

## Evaluation of efficacy of montelukast in rheumatoid arthritis

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### Abstract :

Rheumatoid arthritis is an autoimmune disorder. It is chronic progressive disease resulting in inflammation and deformities of joints. Being an autoimmune disease, there's lacuna in proper management of the disease. Current options like steroids and DMARD'S (disease modifying anti-rheumatic drugs) are the cornerstone in therapy of the disease, but have their own limitation. This study proves efficacy of montelukast in restricting progression of rheumatoid arthritis. Montelukast is an antagonist of leukotriene receptor. Arthritis was induced by two methods according to model by intradermal injection of Complete Freund's Adjuvant and Methylated BSA in right hind paw. Macroscopic, inflammatory, biomarker, X-ray, and radio diagnosis parameters were studied for both models. Hence results of this study clearly indicate that montelukast has promising anti rheumatic activity.

**Keywords:** Montelukast, Rheumatoid arthritis, CFA (Complete Freund's adjuvant).

**Introduction :** Rheumatoid arthritis (RA) is a long lasting autoimmune disorder that primarily affects joints. It typically results in warm, swollen, and painful joints. Pain and stiffness often worsen following rest. Most commonly, the wrist and hands are involved, with the same joints typically involved on both sides of the body. The disease may also affect other parts of the body. This may result in a low red blood cell count, inflammation around the lungs, and inflammation around the heart. Fever and low energy may also be present.<sup>[1]</sup> Often, symptoms come on gradually over weeks to months. While the cause of rheumatoid arthritis is not clear, it is believed to involve a combination of genetic and environmental factors. The underlying mechanism involves the body's immune system attacking the joints. This results in inflammation and thickening of the joint capsule. It also affects the underlying bone and cartilage. The diagnosis is made mostly on the basis of a person's signs & symptoms.<sup>[2,3]</sup>

RA affects between 0.5 to 1% of adults in the developed countries and 5 to 50 per 100,000 people newly developing this condition each year. Onset is most frequent during middle age and women are affected 2.5 times as frequently as men.<sup>[4]</sup>

Montelukast is a selective leukotriene receptor antagonist of the cysteinyl leukotriene CysLT 1 receptor. Montelukast is actively used as an anti asthmatic drug in relevance to allergy.

Montelukast are the selective, reversible inhibitors of the cysteinyl leukotriene-1 receptor there by blocking the effects of cysteinyl leukotriene. These cysteinyl leukotriene and LTB<sub>4</sub>, LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub> are products of the 5 lipoxygenase pathways of arachidonic acid metabolism. This drug is approved for the prophylaxis of asthma.<sup>[5,6]</sup>

### Materials and Methods:

Animals – Male albino rats, weighing 100-150 gram of either sex were obtained from P.D.V.V.P.F's college of pharmacy, Ahmednagar. They were kept in light and temperature controlled room with 12 hours light and dark cycle and fed with commercial pelleted feed and water made available freely.

Experimental protocol was approved by IAEC, Vide: Pharmacology/PG/06/2016.

Drug –

1. Complete Freund's adjuvant – It is a suspension of desiccated killed Mycobacterium tuberculosis (10 mg) in paraffin oil (1 ml). - 'Rajesh chemicals, Mumbai'.
2. BSA (Bovine Serum Albumin) – Vijay chemical industries, Mumbai.
3. Montelukast: 5mg, 10 mg, 15 mg (Test Drug) - yarrow chemicals, Mumbai.
4. Prednisolone: 5mg (standard) - yarrow chemicals, Mumbai.

### Experimental design –

The study comprises two experimental models, Adjuvant induced arthritic model and BSA induced arthritis model.

**Table 1. Adjuvant induced arthritic model**<sup>[7,8,9,10,11]</sup>

Group N=6	Treatment with dose/day	Observation parameters
1	Vehicle control	On 8 <sup>th</sup> day
2	Negative control (plain arthritis) CFA in sub-plantar region by s.c route on 1 <sup>st</sup> day	1) Macroscopic assessment
3	Montelukast treated group CFA on 1st day in sub-plantar region by s.c route, 8th day onwards 5 mg/kg B.W. Montelukast p.o	2)Inflammatory assessment On 25th day
4	Montelukast treated group CFA on 1st day in sub-plantar region by s.c route, 8th day onwards 10 mg/kg B.W. Montelukast p.o	1) Macroscopic assessment 2) Inflammatory assessment
5	Montelukast treated group CFA on 1st day in sub-plantar region by s.c route, 8th day onwards 15 mg/kg B.W. Montelukast p.o	3) Histological assessment 4) Biomarkers for evaluation of disease
6	Standard group CFA on 1st day in sub-plantar region by s.c route, 8th day onwards prednisolone 5 mg/kg B.W. p.o	5)X-ray

