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Antidepressant-like effects of the xanthine oxidase enzyme inhibitor allopurinol in rats. A comparison with fluoxetine

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ABSTRACT

Allopurinol is a xanthine oxidase enzyme inhibitor that is widely used for the treatment of hyperuricemia and gout. The activity of tryptophan 2,3-dioxygenase, which metabolizes tryptophan (TRP), is decreased by xanthine oxidase inhibitors, causing TRP levels in the body to be increased. Increases in TRP levels in the brain might have antidepressant effects. The purpose of this study is to evaluate the antidepressant effects of allopurinol compared to those of fluoxetine, which is a proven antidepressant. Thirty-two Wistar albino male rats were divided into four groups (control, 10 mg/kg fluoxetine, 50 mg/kg allopurinol, 50 mg/kg allopurinol + 10 mg/kg fluoxetine; n = 8 per group), and forced swimming tests were performed before and after 14 days of drug administration. Serotonin, 5-hydroxyindolacetic acid and uric acid levels were measured in blood samples after the final treatment. When allopurinol and fluoxetine were administered separately, a decrease in the duration of immobility and an increased duration of swimming were observed in the forced swimming test. The results showed similar antidepressant efficacies between allopurinol and fluoxetine. However, we found no statistically significant difference in the antidepressant effect of the combined therapy versus single drug therapy.

1. Introduction

Efforts to understand the pathophysiology of depression have resulted in several crucial hypotheses; among the most important of these is the monoamine hypothesis. According to the monoamine hypothesis, a lack or inconsistency of neurotransmitters such as serotonin, dopamine and norepinephrine causes depression (Brown et al., 1990). Of these neurotransmitters, serotonin is the most studied monoamine neurotransmitter with regards to the aetiology of depression and the mechanisms of the antidepressant medications.

The precursor of serotonin is tryptophan (TRP), and the kynurenine pathway metabolizes most of the TRP in the body. The rate-limiting enzymes in the pathway are indole amine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) (tryptophan pyrrolase) (Carlin et al., 1989). Indole amine 2,3-dioxygenase activity is increased by the activation of the immune system (Capuron et al., 2002). Tryptophan 2,3-dioxygenase primarily functions in the liver and is activated by stress-induced corticosteroids (Green and Curzon, 1975). Increased activity of the kynurenine pathway reduces the availability of TRP to be utilized for serotonin synthesis. Recently, a new "5-HT" hypothesis has been suggested, which affirms that the activation of IDO reduces TRP and increases TRP catabolites (Maes et al., 2011). Importantly, it is thought that TRP catabolites lead to depression due to neurotoxic and neuroexcitatory effects (Myint and Kim, 2014). Allopurinol has been shown to counteract the stress-related increases in kynurenine concentrations (Gibney et al., 2014). The role of the kynurenine pathway in depression is further supported by clinical and preclinical studies (Reus et al., 2015). Decreased levels of TRP increased the levels of kynurenine (Gabbay et al., 2010, Maes et al., 2001), and a positive correlation has been observed between the kynurenine/TRP ratio and suicidrality (Bradley et al., 2015). In addition, a reduction in kynurenic acid/quinolinic acid (Savitz et al., 2015) has been found in depressed patients. The activity of tryptophan 2,3-dioxygenase, which metabolizes TRP, is decreased by xanthine oxidase inhibitors, causing TRP levels in the body to increase (Badawy and Evans, 1973, Becking and Johnson, 1967). Allopurinol is a xanthine oxidase enzyme inhibitor that is widely used in the treatment of hyperuricemia and gout. Interestingly, studies have found high levels of xanthine oxidase in patients with depression and panic attacks (Herken et al., 2006, 2007). Together, these data suggest that increases in TRP levels in the brain due to the xanthine oxidase inhibition causing subsequent increases in the levels of serotonin might have antidepressant effects.

Karve et al. recently reported statistically significant antidepressant effects from allopurinol and febuxostat (another hypouricemic medication) when administered to rats subjected to forced swimming tests (Karve et al., 2013). The forced swimming test is a widely used

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experimental assay for depression that was developed to investigate the antidepressant efficacy of medications (Lee et al., 2010, Porsolt et al., 1978).

The purpose of this study is to investigate the antidepressant effects of the xanthine oxidase inhibitor allopurinol and to compare them with those of fluoxetine, a proven antidepressant.

2. Materials and methods

This study was approved by the Adnan Menderes University Animal Experiments Local Ethics Committee for the ethical care and use of animals in research. All animal care and experimental procedures were conducted in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals 1985.

2.1. Animals

A total of 32 (350–400 g weight) Wistar albino male rats, provided by Adnan Menderes University Medical Faculty Experimental Animal Laboratory, were used. The rats were housed with 12 h of light and 12 h of darkness, a constant temperature of 20–22 °C and humidity levels of 45%–55%. Water and feed were supplied ad libitum. The rats were acclimated to the researchers from a young adult age in order to minimize discomfort during the experiments. The experimental animals were randomly divided into four groups after they had been acclimatized to the lab for 7 days (Table 1).

