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Research report

The dual-acting H3 receptor antagonist and AChE inhibitor UW-MD-71 dose-dependently enhances memory retrieval and reverses dizocilpine-induced memory impairment in rats

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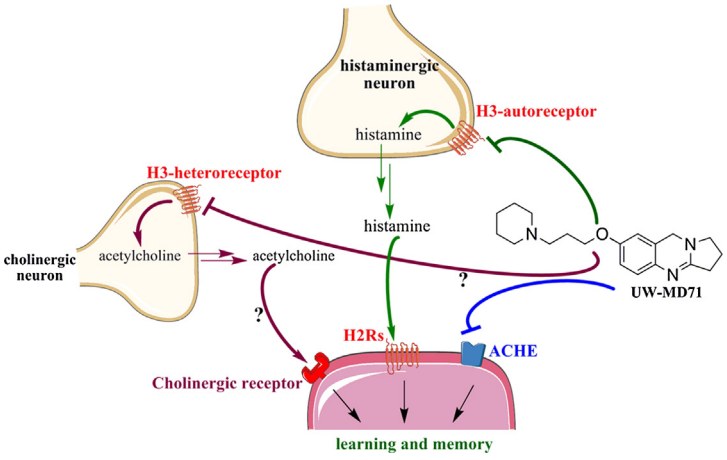
HIGHLIGHTS

- The effects of UW-MD-71, a highly selective and dual-acting H3R antagonist and AChEI, on acquisition, consolidation, retrieval, and dizocilpine-induced amnesia were tested.
- Acute UW-MD-71 administration improved retrieval dose-dependently and ameliorated dizocilpine-induced amnesia.
- The results are the first demonstration for in vivo effectiveness of such a dual-acting ligand and shed light on their mode of action in mediating procognitive effects.

GRAPHICAL ABSTRACT



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ABSTRACT

Both the histamine H3 receptor (H3R) and acetylcholine esterase (AChE) are involved in the regulation of release and metabolism of acetylcholine and several other central neurotransmitters. Therefore, dual-active H3R antagonists and AChE inhibitors (AChEIs) have shown in several studies to hold promise to treat cognitive disorders like Alzheimer's disease (AD). The novel dual-acting H3R antagonist and AChEI 7-(3-(piperidin-1-yl)propoxy)-1,2,3,9-tetrahydropyrrolo[2,1-*b*]quinazoline (UW-MD-71) with excellent selectivity profiles over both the three other HRs as well as the AChE's isoenzyme butyrylcholinesterase (BChE) shows high and balanced in vitro affinities at both H3R and AChE with IC_{50} of 33.9 nM and *h*H3R antagonism with K_i of 76.2 nM, respectively. In the present study, the effects of UW-MD-71 (1.25–5 mg/kg, i.p.) on acquisition, consolidation, and retrieval in a one-trial inhibitory avoidance task in male rats were investigated applying donepezil (DOZ) and pitolisant (PIT) as reference drugs. Furthermore, the effects of UW-MD-71 on memory deficits induced by the non-competitive *N*-methyl-D-aspartate (NMDA)

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Pyrilamine
Zolantidine

antagonist dizocilpine (DIZ) were tested. Our results indicate that administration of UW-MD-71 before the test session dose-dependently increased performance and enhanced procognitive effect on retrieval. However neither pre- nor post-training acute systemic administration of UW-MD-71 facilitated acquisition or consolidation. More importantly, UW-MD-71 (2.5 mg/kg, i.p.) ameliorated the DIZ-induced amnesic effects. Furthermore, the procognitive activity of UW-MD-71 in retrieval was completely reversed and partly abrogated in DIZ-induced amnesia when rats were pretreated with the centrally-acting H2R antagonist zolantidine (ZOL), but not with the CNS penetrant H1R antagonist pyrilamine (PYR). These results demonstrate the procognitive effects of UW-MD-71 in two in vivo memory models, and are to our knowledge the first demonstration in vivo that a potent dual-acting H3R antagonist and AChEI is effective in improving retrieval processes in the one-trial inhibitory avoidance task and provide evidence to such compounds to treat cognitive disorders.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder leading to pronounced cognitive deficits such as disorientation, and impairments in learning and memory functions [1,2]. Despite the fact that much research has been pursued, AD is still not remediable. The difficulty for developing satisfactory therapy of AD lies in the complex pathophysiology of the disease, which involves numerous pathways [3]. Developing novel agents with multiple pharmacological effects have become a promising strategy in today's search for novel treatment options for multifactorial diseases such as AD [3–6]. Pathophysiological changes cover deficiency in cholinergic neurotransmission, defective β-amyloid protein metabolism, abnormalities of glutamatergic, adrenergic, serotonergic and dopaminergic neurotransmission, and the involvement of inflammatory, oxidative and hormonal pathways [7]. Due to the multi-pathogenesis of AD, one of the current strategies to develop novel anti-Alzheimer agents focuses on compounds with multiple activities towards different targets [3–6].

