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Donepezil regulates energy metabolism and favors bone mass accrual☆

Hazem Eimar [a], Sharifa Alebrahim [a], Garthiga Manickam [a], Ahmed Al-Subaie [a], Lina Abu-Nada [a], Monzur Murshed [a,b,c,*], Faleh Tamimi [a,**]

[a] Faculty of Dentistry, McGill University, Montreal, Quebec H3A 0C7, Canada
[b] Faculty of Medicine, McGill University, Montreal, Quebec H3G 1Y6, Canada
[c] Genetics Unit, Shriners Hospital for Children, Montreal, Quebec H3G 1A6, Canada

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ABSTRACT

The autonomous nervous system regulates bone mass through the sympathetic and parasympathetic arms. The sympathetic nervous system (SNS) favors bone loss whereas the parasympathetic nervous system (PNS) promotes bone mass accrual. Donepezil, a central-acting cholinergic agonist, has been shown to down-regulate SNS and up-regulate PNS signaling tones. Accordingly, we hypothesize that the use of donepezil could have beneficial effects in regulating bone mass. To test our hypothesis, two groups of healthy female mice were treated either with donepezil or saline. Differences in body metabolism and bone mass of the treated groups were compared.

Body and visceral fat weights as well as serum leptin level were increased in donepezil-treated mice compared to control, suggesting that donepezil effects on SNS influenced metabolic activity. Donepezil-treated mice had better bone quality than controls due to a decrease in osteoclasts number. These results indicate that donepezil is able to affect whole body energy metabolism and favors bone mass in young female WT mice.

1. Introduction

Bone remodeling is a lifelong process that involves a balance between bone resorption and bone formation. This process is well-regulated, at least, at two different levels: locally and centrally [1]. Locally, bone remodeling is regulated through a direct interaction between osteoblasts and osteoclasts, and by local interactions among these cells and the cells of the immune system [1]. Centrally, bone remodeling is regulated through the hypothalamic–pituitary–thyroid axis and by the common regulators of bone, adipose tissue, and energy metabolism that involve two arms of autonomous nervous system: the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) [2–11].

The activity of the autonomous nervous arms, SNS and PNS, is known to regulate the biochemical process of generating energy needed by the living tissues (energy metabolism). For instance, SNS signaling reduces body weight and bone mass accrual, whereas PNS signaling is associated with an increase in fat deposition and bone mass accrual [6,10–16]. In addition, PNS signaling within the central nervous system (*i.e.*, *locus coeruleus*) is known to down-regulate the SNS tone [11]. Accordingly, it would be logical to expect that the stimulation of the PNS receptors within the central nervous system would down-regulate the SNS, affect the body energy metabolic activities, and enhance bone mass.

One way of stimulating PNS receptors is by the administration of parasympathomimetic agonists such as acetylcholinesterase inhibitors (AChEIs) [17]. AChEIs are group of drugs that increase PNS neurotransmitter acetylcholine levels by inhibiting the action of butyrylcholinesterase in the peripheral tissues and/or acetylcholinesterase in the CNS [18]. Peripheral acting AChEIs are used in the therapy of myasthenia gravis and glaucoma, whereas central acting ACEIs are used for Alzheimer's disease (AD) treatment.

Donepezil is the most common AChEI used for AD treatment [19,20], and its beneficial effects on slowing down the progress of AD is well established [20]. Compared to other types of AChEIs, donepezil has a greater selectivity for acetylcholinesterase than buytrylcholinesterase, due to the presence of N-benzylpiperidine and an indanone moiety in its chemical structure [21,22]. Due to this unique chemical structure, donepezil expresses a more potent effect on the PNS receptors located within the central nervous system with no expected effect on the peripheral nervous system [19,22–26].

Previously, we showed that AD patients who are using donepezil had higher body mass index, lower risk of hip fracture, and faster healing of the hip fracture (if occurred) compared to AD non-users [27,28]. However, these observational studies on non-healthy patients cannot fully determine the exact mechanism by which donepezil promotes bone mass. Accordingly, this study was designed to investigate, in healthy mice, the effects of donepezil on several indicators of energy metabolism and bone mass accrual. Our results demonstrate that daily

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* Correspondence to: M. Murshed, 1529 Cedar Avenue, Montreal, Quebec H3G 1A6, Canada.
** Correspondence to: F. Tamimi, 3640 University Street, Montreal, Quebec H3A 0C7, Canada.
E-mail addresses: monzur.murshed@mcgill.ca (M. Murshed), faleh.tamimimarino@mcgill.ca, fdamimim@farm.ucm.es (F. Tamimi).

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injection of donepezil altered the autonomic mediated-body metabolic activities and promoted bone mass accrual in healthy mice. Besides being an AD drug, donepezil could also be useful for managing energy metabolism problems such as weight loss and osteoporosis.

2. Methods

2.1. Mice

This study was conducted following an animal use protocol approved by the Animal Care Committee of McGill University. Twenty four 5-week-old female wild-type C57BL/6 J mice were purchased form Jackson Laboratory (Bar Harbor, MA). The mice were kept in a 12 h light/dark cycle in 6 cages (4 mice in each cage) with a free access to diet and water, provided *ad libitum*, during the study period. Cages were assigned numbers from 1 to 6 respectively, Mice of cages 1, 3, and 5 ($n = 12$) were treated daily by intraperitoneal injections with donepezil (0.6 mg/kg) [29,30], whereas the rest of mice in cages 2, 4, and 6 ($n = 12$) received daily intraperitoneal injections of 0.9% saline. The treatment time was 4 weeks, from postnatal week 6 to 10.

