Original article

The effect of biologic agents currently used for rheumatoid arthritis on the central nervous system of healthy rats

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Abstract

The aim of this study was to investigate the development of behavioral and memory disorders on healthy adult rats, after chronic, systematic administration of methotrexate and the biologic agents, Tocilizumab (anti-IL6) and Infliximab (anti-TNFa), that are used for Rheumatoid Arthritis. 35 adult male Wistar rats, 12 weeks old, were used in this study. The rats were divided into 5 groups (n=7): a control group (CTRL), which was submitted to tests without receiving any drug, a placebo group (PLC) which received normal saline (i.p.), a methotrexate group (MTX) receiving 0.25mg/kg of the drug (i.p.), an infliximab group (INFL) receiving 6mg/kg of the drug (i.p.), and a tocilizumab group (TCZ) receiving 8mg/kg of the drug (i.p.). The drug infusion was performed weekly. After 30 days of drug administration, behavioral tests were performed to assess the rats' stress levels and memory. The performed behavioral tests were (1) the Elevated-plus maze test, (2) the Elevated-zero maze test and (3) the Olfactory social memory test. The results were analyzed using Oneway-ANOVA and Kruskal-Wallis tests through SPSS 25.0. The MTX group spent significantly less time in the open arms of the mazes, compared to the CTRL group ($P<0.001$) and the PLC group ($p=0.05$), and needed less time during the second encounter compared to the first, when assessed in the olfactory social memory test ($p=0.002$). The TCZ and INFL groups spent more time in the open areas of both mazes compared to PLC and MTX groups ($p=0.033$). Based on the results of this study, the administration of biologic agents improves stress levels and shows a potentially anxiolytic effect, without significantly affecting memory.

Keywords: Rheumatoid Arthritis, Experimental Model, Methotrexate, Tocilizumab, Infliximab

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Introduction

Rheumatoid Arthritis (RA) is the most common rheumatic disease, affecting 1% of the world's population. It is a systemic autoimmune inflammatory disease whose mechanisms of pathogenesis include activation of T-lymphocytes (CD4+) by an antigen and concomitant cytokine release, such as Tumor Necrosis Factor (TNFa-TNFb) and Interleukins (IL-1, IL-2, IL-4, IL-6, IL-8).

The drugs currently used for RA include non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, classic disease modifying anti-rheumatic drugs (DMARDs), and biologic disease modifying drugs, which have been added to the therapeutic agent list this past decade. Some of the most widely used biologic disease modifying drugs are Infliximab (anti-TNFa) and Tocilizumab (Anti-IL-6), while a typical example of a DMARD is Methotrexate (Burmester and Pope, 2017).

Recent clinical trials have supported that DMARD and biologic agent therapy is associated with central nervous system (CNS) disorders. Specifically, Infliximab has been linked to demyelinating diseases (Optic neuritis, chronic inflammatory demyelinating polyneuropathy, Gulliain-Barré syndrome) (Caminero, Comabella and Montalban, 2011; Kaltsonoudis et al., 2014), while Tocilizumab, when co-administered with Methotrexate is believed to increase the risk of depression and anxiety disorders in patients with Rheumatoid Arthritis. (K. and U., 2010; Pinho De Oliveira Ribeiro et al., 2013)

Aim of the study

The widespread use of DMARDs has made the thorough investigation of their effect on different organ systems mandatory. This need is profound especially in their CNS effects, where research data is unclear. The aim of this study is to investigate the development of behavioral and memory disorders on healthy rats, after chronic, systemic administration of Methotrexate and biologic agents Infliximab and Tocilizumab. The above-mentioned mental functions are considered a good indicator of CNS activity and their disorders have been linked to numerous drug adverse effects.

Materials and Methods

Laboratory Animals and Subgroups

Thirty-five (35) adult male Wistar rats were used in this study, all of them being 12 weeks of age. The rats were divided into 5 groups (n=7) as follows: a control group (CTRL), which was submitted to tests without receiving any drug, a placebo group (PLC) which received normal saline (i.p.), a methotrexate group (MTX) receiving 0.25mg/kg of the drug (i.p.) (Bilasy et al., 2015), an infliximab group (INFL) receiving 6mg/kg of the drug (i.p.) (Karson et al., 2013), and a Tocilizumab group (TCZ) receiving 8mg/kg of the drug (i.p.) (Taskin et al., 2016). Drug infusion of the Placebo, MTX and INFL groups was performed weekly. The TCZ group received the drug every two weeks and during the in-between week the rats received normal saline. All infusions were performed intraperitoneally (i.p.), without the use of sedation. After 30 days of drug administration, tests were performed to assess the rats' sense of space, stress levels, memory and learning ability.

