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A calorimetric study on the idebenone–phospholipid membrane interaction

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Abstract

Idebenone is a drug acting at the level of the central nervous system and is largely employed in the treatment of various cerebral diseases. To evaluate in vitro the possible in vivo interaction with biological membranes and to carry out a pre-formulation study for the realization of an idebenone-loaded liposome system, the interaction between this drug and model membranes made up of various naturally occurring phospholipids was investigated. The idebenone–membrane interaction was studied by differential scanning calorimetry (DSC). The presence of the drug noticeably influenced the thermotropic behavior of the membranes, shifting the main transition peak temperature towards lower values and broadening the peak. The extent of the perturbation of the membrane properties is a function of the content of idebenone. In particular, beyond an idebenone molar fraction of 0.09 a phase segregation of the lipid components constituting the bilayers was detected. These features showed that the drug is fully inserted into the bilayer matrix of the various membranes. The idebenone–membrane interaction is enhanced by the presence of negatively charged phospholipids. A kinetic experiment showed that the limiting step in the interaction with a biological substrate could be the diffusion process through the aqueous compartments rather than the interaction with the biological membrane bilayers. The formulation of a liposomal drug delivery system containing idebenone may improve the pharmaceutical and therapeutic properties of the drug.

Keywords: Idebenone; Membrane; Liposome; Differential scanning calorimetry; In vitro interaction

1. Introduction

Idebenone is a new synthesised drug (Fig. 1) (Okamoto et al., 1982) that has been proved to be active at the level of the central nervous system against cerebral disorders elicited by both vascular lesions and neurodegenerative processes (Nagaoka et al., 1989). This drug has been effective in the treatment of experimental cerebral ischaemia and significantly antagonised the decrease in brain ATP-levels and the increase in lactate levels (Na-

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gaoka et al., 1988). After the administration of idebenone in ischaemised rats an increase of the dopamine and acetylcholine cerebral levels, which are drastically reduced by the ischaemic event, was achieved (Narumi et al., 1985; Kakihana et al., 1989). Furthermore, idebenone showed beneficial effects on learning and memory impairment (Nagaoka et al., 1988; Yamazaki et al., 1989). The mechanism of action of this drug is based on the improvement of the cerebral metabolism (Kobayashi et al., 1985). In particular, idebenone is able to accelerate the biosynthesis of ATP by activating the electron-transfer system of mitochondria and, hence, reducing the consumption of non-respiratory oxygen (Okamoto et al., 1985). Thus, idebenone is also able to inhibit the lipid peroxidation of neuronal membranes mainly attributed to the reduced form of the drug (Suno and Nagaoka, 1984; Sugiyama et al., 1985).

Unfortunately, this drug is not very water soluble and the only marketed pharmaceutical formulation is an oral dosage form. The increase of water solubility of drugs can lead to a more rapid and complete adsorption after oral administration. In the attempt to improve idebenone solubility, the preparation and characterisation of inclusion complexes of idebenone with modified cyclodextrins have been recently reported (Ventura et al., 1995; Puglisi et al., 1996a). Another carrier of particular interest for the delivery of drugs at the level of the brain is the liposomal system (Fresta and Puglisi, 1996a; Fresta et al., 1994, 1995). In fact, the liposomal carrier is able to increase the amount of a hydrophilic drug, such as CDP-choline, at the level of the brain after an ischaemic event (Fresta et al., 1995; Fresta and Puglisi, 1996b).

Normally, the passage through the blood brain barrier (BBB) is quite rapid for a drug with a partition coefficient *n*-octanol/water greater than one (Rapoport et al., 1979). On the other hand, for highly hydrophobic molecules, i.e. idebenone, a limiting factor is represented by the drug supply at the level of the brain (serum concentration) and its binding with serum proteins (very high lipophilic drugs), which hampers the passage through the BBB (Robinson and Rapoport 1986). Therefore, the liposomal carrier may improve both the pharmacokinetic and the therapeutic effectiveness of this drug.

In order to realise a suitable liposomal carrier, the interactions occurring between a drug and the liposomal matrix should first be investigated (La Rosa et al., 1992a,b). In the present paper the results of a systematic investigation by differential scanning calorimetry (DSC) on the effects of idebenone on the gel-to-liquid crystalline phase transition of phospholipid membranes are reported. This kind of investigation is of great importance not only as a pre-formulation study for the liposomal carrier, but also to shed light on the type of interaction that may occur between this drug and the phospholipid bilayer of biological membranes.

