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Effect of galantamine on adjuvant-induced arthritis in rats

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ABSTRACT

Stimulation of the vagus nerve suppresses cytokine production and macrophage activation, *via* the interaction of its neurotransmitter acetylcholine (ACh) with the α7 subunit of the nicotinic acetylcholine receptor (α7nAChR), present on neurons and inflammatory cells. The present study aimed to verify the potential anti-inflammatory effect of galantamine against experimental arthritis induced in rats. Fourteen days post adjuvant injection, Sprague-Dawley rats were treated orally with three doses of galantamine (1.25, 2.5 and 5 mg/kg) or leflunomide (10 mg/kg) for 2 weeks and arthritis progression was assessed by hind paw swelling. Additionally, serum biomarkers, *viz*., anti-cyclic citrullinated peptide antibodies (Anti-CCP), tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10) and monocyte chemoattractant protein-1 (MCP-1) were measured. Radiological examination of the hind paws was also carried out to evaluate the degree of joint damage.

Adjuvant arthritis led to a significant weight loss, marked swelling of the hind paw and alteration in the serum levels of anti-CCP, TNF-α, IL-10 and MCP-1. These alterations were associated with significant radiological changes of the joints. Galantamine, in a dose-dependent manner, reduced significantly all biomarkers of inflammation, with the highest dose showing the best beneficial anti-inflammatory effect that was superior in magnitude to the reference drug leflunomide in most of the studied parameters. In conclusion, these results suggest that galantamine may represent a novel, inexpensive and effective therapeutic strategy in the treatment of rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the synovial joints, ultimately leading to progressive and irreversible joint destruction (Firestein, 2003). Early diagnosis and treatment of this ailment reduce joint destruction, preserve function, and improve survival (Kalpakcioglu and Şenel, 2008). Therefore, critical issues, concerning the effect of therapy, are to control symptoms and signs of the disease for prolonged periods, as well as their capacity to retard the damaging effect of inflammation on articular cartilage and bone (Lipsky et al., 2000).

No single agent is completely effective in treating disease pathology and devoid of side effects; consequently, seeking for a safe and effective treatment for RA is mandatory. Current therapies include conventional non-steroidal anti-inflammatory agents, corticosteroids, biological therapies, including the disease-modifying anti-rheumatic-drugs (DMARDs), as methotrexate and leflunomide, besides inhibitors of the signature proinflammatory cytokine tumor necrosis factor-α (TNF-α), as etanercept, adulimumab and infliximab (Abbott and Moreland, 2004).

Recently, it has been demonstrated that the nervous system, *via* an anti-inflammatory reflex of the vagus nerve, plays a profound role in limiting inflammatory responses (Oke and Tracey, 2009), a concept referred to as the "cholinergic anti-inflammatory pathway". The vagus nerve is the extension of the central nervous system (CNS) to visceral organs, including spleen and liver, the major sources of damaging cytokines that are involved in the pathogenesis of RA (McInnes and Schett, 2007; Tracey, 2007). Moreover, stimulation of the vagus nerve, *via* its neurotransmitter acetylcholine, was reported to suppress cytokine production and macrophage activation (Tracey, 2002). Such an effect is mediated by the interaction of acetylcholine with the α7 subunit of the nicotinic acetylcholine receptor (α7nAChR), present on neurons, as well as, on inflammatory cells. The stimulation of α7 nicotinic acetylcholine receptor by acetylcholine or other specific agonists was reported to put down the release of pro-inflammatory

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cytokines (van Maanen et al., 2009; Liu et al., 2010).

Galantamine, a centrally acting reversible competitive acetylcholinesterase inhibitor and a cholinomimetic, has a significant anti-inflammatory effect linked to the accumulated cetylcholine in the synapse (Pavlov et al., 2009b). Apart from inhibiting acetylcholinesterase, galantamine binds allosterically to the α7 nicotinic acetylcholine receptor (Schilström et al., 2006), to produce a selective nicotinic cholinergic enhancement, without affecting the muscarinic acetylcholine receptors (Samochocki et al., 2003).

The curious observation that patients suffering from RA had experienced a marked improvement of their symptoms upon treatment with galantamine (Snorrason and Murray, 2002), made up the idea of the present study. Thus, the current work was carried out to evaluate the possible anti-inflammatory effect of different doses of galantamine in a RA model induced by Freund's adjuvant, in comparison with the conventional anti-rheumatic drug leflunomide.

2. Materials and methods

2.1. Animals

In the present study, adult male Sprague-Dawley rats weighing 170–200 g were used. Rats were kept under observation for at least one week prior to study with free access to food and water. All procedures were performed in accordance to the Research Ethical Committee of the Faculty of Pharmacy, Cairo University, Egypt and comply with the National Research Council's guide for the care and use of laboratory animals (Permit Number: PT 623).

