Available Online at www.jddt.in

ISSN: 2320 - 4230

Journal of Drug Discovery and Therapeutics 2 (13) 2014, 01-05

RESEARCH ARTICLE

Pharmacological evaluation of 2-chloro substituted thiophene compound for anti-inflammatory activity in rats

[*1]Niroop Revannasiddaiah, [2]Surendra Kumar Gondi, [3]Muruganathan Gopal, [4]Revanna Swamy Ankaiah, [5]Muralidhar Pyati

[1]Assistant professor, Department of pharmacology, PES Institute of Medical Sciences and Research, India.

[2]Medical officer, Chittoor, India.

[3]Assistant Professor, Department of Pharmacognosy, P. E. S College of Pharmacy, Bangalore, India.

[4]Professor, Department of Pharmacology, PES Institute of Medical Sciences and Research, India.

[5] Professor and HOD, Department of Pharmacology, Professor and HOD, Department of pharmacology, India.

Received 30 December 2013; Accepted 03 January 2014

ABSTRACT

Introduction: Inflammation is a phenomenon associated with almost every disease process. It has always been a challenge to halt the inflammatory process with various drugs and to identify a drug with least side effects. In this study, 2-chlorothiophene was evaluated for its anti-inflammatory activity as an attempt in pursuit for a new ideal agent. **Methods:** Wistar albino rats were used in the study. Anti-inflammatory activity for acute phase was assessed by carrageenan induced paw edema model and cotton pellet induced granuloma model for chronic inflammation. **Results:** 2-chloro thiophene showed significant dose dependent anti-inflammatory action. At 20 mg/kg dose, it showed 84.62 % reduction in edema volume in acute phase which was superior to indomethacin. In chronic model the test drug at 20mg/kg showed 27.73% reduction in cotton pellet induced granuloma weight. The results were statistically significant with $P<0.05$. **Conclusions:** 2-chloro thiophene was found to possess significant anti-inflammatory properties for both acute and chronic inflammation and can be a promising anti- inflammatory agent.

Key words: *thiophene, 2-chlorothiophene, anti-inflammatory, carrageenan, cotton pellet.*

INTRODUCTION:

The existence of life also has its own misfortunes along with it, like something called as diseases. Almost all diseases are associated with inflammation. Inflammation is present since the origin of life and has helped in protection and evolution of life. [1] Inflammation is a universal phenomenon of defense process and is a complex process which has evolved from primitive life forms. Inflammation underlies a wide variety of physiological and pathological processes. [2] Inflammation is an essential immune response that enables survival during infection or injury and maintains tissue homeostasis under a variety of noxious conditions. Inflammation comes at the cost of a transient decline in tissue function, which can in turn contribute to the pathogenesis of diseases of altered homeostasis. [3] Hence there is a need of anti-inflammatory drugs to bring inflammation to a halt when it is identified that inflammatory responses are being more destructive than beneficial.

Some of the anti-inflammatory drugs in use are non-steroidal anti-inflammatory drugs (NSAIDs), steroids and newer biological agents such anti-TNF. Among all these drugs, NSAIDs are used extensively as anti-inflammatory, and also as over the counter (OTC) drugs. [4] But the NSAIDs have many side effects. The use of NSAIDs, especially over the long term, presents risk of gastritis, gastrointestinal tract bleeding, hepatotoxicity, nephrotoxicity and cardiovascular risks. [5] There are well-known interactions between NSAIDs of all types and other medications like antiplatelet agents [6] and antihypertensives. [7] Also, other agents like Biological agents such as anti-TNF has been associated with increased risk of infection and leukemia. [8]

Hence in the light of all the above developments in the treatment of inflammation, there is always a search for a

*Corresponding author: Niroop Revannasiddaiah | Email: dr.niroop@gmail.com

better, safe and economical drug which fits into existing body of knowledge of NSAIDs. Thiophene derivatives are newer anti-inflammatory agents being investigated recently.[9] A novel fused thiophene derivative was synthesized and was found to have analgesic activity at 15 to 30 mg/kg.[10] As NSAIDs are analgesic and anti-inflammatory drugs, 2-chloro substituted derivative of thiophene compound was investigated for anti-inflammatory activity in this study.