2. Materials and methods

2.1. Chemicals

Synthetic 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine monohydrate (DPPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine monohydrate (DMPC), and 1,2-dipalmitoyl-*sn*-glycerophosphatidic acid disodium salt (DPPA) were purchased from Fluka Chemical (Buchs, Switzerland). The phospholipid purity ($>99\%$) was assayed by two-dimensional thin layer chromatography (TLC) on silica gel plates (Merck, Darmstadt, Germany) loaded with a solution of the lipid in chloroform–methanol (3:1 v/v). TLC was carried out eluting the plate first with a solvent system consisting of chloroform/methanol/5 N ammonium hydroxide (60:3:5 v/v/v) and then with chloroform/methanol/acetic acid/water (12:60:8:2.5 v/v/v/v).

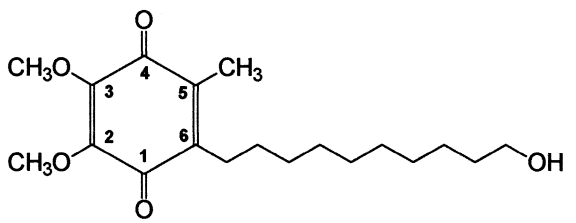

Fig. 1. Chemical structure of idebenone, 6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone.

The phosphorus content of the phospholipid was determined as inorganic phosphate (Bartlett, 1959). Idebenone was kindly provided by Takeda Italia Farmaceutici (Rome, Italy) and used without further purification. Its purity was greater than 99.5% as assayed by HPLC analysis. Double-distilled pyrogen-free water was used. All other reagents and solvents were of analytical grade (Carlo Erba, Milano, Italy).

2.2. Preparation of phospholipid membranes

The phospholipids were solubilised with chloroform in a round-bottomed flask containing 20 g of glass beads (2–3 mm mean size) (Carlo Erba, Italy). Suitable amounts of the drug were co-solubilised with the phospholipids. The organic solvent was evaporated off under nitrogen flow at 35°C by means of a rotavapor, leading to the formation of a thin lipid film on the wall of the vessel and on the surface of the glass beads. Any trace of the organic solvent was removed from the lipid/idebenone films by storage for 24 h at 35°C (Büchi T-51) under vacuum (5.10^2 mmHg). The lipid films (10 mg) were hydrated by adding 200 μl of isotonic phosphate buffer (pH 7.4). The flask was alternatively vortexed and warmed in a water bath at 50°C for 5 min, allowing a complete phospholipid hydration. The glass beads increased the surface area of the dried lipid film, thus enhancing the phospholipid contact with the aqueous solution. The procedure was repeated four times. The liposome suspension was left at room temperature for 1 h to anneal the bilayer structure.

The multilamellar vesicles were extruded through two (stacked) polycarbonate filters (25 mm diameter, Nucleopore, Pleasanton, CA) at 50°C by means of a stainless steel extrusion device (Lipex Biomembranes, Vancouver, B.C.). The extrusion procedure consisted of ten passages of the liposomal suspension through 400 nm polycarbonate filters, followed by another cycle of ten passages through 200 nm filters. The phospholipid recovery after the extrusion procedure was determined to be higher than 94%.

2.3. DSC

The DSC experiments were carried out on samples (40 μl containing ≈ 2 mg of lipid material) sealed in an aluminium pan with a Mettler DSC12E. Indium was used to calibrate the instrument. The detection system was a Mettler Pt100 sensor. The sensor presented a thermometric sensitivity of 56 μV/°C, a calorimetric sensitivity of about 3 μV/mW, and a noise level lower than 60 nV (< 20 μW) peak to peak. The reference was an aluminium pan containing isotonic phosphate buffer. Reference and sample pan masses were always matched to within 5.3% total mass, and usually to within 1.8%. The various samples were submitted to heating and cooling cycles (three times) at a scanning rate of 1°C/min. The data coming from the first scan was always discarded to avoid mixing artefacts. The necessity of periodic recalibration of the system over the course of these experiments may obviate direct comparison between groups of results. For this reason, a control sample, containing the basic phospholipid preparation without any drug, accompanied each set of studies. The endotherm obtained during the second scan of this control sample was used as a reference template for analysis of the accompanying experiments. ΔH values were calculated from peak areas by a Mettler TA89E and FP89 system software (ver. 2.0).

