

Effect of Permeation Enhancers on Permeation Kinetics of Idebenone through the Bovine Buccal Mucosa

Rathi A.A*, Dhamecha D.L, Patel K.A, Saifee M and Dehghan M.H.G

Department of Pharmaceutics, Y.B.Chavan College of Pharmacy, Maulana Azad Education Trusts, Dr. Rafiq Zakaria Campus, Rauza Bagh, Aurangabad, Maharashtra, India 431001

ABSTRACT

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The buccal administration of drugs is attracting considerable attention since it has many advantages like to obtain high levels of drug in blood, circumvent extensive first-pass metabolism and avoid degradation in the gastrointestinal tract by enzymes and bacteria. This system serves as the better solution for the administration of drugs having low bioavailability like idebenone. Idebenone is synthetic analogue of coenzyme Q10 (CoQ10), a vital cell membrane antioxidant administered in friedreich's ataxia, duchenne muscular dystrophy, becker muscular dystrophy, alzheimer's disease, leber's hereditary optic neuropathy, MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis with stroke-like episodes), parkinson's disease mitochondrial myopathies etc.. The drug is practically water insoluble and shows limited bioavailability after oral administration due to a significant degree of first pass metabolism (99%). The low bioavailability of drug demands frequent dosing, hence the aim of the study was to demonstrate *in vitro* enhancement of drug penetration using Keshary – Chien diffusion cell. For demonstration varying concentrations of penetration enhancers and drug were prepared. Different penetration enhancers used were sodium lauryl sulphate, disodium ethylene diamine tetraacetic acid, urea, sorbitol, oleic acid, dimethylsulphoxide, physical mixtures with beta cyclodextrin, hydroxypropyl beta cyclodextrin, and complex of drug with hydroxypropyl beta cyclodextrin through buccal drug delivery system and tested for highest penetration. Drug complex with hydroxyl propyl cyclodextrin showed maximum enhancement ratio of 45.93 when compared with the enhancement ratio of other penetration enhances which ranged from 1.08 to 13.52. Hence, when compared with different penetration enhancers, this inclusion complex of idebenone with HPBCD can itself act as a best penetration enhancer for buccal drug delivery of idebenone. Overall, we can say that Idebenone Inclusion complex with HPBCD is the best way of enhancing the permeation of drug through buccal mucosa.

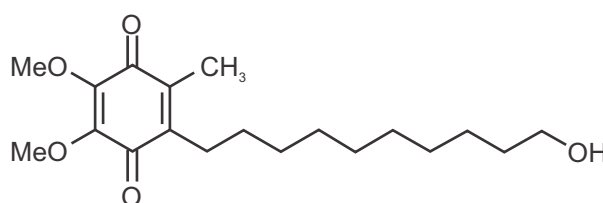
Keywords: Buccal drug delivery, idebenone, Penetration enhancer, idebenone complex.

INTRODUCTION

Idebenone (Fig 1) is a synthetic analogue of coenzyme Q10 (CoQ10), a vital cell membrane antioxidant which is essential constituent of the adenosine-triphosphate (ATP)¹ and is administered in Friedreich's Ataxia, Duchenne Muscular Dystrophy, Becker Muscular Dystrophy, Alzheimer's Disease, Leber's Hereditary Optic Neuropathy, MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis with stroke-like episodes), Parkinson's Disease mitochondrial myopathies etc. Idebenone is rapidly and extensively absorbed following oral administration, with absolute bioavailability of approximately 1-10 % due to a significant degree of first pass metabolism (99%)². Direct access to the systemic circulation through the internal jugular vein bypasses drugs from the hepatic first pass metabolism

leading to high bioavailability^{3,4,5,6}. Oral mucosal permeability of Idebenone is too low to allow plasma concentration to reach therapeutic levels^{7,8}. Therapeutic effective concentration in body can be achieved either by increasing dose or by using penetration enhancers. However, increase in dose may lead to unwanted side effects; hence to achieve therapeutic effective concentration of drug in body we used different penetration enhancers. Buccal permeation⁹ can be improved by various classes of transmucosal and transdermal penetration enhancers such as bile salts, surfactants, fatty acid and derivatives, chelators and cyclodextrins. Cyclodextrins are capable of forming inclusions complexes with many

Fig. 1: Structure of Idebenone.



