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Analgesic effect of Thiozolyl Thiourea Sydnones in albino rats

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Abstract

Pain is a subjective experience; it is a direct response to an untoward event associated with injury and inflammation. Sydnone derivatives have been synthesized with biological interest and reported to possess a wide spectrum of biological and pharmacological activities like antimicrobial, anti-inflammatory, anti-pyretic, antitumor, antiarthritic and antioxidant properties. Rat tail flick method was adopted to study the analgesic activity of various test compounds. Aspirin as standard drug was used for comparison of analgesic activity. The animals were tested for the tail flick response at 30, 60 and 90 minutes after drug administration. A cut off time of 8 seconds was fixed to avoid injury to the animal. Mesoionic compound no's 3, 6, 8 and 9 were evaluated for analgesic activity. The percentage protection for mesoionic test compound no's 3, 6, 8 and 9 were 50, 0, 0 & 33.33 % in comparison to 83.33 % in aspirin group. In addition these mesoionic test compounds were also evaluated for ulcerogenic potential. Mesoionic test compound no's 8 and 9 showed far lesser gastric damage compared to standard drug phenylbutazone.

Keywords: Analgesic, Sydnones, Aspirin, Ulcerogenic, Phenylbutazone

Introduction

Pain is a subjective experience; it is a direct response to an untoward event associated with injury and inflammation.[1] Mediators of pain are Kinnins –bradykinin , kallikrein,prostaglandins ,histamine, lactic acid .Substance p, neurokinin A (NKA), Neurokinin B (NKB) and endogenous tachykinin released in sensory nerve endings cause pain sensation.[2]Pain is of two types: Nociceptive pain arises from stimulation of peripheral pain receptors; neuritic pain is due to inflammation or damage to neural structures.[3]

Drugs used in analgesia[4] are opioid analgesics like morphine, pethidine, fentanyl, NSAIDS: nonselective COX inhibitors, preferential COX 2 inhibitors, selective COX 2 inhibitors, tramadol-a metabolite of antidepressant trazodone, Tricyclic antidepressants-imipramine, amitryptyline, antiepileptics-carbamazepine, gabapentin, dissociative anesthetics – ketamine and local anesthetics- lignoaine also have analgesic activity[5]

They cause analgesia by local mechanisms by decreasing sensation of peripheral nerve endings in response to painful stimulus and inflammation.[1,2] They also have central sub cortical action by raising threshold to pain perception in the CNS.

They have action at supraspinal sites in medulla, midbrain, limbic system and cortical areas and may alter processing and interpretation of pain impulses and send inhibitory impulses through descending pathways to the spinal cord.[1.3]

Mesoionic compounds are dipolar five or six membered heterocyclic compounds in which both the negative and the positives charge are delocalized.[6,7] The most important member of the mesoionic category of compounds is the sydnone ring system. Sydnones[7] are mesoionic compounds having the 1, 2, 3-oxadiazole skeleton bearing an oxygen atom attached to the fifth position. Sydnones are dipolar, pseudo-aromatic heterocycles with a unique variation in electron density around the ring. These characteristics have encouraged extensive study of the chemical, physical, and biological properties of sydnones, as well as their potential applications.[8,9] A large number of sydnone derivatives have been synthesized with biological interest and reported to possess a wide spectrum of biological and pharmacological activities like antimicrobial, anti-inflammatory, analgesic, anti-pyretic, antitumor, antiarthritic and antioxidant properties.[10,11]

Following are some meso-ionic systems having various types of biological activities.[12]

1, 2, 3, 4-oxatriazole-5 amines-Hypotensive agents
1, 2, 3, 4-oxatriazole-5 ones-Hypotensive agents
1, 3 – diazole-4 ones-Antihelminthic
1, 3 –Thiazolo -4 ones-CNS stimulation and anti-inflammatory
1, 3 –thiazol-4 amines-anti inflammatory and CNS stimulation
1, 2, 3 –triazole-4-ones-herbicidal
1, 3, 4-triadiazole-2 amines-sedative
1, 3, 4-thiadiazole-2-thiones-antibacterial

Amongst various N-substituted phenyl sydnones, N-tolylydnones have shown mild analgesic activity besides their marked CNS depression activity.

Animal models for analgesic activity are as follows[13]

Heffner's tail clip method: In this method mechanical stimulus is applied, an artery clip is placed at the root of the tail to apply noxious stimulus in mice. Hot plate method: Animals are placed on the hot plate, which is an electrically heated surface, mice respond by jumping or withdrawal of paws. Radiant heat method: A light beam is focused to the proximal third of the tail, mice tries to pull tail away. Tail warm water immersion method: The distal portion of the tail is immersed in a cup filled with warm water; tail withdrawal reflex of rats is seen. Tooth pulp electrical stimulation method: Rabbits tooth pulp chambers are exposed close to the two front upper incisors, Clamping electrodes are placed into the drilled holes, electrical stimulus is applied, and animals start licking the front tooth pulp.

Materials and methods

Rat tail flick was adopted to study the analgesic activity of test compounds

Chemicals: synthetic Thiazolo thiourea substituted sydnones, carboxymethyl cellulose, gum acacia, aspirin. Aspirin a standard drug was used for comparison of analgesic activity. They were administered orally in the dose of 100mg/kg body weight as fine suspension in gum acacia

Equipments: Dissection set, wooden mouth gag, syringes, skin suturing set, magnifying lens, Analgesiometer (techno).