2.4. Kinetic interaction experiments

To study the interaction between idebenone and membranes as a function of time, a certain amount of idebenone (0.06 molar fraction) was weighed in a vial containing 1 ml of a DPPC liposomal suspension (50 mg/ml). This suspension was incubated either at 20 or 37°C and stirred throughout the duration of the experiment with a magnetic anchor (200 rpm). At predetermined time intervals, 40 μl of this mixture was sealed in an aluminium pan and submitted to DSC analysis. DPPC liposomal suspension was prepared as previously described 2 h before the kinetic experiments. The incubation procedure was carried out under nitrogen to prevent oxidation of the phospholipid.

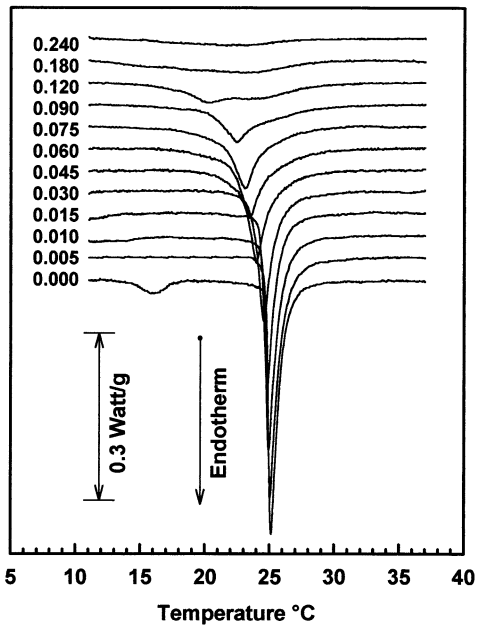

Fig. 2. DSC heating curves of colloidal dispersions of DMPC membranes prepared in the presence of different molar fractions of idebenone (see beside each curve). The furnace heating rate is 1°C/min. The various samples were submitted to DSC analysis 1 h after their preparation.

3. Results and discussion

The evaluation of the liposome thermotropic characteristics allows the investigation of the interactions occurring between a drug and a biological membrane model, such as lecithin vesicles. In fact, the presence of drug molecules in the ordered bilayer structure of the liposome matrix can influence the packing of the phospholipid molecules at the level either of the acyl chains, or the polar head groups or both, depending on their physicochemical properties, thus triggering variations in the transition temperature of the pure lipid and/or changes in the enthalpy of chain melting (O'Learly et al., 1986; Jain, 1988; Puglisi et al., 1995). In order to evaluate the thermotropic properties of lecithin-based vesicles, the DSC analysis, a sensitive and non-perturbing thermodynamic technique, was carried out (Puglisi et al., 1996b).

The presence of idebenone elicited a depression of the main transition peak of DMPC vesicles from the gel state to the liquid crystal phase ($P_\beta \rightarrow L_\alpha$ transition). As shown in Fig. 2, this effect is closely dependent on the concentration of the drug: the higher the molar fraction of idebenone within the DMPC bilayers, the greater the depression of the $P_\beta \rightarrow L_\alpha$ transition. In particular, the disappearance of the pre-transition peak from the gel state to the 'ripple' phase and a broadening of the main transition peak with a reduction of the ΔH values were observed at low idebenone molar fractions. As shown in Fig. 2, the broadening effect was observed up to an idebenone molar fraction of 0.09. A further increase in the drug molar fraction also elicited a splitting of the main transition peak. In fact, as shown in Fig. 3, the DSC curve of the DMPC at 0.12 molar fraction of idebenone can be suitably