2.2. Induction of adjuvant arthritis (AA)

Rat adjuvant arthritis is an experimental model of RA with a proven track record of predictability (Bendele, 2001). Adjuvant arthritis was induced, as mentioned previously (Refaat et al., 2013), by injecting rats, intradermally at the base of the tail, with 0.1 ml suspension of 12 mg/ml heat-killed *Mycobacterium butyricum* (Difco Laboratories Co-USA) in incomplete Freund's adjuvant (Sigma-Aldrich Co-USA). Chronic inflammation was allowed to progress for 14 days; arthritic rats were allocated into 6 groups ($n=8$), besides the non-arthritic healthy control rats, which were assigned as group I. Untreated AA rats signify group II (positive control), while those in groups 3, 4 and 5 were administered galantamine (Gal)1.25, 2.5 and 5 mg/kg, respectively (Woodruff-Pak et al., 2002). The last 6th group serves as the standard group, and the animals were given leflunomide (Leflu) 10 mg/kg (Karaman et al., 2006). Animals in the two control groups receive saline as the vehicle. All treatments were gavaged orally for two weeks until day 27 post-adjuvant injection.

2.3. Assessment of arthritis progression

Hind paw diameter was assessed by caliper measurement of ankle (tibiotarsal) joint width (Bendele, 2001), in both control and test groups on day 0, then every other day till the end of the study. Similarly, the body weight was recorded on day 0, then every other day till the end of the study as a measure of chronic inflammation (Ekambaram et al., 2010).

2.4. Serum parameters

On day 28, rats were deeply anesthetized with diethyl ether and blood was collected from the posterior vena cava through a laparotomy incision. Sera were used to assess the levels of anti-cyclic citrullinated peptide antibodies (Anti-CCP), TNF-α, interleukin (IL)-10 and monocyte chemoattractant protein-1 (MCP-1) using enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen Corporation, Carlsbad California, USA), according to the manufacturers' instructions.

2.5. Tissue parameters

The spleen and liver of all rats were excised, blotted dry, weighed and their mass indices [ratio of organ weight (mg) to body weight (g)] were calculated as further markers of inflammation (Zhang et al., 2014).

2.6. Radiological examination

The left hind paws were promptly amputated at necropsy, kept on ice and examined radiologically by plain X-ray to detect any bone changes (van Maanen et al., 2008). Hind paws were placed over a radiographic cassette containing standard X-ray film at a distance of 90 cm from X-ray source and for 0.1 s exposure time to obtain lateral and oblique views. Based on soft tissue swelling, radiographs were scored on a scale of 0–4 (0=normal with no soft tissue swelling, 1=minimal soft tissue swelling, 2=mild to moderate soft tissue swelling, 3=moderate soft tissue swelling, 4=marked to severe soft tissue swelling). For the degree of joint destruction and periarticular erosive changes, radiographs were also scored from 0–4 (0=no joint destruction, 1=minimal joint destruction, 2=mild to moderate joint destruction, 3=moderate joint destruction, 4=marked to severe joint destruction with evident periarticular erosive changes). All scores were calculated by an observer blinded to the experimental groups.

2.7. Statistical analysis

Values are reported as means ± S.D. ($n=8$). Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey multiple comparison post hoc test. The differences were considered to be significant at $P<0.05$.

3. Results

In this study, the effect of different concentrations of galantamine (Gal; 1.25, 2.5 and 5 mg/kg) was examined in adjuvant arthritis rats and the results were compared with leflunomide (Leflu; 10 mg/kg) as a reference drug. No signs of toxicity were observed with either therapies and all rats survived till the end of experiment.

3.1. Hind paw diameter

Hind paw swelling reveals both inflammatory and arthritic alterations occurring in adjuvant arthritic rats. By day 14 post administration of incomplete Freund's adjuvant, hind paw reached a noticeable large diameter that continued to increase to reach its maximum by day 27. Different treatments administered on day 14 and thereafter caused a gradual decrease in the hind paw size, effect that persists until the end of the experiment period (day 27). Galantamine at the high dose level mediated the optimum effect (Fig. 1A and B).

It is worth mentioning that induction of adjuvant arthritis at the base of the tail resulted in a progressive inflammatory ulceration at the site of injection compared to a little one in all other groups (Fig. 1C). On day 18 of adjuvant injection some brown discoloration appeared in different joints and fingers of arthritic control and remained till the end of the study, which was not the case in any of the treated groups (Fig. 1D).