International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

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SIMULTANEOUS EFFECT OF LEFLUNOMIDE WITH IMIPRAMINE AND FLUOXETINE IN CARRAGEENAN INDUCED ACUTE ARTHRITIC MODEL

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ABSTRACT

Background: Rheumatoid arthritis is a chronic systematic autoimmune inflammatory disease in which patients suffer from aggravation of tissue pain, swelling around the joints and also face its associated depression.

Objectives: To evaluate the effect of simultaneous administration of leflunomide and anti-depressant drugs i.e. imipramine and fluoxetine on arthritis and its associated depression on carrageenan induced arthritic rats.

Methodology: Arthritis was induced by intradermal injection of 0.1ml of 1% carrageenan in right hind paw of female Sprague-Dawley rats. Drugs were given orally on second day after the induction of arthritis. Paw volume was measured by Vernier Caliper to evaluate arthritic progression and forced swimming test was conducted for neurobehavioral analysis.

Results and conclusion: Maximum anti-arthritic activity was observed in carrageenan induced arthritis group receiving leflunomidealone. Leflunomideanti-arthritic activity decreased a little bit with both anti-depressant drugs i.e. fluoxetine and imipramine. Decrease in activity was observed in both rats receiving fluoxetine than in rats receiving imipramine. Furthermore, the forced swimming test also indicatedthat leflunomide affects anti-depressant activity of fluoxetine and imipramine.

Key words: Rheumatoid arthritis, Depression, Leflunomide, Imipramine, Flouxetine

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, immunological disease described by symmetrical inflammation in the joints prompting pain, swelling and stiffness of the joints, ultimately bringing about joint destruction, disability and poor quality of life [1]. Previous studies showed that RA affects patients both physically and psychologically. Comorbid depression is relatable with RA and prompts more awful wellbeing results. Low socioeconomic status, gender, age, race/ethnicity, utilitarian limitation, pain and poor clinical status have all been connected to depression among persons with RA. Systemic inflammation may also be associated with cause or contribute to depression in RA [2]. It was reported earlier that level of tryptophan, the precursor of serotonin, in RA patient decreases due to its increase catabolism by indoleamine 2,3-dioxygenase enzyme (IDO) released from interferon-γ in inflammatory cell. Therefore its availability in brain decreases which ultimately decreases serotonin synthesis in brain. Since serotonin is the neurotransmitter which is involved in mood, consciousness and sleep therefore decrease concentration of serotonin makes the arthritic body depressed [2-7]. Understanding the socioeconomic factors, individual patient characteristics and

biologic causes of depression in RA can lead to a more comprehensive paradigm for targeting interventions to eliminate depression in RA [2].

A diverse group of agents called disease-modifying antirheumatic drugs (DMARDs) and potent anti-inflammatory antibodies that block tumor necrosis factor are widely accepted, respectively, for slowing the progression of RA and alleviating the painful symptoms [8, 9]. Sufficient control of disease activity in rheumatoid arthritis (RA) is achieved in approximately one-third of patients with synthetic disease-modifying antirheumatic drugs (sDMARDs) including methotrexate (MTX), chloroquine (CQ), Leflunomide and sulfasalazine (SSZ) prescribed either as a monotherapy or combination therapy [10]. Previous findings indicate more terrific scores for depression, as a comorbidity of rheumatoid arthritis, increase the rate of suicidal ideation and depression also can worsen general pain, hardships, treatment denial, and prognosis, as well as cause a faster reduction in quality of life. Patients taking biologic DMARDs had the highest rates of depression, anxiety and suicidal ideation among all patients studied [11].

Leflunomide is an isoxazole immune-modulatory agent that inhibits dihydroorotate dehydrogenase (an enzyme involved in pyrimidine synthesis) and leads to decreases in DNA and RNA synthesis and cell proliferation. Leflunomide has been confirmed to be as effective as methotrexate and sulfasalazine in the treatment of rheumatoid arthritis (RA) [12]. Studies showed that RA and its treatment both contributes to develop depression in patients so addition of anti-depressive agent may help RA patient to live joyful life. Among many anti-depressive agent fluoxetine and imipramine have important position [13]. Fluoxetine opens new era of safe and effective treatment for patients with depression [14]. It is a selective serotonin reuptake inhibitor and has little effect on other neurotransmitters [15]. Fluoxetine has shown efficacy in multiple rodent models of depression, including learned helplessness and social isolation models, as well as in the forced swim and tail suspension tests [14, 16]. Imipramine, an iminodibenzyl derivative, is a distant relative of chlorpromazine. Numerous studies suggested that imipramine is of considerable value in the treatment of depression and convulsion therapy [17].