MATERIALS AND METHODS:

The study compound, 2-chlorothiophene was obtained from PES College of pharmacy, Bangalore, India. The LD_{50} values were estimated to be at more than 2 g/kg for rats which is far greater than the maximum testing dose in the current study. Forty eight male Wistar albino rats (*Rattus norvegicus*) weighing around 150 – 250 g were used. The rats were housed in the central animal house of the Department of Pharmacology, PES institute of medical sciences, Kuppam, under suitable conditions of housing, temperature, ventilation and nutrition. Ethics clearance for experiments on animals was obtained from institutional animal ethics committee (IAEC CERTIFICATE: PES IMSR / Pharma / IAEC / 05 / 2010-11). Female rats, rats weighing less than 150 g and more than 250 g and diseased rats were excluded from the study. The methods employed were carrageenan induced paw edema method for acute inflammation and cotton pellet induced granuloma method for chronic inflammation.

Carrageenan induced rat paw edema method: [11]

Four groups each consisting of six randomly allocated male Wistar rats with an average body weight between 150g to 250g were used. The animals were starved overnight. To ensure uniform hydration, all the rats were given 5 ml of water by stomach tube 30 minutes prior to the test. The individual groups of rats were administered control (tween 80)/ standard (indomethacin 10mg/kg) [12]/ test drug (2-chloro thiophene 10mg/kg and 2-chloro thiophene 20mg/kg) per oral in a fixed volume of 1 ml per rat. Thirty minutes later, the rats were challenged by subcutaneous injection of 0.05ml of 1% solution of carrageenan into the plantar region of right hind paw. The paw was marked at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume was measured plethysmographically immediately after injection i.e. at 0 hour, again at 3, 6 hours and eventually 24 hours after challenge. The increase of paw volume after 3, 6 and 24 hours was calculated as percentage compared with the volume measured immediately after injection of the irritant for each animal. The difference of volumes at the various time intervals gives data for the anti-inflammatory effect and the percentage inhibition was compared between control, standard and test drug groups.

Cotton pellet induced granuloma method: [13]

Four groups consisting of six randomly allocated male Wistar rats in a similar manner as in acute model with an average weight between 150g to 250 g were taken and each rat was anaesthetized with ketamine at a dose of 100mg/kg. **[14]** The groin skin was shaved and disinfected with 70% ethanol. Two incisions were made, one in each side of the groin. By a blunt forceps, subcutaneous tunnels were formed and one sterilized cotton pellet weighing 20mg was placed in each groin region and sutured. The animals were treated for 7 days orally with tween 80, standard drug indomethacin at 3mg/kg [12] and respective test drugs as per respective groups. Then on the 8th day, the animals were sacrificed with overdose of anaesthesia, the pellets along with surrounding granuloma was dissected and dried for 24 hours at 65 degree Celsius in hot air oven until the weight remained constant. The net dry weight i.e. after subtracting the weight of the cotton pellets was determined. The average weight of the pellets of the control group, standard group, as well as of the test group was calculated. The percentage reduction in granuloma weight relative to control group and standard group was determined and the results obtained were evaluated statistically.

Statistical tests:

Statistical test applied was one way ANOVA followed by Tukey's multiple comparison tests for both models using Graphpad prism version 5, computer software. A p value of <0.05 was considered to significant.

RESULTS:

Carrageenan induced rat paw edema method:

Table 1 shows the mean paw volume and edema volume of control, standard and test drug groups. Table 2 shows the percentage reduction in edema volume of each drug groups. The standard drug indomethacin at 10mg/kg showed 20%, 57.69% and 61.54% reduction in edema volumes at 3, 6 and 24 hours respectively. The test drug, 2-chlorothiophene showed a dose and time dependent reduction in edema. 2-chlorothiophene at 10mg/kg showed 15%, 53.85% and 69.23% reduction in edema volume at 3, 6 and 24 hours respectively. 2-chlorothiophene at 20mg/kg showed 40%, 69.23% and 84.62% reduction in edema volume at 3, 6 and 24 hours respectively. Figure 1 shows that 2-chlorothiophene at 20mg/kg resulted in highest control of edema than other drug groups; whereas 2-chlorothiophene at 10mg/kg was similar to standard.

