

EXPERIMENTAL STUDY

Donepezil-induced response of Spirulina supplemented rat urinary bladder

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Abstract: *Objective:* At present, very little is known about the effects of donepezil on vascular reactivity. The aim of the present study was to evaluate the responses of rat urinary bladder to donepezil (10^{-10} – 3×10^{-4} M) and the role of Spirulina supplementation in these effects.

Material and methods: Animals were divided into the two groups of six animals in each group. The first group received only distilled water daily as vehicle for six weeks and served as the control. The second group received Spirulina 750 mg kg^{-1} orally, daily for six weeks and served as the spirulina group. Preparations of rat urinary bladder were used from both groups.

Results: Donepezil produced concentration dependent relaxation of rat urinary bladder preparations pre-contracted with KCl. The pIC_{50} value, but not the maximal response of donepezil, was significantly lower ($p < 0.05$) in the Spirulina supplemented group.

Conclusions: These results demonstrated for the first time that spirulina treatment can affect urinary bladder activity (Fig. 1, Ref. 20). Full Text in PDF www.elis.sk.

Key words: donepezil, rat, Spirulina, urinary bladder.

Donepezil hydrochloride is a selective central acetylcholinesterase (AChE) inhibitor, which decreases degradation of acetylcholine (ACh) in the brain, then increases the concentration of ACh in the synaptic cleft (1). This drug is widely used to ameliorate cognitive decline in patients with the Alzheimer's disease (AD) (2, 3), which is thought to be due to a decrease in cholinergic innervation of the cerebral cortex and the basal forebrain (4). Since the bladder is innervated by the parasympathetic cholinergic nerves (5) and neurogenic lower urinary tract (LUT) dysfunction occurs in a subset of patients with AD (6, 7), there has been a controversy that the central AChE inhibitors might exacerbate urinary incontinence in those patients (8).

The activation of the parasympathetic nerves cause the release of ACh, which in turn leads to a muscarinic receptor mediated contraction that empties the bladder. Cholinesterase (ChE) inhibitors have been shown to increase the contractile responses to exogenous ACh or to the electrical stimulation of urinary bladder preparations (9, 10). This implies that ChE activity plays an important role in controlling ACh-induced contraction of the urinary bladder. Clinically, distigmine bromide, a long-lasting ChE inhibitor, has

been used for the treatment of the underactive neurogenic bladder, although controversy exists regarding the effectiveness (11).

Spirulina is a microscopic filamentous blue-green alga (photosynthesizing cyanobacterium) that is rich in proteins, vitamins, essential amino acids, minerals and essential fatty acids and provides one of the few natural food sources of vegetarian vitamin B12. It is produced commercially and sold as a food supplement in health food stores all over the world. Until recently, the interest in Spirulina was mainly for its nutritive value. Over the past few years, however, it has been found to have many additional pharmacological properties. Many preclinical studies and a few clinical studies suggested several therapeutic effects ranging from a reduction of cholesterol and cancer to enhancement of the immune system, an increase in intestinal lactobacilli, reduction of nephrotoxicity by heavy metals and drugs and radiation protection (12–16). In addition, it has been experimentally proven, in vivo and in vitro, that Spirulina was effective for treating certain allergies, anaemia, cancer, hepatotoxicity, viral and cardiovascular diseases, hyperglycemia, hyperlipidemia, immunodeficiency and inflammatory processes, among others. Several of these activities are attributed to Spirulina itself or to some of its components (17). During recent years, an attention has been focused on the antioxidant potential of Spirulina species. It has been found that Spirulina was capable of inhibiting carcinogenesis and organ related toxicity due to its antioxidant properties (16, 18, 19).

Although its various beneficial effects on human health, to our knowledge, there is no in vitro study to explain Spirulina supplementation on smooth muscle reactivity. For this reason, in this in vitro study, we decided to investigate the effects of Spirulina supplementation on rat urinary bladder responses to donepezil.

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Although there are several studies in rat urinary bladder with donepezil, currently, there is an insufficient information.

Materials and methods

Tissue preparations

Male Wistar albino rats weighing 200–250 g bred in Selcuk University Experimental medicine research and application centre were used. The rats housed in polypropylen cages (four per cage) under the standard laboratory conditions with 12 h light/dark cycle and were fed with standard rat chow. The experimental protocol was approved by the Animals Ethics committee of Selcuk University.

Animals were divided into the two groups of six animals in each group. The first group received only distilled water daily as a vehicle for six weeks and served as the control group. The second group received Sorallypirulina 750 mg.kg⁻¹ orally, daily for six weeks and served as the Spirulina group.

At the end of the supplementation period of six weeks, the rats were sacrificed under a deep anesthesia with ketamin (50 mg/kg) and xylazine (10 mg/kg). The lower abdomen was opened and the whole urinary bladder of each rat was removed and rapidly placed in a Petri dish containing Krebs-Henseleit solution (KHS, mM: NaCl 119, KCl 4.70, MgSO₄ 1.50, KH₂PO₄ 1.20, CaCl₂ 2.50, NaHCO₃ 25, Glucose 11), the connective tissues were cut away.

