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Pharmacological effect of a new idebenone formulation in a model of carrageenan-induced inflammatory pain

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ABSTRACT

Considerable evidence demonstrated that the central role of reactive oxygen species and reactive nitrogen species (ROS and RNS) in the development of thermal hyperalgesia is associated to acute and chronic inflammation.

Idebenone (IDE), a synthetic analogue of the endogenous cellular antioxidant coenzyme Q10 (CoQ10), is an active drug in the central nervous system which shows a protection in a variety of neurological disorders. Since it is lipophilic, poorly water soluble and highly bound to plasma proteins, different technological approaches have been explored to increase its solubility and new pharmaceutical properties. Therefore, it has been complexed with HP-β-cyclodextrins (HP) and its efficacy has been assessed in an animal model of carrageenan-induced thermal hyperalgesia.

All male rats used for this study received a subplantar injection of carrageenan into the right hindpaw in the presence or absence of IDE alone and IDE/HP complex. We observed that IDE poorly reduced painful carrageenan effects whereas IDE/HP complex was able to prevent carrageenan-induced hyperalgesia and edema in a dose-dependent manner, reducing spinal MDA levels and protein nitration.

Hence, our results demonstrated that when complexed with HP, idebenone exerts a potent analgesic and anti-inflammatory efficacy.

1. Introduction

After a protracted period of time, inflammation can become chronic from an acute state, by showing injurious symptoms in several pathologies as well as multiple sclerosis, cancer, atherosclerosis, autoimmune disorders and neurodegenerative disease [1,2]

Patients suffering of chronic inflammation can experience relentless pain. Indeed, it is well known that inflammation produces thermal hyperalgesia [3–6].

In the last decades it was proven that reactive oxygen species and reactive nitrogen species (ROS and RNS) helped in the development of inflammatory pain. Superoxide ($O_2^{\bullet-}$, SO) and its downstream signaling mediator, peroxynitrite (ONOO-, PN), have emerged as powerful pronociceptive reactive species [7,8].

SO and $ONOO^-$ are involved, for example, in the central sensitization reported during the spinal activation of the NMDA receptor (NMDAR) [9], in the development of orofacial pain, opiate-induced hyperalgesia and antinociceptive tolerance [10–13] and in the development of thermal hyperalgesia associated with acute and chronic inflammation [14–16].

In particular, superoxide is able to modulate spinal nitration and inactivation of MnSOD providing a critical mechanism that allowed the accumulation of superoxide itself and peroxynitrite during the

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development and maintenance of central sensitization in nociceptive signaling [9,14].

Moreover, NMDA induced PN production modulates glutamate transmission through posttranslational nitration of the glutamine synthase (GS), glutamate transporter GLT1 and NMDAR subunits (GluN1 and GluN2). These processes are involved in the maintenance of thermal hyperalgesia and central sensitization [9].

Mitochondrial genome and proteome are altered by enhanced ROS production, through several mechanisms such as the over-accumulation of malondialdehyde (MDA) and the lipid peroxidation product 4-hydroxynonenal (4-HNE), close to the cellular membranes.

The endogenous antioxidant system or exogenous antioxidants prevents the formation of the mentioned oxidizing species and removes them before they can damage any vital components of cells leading to resolution of inflammation and hyperalgesia [9,14,17,18].

Idebenone (2,3-dimethoxy-5-methyl-6-(10-idroxydecyl)-7 1,4-benzoquinone; IDE), a synthetic analogue of coenzyme Q10 (CoQ10), is a vital antioxidant for cell membranes which can produce a great inhibitory effect to oxidation as well as lipid peroxidation [19]. It is also an essential constituent of the electron transport chain and the consequent production of ATP in the mitochondria. IDE serves as a protection for a wide range of neurological disorders [20] including: trauma, cerebral ischemia, and hypertension-induced vascular lesions. In particular, recent studies demonstrated that in young patients affected by Duchenne muscular dystrophy, Idebenone significantly reduces the loss of respiratory function avoiding the glucocorticoids treatment [21].

Since idebenone is a poor water soluble, lipophilic molecule and highly bound to plasma proteins, several drug-carrier systems have been used to improve the pharmacokinetic profile, the duration of action and lessen toxicity of drugs, such as liposomes, nanoparticles, cucurbituril or cyclodextrins (CDs) [22,23].