**Table 2. BSA induced arthritis model**

Group N=6	Treatment with dose/day	Observation parameters
1	Vehicle control	On 8 <sup>th</sup> day
2	Negative control (plain arthritis) BSA by in subplantar region by s.c. route on 1st day	1) Macroscopic assessment
3	Montelukast treated group BSA on 1st day in subplantar region by s.c. route, 8th day booster dose of BSA and 5 mg/kg B.W. Montelukast p.o	2)Inflammatory assessment On 25th day
4	Montelukast treated group BSA on 1st day in subplantar region by s.c. route, 8th day booster dose of BSA and 10 mg/kg B.W. Montelukast p.o	1) Macroscopic assessment 2) Inflammatory assessment
5	Montelukast treated group BSA on 1st day in subplantar region by s.c. route, 8th day booster dose of BSA and 15 mg/kg B.W. Montelukast p.o	3) Histological assessment 4) Biomarkers for evaluation of disease
6	Standard group BSA on 1st day in subplantar region by s.c. route +8th day booster dose of BSA and prednisolone 5 mg/kg B.W. p.o	5)X-ray

**Results : The above five parameters were evaluated for both the model.**

### 1.1 Macroscopic assessment

The inflammation of paw were observed and noted as per criteria. It is measured on visibility of phases of inflammation, like Erythema, Edematous swelling, and Joint rigidity. This is manual observation. It is done on 8th day after induction of CFA to rats.

**Table 3. Macroscopic assessment**

Sr. No.	Group	Group		
		Erythema	Edematous swelling	Joint rigidity
1	Vehicle control	--	--	--
2	Plain Arthritis	✓	✓	✓
3	Montelukast treated (5 mg)	✓	✓	✓
4	Montelukast treated (10 mg)	✓	✓	--
5	Montelukast treated (15 mg)	✓	--	--
	Standard treated (5 mg) (Prednisolone)	✓	--	--

### 1.2 Inflammation assessment

Plethysmometer and vernier caliper were used to measure inflammation.

**Table 4. Inflammation Assessment**

Sr. No.	Group	(Vernier caliper) Paw edema (mm)	(Plethysmo-meter) Paw edema (ml)
1	Vehicle control	1.025	0.18 ± 0.01
2	Plain Arthritis	8.78 ± 0.015 ##	0.885 ± 0.04 ##
3	Montelukast treated (5 mg)	8.39 ± 0.15 N.S.	0.60 ± 0.006 N.S.
4	Montelukast treated (10 mg)	8.01 ± 0.05 *	0.55 ± 0.09 *
5	Montelukast treated (15 mg)	1.51 ± 0.031**	0.22 ± 0.06**
6	Standard treated (5 mg)	1.4 ± 0.61**	1.20 0.03**

### 1.3 Biomarkers (Modified IFCC method using semi-auto analyzer)

The plasma level of following enzymes was done by using semi-autoanalyzer. The plasma was withdrawn from blood by centrifuge at 4000 RPM.

**Table 5. Biomarkers**







Parameters	Vehicle control (I)	Plain Arthritis (II)	Montelukast treated (5 mg) (III)	Montelukast treated (10 mg) (IV)	Montelukast treated (15mg) (V)	Standard prednisolone treated (5 mg) (VI)
ALT	30 ± 0.014	31± 0.010#	30.5± 0.022 N.S.	31.33± 0.076 N.S.	31± 0.025 N.S.	31.28± 0.51 *
AST	96 ± 0.013	181.3± 0.016##	178.8± 0.028 N.S.	177.6± 0.012 *	99.3± 0.030 **	98.1± 0.015 **
ALP	66 ± 0.017	144.8± 0.021##	141± 0.023 N.S.	133.5± 0.011 **	67.1± 0.013 **	67.1± 0.015 **

N= 6, values are expressed as Mean ± SEM. Comparison were made as follows, # p < 0.05, ## p < 0.01 when compared with normal control. \* p < 0.05, \*\* p < 0.01 when compared with negative control. N.S. - Non significant. (values are compared on 25th day by one way ANOVA Dunnett t test)

**1.4 Histopathology (Motic Photomicrograph 40X resolution)**




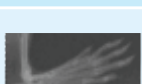
Histopathological study of synovial membrane was done at the end of the study. Paw muscle tissue as well as joints histopathology were done.

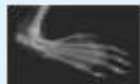

**Table 6. Histopathological assessment**

Sr. No.	Group No.	Histo-pathology	Histological findings
1	Vehicle control		Section from muscle shows no significant pathology
2	Plain Arthritis		Section from muscle shows many inflammatory cells acute as well as chronic with marked edema.
3	Montelukast treated (5 mg)		Section from muscle shows non-significant reduction acute inflammatory cells with edema. No chronic inflammatory cells visualized.
4	Montelukast treated (10 mg)		Section from muscle shows slight reduction acute inflammatory cells with edema. No chronic inflammatory cells visualized.
5	Montelukast treated (15 mg)		Section from muscle shows significant reduction acute inflammatory cells and edema. No chronic inflammatory cells visualized.
6	Standard treated (5 mg)		Section from muscle shows minimal inflammatory infiltrate with no edema.

