EXPERT OPINION

Chemical and technological delivery systems for idebenone: a review of literature production

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Introduction: Idebenone (IDE) is an antioxidant compound, structurally related to coenzyme Q10. Its therapeutic potential is growing in different application areas, as demonstrated by the number of experimental works and patents produced in very recent years.
Areas covered: Cyclodextrin inclusion complexes, liposomes, microemulsions, prodrugs, polymeric and lipid nanoparticles have been explored to achieve different goals, such as topical administration, brain targeting or increasing the bioavailability of this highly lipophilic drug. This review summarizes the results of works published in the last 20 years for the delivery and targeting of this drug.
Expert opinion: A direct comparison of the different carrier systems is not easy and could not even be significant, due to the large variables existing among them. However, the different forms of delivery can help increase idebenone solubility, stability and biochemical activity. Further studies will be developed in order to improve the controlled release and targeting of idebenone.

Keywords: antioxidant activity, cyclodextrins, drug delivery systems, lipoamino acids, liposomes, microemulsions, nanoparticles, prodrugs, SLN, solid lipid nanoparticles

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1. History and properties of idebenone

Idebenone (IDE, 2-(10-hydroxydecyl)-5,6-dimethoxy-3-methyl-2,5-cyclohexa-diene-1,4-dione, according to the IUPAC nomenclature) is a short-chain benzo-quinone, synthetic analog of coenzyme Q10 (CoQ10) (**Figure 1**). It appears like an yellow-orange crystalline, odorless powder, insoluble in water and soluble in most organic solvents, including ethanol, dimethyl sulfoxide (DMSO), chloroform and propylene glycol. Two polymorphs of IDE are known (forms A and B) with different X-ray diffraction patterns and different melting point. It was verified that crystallization leads constantly to form A, whatever the nature of the solvent. Form B is obtained only after melting and re-solidification. Furthermore, the current manufacturing process produces exclusively form A.

The stability results from long-term and accelerated studies indicated that IDE is a very stable substance, in particular in the solid state; after irradiation with a Xenon light source no decomposition was observed in this state. In aqueous buffer solutions degradation was observed only at high pH values.

IDE is characterized by the same benzoquinone ring of CoQ10 with a short hydroxydecyl side chain rather than the isoprenoid side chain of CoQ10.

Both IDE and CoQ10 provide protection and free radical scavenging. Their chemical structure allows to influence the electron balance within mitochondria and to undergo reversible reduction/oxidation (redox) reactions, acting as a potent antioxidant and electron carrier, and increasing the production of cellular adenosine triphosphate (ATP) [1,2].

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Article highlights.

- Idebenone is an interesting drug, due to the wide therapeutic potentialities, moving from the diseases based on alteration of cell redox equilibrium, to other pathological fields, including neurodegenerative diseases and skin protection.
- Different technological approaches have been investigated to increase the bioavailability and solubility of idebenone, a high lipophilic and very low-soluble drug.
- Drug delivery systems prepared with idebenone can also provide a controlled release.
- *In vitro*/*in vivo* biological studies performed on the chemical or technological systems show in many cases an improvement in antioxidant and cell-protective effects.
- Idebenone can receive a beneficial improvement in terms of solubility, stability, and biochemical activity by these various forms of delivery.
- Further nanotechnology strategies to achieve controlled release, vectorization, and targeting of idebenone will be explored, due to the high level of interest for this drug.

This box summarizes key points contained in the article.

In particular, CoQ10 is a vitamin-like molecule involved in the electron transport chain which is an integral part of energy production in living cells [3]. Even if CoQ10 is produced in the body from the amino acid tyrosine and acetyl-CoA, also the synthetic molecule IDE must be supplemented. The interest of researchers in this drug is still strong due to its many advantages compared to CoQ10 in specific situations. Under normal conditions, both idebenone and CoQ10 are effective antioxidants, due to their ability in neutralizing free radicals that cause tissue damage. At low oxygen concentrations (e.g., during a heart attack, stroke, trauma or shock) CoQ10 may act more like a free radical itself. On the contrary, under the same hypoxic conditions that cause CoQ10 to oxidize itself, IDE still prevents free radical damage and maintains relatively normal ATP production. This is one key reason for considering IDE to be much more effective than CoQ10 [4].

In addition, IDE has antioxidant properties, thus it can inhibit lipid peroxidation and protect against reactive oxygen species (ROS)-induced damage in mitochondria, with a consequent protection of membranes from damage [1,5,6].

According to this mechanism, IDE proved cytoprotective after exposure of cultured cells to different noxas [7].

1.1 Pharmacokinetics

After oral administration, IDE is rapidly absorbed by the gastrointestinal system [8]. *In vivo* studies on rats and dogs showed a bioavailability of 91 and 62%, respectively. Plasma peak concentration is reached within 1 h. However, due to a high first-pass metabolism, less than 1% of the administered IDE reaches the systemic circulation. Concentrations of IDE in plasma are considerably lower than the concentrations of metabolites, and detectable in plasma only during a short period of time. Thus, no half-life has been calculated in any species. Administration of IDE suspension in fed condition increased bioavailability considerably in dogs. This was also seen in humans given fat-rich food although the increase in bioavailability was not as pronounced. More than 99% of IDE is bound to plasma proteins. Highest concentrations of radioactivity after oral administration of [^{14}C]IDE were found in liver, kidney, blood, lung, spleen, skeletal muscle, thymus, stomach, and intestine. No accumulation was seen in any tissue studied after repeated dosing. Parent IDE distributed to brain and the highest concentration of IDE and the active metabolite QS-10 was found in cerebellum. Furthermore, IDE was found in the mitochondrial fraction of brain homogenates. Conjugated IDE and metabolites were also detected in brain at concentrations 10 times higher than the parent IDE. Distribution to placenta and fetal tissues was demonstrated; IDE and/or its metabolites were also excreted into milk in moderate amounts.

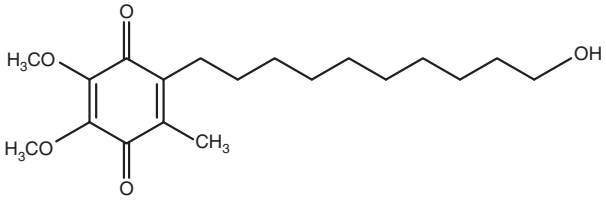
IDE is eliminated mainly after metabolism. Less than 1% of orally administered drug reaches the systemic circulation, due to the extensive first-pass metabolism in liver and intestinal mucosa. The metabolism of IDE includes oxidation and shortening of the side chain resulting in metabolites QS-10, QS-8, QS-6 and QS-4. These metabolites, as well as the parent compound are further modified by conjugation (glucuronidation or sulfatation). The predominant metabolites in human plasma were identified as the sulfate conjugate of IDE and the O-desmethylated glucuronic acid conjugate of QS-10 glucuronide.

As concerns the excretion, 72 h after oral or i.v. administration of [^{14}C]IDE elimination of radioactivity was almost complete. In humans, approximately 80% of the dose is excreted through the urinary path, for the majority in the form of the glucuronic acid and sulfate conjugates of QS-4.

In all studies performed on animals and humans, it was observed that IDE is safe and generally well tolerated. Only mild adverse effects were observed in clinical studies, such as nausea, diarrhea and dyspepsia.

1.2 Pharmacodynamics

In *in vitro* pharmacodynamic studies using isolated mitochondria from heart and brain tissue, the reduced form of IDE inhibited lipid peroxidation and swelling of mitochondria. Almost complete inhibition of lipid peroxidation was observed at a concentration of 200 µM. None of the main metabolites (QS-4, QS-6, QS-10) showed the same potency [9]. A study in the ischemic rat heart model showed that IDE can activate the energy metabolism of cardiac muscle and improve cardiac function after reperfusion. *In vitro* studies further showed that the drug can protect cultured fibroblasts derived from Friedreich ataxia (FRDA) patients from cell-damaging oxidative stress thereby increasing cellular survival. IDE restored enzyme activities of the mitochondrial respiratory chain in heart biopsies of FRDA patients [10].



ALogP: 2.554; PSA: 72.83 Formula: $C_{19}H_{30}O_5$; Formula weight: 338.44

Figure 1. Idebenone structure and physico-chemical data.

In vivo, the systemic effect of IDE was tested in a genetically-modified mouse model of FRDA generated by targeted deletion of the frataxin gene in striated muscle. This study indicated that IDE affects the mitochondrial function and improves cardiac parameters in FRDA mutant mice after the onset of the disease [11]. The protective actions on ischemic cardiac muscle were also investigated in dogs with coronary stenosis. IDE, administered i.v. for 30 min after stenosis was initiated, dose-dependently reduced the S-T interval. In general, this study confirmed that the drug can improve the energy metabolism of the cardiac muscle mitochondria in the ischemic region of the dog heart.

Secondary pharmacodynamic studies, performed in various animal models demonstrated that IDE inhibits the development of stroke and renal vascular lesions associated with severe hypertension. It also exerted a positive effect on neurological deficits related to cerebral ischemia [9]. In normal rats, IDE did not alter brain concentrations of acetylcholine (Ach) or choline in a dose range of 10 – 100 mg/kg, i.p. Pre-treatment with 10 mg/kg IDE, i.p., prevented the decrease in ACh and increase in choline due to ischemia in forebrain regions in rats.

1.3 Toxicology

No safety concerns of relevance for the human situation were observed in animal safety pharmacology studies. IDE (300 – 1000 mg/kg) had no or only minor effects on either the central nervous system or somatic nervous systems [9].