Previously reported about Variations that was observed in anti-inflammatory activity in all leflunomide-metal complexes in comparison when leflunomide was given alone in carrageenan induced acute arthritic model [23]. Arayne *et al* in 2014 reported Leflunomide and meloxicam though reduces RA progression when given alone but in combined therapy produce severe adverse effect. Depression is prominent with RA and therapy with leflunomide and meloxicam [13]. Jacob et al in 2017 reported around 30% of RA patients developed depression within 5 years of the RA diagnosis. The depression risk was higher in women than in men. The current findings also indicate that improved detection and treatment of patients with both RA and depression are important [24].

2. SIGNIFICANCE OF THE PROBLEM

To evaluate the effect of simultaneous administration of leflunomide and anti-depressant drugs i.e. imipramine and fluoxetine on arthritis and its associated depression on carrageenan induced acute arthritic model.

3. METHODOLOGY

Animals

Female Sprague–Dawley rats, weighing 103-142 g (8-10 weeks), kept at $21 \pm 2°C$ on a 12-hour light/dark cycle with free access to

standard laboratory rat food pellets and water, were used for this study under the ethical guidelines of International Association for the Study of Pain in conscious animals [25]. Rats were randomized and grouped (table:4.1) into normal saline treated arthritic rats (CIA saline control), leflunomide treated arthritic rats (CIA lef), imipramine treated arthritic rats (CIA imi), fluoxetine treated arthritic rats (CIA floux), leflunomide along with imipramine treated arthritic rats (CIA lef+imi) and leflunomide along with fluoxetine treated arthritic rats (CIA lef+floux) groups.

Table: 3.1

S. No	GROUPS	TREATMENT
1	CIA control	Saline treated arthritic induced rats
2	CIA lef	leflunomide (lef) 3.2mg/kg
3	CIA imi	imipramine (imi) 10mg/kg
4	CIA floux	flouxetine (floux) 10mg/kg
5	CIA lef+imi	lef-imipramine
6	CIA lef+floux	lef-flouxetine

3.1 Induction of arthritis:

For this study, acute model of arthritis i.e., arthritis induced by carragenan was selected. Arthritis was induced by intradermal injection of 0.1ml of 1% carrageenan in right hind paw of female Sprague-Dawley rats. Paw volume was measured by plethysmomoter to evaluate arthritic progression. Drugs were given orally on second day after the induction of arthritis. Paw volume was measured again by plethysmometer to evaluate arthritic progression and forced swimming test was conducted for neurobehavioural analysis just after giving drug and after 2 hours and then next day.

3.2 Treatment protocol:

Reference standard of leflunomide was granted by Searle Pharmaceutical Pakistan. After the induction of arthritis, treatment was continued for 4 days. Imipramine and fluoxetine were purchased from market. Leflunomide 3.2mg.kg^{-1}, imipramine and fluoxetine were given at a dose of 10mg.kg^{-1} through oral route.

3.3 Clinical assessment of carrageenan induced anti-arthritic activity:

Measurement of rat's right hind paw volume was used to evaluate arthritic severity produced by the carrageenan administration. It was determined by quantitating the change in their paw volume throughout the experiment by the help of plethysmomoter thus any variability of the pattern of swelling of individual limbs can be monitored.

3.4 Neurobehavioral analysis to evaluate depression:

Depression was evaluated by forced swimming test. Anti-depressant drugs i.e. imipramine and fluoxetine was evaluated for treating depression. Behavioral studies of all rats were conducted by forced swimming test. Rats were placed individually in Plexiglas cylinders 46cm in height with a 21cm internal diameter that were filled with water (25°C) to a depth of 30cm. This depth was sufficient to keep adult rats from supporting themselves by placing their paws or tails on the base of the cylinder. The 5 min swim test was used for analysis of behavior. The animal in the tank was evaluated for mobility time i.e. swimming time. Swimming was registered when large forepaw movements displaced the body around the cylinder, more than necessary to merely keep the head above the water, were performed. Immobility was defined as the lack of motion of the whole body consisting only of the small movements necessary to keep the animal's head above the water.

4. STATISTICAL ANALYSIS

Data was analyzed by one-way analysis of variance using SPSS INC software. Tukey's post-hoc test was conducted to determine between group and within the group means differences taking significant level $p < 0.05$.

5. RESULTS

The evidence of clinical tenderness and erythema was observed after 30min of 1% carrageenan administration which showed erythema in joints especially in ankle, metatarsal and interphalangeal joints which is showing consistency with previous studies that carrageenan can induce arthritis [23]. Significant ($p < 0.05$) anti-arthritic activity was observed in group receiving leflunomide alone when compared with all groups receiving no treatment, fluoxetine and imipramine.

One way Anova and post hock tukey's test analysis also showed that simultaneous administration of leflunomide with fluoxetine or imipramine affect their anti-arthritic activity and anti-depressant activity respectively in insignificant ($p > 0.05$) manner.

Anti-depressant effect:

Results showed that depression was induced after carrageenan administration ($p < 0.05$) which persists even after drug administration in control group and group received leflunomide. Imipramine and fluoxetine showed significant improvement in depression ($p < 0.05$) in their respective groups.