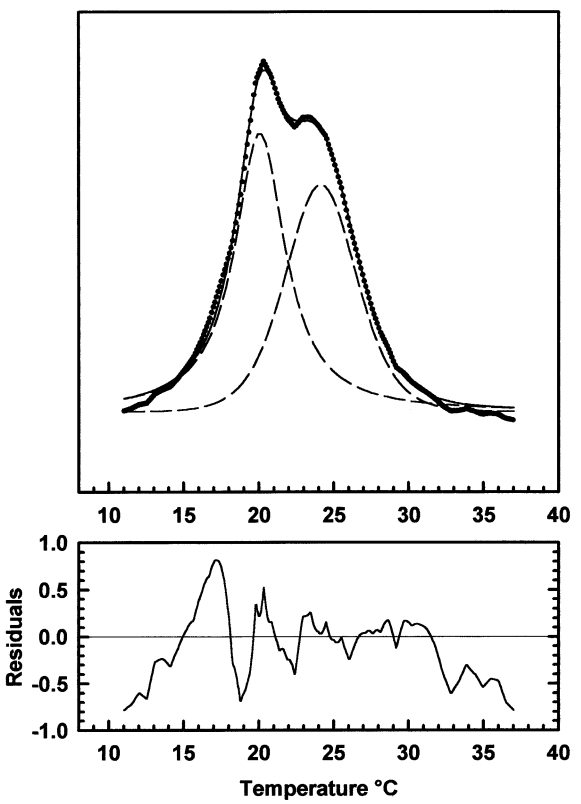

Fig. 3. Computer fitting (solid line) of the experimental DSC curve (filled circles) of DMPC membranes containing idebenone at a molar fraction of 0.12. The deconvolution (dashed line) of the theoretical curve led to two peaks centred at 20.1 and 24.2°C. At the bottom of the figure the residuals of the fitting procedure are shown.

Table 1
Thermotropic parameters of the mesophase transition from the gel state to the liquid crystal phase of colloidal suspensions of DMPC membranes containing different molar fractions of idebenone

Molar fraction	Tm[a] (°C)	Transition range[b]		ΔT 1/2[c] (°C)	ΔH (kcal/mole)	ΔS (cal/mol per K)
		T°C (10%)	T°C (95%)			
0.000	25 2	25.0	26.6	0.7	7.79	26.12
0.005	25.0	24.8	26.5	0.7	7.15	23.99
0.010	24.8	24.4	26.4	0.8	6.62	22.23
0.015	24.5	24.2	26.3	1.0	6.06	20.37
0.030	24.2	24.3	26.7	1.2	5.84	19.65
0.045	23.6	22.2	26.7	1.4	5.70	19.22
0.060	23.0	21.7	26.9	1.7	5.58	18.85
0.075	22.5	21.2	26.8	2.0	5.53	18.71
0.090	22.1	20.8	26.8	3.2	5.49	18.60
0.120	21.6	17.6	27.0	7.3	5.30	18.08
0.180	20.1	11.8	25.8	9.5	2.00	6.79
0.240	—	—	—	—	—	—

These parameters refer to the transition peak of the second DSC scan in heating mode. Each value is the average of three different experiments. The experiments were carried out 1 h after preparation of the DMPC membrane suspensions.
[a] Main transition peak temperature.
[b] Range of the main transition peak of DMPC vesicles from the transition of 10% of the sample to the transition of 95%.
[c] Width half-height of the main transition peak of DMPC membrane.

fitted by two peaks: one curve centred at a lower transition temperature (20.1°C) than the pure DMPC and the other centred at 24.2°C corresponding to the pure phospholipid. This behaviour is characteristic for a phase segregation due to the formation within the vesicle structure of drug-rich phospholipid domains and drug-poor phospholipid domains, similar to other drugs (Balasubramanian and Straubinger, 1994). At the higher drug molar fractions, the drug exclusion from the bilayer and its existence as a solid precipitate was disproved by light microscopy analysis (data not reported). Further proof came from the DSC assay, which showed no endotherm at ≈ 46°C corresponding to idebenone melting as aqueous dispersion.

The progressive reduction of the ΔH values of the transition $P_\beta \rightarrow L_\alpha$ as a function of the idebenone concentration is due to the physicochemical characteristics of the drug. In fact, the high lipophilic character of idebenone allowed the interaction of the molecule with the hydrophobic core of the phospholipid bilayer, noticeably perturbing the packing order of the smectic phase of liposomal vesicles. This interaction triggered a lowering of the transition ΔH values and a broadening of the main peak, due to a reduction of the hydrophobic forces among the acyl chains of the DMPC bilayers. Idebenone was able to intercalate between the various phospholipid molecules and, hence, elicited a reduction of the bilayer co-operativity (Jorgensen et al., 1991). The gradual reduction of co-operativity led to the depression of the $P_\beta \rightarrow L_\alpha$ transition at idebenone molar fractions higher than 0.18 (Fig. 2; Table 1). The gradual co-operativity reduction as a function of idebenone concentration is shown in Table 1 by the T°C (10%) and T°C (95%) values, and by the width half-height values.