In several studies IDE showed no genotoxic potential. It seemed did not display effects on fertility and general reproductive performance, and there was no evidence of embryotoxic or teratogenic potential. Carcinogenity studies in animals also gave safe data, with much higher exposure levels compared to the highest recommended dose in humans [9].

2. Therapeutic applications

Even if in the past IDE has been referred to as a natural compound, like a vitamin, a natural compound or a food/dietary supplement, the U.S. Food and Drug Administration (FDA) has classified it as a prescription medication under the Federal Food, Drug and Cosmetic Act; therefore, IDE requires to be regulated with a formal approval by the Agency for its use on humans [12]. Due to its redox properties and ability to mediate electron transfer to respiratory chain complex III in isolated mitochondria, IDE has been studied and is currently under investigation for the treatment of different mitochondrial and neuromuscular diseases associated with respiratory chain dysfunction [8,13].

IDE was first researched in mid-1980s by Takeda Pharmaceuticals Company Ltd. (Osaka, Japan) [14], and has been available in Japan since 1986 as Avan® for the improvement of cerebral metabolism and psychiatric symptoms in Alzheimer's disease (AD) [12]. AD is a degenerative brain disorder that develops in mid-to-late adulthood causing a progressive and irreversible decline in memory and a deterioration of various other cognitive abilities. AD is characterized by the destruction of neurons and neural connections in the cerebral cortex of the brain and by a significant loss of brain mass. It has been showed that several neurodegenerative diseases are associated with the excessive activation of glutamate receptors, suggesting its contribution to cell vulnerability, in particular astrocytes, through altered glutamate transport [15]. Although some studies demonstrated the efficacy of IDE in AD [4,16], the lack of sufficient evidence avoids its clinical use [4]. IDE has been approved in Italy (Mnesis®, Takeda Italia Farmaceutici; Daruma®, Wyeth Lederle) since the early 1990s for the treatment of myocardiopathy of patients affected by FRDA. It is also developed by Santhera Pharmaceuticals (Liestak, Switzerland) as Catena® and Sovrima®, for the treatment of FRDA and Duchenne muscular dystrophy (DMD), respectively [4]. FRDA is a progressive, multi-system, degenerative disorder caused by mutations in the frataxin gene, characterized in particular by an expansion of GAA triplets. It is very common among Caucasian population and it usually onset in 5 – 25 years old. FRDA is characterized by progressive gait and limb ataxia, dysarthria, areflexia, loss of vibration and position sense, weakness of proximal and distal muscles and abnormal eye movements [16]. As showed by recent studies, IDE is a promising drug for the treatment of neurodegenerative diseases, showing a cryoprotective activity in fibroblasts from patients affected by FRDA [7,17]. In particular, IDE showed to reduce significantly the oxidative DNA damage and prevent iron-induced lipoperoxidation and cardiac muscle

enlargement [18,19]. A double-bind Phase III clinical trial registered IDE safety over a period of 12 months, but highlighted the lack of efficacy in the functional outcome of treated patient with FRDA, compared to those treated with the placebo. However, improvement in the neurological function was observed in a meta-analysis of three Phase II and III clinical studies [4,20]. An improvement of cardiac and respiratory parameters was observed in patients with Duchenne muscular dystrophy (DMD) during a Phase II clinical trial [4,21,22] DMD is a rapidly progressive form of muscular dystrophy that occurs primarily in boys and is caused by a mutation in a gene, called the DMD gene. It is characterized by a progressive loss of muscle function and weakness, which begins in the lower limbs. DMD dystrophy affects approximately 1 in 3500 male births worldwide. Because this is an inherited disorder, risks include a family history of DMD. More recently, IDE efficacy in Leber's hereditary optic neuropathy (LHON) patients has been reported in a double-blind placebo-controlled randomized trial [23]. LHON is a mitochondrial genetic disease that preferentially causes blindness in young adult males and it is characterized by bilateral, painless, subacute visual failure that develops during young adult life. IDE treatment for LHON has been widely studied, suggesting that early and prolonged IDE treatment in patients with acute LHON may significantly improve the frequency of visual recovery thus probably changing the natural course of the disease [24-26]. Another clinical trial of IDE for the treatment of mitochondrial encephalopathy, lactic acidosis and stroke-like episode (MELAS) is still under review [27,28]. It has been suggested long-term safety and potential benefit of oral IDE administration in the prevention of recurrence of stroke-like episodes in adult MELAS patients [29]. Being an antioxidant, IDE may also play a potential role in other conditions which have been linked to mitochondrial dysfunction, such as Parkinson disease (PD), Huntington disease (HD), and amyotrophic lateral sclerosis (ALS) [30]. A recent study showed the efficacy in improving the respiratory function of a patient affected by Leigh syndrome, a subacute necrotizing encephalomyopathy frequently ascribed to mitochondrial respiratory chain deficiency, after treatment with IDE [30,31]. A double-blind, placebo-controlled, Phase I/II clinical trial is currently recruiting participants to study the safety and effectiveness of using IDE in patients with primary progressive multiple sclerosis (PP-MS), an inflammatory disorder of the central nervous system (CNS) that progressively weakens and destroys the pathways of the nervous system [32]. Evidences have been reported about IDE cosmetic application to reduce wrinkles and other signs of aging, by quenching free radicals in the epidermis. In 2005 a clinical trial on 0.5% and 1.0% IDE topical skincare commercial formulations was conducted to establish the topical safety and efficacy in photo-damaged skin [33]. Results of this study showed the efficacy of both formulations in increasing skin hydration, reducing the skin roughness/dryness and fine lines/wrinkles and improving the assessment of photo-damaged skin.

3. Drug delivery systems (DDS) for IDE

Different strategies have been exploited for carrying this active, such as cyclodextrins (CDs), microemulsions, nanoparticles (NP), liposomes and prodrugs, with the aim to increase drug bioavailability, achieve a controlled release and improve its efficacy (**Table 1**).

3.1 Cyclodextrins

The very low water solubility of IDE (0.8 mg/100 ml) and binding with serum proteins cause a reduced absorption and a poor bioavailability at the level of the CNS. For this reason, many strategies have been developed to overcome the limits related to IDE administration. Initially, the feasibility of using CDs as drug carriers to improve drug solubility was studied. CDs are cyclic oligosaccharides composed of glucose molecules, able to include different active compounds into their hydrophobic cavity. In 1995, IDE inclusion complexes with β-cyclodextrin (β-CD) using two different methods, coprecipitation and freeze–drying, were prepared to increase IDE bioavailability [34]. Calorimetric studies and X-ray diffraction analysis were performed on the complexes in the solid state to achieve information about the interaction between IDE and the external surface of β-CD. The dissolution profiles showed a significant increase of the aqueous solubility and dissolution rate of the complexes compared to free IDE, in particular when the complexes were freeze–dried. This could probably due to an increase of drug water solubility, increase of wettability and reduction of drug crystallinity after its complexation.

Due to the nephrotoxicity of β-CD, its inclusion complexes cannot be used for parenteral administration. For this reason, the same authors prepared IDE inclusion complexes with β-CD derivatives, in particular 2,3,6-tri-*O*-methyl-β-CD, 2,6-di-*O*-methyl-β-CD and 2-hydroxypropyl-β-CD using different methods: kneading, coprecipitation, and freeze–drying [35]. The authors verified that complexation significantly increased IDE aqueous solubility, in particular with 2,6-di-*O*-methyl-β-CD and 2-hydroxypropyl-β-CD. A faster dissolution rate was observed for all complexes compared to the free drug (**Figure 2**). The preparation technique selected was shown to influence the dissolution profile of the obtained complex, i.e., the freeze–drying method allowed to obtain an instantaneous dissolution, compared to kneading and coprecipitation methods, due to the formation of amorphous IDE–CD complexes with reduced particle size.

Another recent study has been reported concerning the feasibility to obtain IDE inclusion complex using sulfobutylether-β-cyclodextrin (SBE-β-CD) [36]. SBE-β-CD is a CD derivative currently used in four FDA-approved injectable products. It is able to improve solubility and complexation

Table 1. Events in the development of idebenone drug delivery systems.