Experimental procedure

The preparations were mounted 1 g tension in 25 ml organ baths containing KHS maintained at 37 °C and aerated with 95 % O₂ and 5 % CO₂. Tissues were allowed to equilibrate for 1 h. The responses were recorded isometrically by a force-displacement transducer (BIOPAC MP36, Santa Barbara, California, USA) connected through amplifiers to a ITBS08 Integrated Tissue Bath System (Commat, Ankara, Turkey).

After the equilibration period, the preparations were contracted with 60 mM KCl and then the cumulative concentration-response curves were determined for donepezil (10⁻¹⁰–3x10⁻⁴ M) in both control and Spirulina supplemented rat urinary bladders.

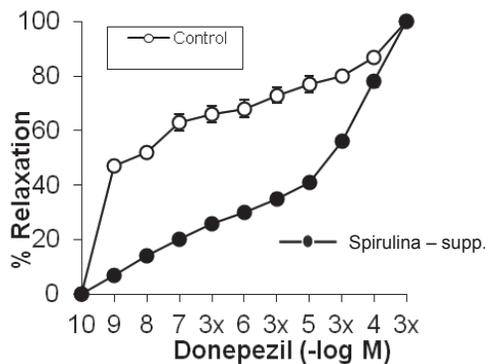


Fig 1. The concentration response curves of donepezil in the control and Spirulina supplemented rat urinary bladders. The preparations were pre-constricted with KCl (60 mM). Each point is the mean \pm SEM of six experiments.

Chemicals

Spirulina platensis powder was obtained from Egert Production of Natural Products LTD, Izmir, Turkey. Donepezil was a gift of Abdi Ibrahim Drug Company, Istanbul, Turkey.

Statistical analysis

Relaxation responses to donepezil were expressed as the percentages of the KCl (60 mM) induced contraction. Concentrations of donepezil causing 50 % of the maximal response (IC₅₀) were calculated from each individual concentration response curve. IC₅₀ values for curves obtained in both control and Spirulina supplemented groups were compared by the Student's t test. The statistical significance was set at p<0.05.

Results

In rat urinary bladder from the control and the Spirulina supplemented rats, KCl (60 mM) produced contractions. The cumulative addition of donepezil (10⁻¹⁰–3x10⁻⁴ M) induced concentration dependent relaxation of rat urinary bladder preparations pre-contracted with KCl in both control and spirulina supplemented rats.

The Figure 1 shows the results of bladders from both the control and the spirulina supplemented rats. In both the control and the Spirulina supplemented rat urinary bladder preparations, donepezil produced 100 % relaxation. The pIC₅₀ value of donepezil was significantly lower (p<0.05) in the Spirulina-supplemented group. PIC₅₀ value was found 8.6 \pm 0.3 and 4.7 \pm 0.2, in the control and the spirulina supplemented rats, respectively. Spirulina-supplementation decreased the sensitivity to donepezil about 1.8 times (p<0.05), as compared to the value obtained in the control group.

Discussion

In the present work, we studied the effects of Spirulina supplementation on donepezil induced responses of rat urinary bladder. To our knowledge, this is the first study to show the effects of Spirulina treatment on the responses of rat smooth muscle in vitro.

Our findings indicated that donepezil induced the concentration dependent relaxation of KCl-precontracted rat urinary bladder. Donepezil is a highly selective inhibitor of AChE. Shimizu et al (10) reported that 100 μ M donepezil induced a contractile effect of rat urinary bladder. In this study, we also investigated the effects of cumulative addition of donepezil (10⁻¹⁰–3x10⁻⁴ M) on rat urinary bladder but we could not observe any contractile effect and we have no explanation for this. As Solntseva et al (20) mentioned, the mechanisms of its action other than AChE inhibition exist. These investigators reported that donepezil was a strong antagonist of the voltage-gated calcium channel. Furthermore, to our knowledge, there are no studies that analyze the effects of donepezil on KCl-dependent contractions.

Spirulina is a photosynthetic, filamentous, spiral-shaped and multicellular edible microbe. It is the nature's richest and most complete source of nutrition. Spirulina has a unique blend of nutrients that no single source can offer. The alga contains a wide

spectrum of prophylactic and therapeutic nutrients that include B-complex vitamins, minerals, proteins, gamma-linolenic acid and the super anti-oxidants such as beta-carotene, vitamin E, trace elements and a number of unexplored bioactive compounds. Because of its apparent ability to stimulate the whole human physiology, Spirulina exhibits its therapeutic functions such as anti-oxidant, anti-bacterial, antiviral, anticancer, anti-inflammatory, anti-allergic and anti-diabetic and plethora of beneficial functions (19). In the present study, we demonstrated for the first time that Spirulina supplementation decreased the sensitivity to donepezil in rat urinary bladder. However, to our knowledge, there is no information regarding the effects of Spirulina supplementation on the urinary bladder activity.

In conclusion, the results of the present study demonstrated for the first time that donepezil induced a relaxation of KCl induced contractions in healthy rat urinary bladder and Spirulina supplementation could induce the responses of urinary bladder to donepezil. Further studies should be performed to clarify the mechanism of Spirulina induced decrease in the sensitivity of donepezil.

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