Simultaneous administration of leflunomide with imipramine or fluoxetine showed

significant improvement in depression condition (p<0.05) which is a little bit more in group received leflunomide+imipramine than in leflunomide+fluoxetine (Table 5.1).

Figure 5.1: Paw Edema

Table 5.1a: Paw edema in CIA rats

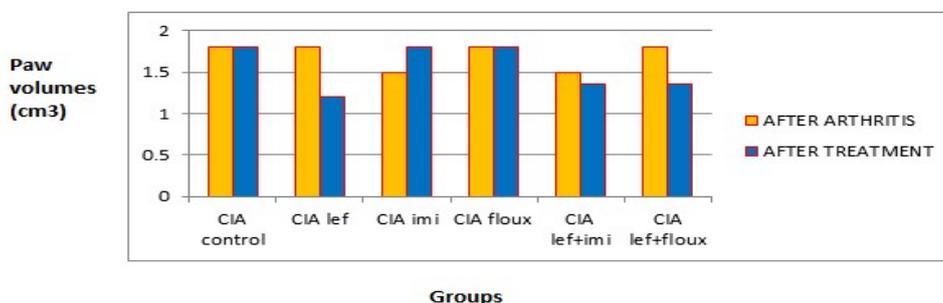| GROUPS	AFTER ARTHRITIS	AFTER TREATMENT
CIA control	1.8	1.8*
CIA lef	1.8	1.2
CIA imi	1.5	1.8*
CIA floux	1.8	1.8*
CIA lef+imi	1.5	1.35
CIA lef+floux	1.8	1.35
One Way ANOVA (df=5)	F=1.067 p>0.05	F=10.400 p<0.05
Values are evaluated by post hock tukey's test ×p<0.05 from leflunomide		

Figure 5.2: Paw Edema assay in CIA rats

Table 5.1 b: Forced swimming test in CIA rats

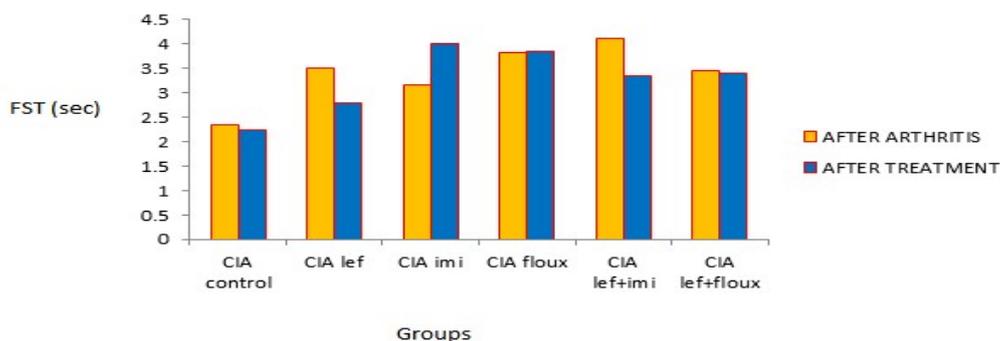| GROUPS	AFTER ARTHRITIS	AFTER TREATMENT
CIA control	2.35	2.24
CIA lef	3.5	2.79
CIA imi	3.15	4.005*+
CIA floux	3.82	3.85*+
CIA lef+imi	4.12	3.36*+
CIA lef+floux	3.44	3.39*+
One Way ANOVA (df=5)	F=6.702 P<0.05	F=13.824 P<0.05
Values are evaluated by post Hoch tukey's test *p<0.05 from control, +p<0.05 from leflunomide		

Figure 5.3: Depression Evaluation

6. CONCLUSION

It is concluded that rheumatoid arthritis patients not only suffer from joint misery but also face its associated depression which exaggerate with leflunomide. In our previous studies it is reported that any anti-depressant drug should be added in such patients regimen[13]. Recent study explores that addition of anti-depressant i.e. imipramine or fluoxetine improve patient depression condition which affects leflunomide anti-arthritic activity insignificantly so this combination can be used simultaneously.

7. FUTURE PERSPECTIVE

With the current national goals of public health research, we want to explore better treatment strategy to make RA patient life healthy both physically and psychologically. We are looking for those treatment combinations which treat RA patient joint associated and depression associated problems. For this purpose, we need to search biochemical factors involve in RA and its associated depression. Estimation of inflammatory markers such as interlukins, interferons, TNF etc and neurochemical substances as monoamines and indolamines in biological samples will help us to find out our target goals.

In the next coming years, rheumatologists might significantly diminish depressive indications in their patients toward tending to the root reason for depression: preventing pain and disability, diminishing systemic aggravation. Furthermore planning evidence-based programs to mitigate the effects of depression in rheumatoid arthritis [26, 27]. This make necessary association to create causal connections that could prompt for new and focused therapies for depression in patients with rheumatoid arthritis.

ACKNOWLEDGEMENTS

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