β-cyclodextrins (β-CD) are cyclic oligosaccharides composed of d-glucose units, linked by −1,4-glucosidic linkages to form a three-dimensional truncated cone. The hydroxyl groups located on the outer surface of CDs make it hydrophilic, whereas the inner cavity is hydrophobic. Thus, CDs have the capability of trapping guest molecules and forming host–guest inclusion complexes mainly driven by hydrophobic or van der Waals interactions. These could enlist them as candidates of novel drug carriers to be used in the pharmaceutical field and to solubilize lipophilic drugs [24–26].

In particular, the complexation of idebenone with modified β – cyclodextrin, hydroxypropyl – β-CD (HP), is able to increase water solubility of IDE up to 600 times [27].

This study was carried out to evaluate the potential pharmacological effect of HP complexed idebenone in an animal model of carrageenan induced thermal hyperalgesia. Hence, IDE/HP complex was able to highlight the analgesic and anti-inflammatory idebenone profile.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (220–250 g) (Envigo), were housed and cared for in accordance with the guidelines of the University of "Magna Graecia", Catanzaro, Italy, as well as complied with the Italian regulations for the protection of animals used for experimental and other scientific purposes (D.L. 26/2014), and with European Economic Community regulations (2010/63/UE).

Idebenone [IDE; 2,3-dimethoxy-5-methyl-6(10-hydroxydecyl)-1,4-benzoquinone, C19H30O5, MW 338.44] was kindly supplied by Wyeth Lederle S.p.A. (Italia). (2-Hydroxypropyl)-β-Cyclodextrin (HP; 0.8 molar substitution, average MW 1460) was purchased from Sigma-Aldrich (St. Louis, MO). Unless specified, all materials were purchased from Sigma-Aldrich (St. Louis, MO). All drugs were dissolved in saline (sodium chloride 0,9%).

2.2. Carrageenan-Induced edema

Rats received a subplantar injection of carrageenan (0.1 ml of a 1% suspension in 0.85% saline) into the right hindpaw. Changes in paw volume were evaluated with a plethysmometer (Ugo Basile, Comerio, Varese, Italy) as previously described [28], and measured up to 6 h post-carrageenan injection. Edema was expressed as the increase in paw volume (milliliters) after carrageenan injection relative to the preinjection value for each animal The animals were sacrificed by decapitation and the lumbar spinal cord (portion from L4 to L6) was removed, immediately frozen in liquid nitrogen and was randomly distributed for further analysis.

Experimental groups

1) Ctrl: animals (n = 15) received an intraperitoneal injection of saline 15 min before an intraplantar saline injection;
2) Carr + saline: animals (n = 15) received an intraperitoneal injection of saline 15 min before carrageenan injection;
3) Carr + IDE: animals (n = 15) received an intraperitoneal injection of IDE (5 mg/kg) 15 min before carrageenan injection;
4) Carr + IDE/HP: animals received an intraperitoneal injection of IDE/HP (1–5–10 mg/kg; n = 15 for each subgroup) 15 min before carrageenan injection;
5) Carr + $FeTMPyP^{5+}$: animals (n = 15) received an intraperitoneal injection of $FeTMPyP^{5+}$ (10 mg/kg) 15 min before carrageenan injection;
6) Carr + HP: animals (n = 15) received an intraperitoneal injection of HP (9 mg/kg) 15 min before carrageenan injection;
7) Saline + HP: animals (n = 15) received an intraperitoneal injection of HP (9 mg/kg) 15 min before an intraplantar saline injection;
8) IDE/HP: animals (n = 15) received an intraperitoneal injection of IDE/HP (10 mg/kg).

The employed doses were choose according with the bibliography [16,29]

2.3. Measurements of hyperalgesia

Thermal hyperalgesic response was evaluated by Hargreaves test as previously described [9,30]. Briefly, rats were individually confined to plexiglas chambers. A mobile unit consisting of a high-intensity projector bulb was positioned to deliver a thermal stimulus directly to an individual hindpaw from beneath the chamber. The withdrawal latency period of injected and contralateral paws was determined to the nearest 0.1 s with an electronic clock circuit and thermocouple and a cutoff latency of 20 s was employed to prevent tissue damage in non-responsive animals. Each point represents the delta change (s) in withdrawal latency (withdrawal latency of contralateral minus withdrawal latency of injected paw) at each time point (2, 3, 4, 5, 6 h after carrageenan injection). Results are expressed as paw withdrawal latency changes (s).

2.4. Rotarod test

Rats were placed on a rotating rod (diameter, 7 cm) turning at 10 rpm. The animals were exposed to the rotarod for one session of 180 s each day for 3 days to adapt the rats to the apparatus. The rats were then injected intraperitoneally with IDE/HP (10 mg/kg)