Year	Context/DDS	Outcomes	Ref.
1995	β-cyclodextrin	Solubility studies with β-CD showed an increase in IDE solubility and the enhancement of dissolution rate on complex with respect to free drug, particularly for freeze–dried complex	[31]
1996	β-cyclodextrin derivatives	DM-β-CD and HP-β-CD significantly increased IDE aqueous solubility and enhanced the dissolution rate of the complexed IDE compared to the free drug	[32]
1998	IDE interaction with biomembrane model	DSC studies showed that IDE-membrane interaction is enhanced by the presence of negatively charged phospholipids	[36]
2000	Self-microemulsifying drug delivery systems	SMEDDS increase the dissolution rate of IDE and its release (more than twofold) compared to conventional dosage form	[41]
2002	Liposomes	Ethanol-induced injury on astroglial cells are mediated by abnormal formation of free radical species and this may represent an useful approach in the treatment of ethanol-related brain disorders	[34]
2002	Nanocapsules	An improvement antioxidant effect was observed when a low IDE concentration was entrapped within Tween 80-coated PECA nanocapsules	[42]
2004	Prodrugs: lipoamino acid esters	*In vivo* studies showed that IDE-LAA conjugates behave as prodrugs releasing IDE rapidly via enzymatic hydrolysis *in vitro* in serum and liver homogenate, resulting advantageous after absorption through the cornea into the anterior chamber of the eye	[54]
2004	Prodrugs: alkylamino acids	Derivatization of IDE molecule at the level of the hydroxyl group in the side alkyl chain preserved its biological activity. Short alkyl chain conjugates showed a greater capacity to scavenge free radicals	[55]
2004	Liposomes	The entrapment of IDE in PEGylated liposomes improved its effect on ethanol-induced injury, probably due to the greater drug bioavailability at a cellular level	[35]
2006	Solid lipid nanoparticles	SLN prepared by the solvent injection technique guarantee IDE prolonged release ensuring its protective effect against oxidative cell damage.	[47]
2006	Prodrugs synthesis and interaction with biomembrane models	IDE-LAA conjugates interacted at different levels with respect to pure IDE with DMPC bilayers.	[56]
2006	O/W microemulsions	IDE delivery from PIT O/W microemulsions can be controlled by changes in the proper combinations of oil phase lipids and emulsifiers	[38]
2010	Chitosan nanoparticles	IDE incorporation into chitosan and N-carboxymethyl-chitosan NP prepared by spray-drying increased drug stability and antioxidant activity, decreasing its irritative effect on the mucous membrane.	[43]
2011	Liposomal prodrugs	Experimental *in vitro* data suggested that high affinity shown by the lipophilic IDE derivatives for the liposomal carriers negatively affects their biological activity.	[57]
2011	Solid lipid nanoparticles	SLN prepared by the PIT method can be used for the brain targeting of IDE to overcome the BBB and increase the drug efficacy	[46]
2012	Nanostructured lipid carriers	NLC for topical application can significantly improve IDE skin permeation and stability.	[52]
2012	Solfobutylether-β-cyclodextrin	SBE-β-CD enhanced IDE aqueous solubility and dissolution rate, avoiding toxicity effects associated with the parent or certain other alkylated CDs	[33]
2012	Solid lipid nanoparticles	*In vitro* permeation studies across a model of BBB highlighted that IDE-loaded SLN prepared by the PIT method are a promising drug delivery system for administration to the brain	[49]
2012	Solid lipid nanoparticles	*In vitro* skin permeation/penetration showed no permeation of IDE from SLN, while penetration was related to the primary surfactant selected	[50]

avoiding the toxicity related to alkylated CDs. This study showed a considerable improvement in IDE solubility due to the formation of 1:1 SBE-β-CD complex. Moreover, a significant enhancement on dissolution rate has been observed, thus encouraging the use of the complex as a new drug delivery product. In particular, it has been observed a linear increase of IDE solubility with increasing concentration of SBE-β-CD at different temperature (300, 310, and 320 K). The value for the stability constant was found to decrease when the heat was provided to the system in the order 300 K > 310 K > 320 K, highlighting the presence of an exothermic process in all systems.

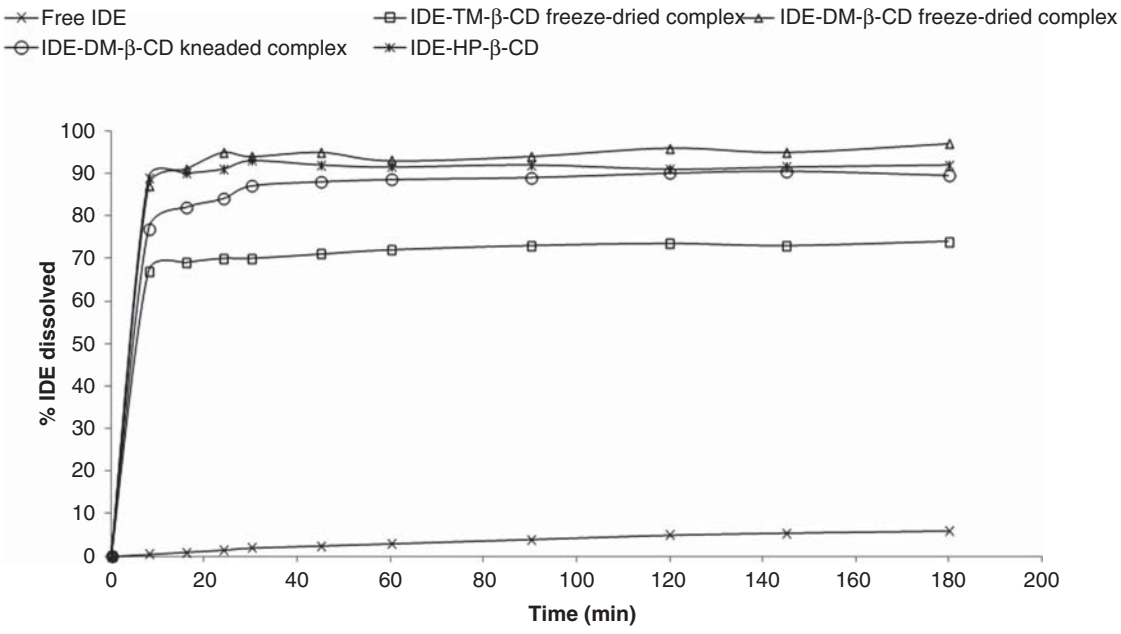


Figure 2. Dissolution profiles of free and complexed IDE in buffer solution (pH 1.1) and at 37 ± 0.5°C. IDE; IDE-HP-β-CD; IDE-TM-β-CD freeze–dried complex; IDE-DM-β-CD kneaded complex; IDE-DM-β-CD freeze–dried complex.
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Table 2. Effects of IDE-loaded liposomes on LDH release, GS activity and cell viability (MTT test) in ethanol-injured (100 μM) primary rat cortical astrocyte cultures.

Sample	LDH	MTT	GS
Control	9.5 ± 0.8	0.800 ± 0.020	100 ± 4
Ethanol treatment	39.0 ± 2.1	0.590 ± 0.028	42 ± 3
IDE 0,5 μM	37.8 ± 1.9	0.607 ± 0.025	51 ± 3
IDE 5 μM	15.9 ± 1.2	0.662 ± 0.016	65 ± 5
IDE 25 μM	5.7 ± 2.1	0.705 ± 0.013	80 ± 4

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3.2 Liposomes

Among the different strategies employed to improve the pharmacokinetics of poorly soluble drug, such as IDE, leading to a more rapid and complete absorption, liposomal encapsulation can be pursued. In this respect, the advantage of using liposomally-entrapped IDE on free radical overproduction in primary cortical rat astroglial cells has been demonstrated [37]. CNS is known to be damaged by ethanol consumption: *in vitro* studies showed that chronic treatment of astroglial cell cultures with ethanol leads to an increased glutamate uptake and imbalance in extracellular glutamate equilibrium. Addition of liposomal IDE significantly reduced the ethanol-induced injury of astrocytes, as shown by the decrease of ethanol-induced lactate dehydrogenase (LDH) release, restoration of glutamine synthase activity and cell viability enhancement in respect to ethanol-exposed cells untreated or incubated with empty liposomes (**Table 2**).

IDE also dose-dependently antagonized the ethanol-induced oxidative stress in astroglial cell cultures: reduction of both Heat Shock Protein 70 kDa (HSP70) and malonyl dialdehyde (MDA) formation was observed, indicating that the liposomal drug exerted a protective effect on astroglial cells via an antioxidant mechanism [37].

The potentiality of IDE-loaded large unilamellar vesicle (LUV) prepared by the extrusion technique has also been studied by the same research groups [38]. An improved activity of IDE on ethanol-induced injury in primary cortical astrocyte cultures was registered in the liposomal formulation, compared to free IDE, most probably related to the greater bioavailability of the drug at a cellular level. The greater effectiveness of IDE-loaded pegylated liposomes on both biological response and effective therapeutic dose, encourage the production of liposomal formulations with a low drug dosage, with the consequent advantages of improving drug antioxidant activity and reducing drug-induced toxicity on cell membranes. Microscopic evaluation of cell morphology after exposure of astrocyte cultures to either free or liposomal IDE suggested that at lower concentrations (5 μM) the drug does not affect cells integrity, whereas at 50 μM both drug forms showed damaging effects on the cell growing (**Figure 3**).

The interaction of IDE with different naturally occurring phospholipids was investigated by differential scanning calorimetry (DSC) [39]. It represents an useful technique to study the nature and level of the effects exerted by a biologically active compound on lipid-based biomembrane models [40]. DSC experiments performed

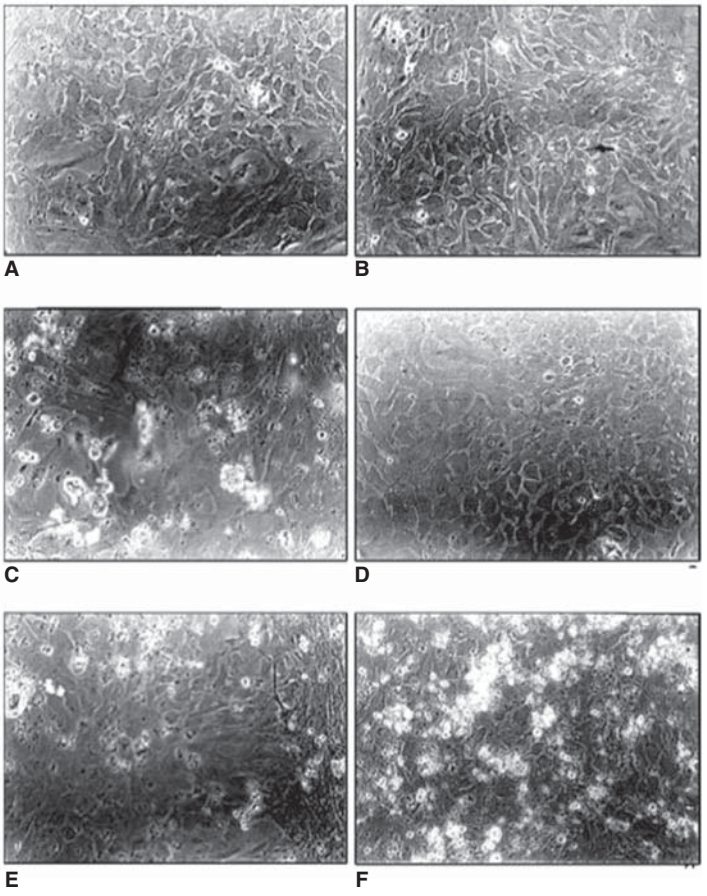

Figure 3. Microscopy pictures of primary cortical astrocyte cultures: (A) untreated cells (control); **(B, C)**: cells treated with 5 μM or 50 μM free IDE; **(D, E)**: cells treated with 5 μM or 50 μM pegylated liposomal IDE; and **(F)**: cells incubated with empty (unloaded) liposomes.