The presence of idebenone also influenced the transition temperature which shifted towards lower values, as reported in Table 1. The transition temperature is normally influenced when drug–phospholipid interactions occur also at the level of the polar heads of the phospholipid molecules. In the case of idebenone, the only polar group present in the molecule is the primary alcoholic group (Fig. 1), which could be able to interact with the phosphocholine moiety of the DMPC molecules. Probably, the presence of this

group in the hydrophilic zone of the bilayer, besides the perturbing action at the level of the lipid bilayer core, is responsible for the shift of the transition temperature. In fact, in the case of molecules, i.e. cholesterol, whose interaction is mostly confined to the level of the hydrophobic zone of the bilayers, no remarkable shift of the transition temperature has been reported (Estep et al., 1978).

In order to evaluate the influence of the length of the acyl chain on the drug–vesicle interaction, DSC experiments were also carried out with vesicles of DPPC. In this case, no substantial change of the type of interaction occurring between the phospholipid matrix of the bilayers and idebenone was observed. Only the intensity of the phenomenon was different. The perturbation of the thermotropic parameters of the DPPC vesicles as a function of the idebenone molar fraction is less strong than for DMPC. The depression of the main transition peak is less evident, particularly for the systems with a low molar fraction of idebenone (Fig. 4; Table 2). In fact, the pre-transition peak can be still detected at an idebenone molar fraction of 0.005, although less intense than the pure DPPC. In the case of DMPC, the $L_\beta \rightarrow P_\beta$ transition disappears at drug molar fractions lower than 0.005 (data not reported). Furthermore, the phase segregation, occurring at an idebenone molar fraction of 0.09 in the case of DMPC vesicles, occurred at a higher molar fraction (0.18) for the system DPPC-idebenone. The co-operativity of the DPPC peak is also maintained up to higher molar fractions of idebenone (Table 2). These findings may be due to the longer DPPC acyl chains, which increase the hydrophobic part of the bilayer allowing a higher solubility of idebenone within the phospholipid matrix of the vesicles. Namely, the drug would be placed in the ordered structure of DPPC vesicle bilayers in a more suitable way than for the DMPC vesicles.

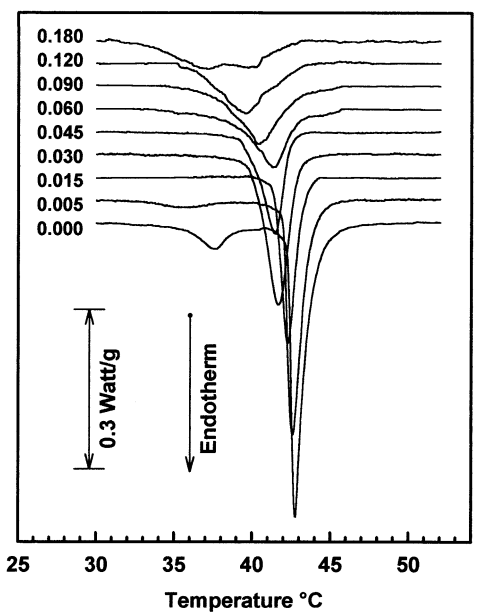

Fig. 4. DSC heating curves of colloidal dispersions of DPPC membranes prepared in the presence of different molar fractions of idebenone (see beside each curve). The furnace heating rate is 1°C/min. The various samples were submitted to DSC analysis 1 h after their preparation.

The influence of the presence of charged phospholipids in the lipid matrix on the drug membrane interaction was also evaluated with a vesicle colloidal suspension made up of DPPC–DPPA (8:2 molar ratio). In this system, the presence of DPPA ensures a negative charge along the bilayer surfaces. The DPPC–DPPA (8:2 molar ratio) system did not show the same thermotropic profile observed for pure DPPC, although DPPA contains the same acyl chains of DPPC.

