstate adjusted to pH 7.0. Using filter paper, the excess staining solution was removed of the grid, and the sample was observed with a Philips 208S transmission electron microscope operated at 80 kV. (12)

In-vitro drug release of the nasal gels and release kinetics: A membrane-less model was applied to the USP type II dissolution apparatus to determine the drug release profile in which the prepared NGs were introduced directly to the dissolution media (200 ml simulated nasal fluid (SNF) with 1% Tween 80) without dialysis membrane (13). This approach can be achieved by using a 5ml test tube of 1cm diameter that is thermally equilibrated at 34°C in a water bath, as shown in figure (2), then 0.5 gram of tested NG equivalent to 10mg IDB was loaded into the tube and left 10min to ensure complete sol-gel transformation. The tube was fixed to the wall of the dissolution jar using double-sided adhesive tape. Aliquots of 1ml were withdrawn at different periods (0, 0.25, 0.5, 1.1.5, 2.2.5, and 3h) and were filtered using 0.45µm syringe filter. The quantity of the drug in each sample was measured using the UV-visible spectrophotometer. The media in the acceptor chamber was replenished with the same amount of fresh PBS solution each time to preserve sink conditions (14).

Figure (2): Membrane-less model dissolution test.

Ex-vivo transmucosal permeation through sheep nasal mucosa

Transmucosal permeation through the anterior turbinate mucosa of a recently sacrificed sheep was done to evaluate the permeation parameters after the formulation as NG. The excised mucosa was cut, washed with phosphate buffer saline (PBS) and preserved and cooled at 4°C until mounted on a 20ml vertical Franz-diffusion cell that had an effective permeation surface area of 3.14 cm². The Franz cell was kept in a thermo-regulated water bath at 34°C to simulate the temperature of the nasal cavity. The lower acceptor chamber was filled with PBS with 1% Tween 80) without any entrapped air bubbles and stirred at 60 rpm using a magnetic stirrer. The excised mucosa was fixed in such a way that the upper part was facing the donor chamber and was left for 30 min for equilibrium. An amount equal to 0.5g of NG, which is equivalent to 10mg IDB, was applied into the donor chamber. Aliquots of 1ml from the receptor chamber were withdrawn at predetermined points of time (0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, and 3.0h), filtered using 0.45µm syringe filter and its IDB concentration was measured using UV-visible spectrophotometer. The media in the acceptor chamber was replenished with the same amount of fresh PBS solution each time to preserve sink conditions (15).

Data analysis of ex-vivo transmucosal permeation of nasal gels

The permeation profile across the nasal mucosa was constructed by plotting the cumulative amount of IDB permeated per unit area (Q, µg/cm²) on the Y-axis against its corresponding time (t, h) on the X-axis (16). This plot was used to obtain the permeation rate or transmucosal flux of IDB at the steady state (Jss, µg/cm²/h), which was obtained from the slope of the straight linear portion of the regression line equation (1). The lag time (Tlag) was obtained from the intercept of the straight line that represents steady-state regression with the time axis. In contrast, the permeability coefficient (PC, cm/h), was obtained by dividing the ratio of drug flux (Jss) by the initial concentration (C0) of IDB in the tested NG formula equation (2) and finally, the
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enhancement ratio (Er), which was obtained by dividing the permeation rate or flux (Jss) of IDB from the tested NG formula by the flux (Jss) of the pure IDB oily dispersion (control) equation (3).

\[
\text{Jss} = \frac{dQ}{dt} \quad \text{Equation (1)} \\
\text{PC} = \frac{\text{Jss}}{C_d} \quad \text{Equation (2)} \\
\text{Er} = \frac{\text{Jss of IDB (formulation)}}{\text{Jss of IDB (control)}} \quad \text{Equation (3)}
\]

Nasal mucosal histopathology study:
Histopathological studies were done on sheep nasal mucosa to study the direct effect of the formulation application on mucosa when administered intranasally. Sheep nasal mucosa was obtained from a recently sacrificed animal from a local slaughterhouse. The excised mucosa was placed in PBS, cleaned, and cut into nine symmetrical pieces. The formulation was applied to 3 pieces, and the positive control, 70% isopropyl alcohol, was applied to another 3 pieces, while the negative control (PB pH= 6.5) was introduced to the last three pieces. After 1 hour of treatment, all samples were washed using PBS and stored in 10% formalin for 2h. Next, all samples were kept in 70% ethanol at 4°C for dehydration. The samples were then cut into 5μm thickness by using a microtome and were stained with a combination of hematoxylin and eosin (H&E) dye to observe any damage under an optical microscope.

