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Taraxerol as a possible therapeutic agent on memory impairments and Alzheimer's disease: Effects against scopolamine and streptozotocin-induced cognitive dysfunctions

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder associated with cognitive impairment and cholinergic neuronal death, characteristic of the effect of time on biochemical neuronal function. The use of medicinal plants as an alternative form of prevention, or even as a possible treatment of AD, is therefore interesting areas of research, since the standard drugs have many side effects. Taraxerol (TRX) is a triterpene that has been isolated from several plant species, and its various pharmacological properties have already been identified, such the acetylcholinesterase (AChE) inhibition activity in vitro. There is a lack of information in literature that confirms the effect of TRX in an animal AD-like model. Seeking to fill this gap in the literature, in the present work we assessed the effect of TRX on AChE activity in the animals' encephalon and hippocampus. We also investigated the effect of TRX (1.77 μM/side, 0.5 μL) isolated from leaves of *Eugenia umbelliflora* Berg. on aversive memory impairments induced by scopolamine (2 μg/side, 0.5 μL) infused into rat hippocampus, and the effect of TRX (0.89 and 1.77 μM/side, 0.5 μL) on aversive memory impairments induced by streptozotocin (STZ) (2.5 mg/mL, 2.0 μL) infused i.c.v. into mice, using the step-down inhibitory avoidance task. We found that TRX significantly inhibited AChE activity in the animal's hippocampus. Furthermore, TRX significantly improved scopolamine and STZ-induced memory impairment. Taking together, these results confirms its AChE activity inhibition in animals and indicate that TRX has anti-amnesic activity that may hold significant therapeutic value in alleviating certain memory impairments observed in AD.

1. Introduction

Ageing is a process that leads to specific structural, biochemical and functional changes in multiple organs, including the brain [1]. This event can represent the major risk factor for the development of age-related brain disorders like Alzheimer's disease (AD) and Parkinson's disease [2]. AD is the most common form of dementia, affecting nearly 35% of the population aged over 65 years in the late-onset form; despite it also affects up until 6% of the patients under 60 years in the early-onset form [3–5]. AD is characterized by progressive memory loss, cognitive impairment and personality changes due to neurodegeneration in the hippocampus and frontal cortex [6–8]. The most pronounced neurochemical abnormality in AD patients is a decline in cholinergic function in the central nervous system, besides the altered redox homeostasis [8,9].

The treatment of AD is focused on bringing symptomatic benefits and slowing the progression of the disease [10]. Early clinical studies of cholinesterase inhibitors aimed to activate the central cholinergic system and alleviate cognitive deficits in AD. Acetylcholinesterase (AChE) inhibitors are commonly used drugs in the treatment of AD as traditional nootropic medicaments [11], such as tacrine, donepezil, rivastigmine, and galantamine. These compounds have been shown to significantly improve cognitive function in AD [10,12]. However, they are known to have limitations for clinical use, due to their unfavorable side-effects [13,14].

With regard to the use of medicinal plants to prevent or treat AD, it has become an interesting area of research. Essa et al. [15] and Howes and Houghton [16] demonstrated that herbal medicines containing

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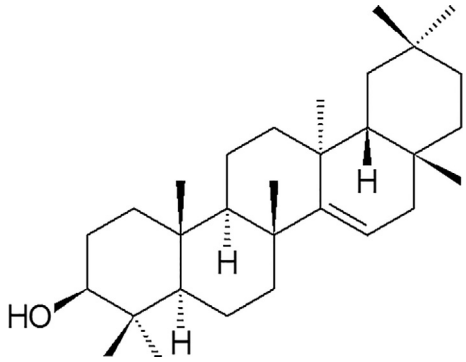

Fig. 1. Structure of Taraxerol (TRX).

phytochemicals can modify brain aging. In addition, recent studies suggest that the neurobiological effects of phytochemicals from medicinal herbs may contribute to clinical benefits in vitro and in vivo models of AD [5,17,18]. Taraxerol (TRX) (Fig. 1), is a triterpene that has been isolated from several plant species, including *Vaccinium oldhamii* [19] and *Eugenia umbelliflora* [20]; and shows a steroidal nucleus. Khanra et al. [21] demonstrated that TRX decreased acute inflammation through the inhibition of NF-κB signaling; Naika et al. [22] denoted inhibition of a GSK-3β protein by TRX and a low toxicity in docking and dynamics studies. Yao et al. [23] mentioned that TRX interferes on the Akt and TAK1 activation, inhibiting the LPS-induced IL-6, IL-1β, iNOS, COX-2 and TNF-α expressions. Finally, Lee et al. [19] showed that TRX exhibited anti-AChE activity.

So, in order to determine the anti-AChE activity of TRX isolated from leaves of *Eugenia umbelliflora* Berg. and its effect on aversive memory, we induced sporadic AD in rats and mice by scopolamine and streptozotocin (STZ) and evaluates its aversive memory on step-down inhibitory avoidance test.