As shown in Fig. 5, the pre-transition peak $L_{\beta 1} \rightarrow P_{\beta 1}$ disappeared due to the presence of DPPA in the DPPC bilayer. The main transition peak already presented a noticeable reduction of co-operativity (Table 3) at an idebenone molar fraction of 0.03. The co-operativity reduction is linked to a phase segregation, which can be easily observed at the 0.03 molar fraction (Fig. 5). Contrary to DMPC and DPPC systems the ΔH value of the main transition peak rapidly decreased following the presence of idebenone, even at low concentrations. The T_m values were also shifted towards lower temperatures. These features are probably due to an interaction between idebenone and DPPA. This kind of interaction could be due to an influence both at the level of the acyl chain constituting the hydrophobic zone of the bilayers, similarly to DPPC and DMPC, and at the level of the polar headgroups of DPPA. In fact, the pres-

Table 2
Thermotropic parameters of the mesophase transition from the gel state to the liquid crystal phase of colloidal suspensions of DPPC membranes containing different molar fractions of idebenone

Molar fraction	Tm[a] (°C)	Transition range[b]		ΔT 1/2[c] (°C)	ΔH (kcal/mole)	ΔS (cal/mol per K)
		T°C (10%)	T°C (95%)			
0.000	42.2	41.7	43.5	0.9	8.27	26.24
0.005	42.1	41.8	43.7	0.9	7.89	25.04
0.015	41.9	41.3	43.2	1.2	6.73	21.37
0.030	41.5	41.2	43.6	1.4	6.24	19.84
0.045	41.0	39.6	42.5	1.6	5.71	18.18
0.060	40.7	39.0	42.2	2.3	5.45	17.37
0.090	39.7	37.7	42.4	2.8	5.32	17.01
0.120	38.8	36.7	41.5	3.6	4.99	16.00
0.180	36.2	33.8	40.8	5.9	3.66	11.83

These parameters refer to the transition peak of the second DSC scan in heating mode. Each value is the average of three different experiments. The experiments were carried out 1 h after preparation of the DPPC membrane suspensions.
[a] Main transition peak temperature.
[b] Range of the main transition peak of DPPC vesicles from the transition of 10% of the sample to the transition of 95%.
[c] Width half-height of the main transition peak of DPPC membrane.

ence of an alcoholic group in the idebenone molecule may ensure the formation of hydrogen bonds with the polar DPPA headgroups (La Rosa et al., 1992a; Puglisi et al., 1992). Contrarily, the choline groups of DPPC and DMPC do not form hydrogen bands. These different properties could be responsible for the particular behaviour of the DPPC–DPPA system. The idebenone–DPPA interaction could lead to the formation of rich domains in DPPA–idebenone and, hence, of vesicular structures constituted by heterogeneous bilayers. This hypothesis can justify the presence of two peaks in the DSC curves of DPPC–DPPA even in the presence of low idebenone molar fractions (Fig. 6). One peak is centred at a higher temperature (43 9°C) and may be attributed to the transition $P_\beta \rightarrow L_\alpha$ of the DPPA-rich domains, whereas the other peak centred at 41.9°C may be due to the transition of DPPC-rich domains. Increasing the idebenone amount (molar fraction 0.09), a further segregation was observed, leading to the formation of three different phospholipid domains within the liposomal matrix (Fig. 6). A similar trend was observed for phospholipid mixtures containing DPPS, which is also able to form hydrogen bonds with idebenone (data not reported).

In order to explore possible interactions with biomembranes as a function of time, the kinetics of interaction between the DPPC vesicles and idebenone were investigated. The DSC scan of pure DPPC at time 0 did not present any particular change. As shown in Fig. 7, just after 1 h the system DPPC–idebenone was characterised by a different thermotropic profile. Namely, the pretransition peak $L_\beta \rightarrow P_\beta$ disappeared, the ΔH value of the main transition peak $P_\beta \rightarrow L_\alpha$ slightly decreased and a second peak centred at ≈ 46.8°C was observed and attributed to drug fusion. This new thermotropic profile of the DPPC–idebenone system was maintained throughout the duration of the experiment (≈ 24 h), even if with some variation of the intensity of the two peaks. In particular an intensity reduction of the two peaks was observed as a function of time. Furthermore, a gradual broadening of the peak $P_\beta \rightarrow L_\alpha$ with a reduction of co-operativity and ΔH was observed. This trend was elicited by the interaction between DPPC vesicles and idebenone, which is responsible for the depression of the $P_\beta \rightarrow L_\alpha$ peak (Fig. 7). Following the interaction with DPPC membranes, the amount of free idebenone present in the DSC crucible gradually decreased as a function of time, and hence a reduction of the ΔH value of the