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on LUV prepared with dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC) or DPPC-dipalmitoylphosphatidic acid (DPPC-DPPA) showed that IDE interacts with the membrane model, in particular in the presence of negatively charged phospholipids, which are particularly abundant in the brain. A slow drug–membrane interaction was observed in kinetic experiments of transfer from an aqueous medium to preformed liposomes, due to IDE-limited water solubility; for this reason, using a liposomal system can efficaciously improve the bioavailability of this compound. The above drug–membrane interaction studies demonstrated that IDE can be solubilized within the bilayer structure of phospholipid vesicles, allowing the formation of a drug delivery system able to be administered intravenously. Noteworthy, this study also evidenced that drug molar fractions higher than 0.06 in respect of phospholipids can lead to a phase segregation, i.e., a non-uniform distribution of the drug in the system and the formation of drug aggregates within the phospholipid bilayers.

3.3 Microemulsions

It has been recently reported the feasibility to prepare microemulsions (MEs) containing low percentages of surfactants using the phase inversion temperature method, for the controlled delivery of IDE after topical administration [41]. MEs are transparent, thermodynamically stable, low viscosity and isotropic dispersions composed by an aqueous phase, an oil phase, and an emulsifier system [42]. They represent an interesting carrier for topical application due to their ease of manufacturing, ability to incorporate a wide range of compounds and to enhance drug skin penetration [43]. Montenegro *et al.* obtained different IDE release profiles depending on oil phase lipophilicity and type of surfactant used [41]. Furthermore, IDE release compared to those of naproxen and butylmethoxydibenzoylmethane elucidated that drug release from PIT (Phase inversion Temperature) MEs is inversely related to the drug lipophilicity, since the higher the drug partition coefficient, the lower the amount of drug released at the end of the experiment. A

Table 3. Evaluation of ROS production (a), DNA damage (COMET assay) (b), and LDH release (c) of stress-induced, non-immortalized human fibroblasts.

Sample	% LDH release	Mean length tails/100 cells (a.u.)	pmol DCF/mg proteins
C	14 ± 1.1	0.2 ± 0.05	7 ± 0.5
DEM	36 ± 1.6	1.5 ± 0.15	30 ± 2.1
A	32 ± 1.8	1.6 ± 0.15	28 ± 1.9
B	20 ± 2.1	0.5 ± 0.16	13 ± 1.5
H_2O_2	54 ± 3.2	3.5 ± 0.15	43 ± 1.0
DEM	42 ± 1.5	3.3 ± 0.15	38 ± 2.3
E	23 ± 2.7	2.1 ± 0.25	28 ± 2.1

C, untreated control cells; DEM, cells treated with diethyl malonate (DEM) (0.5 mM) for 60 min; A, DEM-stressed cells treated with free IDE; B, DEM-stressed cells treated with IDE-loaded PECA NC; H_2O_2, cells treated with 0.1 mM hydrogen peroxide for 30 min; D, H_2O_2-stressed cells treated with free IDE; E, H_2O_2-stressed cells treated with IDE-loaded PECA NC.
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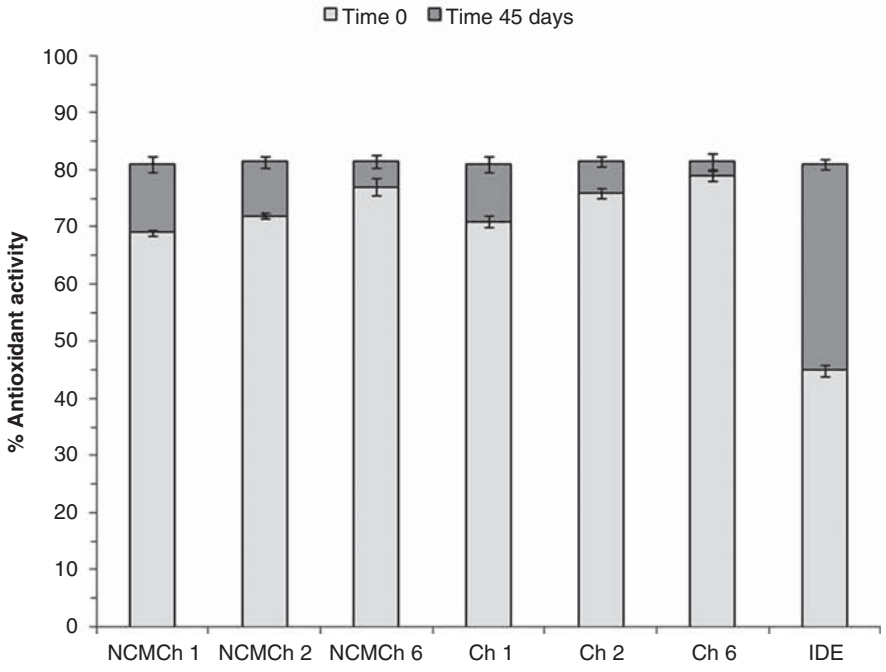

Figure 4. Percentage of antioxidant activity ($ABTS^+$) as a function of time for samples of N-CMCh1, N-CMCh6, Ch1, Ch2, Ch6 (10 nM) and IDE concentration (10 nM). Zero time and 45 days refer to the times of the stability study at 40°C and 75% moisture.
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self-microemulsifying drug delivery system (SMEDDS) containing IDE has also been developed to increase the dissolution rate and consequently improve the oral bioavailability [44]. SMEDDS is an anhydrous system of MEs which requires only very low free energy to form an emulsion, with the consequent spontaneous formation of an interface between the oil droplets and water. These systems have been developed to overcome the limit of MEs, i.e., the high water content. The release experiments performed on various SMEDDS prepared with different oils, such as Labrafac™ lipo, Labrafil® 2125, Labrafac™ hydro and Labrafil® 2609, showed a significant increase (more than twofold) of IDE release from SMEDDS containing Labrafac™ hydro (42.9%) and Labrafil® 2609 (44.5%), compared to a conventional dosage form [44].

3.4 Nanoparticles

3.4.1 Nanocapsules

Nanocapsules (NC) represent interesting drug delivery systems due to their ability to protect drugs from biodegradation and to promote drug targeting. Moreover, the encapsulation into polymeric shell NC represents an interesting strategy to improve the water solubility of poorly water-soluble drugs. On the basis of these considerations, Palumbo *et al.* investigated the protective antioxidant effect of