To further assess the effect of donepezil on the sympathetic regulation of bone, we assessed the effect of a sympathetic agonist on the bone phenotype of donepezil-treated mice. Briefly, 6 week-old C57BL/6 females were injected with 0.6 mg donepezil/kg body weight/day alone ($n = 5$), or in combination with 6 mg isoproterenol/kg body weight/day ($n = 5$) for 4 weeks.

2.2. Locomotor activity

Open field box ($30 \times 30 \times 60$ cm) was used to assess the locomotor activity of the treated mice groups. The floor of the box was divided into 9 squares and number of squares crossed by each mouse was counted for 5 min [31].

2.3. Body metabolites and bioassays

Body weight and intra-abdominal fat weight were assessed for each mouse. Intra-abdominal fat was collected from epididymal and retroperitoneal region. Serum leptin (Life Technologies, Gaithersburg, MD), serum insulin (B-Bridge International, BioCat, Cupertino, CA), and urinary epinephrine (Elisa kit, BlueGene, Biotech, Shanghai, China) levels were measured using commercially available ELISA kits. Serum levels of receptor activator of nuclear factor κB ligand (RANKl) and osteoprotegerin were measured using antibody-based ELISA kits (abcam, Toronto, ON).

2.4. 3D-micro computed tomography (μCT) analysis

μCT analyses were performed as previously described [32]. Briefly, left tibiae of each mouse was scanned by SkyScan 1072 (Bruker–Microct, Kontich, Belgium) machine. Bone-analysis software (Version 2.2f, Skyscan, Kontich, Belgium) was used to calculate the following 3D morphological parameters: bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), and cortical thickness (Ct.Th) [33].

2.5. Dual X-ray absorptiometry

PIXIMUS bone densitometer (GE Medical Systems, Schenectady, NY, USA) was used to assess bone mineral density (BMD) of left tibia collected from different treatment groups. The parameters were adjusted according to previous work in the field [32].

2.6. Mechanical testing

A three-point breaking test was performed on the midshaft of the right tibiae obtained from all mice as previously described [32]. Briefly, a commercial bench-mounted vertical tensile/compression tester, the Instron 5569 (Instron Corp., Canton, MA, USA), was used. The span of two support points was 10 mm, and the deformation rate was 1.0 mm/min. Stiffness and ultimate force were calculated from the resulting load-displacement curves.

2.7. Histomorphometric analyses

Histomorphometric analyses were performed as previously described [34,35]. Briefly, calcein solution (0.25% calcein and 2% $NaHCO_3$ dissolved in 0.15 M NaCl) was injected twice i.p. (10 μl/g body weight) at an 8-day interval. Mice were euthanized 2 days after the second calcein injection. Lumbar vertebrae, collected from the treated-mice, were fixed for 24 h in 4% PFA/PBS, dehydrated in graded ethanol series, embedded in methyl methacrylate resin, and sectioned (7-μm thickness) [34]. The undecalcified sections of the lumber vertebra were stained by von Kossa/van Gieson, toluidine blue, and tartrate-resistant acid phosphatase (TRAP). Stained bone sections were analyzed for bone volume-to-tissue volume ratio (BV/TV), osteoblast count, osteoclast count, and bone formation rate (BFR) using the Osteomeasure software (Osteometrics Inc., Atlanta, GA). Images were taken using light microscope (DM200; Leica) adjusted at $2.5 \times 20 \times$ or $40 \times$ objective. All histological images were captured using a camera (DP72; Olympus), acquired with DP2-BSW software (XV3.0; Olympus), and processed using Photoshop (Adobe).

2.8. Statistical analyses

All results are shown as descriptive outcomes (mean ± standard deviation (SD)). Normality of the data was checked by the Shapiro–Wilks statistical test. Statistical analyses were performed by Student's two-tailed unpaired t-test. In all experiments, a value of $p < 0.05$ was considered significant as indicated by a single asterisk.

3. Results

3.1. Donepezil alters energy metabolism

We compared the whole-body energy metabolism of donepezil-treated mice with a group of mice treated with saline (control). The following indicators of the body energy metabolism were assessed: locomotor activity, weight changes, intra-abdominal visceral fat, leptin and insulin serum levels, and urinary epinephrine (SNS neurotransmitter) level.

We observed that donepezil-treated mice had lower locomotor activity (a relative measure of gross motor activity [36]) than the controls (Fig. 1A). In comparison to saline-treated mice, donepezil-treated mice had higher body weight and body fat that accompanied with increased serum levels of leptin and insulin (Fig. 1B–E), most likely due to the up-regulation of the PNS and suppression of the SNS. This donepezil-induced suppression of the SNS was further confirmed by the lower levels of urinary epinephrine content in the donepezil-treated mice compared to controls (Fig. 1F). These results may indicate that donepezil, an AChEI acting on the CNS, is able to affect energy and body metabolism in mice.

3.2. Donepezil favors bone mass accrual

We compared the bone phenotypes of donepezil-treated mice with that of saline-treated mice. The physical properties of the long bones (tibia) were determined using μCT, DXA, and 3-point bending mechanical test. As shown in Fig. 2, the administration of donepezil in