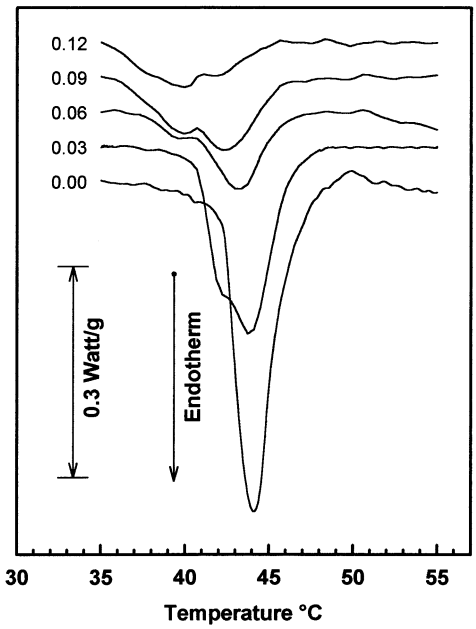

Fig. 5. DSC heating curves of colloidal dispersions of DPPC–DPPA (8:2 molar ratio) membranes prepared in the presence of different molar fractions of idebenone (see beside each curve). The furnace heating rate is 1°C/min. The various samples were submitted to DSC analysis 1 h after their preparation.

fusion peak of the drug was also detected. In fact, only a variation of the intensity and not of the shape of the peak was observed (Fig. 7).

The interaction between idebenone and the DPPC vesicles may be due to a direct contact of the membranes with the dispersed drug rather than to a diffusion process of idebenone through the aqueous phase where this molecule is highly insoluble. In particular, it was possible to calculate the amount of idebenone that is taken up by the liposomal colloidal suspension as a function of time. In fact, the ΔH value of the endotherm peak referring to the drug melting is directly proportional to the amount of idebenone still present as solid dispersion. Therefore, carrying out a calibration straight line, it was possible to evaluate the remaining drug and hence the amount of drug taken up by the liposome system. It was calculated that 64 and 81% of the added drug amount was inglobated in the bilayers of the DPPC membranes after 24 h of incubation at 20 and 37°C, respectively. As shown in Fig. 7, the drug–membrane interaction was more intensive when the experiments were carried out at 37°C compared with 20°C.

4. Conclusion

The findings, here reported, show that the drug can interact with the membranes particularly in the presence of negatively charged phospholipids, in which the brain is particularly rich. Idebenone

Table 3
Thermotropic parameters of the mesophase transition from the gel state to the liquid crystal phase of colloidal suspensions of DPPC–DPPA (8:2 molar ratio) membranes containing different molar fractions of idebenone

Molar fraction	Tm[a] (°C)	Transition range[b]		ΔT 1/2[c] (°C)	ΔH (kcal/mole)	ΔS (cal/mol per K)
		T°C (10%)	T°C (95%)			
0 000	43.6	42.6	46.0	2.2	10.61	33.50
0.015	43.6	41.7	46.6	2.6	9.89	31.23
0.030	43.5	41.5	46.7	3.1	9.35	29.53
0 060	43.0	41.2	44.6	3.9	8.98	28.75
0.090	41.1	37.6	43.8	5.5	7.91	25.18
0.120	39.6	34.2	43.6	6.1	3.63	11.49

These parameters refer to the transition peak of the second DSC scan in heating mode. Each value is the average of three different experiments. The experiments were carried out 1 h after preparation of the DPPC–DPPA membrane suspensions.
[a] Main transition peak temperature.
[b] Range of the main transition peak of DPPC-DPPA vesicles from the transition of 10% of the sample to the transition of 95%.
[c] Width half-height of the main transition peak of DPPC–DPPA membrane.