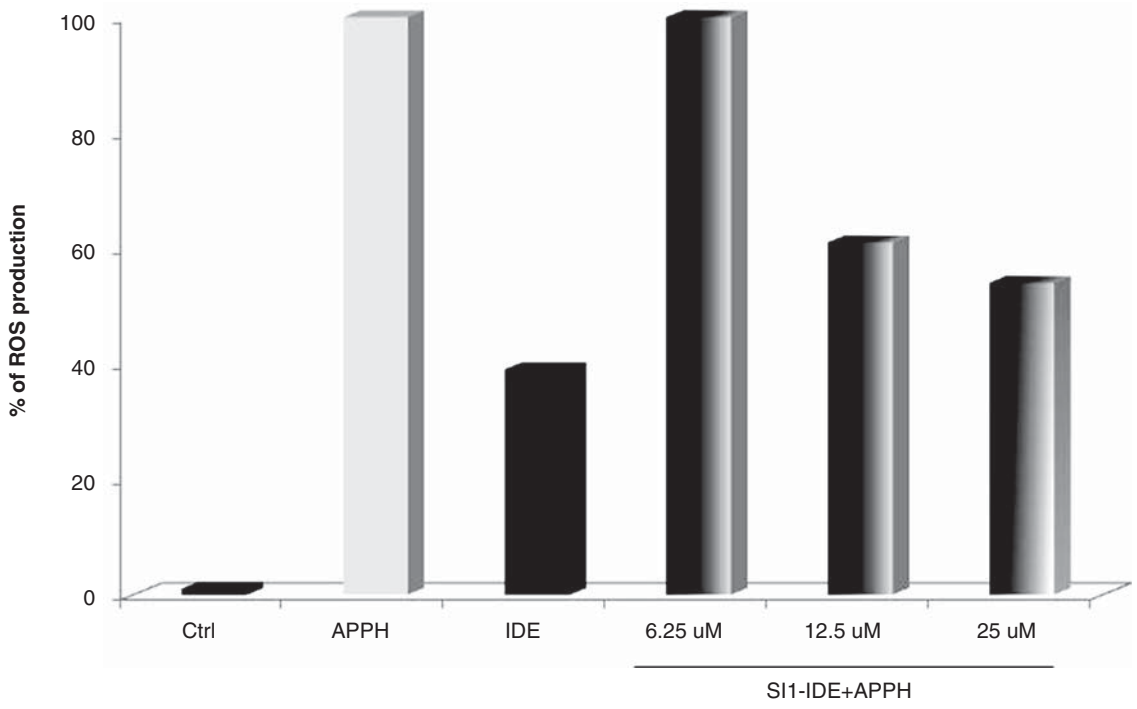

Figure 5. Inhibition of APPH-induced oxidative damage in astrocyte cultures exerted by free IDE or loaded in SI1-SLN.
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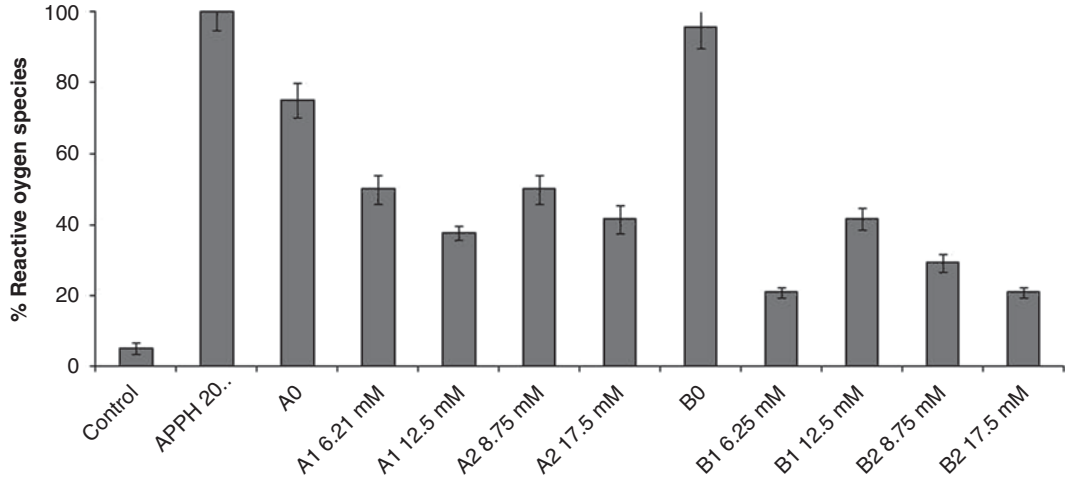

Figure 6. Inhibition of 2,2′-azobis-(2-amidinopropane)dihydrochloride (APPH)-induced oxidative damage in astrocyte cultures by different concentrations of IDE-loaded solid lipid nanoparticles.
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IDE-loaded polyethyl-2-cyanoacrylates (PECA) NC prepared by the interfacial polymerization method for oral administration [45]. The size of the PECA-NC could be controlled within a certain interval by regulating the concentration of the selected surfactant, Tween® 80. In particular, increasing Tween® 80 concentration up to 3% w/v allowed to reduce the size of the NC, improving IDE water solubility. The encapsulation of IDE into PECA-NC exerted a greater protective antioxidant effect compared to the free drug, as confirmed by *in vitro* ROS test performed on human fibroblasts (**Table 3**). The drug delivery system showed a greater efficacy also regarding the reduction of DNA fragmentation caused by diethyl maleate (DEM) or H_2O_2 treatment (**Table 3**). In particular, the results of the COMET assay highlights that

the efficacy of IDE encapsulated into PECA-NC was much more higher than the free drug concerning the reduction of DNA fragmentation. The cytotoxicity of the nanocarrier, evaluated measuring the activity of LDH, showed an increase of LDH leakage in the case of stress-inducing molecule treatment. As reported in **Table 3**, the results of the LDH release test were similar to those obtained in the evaluation of ROS production and LDH release test. In fact, IDE-loaded PECA-NC was able to significantly reduce the LDH release while the free drug had no significant effect (**Table 3**).

These results provide further important evidence about the antioxidant effect of IDE, which is exerted particularly at the mitochondrial level. IDE effects can be improved by its encapsulation into PECA-NC, which allow an easier passage through biological barriers, reducing the binding between drug and serum proteins and reducing the concentration of IDE to be used.

3.4.2 Polymeric nanoparticles

The incorporation of IDE into polymeric NP has been reported as a strategy to decrease the undesired effect of the drug after topical or nasal application. In particular, chitosan (Ch) and N-carboxymethylchitosan (N-CMCh) NP cross-linked with tripolyphosphate (TPP) and colloidal silicon dioxide have been prepared by spray-drying [46], which is a rapid single-step process commonly used to dry aqueous systems even if contain heat-sensitive materials, into sub-micron solid system. Ch is a widely used compound for the preparation of long-term sustained drug release microparticles, due to its biocompatibility, biodegradability and ability to undergo structural modification [47] such as the cross-linking with TPP, that improves the chemical and mechanical resistance. The amount of IDE loaded into Ch or N-CMCh NP varied in function of the polymer–drug ratio and allowed to obtain different particle size, related to the amount of TPP cross-linking. The mucous membrane irritation test showed a significant ($P < 0.05$) decrease in drug reactivity when loaded into these NP, compared to the free drug. Moreover, a strong increase in drug stability and enhancement of the protective *in vitro* antioxidant activity were observed. **Figure 4** shows that even if at time zero both the free drug and IDE-loaded into Ch or N-CMCh NP were efficient, after 45 days of storage at 40°C a significant loss of the antioxidant efficiency was observed for the free drug, while its incorporation into polymeric nanoparticles preserved its antioxidant efficiency.

3.4.3 Lipid nanoparticles (SLN and NLC)

Among the various colloidal DDS developed as carriers for IDE, solid lipid nanoparticles (SLN) are regarded as a promising solution, due to their many advantages such as good tolerability, improved drug stability, drug targeting, and increasing drug bioavailability [48]. The possibility to prepare SLN by a solvent injection method (SI-SLN) and dilution of a microemulsion (ME-SLN), as alternative techniques to the most diffused high pressure homogenization method, has been reported [46-48].

The SI procedure was operated by the so-called Quasi-emulsion solvent diffusion (QESD) method [49]; the technique, applied for the first time to this carrier system, allowed to prepare IDE-loaded SLN with reduced particle size compared to ME-SLN, characterized by a narrow size distribution and good stability, with the advantage of using reduced amount of required surfactants [50]. *In vitro* release experiments assessed by dialysis showed a prolonged IDE release for both the preparation, with a more evident burst-effect for the SI-SLN. The use of palmitic acid as lipid matrix instead of stearic acid allowed to obtain a good dose-dependent protective effect against damaged oxidative cell. *In vitro* biological studies to determine the protective effects against 2,2'-azobis-2-amidinopropane dihydrochloride (APPH)-induced ROS production were performed using cultures of newborn rat astrocytes (**Figure 5**). IDE-loaded SI-SLN were more effective in inhibiting APPH-induced LDH release in primary cultures of astrocytes compared to the free drug, but a reduction in antagonizing ROS production was observed, probably due to the effect of the lipid matrix to synergize the cell damage induced by APPH.

Recently, it has been verified the feasibility to obtain SLN by the PIT method, previously used for the preparation of O/W MEs, using cetyl palmitate as lipid solid at room temperature [51]. IDE-loaded SLN were characterized by mean particles size below 100 nm. A different loading capacity and *in vitro* drug release profiles were observed related to the different chemical structure and properties of the selected primary surfactant. All prepared SLN did not show any cytotoxic effect compared to the controls, as confirmed by the MTT test. *In vitro* biological tests performed on primary cultures of astrocytes obtained from rat cerebral cortex showed a strong antioxidant effect in inhibiting ROS production (**Figure 6**). The same effect was observed in antagonizing the toxicity of APPH-induced LDH release. A significant effect of the type of primary surfactant was observed on both technological and biological properties.

In a very recent study, it has been evaluated the *in vitro* permeation of IDE loaded into SLN, prepared by the above PIT method, across MDCKII-MDR1 cell monolayer, selected as a model of the blood–brain barrier (BBB) [52]. These experiments showed that IDE permeates via a transcellular pathway. In particular, drug permeability across MDCKII-MDR1 cell monolayers from the SLN carrier was 0.40 – 0.53-fold lower compared to the free drug and no significant difference was observed comparing IDE permeation from the various tested SLN formulations.