2. Materials and methods

2.1. Plant material and isolation of taraxerol

Leaves of *Eugenia umbelliflora* Berg. were collected in May 2006 in the State of Santa Catarina, Brazil, and identified by Prof. Oscar Benigno Iza (UNIVALI, Itajaí-SC, Brazil). A voucher specimen was deposited at the Barbosa Rodrigues Herbarium (Itajaí-SC, Brazil) under number VC Filho 50. The phytochemical procedures for TRX isolation are reported elsewhere by Meyre-Silva et al. [20].

2.2. Animals

Male Wistar rats (250–300 g) and male Swiss albino mice (25–30 g) were used in this study. The animals were housed in groups, with access to water and food *ad libitum*, kept under a 12 h light–dark cycle (lights on at 7:00 am) and controlled temperature (23 ± 1 °C). For each experiment, the animals were divided into 10 treatment groups (n = 10) and twenty-four hours before the experiments they were transferred to the laboratory, where they were kept for an acclimatization period. All the experiments were conducted in accordance with international standards of animal care, and were authorized by the Ethical Committee for Animal Care of the University of UNIVALI (Protocol number 232/10). The minimum number of animals and the duration of observation required to obtain consistent data were employed. Each animal was used only once and, the behavioral experiments were conducted from 9:00 h to 17:00 h.

2.3. Drugs and treatments

The following substances were used: scopolamine, streptozotocin, 5,5-dithium acid-bis-2-nitrobenzoic acid (DTNB) and sucrose (Chromate) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The reagents (methanol, hexane, dichloromethane and ethyl acetate) used to obtain the TRX are commercial grade, purchased from VETEC (RJ, BR) and LABSYNTH (SP, BR). Tris was purchased from LABSYNTH (SP, BR); phosphate (potassium chromate), acetylthiocholine and naphthyl acetate were purchased from Acros Organics (Geel, BE), ketamine (Vetbrands, FL, USA), xylazine (Agener União, SP, BR), lidocaine with epinephrine 2% (Cristália, SP, BR), self-polymerizing acrylic (Clássico, SP, BR), Fast Blue Salt B (Fluka, BE, CH).

All drugs were dissolved in saline (except TRX which was dissolved in artificial cerebrospinal fluid prepared as described by Wang et al. [24] and were infused at room temperature). The dose of scopolamine used in this study was determined from a preliminary study based on the doses used by Izquierdo et al. [25] and Gonzalez et al. [26]. The other doses used were determined based on pilot experiments and on previous studies showing the effect of TRX on learning, memory and other behavioral and physiological variables. Due to the low yield of the compound, the TRX was infused directly in the hippocampus and ventricles (i.c.v.) of rats and mice, respectively.

2.4. In vitro assay methods: acetylcholinesterase activity

For this experiment, rats were killed by decapitation and the brain was quickly removed and placed on an inverted Petri dish on ice. The total brain and hippocampus were dissected, weighed and homogenized in 10 volumes of a medium containing 10 mM Tris-HCl buffer, pH 7.2, and 160 mM sucrose. The total homogenate was centrifuged at 1000 × *g* in a refrigerated (0–4 °C) centrifuge for 10 min to yield a low-speed supernatant (S1). The hippocampus was homogenized in 20 volumes of a medium (10 mM Tris-HCl buffer, pH 7.2, and 160 mM sucrose) and the homogenate was centrifuged at 1000 × *g*/15 min/4 °C. The S1 fraction was used to determine the AChE activity [27]. The protein concentration was determined in samples using bovine serum albumin as standard [28].

AChE activity was carried out as previously described by Ellman et al. [29] with some modifications. The assay medium contained 900 μL of 24 mM K^+-phosphate buffer, pH 7.2, 50 μL of 1 mM 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), 50 μL of supernatant solution S1 (0.2–0.4 mg protein), and 10 μL of TXR (0.45, 0.89 and 1.77 μM). The reaction was started adding 25 μL of 0.8 mM of acetylthiocholine as substrate and monitored for 4 min at 412 nm in a spectrophotometer at room temperature (25 °C). All samples were run in duplicate. The AChE activity was expressed in μmol hydrolyzed acetylcholine/h/mg protein. These doses were based on a pilot study conducted in our laboratories.

2.5. Pharmacological assays, surgery and intrahippocampal infusions

The implant of cannulas was performed according to Cammarota et al. [30] with minor modification. The rats were deeply anesthetized with thiopental (30–50 mg/kg, i.p.), and 27-gauge cannulas were stereotaxically aimed 1.0 mm above the stratum pyramidale of the dorsal CA1 region of the hippocampus (coordinates described by Paxison and Watson [31]: anterior, 4.3; lateral, ± 4.3; ventral, 2.6). The drugs were delivered using a 30-gauge infusion cannula, connected by a polyethylene tube in a 1 μL microsyringe. Infusions were performed over 30 seconds (s), first on the left side and then on the right side; the infusion cannula was kept in place for an additional 1 min to minimize backflow of the injectant. The animals were allowed to recover from surgery for 4 days before being submitted to behavioral tests. The placement of infusion cannulas was verified post-mortem: four hours after the behavioral test, the animals were killed by decapitation and 0.5 μL of a 4% methylene-blue solution was infused through the implanted cannula and the extension of the dye 30 min thereafter was taken as indicative of diffusion of the vehicle (artificial CSF/0.5 μL) or drug previously given. Only data from animals with correct cannula implants