Table 1: Mean paw volume in ml and edema volume in ml of control, standard, 2-chlorothiophene 10mg/kg and 2-chlorothiophene 20mg/kg groups at 0, 3, 6 and 24 hours.

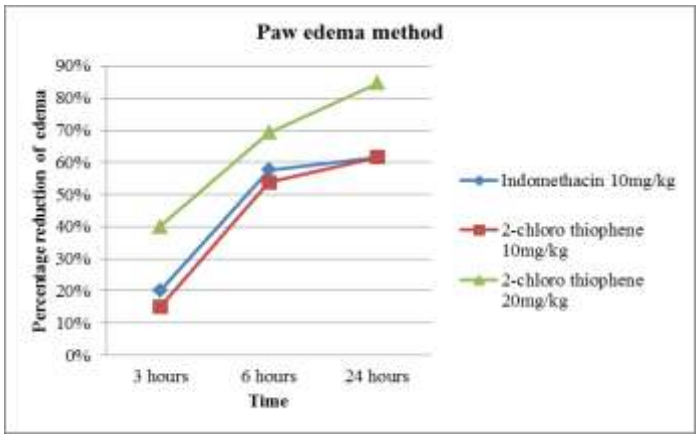
Group	Mean Paw volume at 0 hours (ml)	Edema volume at 0 hours	Mean Paw volume at 3 hours	Edema volume at 3 hours	Mean Paw volume at 6 hours	Edema volume at 6 hours	Mean Paw volume at 24 hours	Edema volume at 24 hours
Control Tween- 80	0.55 ±0.02*	-	0.75±0.03*	**0.2**	0.81±0.03*	**0.26**	0.68±0.01*	**0.13**
Standard Indomethacin 10mg/kg	0.76±0.04*	-	0.92±0.06*	**0.16**	0.87±0.04*	**0.11**	0.81±0.05*	**0.05**
2-chlorothiophene 10mg/kg	0.34±0.03*	-	0.51±0.07*	**0.17**	0.46±0.05*	**0.12**	0.39±0.03*	**0.05**
2-chlorothiophene 20mg/kg	0.42±0.03*	-	0.54±0.04*	**0.12**	0.50±0.04*	**0.08**	0.44±0.04*	**0.02**

* P<0.001

Table 2: Percentage reduction in edema at 3, 6 and 24 hours in standard, 2-chlorothiophene 10mg/kg and 2-chlorothiophene 20mg/kg groups.

Time	Standard (indomethacin 10mg/kg)	2chloro thiophene 10mg/kg	2chloro thiophene 20mg/kg
at 3 hours	20%	15%	40%
at 6 hours	57.69%	53.85%	69.23%
at 24 hours	61.54%	61.54%	84.62%

Figure 1: Line graph showing percentage reduction of edema in different groups.



Chronic model: Cotton pellet induced granuloma method:

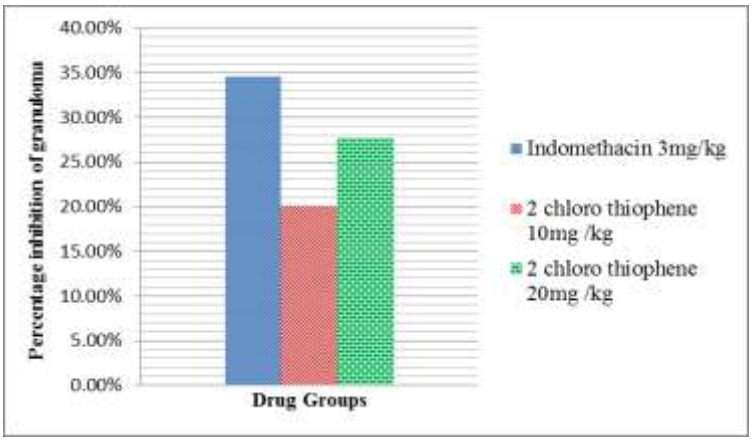
As shown in table 3, the weight of cotton pellet granuloma was highest in control group with 107 ± 2.66 mg. Indomethacin treated group showed least granuloma weight of 70 ± 1.88 mg. The test drug, 2-chlorothiophene at 20mg/kg showed 77.33 ± 3.69 mg and 2-chlorothiophene at 10mg/kg showed 85.52 ± 3.42 mg. Indomethacin at 3mg/kg showed maximum percentage reduction in cotton pellet granuloma weight of 34.58% whereas the test drug, 2-chlorothiophene at 10mg/kg showed 20.07% reduction and 2-chlorothiophene at 20mg/kg showed 27.73% reduction. Figure 2 depicts the values on a bar graph.