In vitro permeation/penetration experiments were also performed on IDE-loaded SLN prepared with different non-ionic primary surfactants, using pig skin and Franz-type diffusion cells [53]. As shown in **figure 7**, no permeation occurred from all the tested SLN, while the concentration of IDE penetrated into the different depended on the amount of IDE loaded into the systems and on the type of the primary

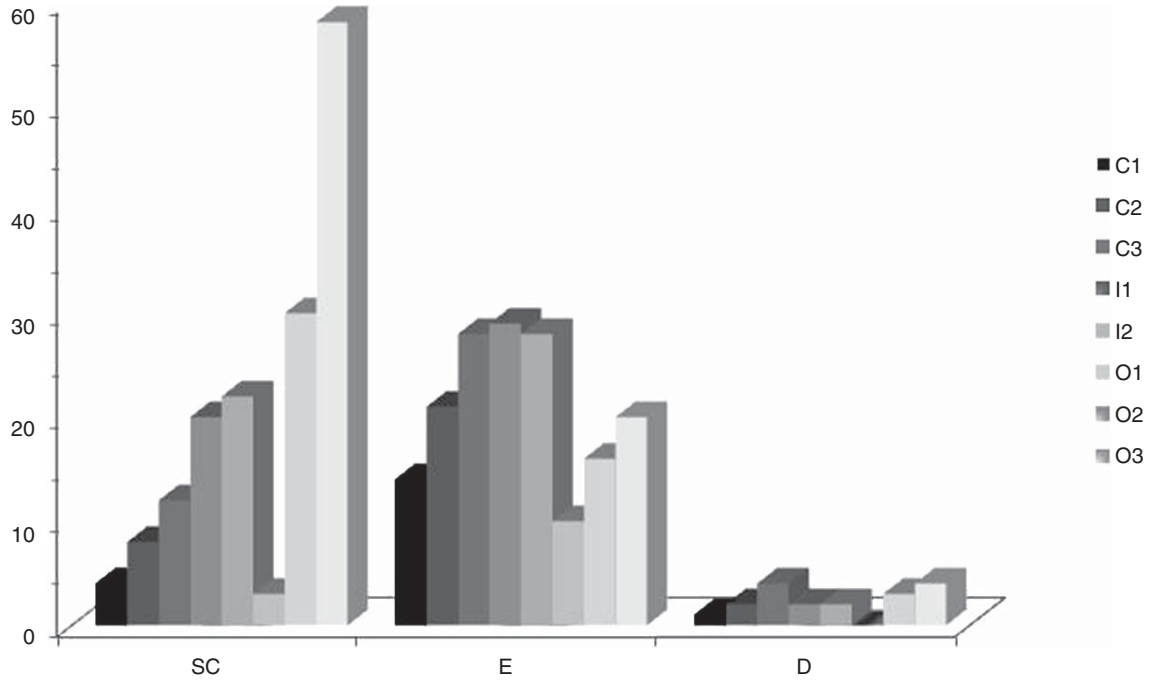

Figure 7. *In vitro* skin penetration of IDE from IDE-loaded SLN prepared with different primary surfactants: Ceteth-20 (SLN C1, C2, C3), Isoceteth-20 (SLN I1, I2), Oleth-20 (SLN O1, O2, O3).

Modified from [53] with permission of Elsevier

D: Dermis; E: Epidermidis; SC: Stratum corneum

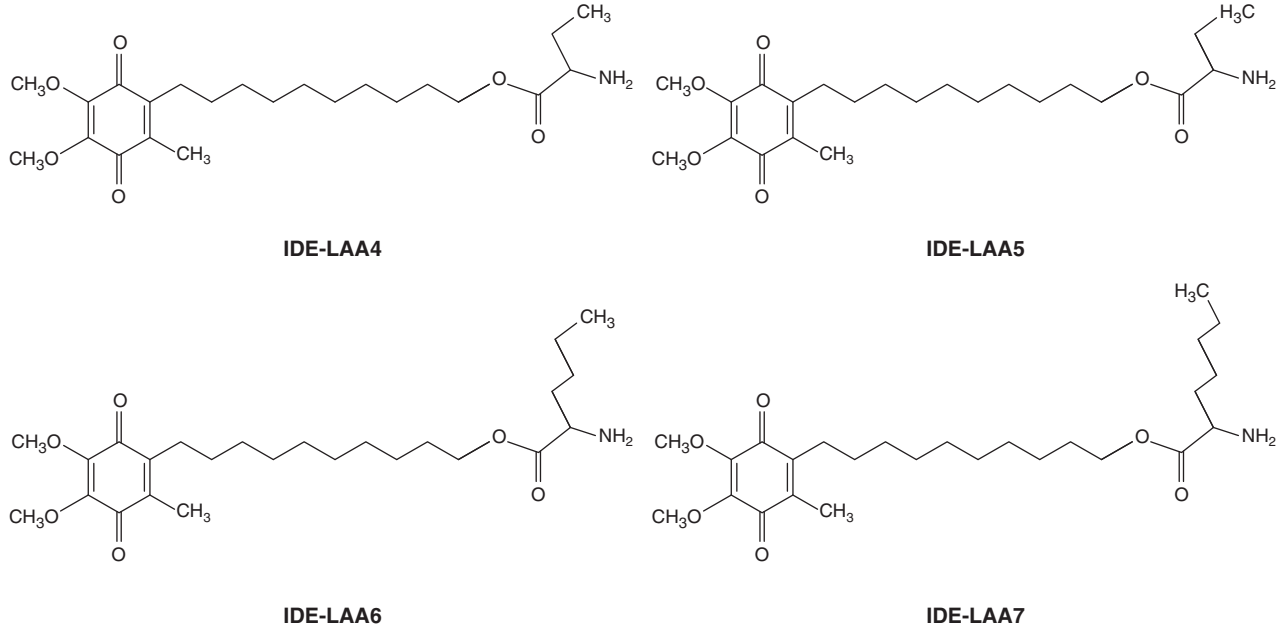

Figure 8. Structure of IDE conjugates with alkyl-α-amino acids.

surfactant used. In particular, Ceteth-20® and Isoceteth-20® produced the highest drug content in the epidermis (formulations C1, C2, C3, I1 and I2), while IDE distribution into the upper layers from SLN containing Oleth-20® (SLN O1-O3) was related to the amount of drug loaded in the nanoparticles (**Figure 7**) [53].

A second generation of lipid nanoparticles, called nanostructured lipid carriers (NLC), has been introduced as drug

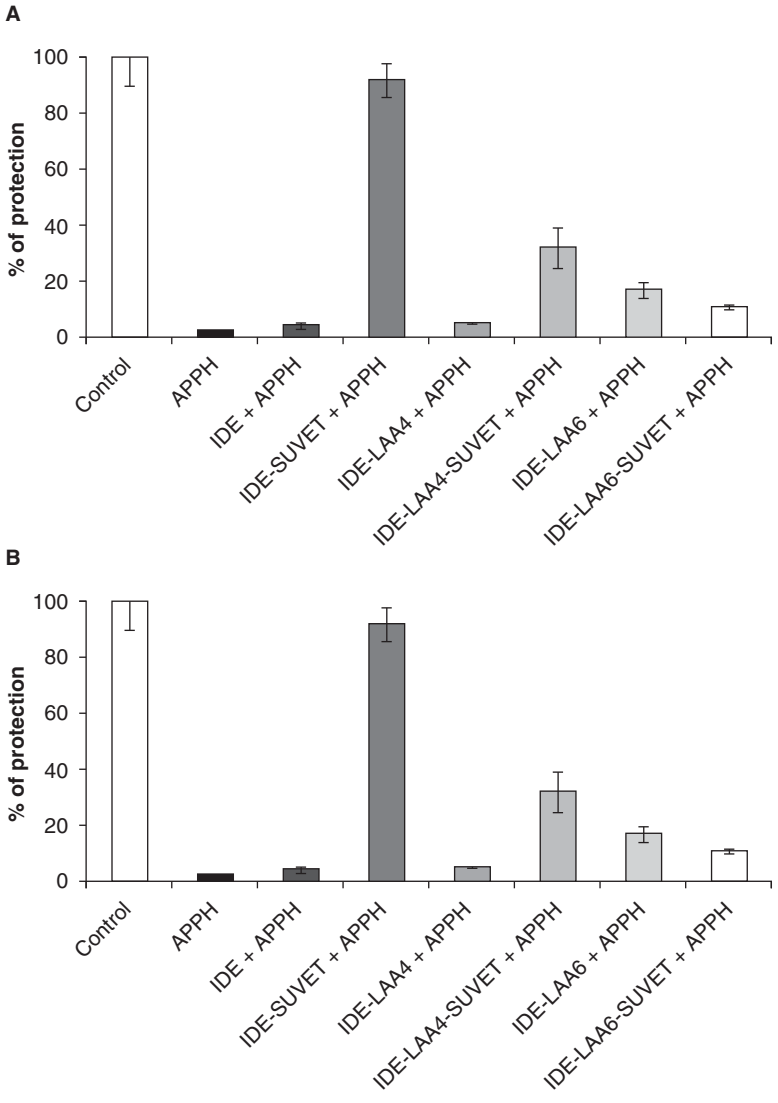

Figure 9. Protection percentage of oxidative damage in rat astrocytes treated with APPH in the presence of IDE or IDE-LAA4 and IDE-LAA6 prodrugs, either free or encapsulated in neutral (A) or negative SUVETs (B). Values express the percent reduction of ROS levels.

Modified from [60] with permission of Informa Healthcare

vectors due to their numerous advantages, such as: increasing loading capacity of the active compound, minimizing the expulsion of the active compound during storage and its protection from chemical degradation [54]. A significant improvement of IDE chemical stability was obtained when the drug was loaded into NLC prepared by the modified high-shear homogenization and ultrasound method, compared to nanoemulsions and to a drug oil solution [55].

The *ex vivo* skin permeation studies highlighted that incorporation into NLC significantly enhanced the skin penetration of IDE, compared to both the nanoemulsion and oil solution. Due to the small particle size (<100 nm), the high formulation stability and the observed penetration-enhancing ability of this carrier, IDE-loaded NLC have been proposed for the topical application of this active.