Behavioral Tests

The behavioral tests that were performed included the Elevated-plus maze test, (Braun et al., 2012) the Elevated zero maze test (Tucker and

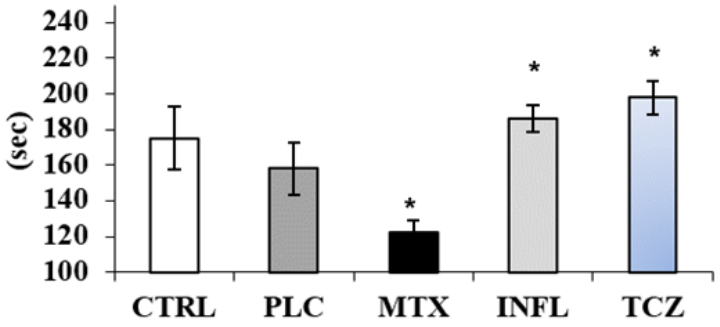

Figure 1: Time spent in open arms during the Elevated Plus Maze test. Group CTRL= control (n=7), Group PLC=placebo (n=7), Group MTX=methotrexate (n=7), Group INFL=infliximab (n=7), Group TCZ=tocilizumab (n=7). Mean values ± SEM. **P* < 0.05; ***P* < 0.01, significantly different

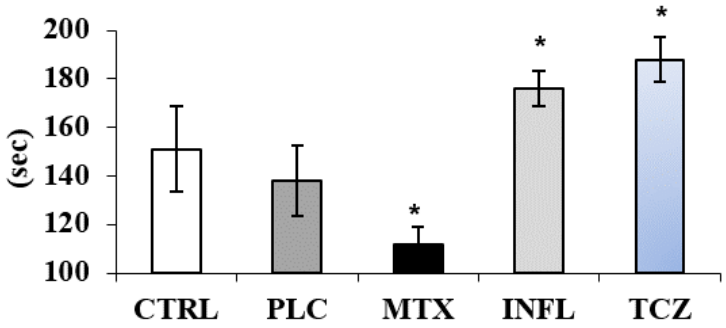

Figure 2: Time spent in the open arms during the Zero Maze test. Group CTRL= control (n=7), Group PLC=placebo (n=7), Group MTX=methotrexate (n=7), Group INFL=infliximab (n=7), Group TCZ=tocilizumab (n=7). Mean values ± SEM. **P* < 0.05; ***P* < 0.01, significantly different

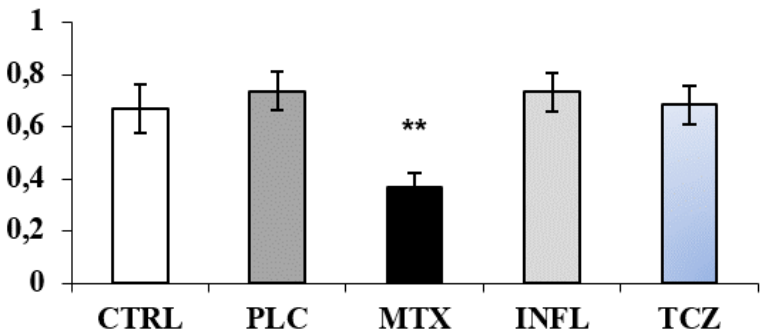

Figure 3: Ratio of First to Second exposure time in the Olfactory Social Memory Test. Group CTRL= control (n=7), Group PLC=placebo (n=7), Group MTX=methotrexate (n=7), Group INFL=infliximab (n=7), Group TCZ=tocilizumab (n=7). Mean values ± SEM. **P* < 0.05; ***P* < 0.01, significantly different

McCabe, 2017) and the Olfactory social memory test (Dantzer et al., 1987). The Elevated-plus maze test was used to assess increased stress levels, in combination with memory. It is comprised of an elevated maze made of Plexiglas with two, white colored, open arms (each 45×10 cm) and two closed, black colored arms with the same dimensions. The elevated zero maze test was performed to assess stress and memory. It is comprised of an elevated, round maze made of Plexiglas, 65cm above the floor, with a diameter of 105cm which was split into quarters. Two of the quarters are open and two of them are closed. The open quarters of the maze have a 1cm high barrier on each edge to prevent rats from falling. The olfactory social memory test was used to assess memory disorders and investigate whether the differences found in the previous tests were caused by stress or memory impairment. All the above tests were performed between 14:00 and 18:00, in standardized lighting, temperature and humidity conditions, in a dark testing room lit by a red-light emitting bulb. The data resulting from the above tests were analyzed using Oneway-ANOVA and Kruskal-Wallis tests through SPSS 25.0.

Results

The animals receiving TCZ and INFL spent a greater amount of time in the open areas of both mazes compared to PLC and MTX groups ($p=0.033$), (Figure 1 and Figure 2), while there was no statistically significant difference when comparing the results of the olfactory social memory test (Figure 3). The animals from the MTX group spent significantly less time in the open arms of the mazes compared to the Control group ($p<0.001$) and the Placebo group animals ($p=0.05$) (Figure 1 and Figure 2). Additionally, the animals receiving MTX needed less time during the second encounter compared to the first, when assessed in the olfactory social memory test. This result suggests that there was no memory dysfunction ($p=0.002$) (Figure 3).

Conclusion

The animals received drug therapy for a period of 30 days. It is suggested that 13,8 days in the lifespan of a rat are equivalent to a year in the life of a human. This means that the study mimicked a patient's chronic exposure to these drugs, lasting longer than 2 years (Sengupta et al. 2012). MTX infusion resulted in less time spent in the open arms of the mazes without affecting memory, a result that is justified by the reported adverse effects of the drug, even in patients receiving low doses. The results of the study showed that administration of biologic agents eased the stress caused by a stressful stimulus (exploration of the open arms of the mazes), without affecting memory. The effects of INFL and TCZ in the above behavioral tests suggest a potentially anxiolytic effect of these agents.

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