Results
Thermal properties of nasal gels and temperature of gelation
The prepared NGs that were made of P407 as gel forming polymer and P188 as Tgel modulator were translucent and free from flocculation. Poloxamer 407 has a minimum concentration equal 15% (18).

Determination of mean globule size, polydispersity index, and zeta potential
Mean globule size (MGS) obtained from Zetasizer were 42.0, 54.56, and 45.65nm with zeta potential values -6.4, -3.2 and -2.8 mV, respectively.

Table (3): Temperature of gelation, mean globule size, and zeta potential and pH of the Prepared NGs

<table>
<thead>
<tr>
<th>NG-code NE(P407:P188)</th>
<th>NG1 (10:1)</th>
<th>NG1 (10:2)</th>
<th>NG1 (10:3)</th>
<th>NG2 (10:1)</th>
<th>NG2 (10:2)</th>
<th>NG2 (10:3)</th>
<th>NG3 (10:1)</th>
<th>NG3 (10:2)</th>
<th>NG3 (10:3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tgel (°C)</td>
<td>31.5</td>
<td>31.2</td>
<td>31.8</td>
<td>31.1</td>
<td>31.2</td>
<td>31.5</td>
<td>30.3</td>
<td>32.0</td>
<td>31.7</td>
</tr>
<tr>
<td>MGS (nm)</td>
<td>144.8</td>
<td>165.4</td>
<td>154.1</td>
<td>182.2</td>
<td>253.5</td>
<td>163.7</td>
<td>243.9</td>
<td>186.5</td>
<td>185.5</td>
</tr>
<tr>
<td>PDI (0-1)</td>
<td>0.31</td>
<td>0.12</td>
<td>0.26</td>
<td>0.24</td>
<td>0.27</td>
<td>0.17</td>
<td>0.25</td>
<td>0.19</td>
<td>0.23</td>
</tr>
<tr>
<td>ZP (mv)</td>
<td>0.2</td>
<td>-3.4</td>
<td>-2.7</td>
<td>0.5</td>
<td>-4.64</td>
<td>-2.32</td>
<td>-5.54</td>
<td>-2.42</td>
<td>-5.53</td>
</tr>
<tr>
<td>pH</td>
<td>5.8±0.13</td>
<td>5.9±0.08</td>
<td>5.7±0.09</td>
<td>5.8±0.13</td>
<td>5.78±0.11</td>
<td>5.95±0.08</td>
<td>5.68±0.1</td>
<td>5.7±0.11</td>
<td>5.8±0.09</td>
</tr>
</tbody>
</table>

In-vitro drug release of the nasal gels and release kinetics
Nasal gels can maintain their integrity at 34°C therefore NGs were introduced directly to the dissolution media without barrier. Formula NG1(10:3) showed the best release profile with almost complete drug release at minute 120 as shown in figure (3).

Data analysis of ex-vivo transmucosal permeation of selected nasal gel
The outcome of the permeation study is shown in Figure (5), in which there is an appreciable difference in permeation profile between the formula NG1(10:3) and the oil dispersion of the drug as a control that was obtained from our previous work. The permeation was calculated as enhancement ratio, which was equal to 20.3 times with a permeation rate equal to 28.48 μg/cm²/h, lag time equal to 0.57h, and permeation coefficient of 14.24*10⁻⁶ cm²/h.
Time (h)

0 0.5 1 1.5 2 2.5 3 3.5
Cumulative permeation (µg/cm²)

Control (NG)
Test (NG)

Figure (5): Cumulative IDB permeated per cm² versus time profile of NG1(10:3).

As mentioned earlier, the mucociliary wash time is between 12-15min. Therefore, the transition process from sol to gel is anticipated to prolong the dose residence time more than this period as well as beyond the lag time obtained from the ex-vivo permeation study (0.57h). The longer dose residency time serves to offer a better chance for the drug to be absorbed sufficiently.