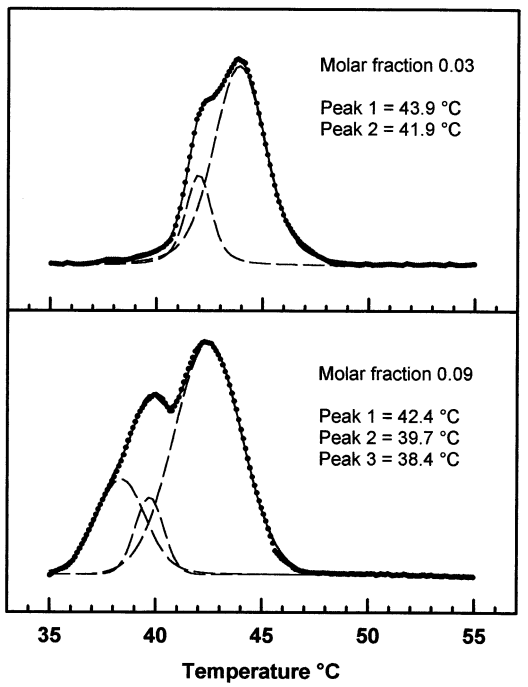

Fig. 6. The experimental DSC curves of DPPC–DPPA (8:2 molar ratio) membrane prepared in the presence of different molar fraction of idebenone are suitably theoretically fitted. The deconvolution showed the presence of various peaks due to idebenone–phospholipids phase segregation. Filled circle, experimental points; solid line, theoretical fitting; broken line, deconvolved peaks. The fitting procedure presented a r^2 coefficient of 0.9987.

is potentially able to interact with neuronal biological membranes, which represent its therapeutic target site. The kinetic experiment showed a slow drug–membrane interaction due to the poor water solubility of the drug, which hampers a rapid diffusion through the aqueous compartments. These features justify the low bioavailability of idebenone. Thus, the realisation of a carrier system, such as the liposomal carrier (Fresta et al., 1995; Fresta and Puglisi, 1996a), can be of particular interest because it can improve the drug bioavailability and selectivity towards the target site, while reducing the drug-serum protein binding, which is a limiting factor in the treatment of diseases at the level of the CNS.

To obtain a liposomal system which can work as a biological carrier of idebenone, it is necessary to incorporate the drug within the phospholipid matrix of the carrier, avoiding drug molar fractions higher than 0.06. In fact, above this value a phase segregation of the liposome phospholipid matrix was observed. The lipid segregation may lead to a loss of the stability and integrity of the liposomal carrier particularly when administered in vivo. Furthermore, the liposomal colloidal formulation may ensure the possibility of i.v. administration, which is of noticeable interest to improve the therapeutic characteristic of idebenone and to extend the clinical use to extreme situations, i.e. cerebral ischaemia, cerebral oedema, traumatic events, and cerebrovascular lesions.

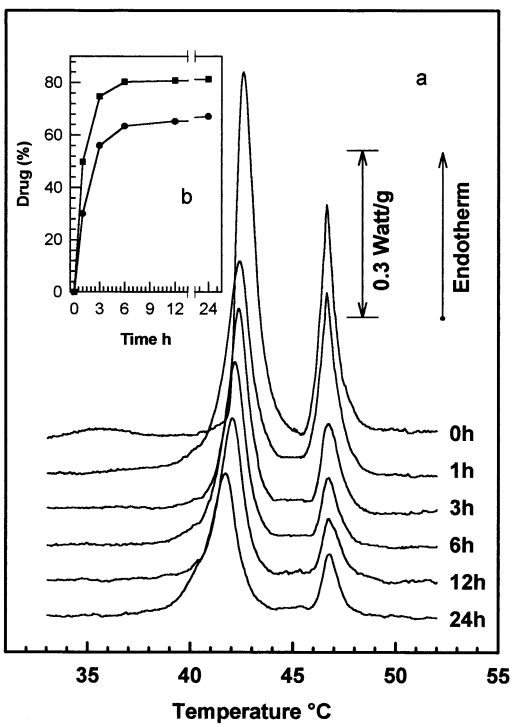

Fig. 7. Kinetic experiment of the interaction between idebenone and a colloidal suspension of DPPC membranes dispersed in isotonic phosphate buffer (pH 7.4). The drug amount added to the vesicle suspensions correspond to a molar fraction of 0.06. DSC scans of DPPC vesicles incubated with idebenone at a temperature of 37°C (graph a). Percentage of drug amount inglobated within the phospholipid matrix of DPPC membranes as a function of time (graph b). The experiment was carried out at 20 (filled circle) and 37°C (filled square).

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