Biomedicine & Pharmacotherapy 86 (2017) 271–278

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Agmatine ameliorates adjuvant induced arthritis and inflammatory cachexia in rats

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ARTICLE INFO

Article history:
Received 14 October 2016
Received in revised form 28 November 2016
Accepted 9 December 2016

Keywords:
Agmatine
Complete freud adjuvant
Arthritis
Cachexia
Interleukin
TNF-α

ABSTRACT

The present study investigated the pharmacological effect of agmatine in Complete Freud Adjuvant (CFA) induced arthritis and cachexia in rats. The rats were injected with CFA (0.1 ml/rat) to induced symptoms of arthritis. Day 8 onwards of CFA administration, rats were injected daily with agmatine for next 7 days, and arthritis score, body weights and food intake were monitored daily (g). Since cachexia is known to produce severe inflammation, malnutrition and inhibition of albumin gene expression, we have also monitored the total proteins, albumin, TNF-α and IL-6 levels in arthritic rats and its modulation by agmatine. In the present study, CFA treated rats showed a progressive reduction in both food intake and body weight. In addition analysis of blood serum of arthritis animals showed a significant reduction in proteins and albumin and significant elevation in tumor necrosis factor (TNF)-α and Interleukins (IL)-6. Chronic agmatine (20–40 mg/kg, ip) treatment not only attenuated the signs of arthritis but also reverses anorexia and body weight loss in CFA treated rats. In addition, agmatine restored total protein and albumin and reduces TNF-α and IL-6 levels in arthritis rats. These results suggest that agmatine administration can prevent the body weights loss and symptoms of arthritis via inhibition of inflammatory cytokines.

1. Introduction

Cachexia syndrome exhibits significant loss of body weight, muscle atrophy, fatigue, weakness and chronic loss of appetite. It is a devastating condition and occurs in many chronic pathological processes including cancer, renal failure, HIV infection and in chronic inflammatory illness such as rheumatoid arthritis [1–4]. Rheumatoid arthritis is usually associated with accelerated protein breakdown [5,6] leading to increase morbidity and premature mortality [7]. Increasing evidence from both animal and clinical studies suggests that an inflammatory response, mediated by a dysregulated production of pro-inflammatory cytokines, plays a role in the genesis of cachexia. However, the mechanisms leading to cachexia remain largely unclear.

Agmatine, an endogenous amine is synthesized through decarboxylation of L-arginine by arginine decarboxylase (ADC). It is a putative neurotransmitter [8,9] and exhibits biological effects by interacting with several receptors. Agmatine activates α_2-adrenoceptors and imidazoline receptors [9,10], and antagonize N-methyl D-aspartate (NMDA) receptors [11]. Additionally, it competitively inhibits nitric oxide (NO) synthase [12]. In experimental studies, agmatine showed a variety of pharmacological effects including anticonvulsant, anxiolytic, antinociceptive, antidepressant, and neuroprotective effects [9,10,13–20]. Several studies have reported that agmatine blocks spinal nociceptive reflexes, prevents inflammation, spinal cord injury and nerve injury induced pain [21,22]. Further, agmatine also attenuates mechanical hypernociception induced by Complete Freund's Adjuvant (CFA) in mice [23] and streptozotocin induced diabetic neuropathy in rats [22]. In fact, a recent clinical trial confirm that agmatine is safe and effective for treating pain and improving quality of life in patients suffering from lumbar disk-associated radiculopathy [24]. However, the information pertaining to involvement of agmatine in chronic inflammatory state like rheumatoid arthritis is much limited. In view of complimentary role of agmatine in pain and inflammation, therefore we hypothesized that agmatine may play role also in inflammatory cachexia in adjuvant-induced arthritis in rats.

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http://dx.doi.org/10.1016/j.biopha.2016.12.039
0753-3322/

The present investigation was undertaken to define the role of agmatine in inflammation and weight loss associated with adjuvant induced arthritis in rats. The arthritis was induced by CFA (0.1 ml/rat). Day 8 onwards of CFA administration, rats were injected daily with agmatine for next 7 days, and food intake and body weight were monitored daily (g). Since cachexia is known to produce severe inflammation, malnutrition [25–27] and inhibition of albumin gene expression [28] we have also monitored the total proteins, albumin, TNF-α and IL-6 levels in arthritic rats and its modulation by agmatine.

2. Materials and methods

2.1. Subjects

Adult male Sprague-Dawley rats were used. Initially they were group housed in acrylic cages under constant room temperature (25 ± 2 °C), relative humidity (50 ± 5%), and maintained under a controlled 12:12 h light-dark cycle (lights on at 0700 h). Food and water were available *ad libitum*. During the experimental protocol rats were housed individually. All experimental procedures were approved by the Institutional Animal Ethical Committee and executed in strict accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Govt. of India.