*Address for Correspondence:

Rathi A.A, Department of Pharmaceutics, Y.B.Chavan College of Pharmacy, Maulana Azad Education Trusts, Dr. Rafiq Zakaria Campus, Rauza Bagh, Aurangabad, Maharashtra, India 431001

Email: amit.pharmaceutics@gmail.com

drugs^{10,11}. Drug-cyclodextrin complexes can improve the clinical usage of drugs by increasing their aqueous solubility, dissolution rate and pharmaceutical availability. Solubilization of poorly water soluble drugs by complexation with cyclodextrins and then delivery via the buccal or sublingual mucosa may be advantageous for increasing drug absorption.¹¹ Hence, the objective of the present study was to demonstrate *in vitro* enhancement of drug penetration using Keshary-Chien diffusion cell. The study also involves the comparison between different penetration enhancers and the cyclodextrin complexes on penetration enhancement of Idebenone.

MATERIALS AND METHODS

Materials: Idebenone and cyclodextrins were obtained as gift sample from International Specialty Products (India) Pvt. Ltd. Hyderabad. Other chemicals used were of analytical grade. Different penetration enhancers like Sodium lauryl sulphate^{12, 13} (SLS), EDTA, Urea, Sorbitol, Oleic acid^{14, 15}, DMSO^{16,17} and Cyclodextrin¹⁸ were used.

Methods:

Solubility of Idebenone: Different solvents like ethanol, methanol, phosphate buffer pH6.8 and pH7.4, hexane, ether, octanol, etc were used to determine the solubility of Idebenone. 5 ml of each solvent was saturated with Idebenone. These solutions were allowed to stir for 24 hr with the help of magnetic stirrer. Then contents were filtered through 0.2 micron filter and analyzed by UV spectrophotometer at 282 nm.^{19,20}

Drug diffusion study through bovine buccal mucosa: *In vitro* permeation studies of Idebenone through the bovine buccal mucosa was performed using Keshary – Chien diffusion cell at 37° C. Bovine buccal mucosa was obtained from a local slaughter house and used within 3 hrs of slaughter. The mucosa was stored in normal saline solution upon collection. The epithelium was separated from underlying connective tissue with surgical scissor and buccal mucosa was clamped between donor and receiver compartment of Keshary – Chien diffusion cell. The temperature was maintained at 37°C ± 10°C by a jacket surrounding the receiver chamber that was stirred with a magnetic bead (maintained at 200 rpm ± 25). After the buccal membrane was equilibrated with phosphate buffer pH 7.4 solutions in both the chambers for half an hour then donor chamber solution was replaced with drug solution⁴.

To solve the problem of drug saturation we modified the method by completely replacing the sample from receptor chamber (which had alcohol) with fresh phosphate buffer ph

7.4 (prewarmed at 37° C in dissolution apparatus). The amount of Idebenone permeated through the buccal mucosa was then determined by measuring the absorbance at 282 nm using JASCO V-630 spectrophotometer.¹³

The experiments were performed in triplicate (n= 3) and the mean value was used to calculate the permeability coefficient. The cumulative amount of Idebenone permeated per unit skin surface was plotted against time and the slope of the linear portion of the plot was estimated as steady state flux (µg/cm²/h).

$$Kp = \text{slope} \times Vd / S$$

Where, Vd = volume of donor solution, S = surface area of tissue (for bovine 2.54cm²)

The permeability coefficient (Kp) was calculated by using the following equation.

$$Kp = Jss / CV$$

where, Jss is the steady state flux and CV is the initial concentration of Idebenone in donor compartment.

$$ER = Kp \text{ of drug with enhancer} / Kp \text{ of drug alone}$$

Where, ER is enhancement ratio.

Preparation of physical mixtures of drug with different penetration enhancers: For physical mixture-penetration enhancers (DMSO, SLS, EDTA, urea, sorbitol, oleic acid) were used in 4%w/w of pure drug and for inclusion complexes, 1:1 stoichiometric quantities of drug with βCD and HPβCD were used.

Preparation of Drug Complexation with Hydroxy Propyl β Cyclodextrin (HPβCD): Phase solubility studies were performed according to the method reported by Higuchi and Connors. An excess of drug was added to all vials containing 5ml portions of variable amount of HPβCD (0, 2, 4, 6, 8 and 10 mM). All the above solutions were subjected to sonication for 30 minutes and were allowed to stand for 24 hrs at room temperature (~25 °C) without disturbance to attain saturation equilibrium. These supersaturated systems were carefully filtered through Whatmann filter paper (#41) and were analyzed by uv spectrophotometer at 282 nm. Appropriate dilutions were done with dilution fluid (phosphate buffer pH 6.8 and ethanol in the ratio of 8:2). Solubility of Idebenone in every HPβCD solution was calculated, and phase solubility diagram was drawn between solubility of Idebenone and concentration of HPβCD. The apparent stability constant (Kc) was calculated by using formula.²¹

$$\text{Stability constant (Kc)} = \text{Slope} / So (1 - \text{Slope})$$

Where, So= Aq. Solubility of Idebenone

Preparation of Drug complex by kneading method:

Stoichiometric quantities (i.e. 1:1) of Idebenone and HPβCD were weighed. HPβCD was added to the mortar, and a required quantity of 1:1 of ethanol and water was added while triturating to get semisolid consistency. Then slowly drug was incorporated into the slurry, and trituration was continued further for 45 min. The slurry was dried at room temperature for 24 hrs, pulverized and passed through sieve # 72 and stored in desiccators until used for further study.²¹

Characterization of Drug Cyclodextrin Inclusion Complexes:

Inclusion complexes were characterized by DSC graphs. DSC curves of drug and its inclusion complex were recorded using Shimadzu DT-40 thermal analyzer. The samples were heated at about 40°C at a rate of 10°/ min in the open pan using alumina as a reference material.²²

RESULTS AND DISCUSSION

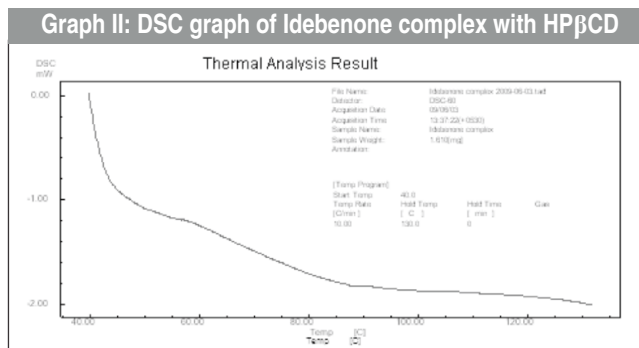
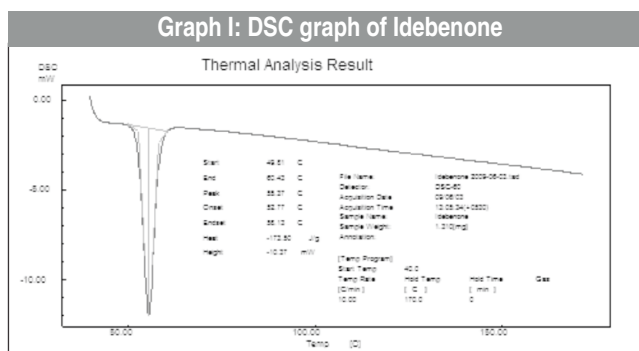
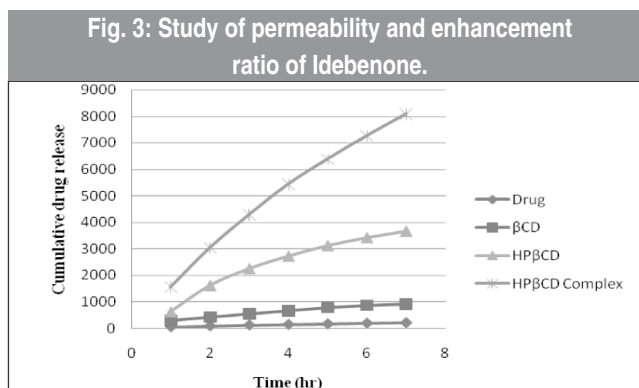
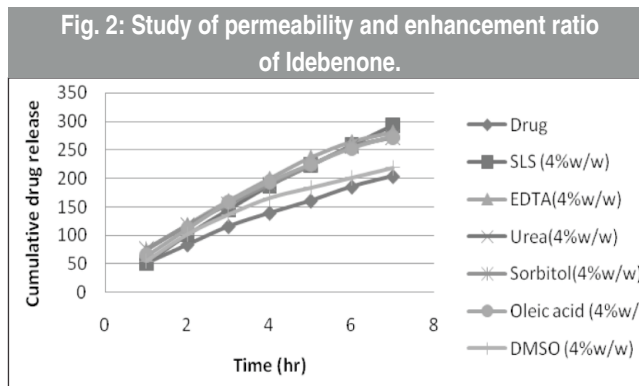
The solubility of Idebenone is as shown in table 1.

Initially study has been done by simple method¹³ but results were not obtained due to the fast saturation observed in the receptor compartment due to the very less solubility of drug in receptor medium. The literature suggests that use of alcohol can solve this problem so the studies were done using alcohol in the receptor compartment, but as alcohol acts as penetration enhancer it was found that increase in concentration of alcohol increases the penetration of drug, (This corroborates with the result of Nuntakan Suwanpidokkul et al).¹³ Ethanol in receptor or donor compartment modifies the cell structure of diffusion membrane and acts as penetration enhancer²³.

DSC graphs I and II confirms the formation of Idebenone inclusion complex with HPβCD. The absence of the sharp endotherm (DSC thermogram II) at about 53°C indicates that idebenone has been completely entrapped in HPβCD.