3.5 Prodrugs

IDE conjugation with lipoamino acids (LAAs) has been proposed as alternative technological strategy to increase drug lipophilicity and promote its penetration through biological membranes and barriers. LAAs are α-amino acids bearing an alkyl side chain whose structure and length can be modified to obtain different physico-chemical properties [56]. Pignatello *et al.* prepared IDE ester conjugates using short and medium alkylamino acids from butyric to heptanoic residue (four to seven carbon atoms) (**Figure 8**) [57,58].

This study evidenced that chemical derivatization of IDE molecule at the level of the hydroxyl group in the side alkyl chain did not suppress its biological activity, since all the lipophilic prodrugs exhibited interesting *in vitro* antioxidant properties, expressed as their capacity to scavenge free radicals. In particular, the esterification of IDE molecule with short 2-α-aminoacyl chains (four to five carbon atoms) improved the scavenging effect on superoxide anion (inhibition of xanthine oxidase activity) ability to bleach the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and in the lipid peroxidation assay compared to the parent IDE [58]. The chemical and enzymatic hydrolysis evaluation of IDE-LAA conjugates showed their ability to rapidly release the drug via enzymatic hydrolysis *in vitro* in serum and liver homogenate [58]. Therefore, IDE-LAA conjugates behave as prodrugs when administered *in vivo*. An high enzymatic degradation rates could result in a poor availability of the intact conjugates, thus representing a limit for their uptake across the BBB. However, the rapid conversion of the conjugates into the active drug would represent an advantage for treating oxidative eye diseases.

An interesting calorimetric study on IDE prodrugs was described to evaluate their interaction with a biomembrane model [59]. DSC experiments performed using DMPC multilamellar liposomes (MLVs) as a biomembrane model showed a greater ability of IDE-LAA conjugates, with respect to the free drug, to change the phospholipid main transition temperature (Tm), due to a different site of interaction within the DMPC bilayers. Thus, an increased affinity for cell membranes was observed when IDE was conjugates with the alkylamino acids, while only a weak interaction at the level of liposome surface was observed for free IDE [59].

The influence of the increased amphiphilicity of IDE-LAA prodrugs on their loading into neutral (DPPC-Chol) or negatively charged (DPPC-Chol-DPPS) unilamellar liposomes, prepared by thin-layer evaporation followed by membrane extrusion (SUVETs) was also evaluated [60]. These liposomal carriers showed a high loading efficiency for IDE, even if they were not able to efficiently release the encapsulated drug *in vitro*. On the other hand, IDE-LAA prodrugs encapsulation in liposomes improved their *in vitro* protective activity against oxidative damage in primary cultures of murine astrocyte cells (**Figure 9**) [60].

4. Expert opinion and conclusions

A recent PubMed interrogation with the term "idebenone" returned more than 100 hints only in the last five years. This proofs the actual and active interest on this drug, whose therapeutic potentialities are moving from the diseases based on alteration of cell redox equilibrium, to other pathological fields, including neurodegenerative diseases and skin protection. In this regard, the cosmeceutical industry also has recently shown interest on IDE.

Our research group and scientists who collaborated with us in the last two decades, as well as other researchers have largely explored the possibility of encapsulating IDE in different colloidal vectors, from liposomes to nanoparticles, to microemulsions and SLN, as well as to chemical modify IDE molecule through the strategies of prodrugs and CDs. Actually, we often used this compound essentially as a model drug, because of its peculiar physico-chemical properties (e.g., very low solubility and high lipophilicity) and good analytical features. However, most of these studies were associated with an *in vitro*/*in vivo* biological evaluation of the produced chemical or technological systems, which always demonstrated that the approaches used did not affect the pharmacological potential of IDE and, in many cases, even improved its antioxidant and cell-protective effects.

A direct comparison of the different carriers studied is not easy and could not even be significant, due to the large variables existing among them. However, a general conclusion that can be drawn by this review is that IDE can receive a beneficial improvement in terms of solubility, stability and biochemical activity by these various forms of delivery. The actual high level of interest for this drug thus would prompt to deepen these studies, and explore further nanotechnology strategies for a controlled release, vectorization, and targeting of IDE.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Sugiyama Y, Fujita T, Matsumoto M, et al. Effects of idebenone (CV-2619) and its metabolites on respiratory activity and lipid peroxidation in brain mitochondria from rats and dogs. J. Pharmacobiodyn 1985;8:1006-17
2. James AM, Cochemé HM, Smith RA, Murphy MP. Interactions of mitochondria-targeted and untargeted ubiquinones with the mitochondrial respiratory chain and reactive oxygen species. Implications for the use of exogenous ubiquinones as therapies and experimental tools. J. Biol. Chem 2005;280:21295-312
3. Ernster L, Dallner G. Biochemical, physiological and medical aspects of ubiquinone function. Biochem Biophys Acta 1995;1271:195-204
4. Firuzi O, Miri R, Tavakkoli M, Saso L. Antioxidant therapy: current status and future prospects. Curr Med Chem 2011;18:3871-88

• **An excellent review on antioxidant therapy.**

5. Rauchovà H, Vrbacky M, Bergamini C, et al. Inhibition of glycerophosphate-dependent H_2O_2 generation in brown fat mitochondria by idebenone. Biochem Biophys ResCommun 2006;339:362-6
6. Suno M, Nagaoka A. Inhibition of lipid peroxidation by idebenone in brain mitochondria in the presence of succinate. Arch Gerontol Geriatr 1989;8:291-7
7. Jauslin ML, Wirth T, Meier T, Schoumacher F. A cellular model for Friedreich ataxia reveals small-molecule glutathione peroxidase mimetics as novel treatment strategy. Hum Mol Genet 2002;11:3055-63
8. Becker C, Bray-French K, Drewe J. Pharmacokinetic evaluation of idebenone. Expert Opin Drug Metab. Toxicol 2010;6:1437-44

•• **A good review covering the most recent publications in this field.**

9. EMEA/387246/2009 CHMP Assessment Report for Sovrima. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/000908/WC500070576.pdf [Last Accessed 31 July 2012]
10. Haefeli RH, Erb M, Gemperli AC, et al. NQO1-Dependent Redox Cycling of Idebenone: effects on Cellular Redox Potential and Energy Levels. PLoS One 2011;6:e17963
11. Lagedrost SJ, Sutton MJ, Cohen MS, et al. Idebenone in Friedreich ataxia cardiomyopathy-results from a 6-moth phase III study (IONIA). Am Heart J 2011;161:639-45
12. Meier T, Buyse G. Idebenone: an emerging therapy for Friedreich ataxia. J. Neurol 2009;256:25-30
13. Giorgio V, Petronilli V, Ghelli A, et al. The effects of idebenone on mitochondrial bioenergetics. Biochim Biophys Acta 2012;1817:363-9
14. Suno M, Nagaoka A. Inhibition of lipid peroxidation by a novel compound (CV-2619) in brain mitochondria and mode of action of the inhibition. Biochem Biophys Res Commun 1984;125:1046-52
15. Meldrum BS. Glutamate as a neurotrasmitter in the brain: review of physiology and pathology. J Nutr 2000;130:1007S-15S
16. Mancuso M, Orsucci D, Choub A, Siciliano G. Current and emerging treatment options in the management of Friedreich ataxia. Neuropsychiatr Dis Treat 2010;6:491-9
17. Seznec H, Simon D, Monassier L, et al. Idebenone delays the onset of cardiac functional alteration without correction of Fe-S enzymes deficit in a mouse model for Friedreich ataxia. Hum Mol Gen 2004;13:1017-24
18. Rustin P, Von Kleist-Retzow JC, Chantrel-Groussard K, et al. Effect of idebenone on cardiomyophathy in Friedreich's ataxia: a preliminary study. Lancet 1999;354:477-9
19. Shulz JB, Dehmer T, Schools L, et al. Oxidative stress in patients with Friedreich ataxia. Neurology 2000;55:1719-21
20. Idebenone Friedreich Ataxia. ClinicalTrials gov A service of the U S National Institutes of Health. 2012.Available from: http://www.clinicaltrials gov/ct2/results?term=idebenone+Friedreich+ataxia+ [Last accessed 21 May 2012]
21. Buyse GM, Goemans N, van den Hauwe M, et al. Idebenone as a novel, therapeutic approach for Duchenne muscular dystrophy: results from a 12 month, double-blind, randomized placebo-controlled trial. Neuromuscul Disord 2011;21:396-405
22. Efficacy and Tolerability of Idebenone in Boys With Cardiac Dysfunction Associated With Duchenne Muscular Dystrophy (DELPHI). 2012.ClinicalTrials gov A service of the U S National Institutes of Health. Available from: http://www.clinicaltrials gov/ct2/results?term=idebenone+Duchenne+muscular+dystrophy+ [Last accessed 21 May 2012]
23. Klopstock T, Yu-Wai-Man P, Dimitriadis K, et al. A randomized placebo-controlled trial of idebenone in Leber's hereditary optic neuropathy. Brain 2011;134:2677-86
24. Carelli V, La Morgia C, Valentino ML, et al. Idebenone treatment in Leber's hereditary optic neuropathy. Brain 2011;134:e188
25. Sabet-Peyman EJ, Khaderi KR, Sadun AA. Is leber hereditary optic neuropathy treatable? Encouraging results with idebenone in both prospective and retrospective trials and an illustrative case. J Neuroophtalmol 2012;32:54-7
26. Mashima Y, Kigasawa K, Wakakura M, Oguchi Y. Do idebenone and vitamin therapy shorten the time to achieve visual recovery in Leber hereditary optic neuropathy? J Neuroophtalmol 2000;20:166-70
27. Study of Idebenone in the Treatment of Mitochondrial Encephalopathy Lactic Acidosis & Stroke-like Episodes (MELAS). ClinicalTrials gov A service of the U S National Institutes of Health. 2012.Available from: http://www.clinicaltrials.gov/ct2/show/NCT00887562?term=idebenone+MELAS&rank=1 [Last accessed 21 May 2012]
28. Douglas KS. Treatment of mitochondrial electron transport chain disorders: a review of clinical trials over the past

decade. Mol Genet Metab 2010;99:246-55

• **A recent review of the clinical features and therapeutic approaches of the mitochondrial electron transport chain disorders.**