Involvement of histamine receptors (HRs) in the cognitive process has long been confirmed, especially of H3Rs. H3Rs are primarily expressed in the CNS. While activation of H1R and H2R mediates slow excitatory postsynaptic potentials, H3Rs are coupled to Gi/Go-proteins and act as auto-receptors that control the synthesis and release of histamine with high constitutive activity. In addition, H3Rs functioning as hetero-receptors can also control the release of other neurotransmitters like acetylcholine, glutamate, GABA, norepinephrine, serotonin, dopamine in variable brain regions [8,9]. Interestingly, blocking central H3Rs has been proposed to enhance the cortical fast rhythms closely associated with cognitive behaviors [9]. Several drug candidates that block the H3R have shown to increase the release of ACh, and therefore alleviate symptoms and slow the progression of AD in clinical trials [10–12]. Moreover, recent studies revealed that prototypical H3R inverse agonists, e.g. thioperamide or pitolisant, improved multiple aspects of memory processing in an inhibitory avoidance task in rodents [13,14]. Notably, the current treatment of AD still relies on the three (single-targeting) approved acetylcholinesterase inhibitors (AChEIs), namely galantamine, donepezil, and rivastigmine, and the *N*-methyl-D-aspartate (NMDA) receptor antagonist memantine [15–17]. Considering that both H3R and AChE are involved in the regulation of the amount of a variety of central neurotransmitters including histamine and acetylcholine, dual-active H3R antagonists and AChEIs have been developed by several groups [18–21], but the affinities at the two-targets were never balanced into an identical concentration range. Even more important, to our knowledge the behavioral data of such dual-acting compounds have not been published until now, and therefore such compounds still lack their proof of principle in vivo. Notably, both AChE and butyrylcholinesterase (BChE) continue to be very attractive as biological targets, not only in light of the still actual cholinergic hypothesis of AD but also due to their secondary functions, which include mediation of processing and deposition of β-amyloid peptide considered crucial for the development of this disease. Interestingly, ChEs are found in close connection to β-amyloid forming a complex, especially in neurons and glia as well as in neuritic plaques and tangles in the human brain in AD patients. Moreover, recent studies have shown that both cholinesterases are involved in AD pathology and cognition in normal brains [17,22–24].

In the present study, we describe the in vivo effects of a recently described novel dual-acting H3R antagonist and AChEI, UW-MD-71 (7-(3-(piperidin-1-yl)propoxy)-1,2,3,9-tetrahydropyrrolo[2,1-*b*]quinazoline; Fig. 1). It belongs to the quinazoline class of compounds and was developed and characterized as a highly hH3R-selective (over hH1R, hH2R and hH4R) antagonist and as a reversible and competitive inhibitor of AChE [25,26]. Both biological targets are blocked and inhibited in the two-digit nanomolar range (Fig. 1) [25,26]. In the present study, we investigated the compound's effects on acquisition, consolidation and retrieval processes in a one-trial inhibitory avoidance paradigm in Wistar rats. Moreover, the effects of UW-MD-71 on the memory deficits induced by the non-competitive *N*-methyl-D-aspartate (NMDA) antagonist dizocilpine (DIZ) were tested. Given that motor activity could mask the effects of UW-MD-71 on learning and memory, we also used an open-field test to evaluate activity and anxiety in the same animals.

2. Material and methods

2.1. Animals

Male Wistar rats (bred at the Central Animal Facility of the UAE University) at a body weight of 180–200 g were used. All animals were maintained in an air-conditioned room with controlled temperature (24 ± 1 °C) and humidity (55 ± 15%) under a 12-h light/dark cycle (lights on at 07:00 h). The animals were given free access to food and water. Experiments were conducted between 9:00 and 15:00 h, and all procedures were performed in accordance with the guidelines of the European Communities Council Directive of 2010 (2010/63/EU) and were approved by the Institutional Animal Ethics Committee of College of Medicine and Health Sciences/United Arab Emirates University (A30-13). Naïve rats were handled for a week before drug treatment began.

2.2. Drugs

The brain penetrant H1R antagonist pyrilamine (PYR), the brain penetrant H2R antagonist zolantidine dimaleate (ZOL), donepezil hydrochloride (DOZ), and dizocilpine hydrogen maleate (DIZ) were purchased from Sigma–Aldrich (St. Louis, Missouri, USA). The H3R