All the penetration enhancer (except cyclodextrin) showed 1-2 times enhancement ratio (fig 2 and 3, Table 2) but use of cyclodextrin enhances the ratio by 4 to 46%. Low aqueous

| Sr.no | Solvent | Solubility |
|-------|--|----------------|
| 1 | Water | 0.021 mg/ml |
| 2 | Phosphate buffer pH 6.8 | 0.037mg/ml |
| 3 | 10% ethanol in phosphate buffer pH 6.8 | 0.074mg/ml |
| 4 | 20% ethanol in phosphate buffer pH 6.8 | 0.126mg/ml |
| 5 | 30% ethanol in phosphate buffer pH 6.8 | 0.300mg/ml |
| 6 | DMSO, Methanol, Ethanol and Acetone | freely soluble |



solubility of idebenone results in limited amount of drug dissolution inside the hydrated polymeric matrices but the Idebenone complex dissolves easily in a hydrated polymeric environment (Table 3), resulting in a higher diffusional driving force and faster drug release. Incorporation of HPβCD in the matrix improved the drug solubility and

Table 2: Effect of permeation enhancer on permeability coefficient and flux of Idebenone

| Sr. No. | Enhancer | Permeability coefficient (cm/hr) | Flux (J_{ss}) $\mu\text{g}/\text{cm}^2/\text{hr}$ | Enhancement Ratio (ER) |
|---------|-----------------------|----------------------------------|---|------------------------|
| 1 | Pure drug | 0.32 | 32.21 | 1 |
| 2 | SLS (4%w/w) | 0.50 | 49.5 | 1.54 |
| 3 | EDTA(4%w/w) | 0.45 | 44.85 | 1.39 |
| 4 | Urea(4%w/w) | 0.43 | 42.5 | 1.32 |
| 5 | Sorbitol(4%w/w) | 0.42 | 41.8 | 1.30 |
| 6 | Oleic acid (4%w/w) | 0.47 | 47.32 | 1.47 |
| 7 | DMSO (4%w/w) | 0.35 | 34.84 | 1.08 |
| 8 | β CD | 1.28 | 127.54 | 3.96 |
| 9 | HP β CD | 4.36 | 435.62 | 13.52 |
| 10 | HP β CD Complex | 14.80 | 1479.47 | 45.93 |

Table 3: Effect of alcohol on drug diffusion kinetics.

| Time (hrs) | Absorbance | Drug diffused ($\mu\text{g}/\text{ml}$) | Absorbance | Drug diffused ($\mu\text{g}/\text{ml}$) | Absorbance | Drug diffused ($\mu\text{g}/\text{ml}$) |
|------------|------------|---|------------|---|------------|---|
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.50 | 0.30 | 6.59 | 0.30 | 6.58 | 0.18 | 4.18 |
| 1.00 | 0.32 | 7.01 | 0.37 | 8.13 | 0.33 | 7.33 |
| 1.50 | 0.37 | 8.12 | 0.43 | 9.42 | 0.42 | 9.24 |
| 2.00 | 0.37 | 8.18 | 0.60 | 12.79 | 0.59 | 12.65 |
| 2.50 | 0.30 | 6.67 | 0.52 | 11.14 | 0.77 | 16.28 |
| 3.00 | 0.29 | 6.44 | 0.50 | 10.77 | 0.83 | 17.65 |
| 4.00 | 0.28 | 6.22 | 0.50 | 10.80 | 0.83 | 17.61 |
| 5.00 | 0.26 | 5.91 | 0.52 | 11.22 | 1.00 | 21.06 |
| 6.00 | 0.27 | 5.97 | 0.51 | 11.07 | 1.03 | 21.68 |
| 7.00 | 0.23 | 5.29 | 0.48 | 10.31 | 1.19 | 25.02 |
| 8.00 | 0.22 | 5.01 | 0.47 | 10.10 | 1.19 | 25.12 |

dissolution rate. Cyclodextrins (CDs) enhance the bioavailability of idebenone by increasing the drug solubility, dissolution and /or drug permeability. CDs increase the permeability of idebenone by making the drug available at the surface of biological barrier i.e., mucosa, from where it partitions into the membrane without disrupting the lipid layers of the barrier.²⁴

CONCLUSION

It was concluded that the Idebenone complex with HP β CD shows good flux and enhancement ratio. When compared with different penetration enhancers, this inclusion complex of idebenone with HP β CD can itself act as a penetration enhancer for buccal drug delivery of idebenone. The complex gave best enhancement ratio (45.93) when compared to that of all other penetration enhancers (1-13.52). Hence, we can conclude that Idebenone Inclusion complex

with HP β CD is the best way of enhancing the permeation of drug through buccal mucosa.

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