29. Lekoubou A, Kouamé-Assouan AE, Cho TH, et al. Effect of long-term oral treatment with L-arginine and idebenone on the prevention of stroke-like episodes in an adult MELAS patient. Rev Neurol (Paris) 2011;167:852-5

30. Orsucci D, Mancuso M, Ienco EC, et al. Targeting mitochondrial dysfunction and neurodegeneration by means of coenzyme Q10 and its analogues. Curr Med Chem 2011;18:4053-64

31. Haginoya K, Miyabayashi S, Kikuchi M, et al. Efficacy of idebenone for respiratory failure in a patient with Leigh syndrome: a long-term follow-up study. J Neurol Sci 2009;278:112-14

32. Double Blind Placebo-Controlled Phase I/II Clinical Trial of Idebenone in Patients With Primary Progressive Multiple Sclerosis (IPPoMS). ClinicalTrials gov A service of the U S National Institutes of Health. 2012.Available from: http://www.clinicaltrials gov/ct2/show/NCT00950248?term=idebenone+primary+progressive+multiple+sclerosis&rank=1 [Last accessed 21 May 2012]

33. Mc Daniel DH, Neudecker BA, Di Nardo JC, et al. Clinical efficacy assessment in photodamaged skin of 0.5% and 1.0% idebenone. J Cosmet Dermatol 2005;4:167-73

34. Ventura CA, Fresta M, Giovinazzo C, Puglisi G. Solid state characterization and in solution studies of idebenone-β-cyclodextrin inclusion complex. Acta Technologiae et Legis Medicamenti 1995;VI:55-66

•• **IDE complexation with β-cyclodextrin increase IDE bioavailability due to the increase of drug water solubility, wettability, and reduction of drug crystallinity.**

35. Puglisi G, Ventura CA, Fresta M. Preparation and physico-chemical study of inclusion complexes between idebenone and modified β-cyclodextrins. J Inclusion Phenom Mol Recognit Chem 1996;24:193-210

•• **Complexation with 2,6-di-O-methyl-β-CD and 2-hydroxypropyl-β-CD using the freeze–drying method significantly increased IDE aqueous solubility.**

36. Cannavà C, Crupi V, Guardo M, et al. Phase solubility and FTIR-ART studies of idebenone/sulfobutylether β-cyclodextrin inclusion complex. J Inclusion Phenom Mol Recognit Chem. In press 2012

•• **IDE inclusion complex can be obtained using sulfobutylether-β-cyclodextrin.**

37. Muscoli C, Fresta M, Cardile V, et al. Ethanol-induced injury in rat primary cortical astrocytes involves oxidative stress: effect of idebenone. Neurosci Lett 2002;329:21-4

•• **Results show the advantage of using liposomally-entrapped idebenone on free radical overproduction in primary cortical rat astroglial cells.**

38. Paolino D, Iannone M, Cardile V, et al. Tolerability and improved protective action of idebenone-loaded pegylated liposomes on ethanol-induced injury in primary cortical astrocytes. J Pharm Sci 2004;93:1815-27

•• **These results encourage the application of pegylated liposome in the delivery of idebenone to the central nervous systems.**

39. Fresta M, Ventura CA, Mezzasalma E, Puglisi G. A calorimetric study on the idebenone-phospholipid membrane interaction. Int J Pharm 1998;163:133-43

•• **A very interesting study of the interaction between idebenone and biomembrane.**

40. Pignatello R, Musumeci T, Basile L, et al. Biomembrane models and drug-biomembrane interaction studies: involvement in drug design and development. J Pharm Bioall Sci 2011;3:4-14

•• **Comprehensive review of differential scanning calorimetry application to study drug–biomembrane interaction.**

41. Montenegro L, Carbone C, Condorelli G, et al. Effect of oil phase lipophilicity on in vitro drug release from O/W microemulsions with low surfactant content. Drug Dev Ind Pharm 2006;32:539-48

•• **This technological study highlights the promising application of microemulsions in providing a controlled drug release.**

42. Schmalfub U, Neubert R, Wohlrab W. Modification of drug penetration into human skin using microemulsions. J Control Release 1997;46:279-85

43. Gasco MR. Microemulsions in the pharmaceutical filed: perspective and application. In: Solans C, Kunieda H, editors. Industrial applications of microemulsions. Marcel Dekker; New York: 1997. p. 97-122

44. Kim HJ, Yoon KA, Hahn M, et al. Preparation and in vitro evaluation of self-microemulsifying drug delivery systems containing idebenone. Drug Dev Ind Pharm 2000;26:523-9

45. Palumbo M, Russo A, Cardile V, et al. Improved antioxidant effect of idebenone-loaded polyethyl-2-cyanoacrylate nanocapsules tested on human fibroblasts. Pharm Res 2002;19:71-8

•• **The antioxidant effect of idebenone can be improved by its encapsulation into polyethyl-2-cyanoacrylate nanocapsules.**

46. de Amorim CM, Couto AG, Netz DJA, et al. Antioxidant idebenone-loaded nanoparticles based on chitosan and N-carboxymethylchitosan. Nanomedicine 2010;6:745-52

•• **The nanoparticle formulation shows a lower mucous membrane irritation compared to the free drug, which was highly irritative.**

47. Yang CH, Huang KS, Lin YC. Using a cross-flow microfluidic chip and external crosslinking reaction for monodisperse TPP-chitosan microparticle. Sens Actuators B 2007;12:510-16

48. Muller RH, Mader K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of the art. Eur J Pharm Biopharm 2000;50:161-77

• **A good review on solid lipid nanoparticles.**

49. Pignatello R, Ricupero N, Bucolo C, et al. Preparation and characterization of Eudragit Retard nanosuspensions for the ocular delivery of cloricromene. AAPS PharmSciTech 2006;7:E27

50. Stancampiano AH, Acquaviva R, Campisi A, et al. Technological and biological characterization of idebenone-loaded solid lipid nanoparticles prepared by a modified

solvent injection technique. J Biomed Nanotechnol 2006;2:253-60

• **A new method for the preparation of SLN, the Quasi-emulsion solvent diffusion technique, is proposed in this work.**

51. Montenegro L, Campisi A, Sarpietro MG, et al. In vitro evaluation of idebenone-loaded solid lipid nannoparticles for drug delivery to the brain. Drug Dev Ind Pharm 2011;37:737-46

• **IDE release is dependent on the type of the primary surfactant used for the preparation of the SLN.**

52. Montenegro L, Trapani A, Latrofa A, Puglisi G. In vitro evaluation on a model of blood brain barrier of idebenone-loaded solid lipid nanoparticles. J Nanosci Nanotechnol 2012;12:330-7

•• **A relevant article due to the *in vitro* study performed on a model of blood brain barrier.**

53. Montenegro L, Sinico C, Castangia I, et al. Idebenone-loaded solid lipid nanoparticles for drug delivery to the skin: in vitro evaluation. Int J Pharm 2012;434:169-74

•• **Detailed skin penetration and permeation study.**

54. Teeranachaideekul V, Souto EB, Junyaprasert VB, Muller RH. Cetyl palmitate-based NLC for topical delivery os Coenzyme Q10 - development, physicochemical characterization and in vitro release study. Eur J Pharm Biopharm 2007;67:141-8

55. Li B, Ge ZQ. Nanostructured lipid carriers improve skin permeation and chemical stability of idebenone. AAPS PharmSciTech 2012;13:276-83; (2006) 299:626-35

• **Comparison between NLC, nanoemulsions and a drug oil solution.**

56. Toth I. A novel chemical approach to drug delivery: lipidic amino acid conjugates. J Drug Target 1994;2:217-39

57. Pignatello R, Puleo A, Acquaviva R, et al. Lipophilic conjugates of idebenone with short and medium alkylamino acids: synthesis and antioxidant activity. J Drug Del Sci Tech 2004;14:181-6

•• **An interesting chemical approach for improving the activity of idebenone.**

58. Puleo A, Niemi R, Jarvinen T, et al. Chemical and enzymatic stability evaluation of lipoamino acid esters of idebenone. Eur J Pharm Biopharm 2004;57:343-6

•• **These data strongly suggest that the esterification of idebenone with short 2-α-aminoacyl chains can improve the scavenging effect compared to the parent IDE.**

59. Pignatello R, Intravaia VD, Puglisi G. A calorimetric evaluation of the interaction of amphiphilic prodrugs of idebenone with a biomembrane model. J Colloid Interface Sci 2006;299:626-35

•• **An interesting calorimetric study on prodrugs of idebenone.**

60. Pignatello R, Acquaviva R, Campisi A, et al. Effects of liposomal encapsulation on the antioxidant activity of lipophilic prodrugs of idebenone. J Liposome Res 2011;21:46-54

•• **IDE-LAA prodrugs encapsulation in liposomes improves *in vitro* protective activity against oxidative damage in primary cultures of murine astrocyte cells.**

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