2.2. Experimental design

Arthritis was induced in rats by subplannter injection of CFA (0.1 ml/rat) in the right hind paw. Rats receiving CFA did not show any sign of acute toxicity. Control animals were injected with 0.9% saline. On day 8 after adjuvant injection, these rats were divided in treatment groups (n = 6 rats/group) and injected daily with saline or agmatine 10, 20 and 40 mg/kg, intraperitoneally upto day 15. The animals were weighed daily. The injections were given daily in between 0900 and 1000 h and animals were subjected to measurement of arthritis score [29] as described below. Thereafter they were shifted to their cages and the pre-weighed food pellets were placed inside the cage hopper. The food consumed by rats was quantified by weighing leftover food in the hopper.

2.3. Arthritis score

Evaluation of arthritis severity was performed by measuring the arthritis index of each animal, which was scored by grading each paw from 0 to 4, as described previously [29]. Grading was determined as follows:

Score	Signs
0	No erythema or swelling
1	Slight erythema or swelling of one or more digits
2	Swelling of the entire paw
3	Erythema and swelling of the ankle
4	Ankylosis, incapacity to bend the ankle

The severity score was the sum of the arthritis scores of the right hind limb, maximum upto 4. On day 15 after adjuvant injection blood was withdrawn by retro-orbital method for biochemical analysis.

2.4. Paw volume

The paw volumes of all animals were measured daily till day 15 using a plethysmometer (VJ instrument, India). The change in paw volume was measured as the difference between the final and initial paw volumes.

2.5. Effect of adjuvant arthritis on the serum biochemical parameters

On day 15 after saline or CFA treatment, the blood samples were obtained by retro-orbital method from saline and agmatine treated rats (n = 6/group). Total serum proteins, albumin were measured by Biuret test. Serum TNF-α and IL-6 levels were measured by enzyme-linked immunosorbent assay (ELISA) technique according to manufacturers protocol.

2.6. Statistical analysis

The data are presented as mean ± SEM. The behavioral and biochemical data were analyzed by two-way analysis of variance (ANOVA) and individual means were compared by post-hoc Bonferroni multiple comparison test. The values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Effect of agmatine on adjuvant induced arthritis score

While arthritis score did not change upto day 4 following subplantar CFA administration (0.1 ml/rat), external signs of arthritis started to increases from day 5 onwards and on day 15 of the protocol 100% rats showed the occurrence of arthritis. [CFA treatment $F(1, 144) = 384.55$, $P < 0.001$; duration in days $F(15, 144) = 8.79$, $P < 0.001$; and interaction treatment × days $F(15, 144) = 8.79$, $P < 0.001$] (Fig. 1A).

As depicted in Fig. 1B, daily treatment with agmatine (20 and 40 mg/kg, ip) starting from post day-8 following CFA injections, progressively reduced the arthritis score in rats as compared to the saline treated animals. Application of two-way ANOVA showed significant interaction [$F(45, 352) = 1.45$, $P < 0.05$] between variables like agmatine treatment [$F(3, 352) = 8.07$, $P < 0.001$] and days [$F(15, 352) = 27.42$, $P < 0.001$]. Application of post hoc Bonferroni multiple comparison test revealed significant recovery of adjuvant arthritis on post-arthritis days 12 ($P < 0.05$), 13 ($P < 0.05$), 14 ($P < 0.05$) and 15 ($P < 0.01$) by agmatine 40 mg/kg and by 20 mg/kg on day 14 ($P < 0.05$) and 15 ($P < 0.05$) of the protocol. On the other hand, agmatine (10 mg/kg) did not significantly improve arthritis score in rats.

3.2. Effect of agmatine on adjuvant induced increased paw volume

As demonstrated in Fig. 2A, CFA treated rats showed significant increase in the paw volume on day 5 of administration and remained sustained high till the end of the protocol. In the primary phase of the arthritis i.e. form day 1 – 4, non-significant but progressive increased in the paw volume was observed. Two-way ANOVA revealed a significant main effect of CFA treatment [CFA treatment- $F(1, 176) = 261.64$, $P < 0.001$; duration in days- $F(15, 176) = 5.18$, $P < 0.001$; and interaction treatment × days- $F(15, 176) = 5.54$, $P < 0.001$].

As shown in Fig. 2B, daily treatment with agmatine (20 and 40 mg/kg) decreased the paw volume in rats as compared to the saline treated animals. Two-way ANOVA showed significant interaction [$F(45, 368) = 1.12$, $P = 0.29$] between variables like agmatine treatment [$F(3, 368) = 8.87$, $P < 0.001$] and days [$F(15, 368) = 17.95$, $P < 0.001$]. Application of post hoc Bonferroni multiple comparison test revealed significant decreased in paw volume on post-arthritis day 15 by agmatine 20 mg/kg and from days 13 onwards by agmatine 40 mg/kg. The lower dose of agmatine (10 mg/kg) did not significantly improve paw volume in arthritic rats.