2.2. Performing forced swimming test and scoring

On the first day of the experiment, the rats were placed individually into a tank 40 cm high and 25 cm in diameter that contained 23 °C water at a depth of 30 cm and were left to spend 15 min in the tank alone. The water in the tank was changed between animals. After being dried, the rats were then placed back in their cages. Twenty-four hours after familiarization, the forced swimming test was performed. A high-resolution camcorder (Samsung HMX-QF30 full HD) recorded the 5 min following the first minute of contact with the water. After being dried, the rats were placed back in their cages. This same process was performed for all animals. Climbing, swimming and immobility durations were determined via camcorder recordings. The 5-min recordings were uniformly divided into 5-s intervals, and each interval was classified according to the dominant activity performed during the interval (Cryan et al., 2002, Gibney et al., 2014, Porsolt et al., 1977). The duration in seconds of each activity exhibited by the animal during the forced swimming test was determined by multiplying the number of the intervals per corresponding activity type by 5.

2.3. Drugs used in the experiment

Allopurinol (Sigma–Aldrich, St. Louis MO) at a dose of 50 mg/kg was prepared daily and administered via intraperitoneal (ip) injection. Fluoxetine was prepared according to Brandes et al., and a 10 mg/kg dose was administered via ip injection (Brandes et al., 1992). The control group was injected with distilled water at 1 ml/kg ip.

Table 1
Experimental groups.

Drug groups	Dose and administration route	Duration
Distilled water (Control) (n = 8)	1 ml/kg ip	14 days
Fluoxetine (n = 8)	10 mg/kg ip	14 days
Allopurinol (n = 8)	50 mg/kg ip	14 days
Fluoxetine + allopurinol (n = 8)	10 mg/kg + 50 mg/kg ip	14 days

2.4. The experimental procedure

The initial weights of the rats were measured, and proper medication doses were determined accordingly. Prior to administration of the drug, all animals were subjected to the forced swimming test. Then, the drugs were administered via ip injection for 14 days. The rats were again subjected to the 15-min familiarization swim 1 day before the last dose. After 24 h, their weights were measured following the last injection, and the 5-min swimming test was performed. All tests were recorded by camcorder, and these recordings were used by a blind researcher after the experiments were finalized to estimate the durations of immobility, swimming and climbing activities. After the swimming test, intracardiac blood samples were collected while the rats were under anaesthesia with 50 mg/kg ketamine and 10 mg/kg xylazine. Serotonin, 5-hydroxyindolacetic acid (5-HIAA) and uric acid levels were examined in the blood samples.

2.5. Analysis of 5-HIAA, serotonin and uric acid concentrations

The concentrations of 5-HIAA and serotonin were determined using commercially available rat-specific ELISA assays. Rat 5-HIAA/5-Hydroxyindolacetic acid (Catalogue No: E-EL-R1098 Lot No: AK0014JUL26028), and Rat Serotonin/5-Hydroxytryptamine/5-HT (Catalogue No: E-EL-0033 Lot No: AK0014JUL26027; both from Elabscience, WuHan, P.R.C) kits we used. The assays were performed according to the manufacturer's instructions. The assay principle is a competitive ELISA with colorimetric detection performed using an ELISA microplate reader (Epoch Microplate Spectrophotometer SN:242136, Biotek, Winooski, VT, USA) at 450 nm.

The concentrations of uric acid were analysed using an automated chemistry analyser (Tokyo Boeki TMS 1024, SN 1001340101, Japan). A commercially available uric acid test kit with control sera (Uric Acid Ref No: 1001011 Lot No: 325, Spintrol “H” Normal Ref: 1002120 Lot No: 3576, SpinReact, Girona, Spain), which is based on enzymatic–colorimetric detection, was used according to the manufacturer's instructions.

2.6. Statistical analysis

SPSS 20.0 for Windows was used to analyse the data (IBM, 2011). The normality of the distribution was evaluated using the Kolmogorov–Smirnov test. The data, which were found to be normally distributed, were analysed using a paired *t*-test, one-way analysis of variance (ANOVA) and two-way mixed ANOVA. A paired *t*-test was applied for self-comparison within each group before and after drug administration. Behaviour patterns (immobility and swimming) were analysed using a mixed-design ANOVA with a within-subject factor of time (pre/post-drug administration) and a between-subject factor of groups (control, fluoxetine, allopurinol, fluoxetine + allopurinol). An a priori multiple comparison test (Bonferroni *t*-test) was used when the ANOVA results were significant. One-way ANOVA was used to analyse the uric acid levels. If the variance was considered non-homogeneous, Tamhane's post hoc test was used. The Kruskal–Wallis (KW) H test was used as a non-parametric test for blood serotonin and 5-HIAA levels. A two-tailed *p* value of less than 0.05 was considered statistically significant.

3. Results

There was no difference between the mean weights of the animals at the start of the experiment. The increase in the weights of the rats in the allopurinol group ($p = 0.003$) was similar to that of the control group ($p = 0.016$) during the drug administration period. No significant weight increase was detected in the fluoxetine group ($p = 0.68$).