**2.3 X-ray analysis of joints**




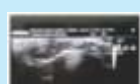

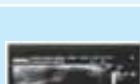
**Table 7. X-ray**

Sr. No.	Group	Radiograph findings	X-ray	Impression
1	Vehicle control	The Inter-tarsal and Meta-tarso-phalyngeal joints are not satisfactorily demonstrated.		Normal legs without deformities
2	Vehicle control	The Inter-tarsal and Meta-tarso-phalyngeal joints are not satisfactorily demonstrated.		Deformities are seen in legs.
3	Montelukast treated (5 mg)	The Inter-tarsal and Meta-tarso-phalyngeal joints are not satisfactorily demonstrated.		No significant reduction in bone deformities is noted.
3	Montelukast treated (5 mg)	The Inter-tarsal and Meta-tarso-phalyngeal joints are not satisfactorily demonstrated.		No significant reduction in bone deformities is noted.

Sr. No.	Group	Radiograph findings	X-ray	Impression
5	Montelukast treated (15 mg)	The Inter-tarsal and Meta-tarso-phalyngeal joints are not satisfactorily demonstrated.		Slight deformities are seen.
6	Standard treated (5 mg)	The Inter-tarsal and Meta-tarso-phalyngeal joints are not satisfactorily demonstrated.		Insignificant deformities are seen.

**2.4 Ultrasound (High Resolution Musculoskeletal Ultrasound)**

**Table 8. Ultrasonography**

Sr. No.	Group	HR-MSK-USG	USG	Impression
1	Vehicle control	The Inter-tarsal and Meta-tarso-phalyngeal joints are well demonstrated. No effusion / synovial thickening / articular cartilage changes seen.		Normal Study
2	Plain Arthritis	The Inter-tarsal and Meta-tarso-phalyngeal joints are well demonstrated. Moderate (++++ effusion and synovial thickening is seen.		Early Changes of Rheumatoid Arthritis.
3	Montelukast treated (5 mg)	The Inter-tarsal and Meta-tarso-phalyngeal joints are well demonstrated. Minimally Reduced effusion (+) and synovial thickening is seen.		Mild Resolution of changes of RA.
4	Montelukast treated (10 mg)	The Inter-tarsal and Meta-tarso-phalyngeal joints are well demonstrated. Minimally Reduced effusion (++) and synovial thickening is seen.		Mild Resolution of changes of RA.
5	Montelukast treated (15 mg)	The Inter-tarsal and Meta-tarso-phalyngeal joints are well demonstrated. Minimally Reduced effusion (+++) and synovial thickening is seen.		Mild Resolution of changes of RA.
6	Standard treated (5mg) (Prednisolone)	The Inter-tarsal and Meta-tarso-phalyngeal joints are well demonstrated. Markedly Reduced effusion (+) and synovial thickening is seen.		Significant Resolution of Changes of RA.

**Discussion :**

Rheumatoid arthritis is an autoimmune disorder, though it can't be cured but progression of disease can be restricted to improve the quality of life. In this study, we investigated the anti-arthritic effect of montelukast using Complete Freund's Adjuvant's Induced Arthritis. Results showed that 15 mg of montelukast significantly

inhibited the inflammation of paws; also it normalized biomarkers enzymes levels, suggesting its possible role in ameliorating.<sup>[12,13,14]</sup> In macroscopic studies montelukast showed dose dependant decrease in inflammation suggesting its possible involvement by inhibiting inflammatory mediators in membrane phospholipids pathway. Profound elevations in serum ALP and AST could have arisen from extra hepatic sources such as acute MI or bone disease and remodeling because ALT levels are almost normal suggesting involvement of bone lesions as arthritis. Histopathology of samples from test drug treated rats confirms that drug restricts progression of disease; because number of acute inflammatory cells is decreased in dose dependant manner where as chronic inflammatory cells are rarely seen in treated rats. The ultrasound result revealed highest dose of montelukast preserved synovial membrane integrity more significantly than prednisolone possibly by inhibiting decrease in hyaluronic acid due to inflammatory fluid.<sup>[15,16]</sup> Which is concurred from x-ray diagnosis of paw of the animals showing decrease in bone deformities but ameliorate progression of bone density as seen in x-rays.

**Conclusion :** Above studies suggest that montelukast as a probable candidate for its use as disease modifying agent in progression of rheumatoid arthritis.

**Acknowledgements :** The authors are thankful to Dr. Sushil Kachewar, Professor and Head of Department of Radiology, P.D.V.V.P.F's Medical College, for his valuable help in radio diagnosis. They wish to extend their gratitude to Dr. S.H. Khaparde, Department of Pathology, P.D.V.V.P.F's Medical College for histopathological studies.

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