Table 3: Mean dry weight and percentage reduction in cotton pellet granuloma in control, standard and test drug groups in all groups.

Group	Mean dry weight ± S.D (in mg)	Percentage reduction in granuloma weight
Control	107.0 ± 2.66*	-
Standard	70.00 ± 1.88*	34.58 %
2 chloro thiophene 10mg /kg	85.52 ± 3.42*	20.07 %
2 chloro thiophene 20mg /kg	77.33 ± 3.69*	27.73 %

*P<0.001

Figure2: Bar chart showing percentage reduction in standard, 2-chloro thiophene 10mg/kg and 20mg/kg groups.



DISCUSSION:

The most widely used tests for screening anti-inflammatory compounds are carrageenan induced paw edema method [15] and cotton pellet induced granuloma method. Previous studies have also shown that thiophene derivatives have anti-inflammatory action. [9, 16] Hence, 2-chlorothiophene was evaluated for anti-inflammatory activity. In carrageenan induced edema model, anti-edematous effect of drugs is evaluated. Occurrence of edema secondary to carrageenan injection serves as an index of acute inflammatory changes and can be determined from differences in the paw volume measured immediately, at 3 , 6 and 24 hours. In cotton pellet induced granuloma model, the dry weight of cotton granuloma indicates the amount of granulomatous tissue developed due to chronic inflammation. A drug with anti-inflammatory properties is expected to reduce the granulomatous tissue.

In carrageenan induced edema model, 2-chloro thiophene at 20 mg/kg has shown maximum percentage inhibition of 40 % at 3 hours, 69.23 % at 6 hours and 84.62 % at 24 hours compared to indomethacin at all three readings. 2-chloro thiophene at 10 mg/kg has shown lesser percentage inhibition than indomethacin at 3 and 6 hours but has shown equal response as that of indomethacin at 24 hours which is 61.54 %. So, the observations indicated that 2-chloro thiophene at 20 mg/kg dose action was superior and had started earlier compared to indomethacin and 2-chloro thiophene at 10 mg/kg.

In cotton pellet method, Indomethacin at 3 mg/kg dose has shown maximum response of 34.58 % inhibition of granuloma weight when compared to 2-chlorothiophene at 10 mg/kg which has shown 20.07 % inhibition and 2-chlorothiophene at 20 mg/kg which has shown 27.73 % inhibition. Among the test drugs, 2-chlorothiophene at 20 mg/kg has shown greater granuloma inhibitory response than 2-chlorothiophene at 10 mg/kg. Hence the comparative study indicates that indomethacin at 3 mg/kg was more effective in chronic inflammation when compared to test drugs. However, 2-chlorothiophene at 20 mg/kg has shown 27.73 % granuloma inhibition which is comparable to indomethacin.

CONCLUSION:

In carrageenan induced paw edema method, the test drug 2-chlorothiophene, has inhibited the edema induced by carrageenan in rats significantly in a dose of 20mg/kg

and to a greater extent when compared to indomethacin at 10 mg/kg. In cotton pellet granuloma method, though 2-chlorothiophene at 20mg/kg has produced good anti-inflammatory effect, it was lesser when compared to the indomethacin at 3mg/kg. At the end of the study it is concluded that the test drug, 2-chloro thiophene which was investigated, does possess significant anti-inflammatory activity.

LIMITATIONS:

In acute model, the study could have been further conducted beyond 24 hours to further follow the duration of anti-inflammatory responses of all drugs. In chronic model, the test drugs were given as a single daily dose. Perhaps, more frequent dosing would have further shown more effective results. The adverse effects of the drugs must have been included for the study. Further comprehensive studies must be carried out in different species of animals to evaluate pharmacokinetic and pharmacodynamic properties of the test drug.

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