Histopathological study after nasal mucosa application

Histopathological studies’ outcomes are shown in Figure (6), where the effect of direct application of NG1(10:3) is shown in (D). Comparing (D) with PBS-treated negative control that has shown almost no destructive effect (B) and propyl alcohol-treated positive control that had a completely destructive effect (C), revealed a minor inflammatory response that was characterized by edematous serous infiltration of fluids and inflammatory cells. The cilia of viable cells were retained normally without ciliary toxicity after an hour of the formula application. However, direct toxicity outcome may offer basic information on the total histopathological changes since it was carried on excised nasal mucosa in which both the mucociliary clearance and blood perfusion are absent.

Discussion:

Below this, no gel structure can be formed; therefore, no gel could be anticipated at the proposed range in this study. However, a remarkable outcome in this study was the formation of thermosetting gel at lower concentrations of P407 equal to 8%, unlike the observed effective concentrations (15-30%) that have been used in many previous studies (19,20). Nasal gels of P407 concentration between (10-12%), regained their ability to form thermo-triggered gels upon the addition of the drug-loaded NE. Consequently, the viscoelastic and thermal properties of the obtained NG were characterizable, as shown in Table (2). The Tgel of the NGs that were prepared of P407 at 11&12% w/w were below 30°C therefore, out of 27 formulas, 18 were excluded from the study, and only nine NGs at P407 equals 10%, shown in table (2), were adapted. Poloxamers have a surface activity that enables them to aggregate in the micellar conformation of a hydrophobic core at concentrations above their critical micellar concentrations (CMC), which actually represents the critical gel concentration (CGC) at a temperature exceeding the critical micellar temperature (CMT). Cremophore EL is a well-known surfactant that is manufactured by the polyethoxylation of 35 moles of castor oil, which results in a mixture of different polyethoxylated long chains of (free fatty acids, mono, di, and tri fatty acid glycerol esters and glycerols) as well as unmodified castor oil residuals. This diversity in the composition of long alkyl chain compounds offers the possibility to manipulate and enhance the hydrophobic core radius of poloxamer micelles, resulting in a more hydrophobic polymeric aggregation. This heterogeneous micellar aggregation can be easily separated from the aqueous phase, forming a three-dimensional gel phase at a lower CMC value(21,22). This reduction in the CMC leads to an overall reduction in copolymer concentration used in the formulation and, consequently, their toxicity. Furthermore, the addition of the NE to four parts poloxamer HG results in a great reduction (dilution to one-fifth) in total surfactant (CrEL) concentration to less than 10%, as well as other NE components (IDB and TC). This reduction in all components helps to decrease the direct toxicity and irritation that could be caused by the surfactant or the drug itself to the nasal mucosa(23). The low globular size indicates the efficiency of the spontaneous emulsification method used for preparation to obtain microemulsion (ME) with MGS below the referential value of 100nm at low PDI. Correspondingly, the results that are related to the prepared NGs showed that the prepared gels didn’t retain the globule size below 100nm with a slight increment in diameter and PDI after the incorporation into a gel, as shown in Table (3) due to the formation of NEs. Generally, particles with zeta potentials more than +30 mV or less than −30 mV are normally considered stable. Results obtained from measuring the zeta potential varied between (+0.5 and -5.54), which are considered low in defining the stability of NEs within the final product, which is maintained by the steric effect imparted by the surfactant side chains rather than electrostatic
stabilization(24). The permeation-enhanced results are attributed to several reasons, among which is the tremendous increase in the total surface area of the dispersed oil phase, which can profoundly increase the soluble species of the drug that is permeable across the epithelial membrane(26). In addition, the ultra-fine drug-loaded oil droplets formed by the emulsifying agent’s action and, due to their size and nature, can easily permeate through the cellular membrane and cross tight junctions. Another mechanism that NE could offer is the permeation enhancement effect of the surfactant that contributes to the higher diffusion rate.

Conclusions
Idebenone can be formulated as NE-based thermosensitive NG that can maintain pharmaceutical stability and appreciable transmucosal permeability at a dose of 5mg/0.25g with a permeation rate equal to 28.48 µg/cm²/h, especially when considering the good surface area of the nasal cavity 150-160cm² therefore more than 50% of the administered dose can be delivered. Therefore, an increased bioavailability is anticipated through nasal administration of idebenone as nanoemulsion. The basic direct histopathological tests showed retained histological features of the respiratory epithelial mucosa; therefore, further studies on viable animal models are recommended.

Author Contribution
Hussein J. Kadhim: MSc Student
Khalid K Al-Kininani: Supervisor

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