Sr. no.	Name of compound
1	4(4'(3-P-Phenyl Sydnonyl)Thiozolo-2-Aminophenyl Thiourea
2	4(4'(3-P-Phenyl Sydnonyl)Thiozolo-2-Amino P.Chlorophenyl Thiourea
3	4(4'(3-P-Phenyl Sydnonyl) Thiozolo-2- Amino P. Bromophenyl Thiourea
4	4(4'(3-P-Phenyl Sydnonyl)Thiozolo-2-Amino Benzyl Thiourea
5	4(4'(3-P-Chlorophenyl Sydnonyl)Thiozolo-2-Amino Phenyl Thiourea
6	4(4'(3-P-Toly Sydnonyl)Thiozolo-2-Amino P.Chlorophenyl Thiourea
7	4(4'(3-P-Toly Sydnonyl)Thiozolo-2-Amino -Benzoyl Thiourea
8	4(4'(3-P-Chlorophenyl Sydnonyl)Thiozolo-2-Amino P.Chlorophenyl Thiourea
9	4(4'(3-P-Chlorophenyl Sydnonyl)Thiozolo-2-Amino P.Bromophenyl Thiourea
10	4(4'-(3-P-Chlorophenyl Sydnonyl)Thiozolo-2-Aminoallyl Thiourea

Study of analgesic activity

The method was adopted by D'Amour and Smith[14].which was modified by Davis et al[15] and standardized by U K Seth et al[16]. Analgesiometer (techno) was used in this method. 60 albino rats of either sex weighing between 100-150 g were chosen. The rat was placed in a metal box through which its tail protruded out and could flick its tail. The rate of inflow of water into the water jacket of Analgesiometer was so adjusted that when the wire was heated the water at the inlet and outlet of water jacket registered the same temperature. The temperature of the wire is so adjusted that the animal reacts in 5 seconds. This is the reaction time for the animal. In other words, it is the time interval between the switching on the heating of wire and the flicking of animals tail. The tail of the rat was placed over the nichrome wire. The rat was allowed some time to settle down. Both stop watch and heating switch were switched on simultaneously. When the animal flicked its tail the stop watch was stopped and current was switched off. The time in the stop watch read the reaction time of the animal. Only those rats which responded within 5 seconds were selected, hence 36 rats were selected. These animals were fasted for 18 hrs and divided into six groups of six each. The first five groups received orally mesoionic compounds No's 3, 6, 8, 9 and aspirin respectively in the dose of 100mg/kg body weight in 1ml of 4% gum acacia and the sixth group received only 1 ml of 4% gum acacias. We were not able to test the analgesic activity of the other test compounds due to lack of sufficient amount of compounds. The animals were tested for the tail flick response at 30, 60 and 90 minutes after drug administration. A cut off time of 8 seconds was fixed to avoid injury to the animal. The results are shown in the table no I and II.

Investigation of the ulcerogenic potential of the new compounds

Gastrointestinal and renal effects are the most common side effects produced by the presently available non steroidal anti-inflammatory drugs. Hence it was thought appropriate to study at least the gastrointestinal effects of the new compounds that were screened.

The method adopted was essentially that of Waltz D et al[17]. Twenty four albino rats of either sex weighing 100-150 gms were selected for the study .The rats were fasted for 18 hrs before the drug administration. They were divided into four groups of six each. First three groups received orally compound no's 8, 9 and phenylbutazone respectively in the dose of 100mg/kg body weight in 1ml of 4% gum acacia. Fourth group served as control. The rats were not allowed food but water was allowed at their liberty. Eight hours after the drug administration they were sacrificed. Their stomach opened along the greater curvature and examined for hemorrhages with the help of hand lens. The severity was graded as under.

0 - No lesions

1+ One or two linear small hemorrhages (streaks) or pin point hemorrhages
2+ One or two wider hemorrhagic areas (small tablet size) or great number of linear hemorrhages (more than two)
3+ More than two wider hemorrhagic areas
4+ Wider hemorrhagic areas with clots of blood
5+ Hemorrhagic areas with clots of blood with perforations

Results

As various NSAIDS show considerable analgesic activities, besides their anti-inflammatory property. Hence we evaluated some mesoionic test compounds from this series for their analgesic activity by using rat tail flick test. Techno analgesiometer was used for this test, where in a nichrome wire was heated and the heat radiation caused the rat to flick its tail. Generally animals showing reaction time greater than 8 seconds were considered having positive and complete analgesia. Compound no's 3, 6, 8 and 9 were taken for this test. We were not able to test the analgesic activity of the other compounds due to lack of sufficient amount of compounds. The results are shown in table I & II below. The percentage protection for mesoionic test compound no's 3, 6, 8 and 9 were 50, 0, 0 & 33.33 % in comparison to 83.33 % in aspirin group. In addition these mesoionic test compounds were also evaluated for ulcerogenic potential. Mesoionic test compound no's 3 and 9 showed far lesser gastric damage compared to standard drug phenylbutazone. The results are shown in table III below.

TABLE I: Showing the rat tail flick results. Reaction time in seconds after drug administration (30,60,90 minutes)

	Control			Aspirin			Compound 3			Compound 6			Compound 8			Compound 9		
Rat no	**30 min**	**60 min**	**90 min**	**30 min**	**60 min**	**90 min**	**30 min**	**60 min**	**90 min**	**30 min**	**60 min**	**90 min**	**30 min**	**60 min**	**90 min**	**30 min**	**60 min**	**90 min**
1	4	4	4	6	6.5	8	7	8	8	5	5.5	6	6	6	6	7.5	7.5	7
2	5	4	5	7.5	8	8	5.5	6	6	6	6.5	5.5	5	5.5	5	8	7.5	7.5
3	5	5	5	8	8	8	6.5	6.5	6.5	5	5.5	5.5	6	5.5	5.5	7	7	8
4	5	5	5	6.5	7	7.5	7	7.5	8	5	5.5	6	6.5	6.5	6	6.5	7	8
5	4	5	5	7	7.5	8	7.5	8	8	5.5	5.5	6	7.5	7	7	5	5.5	5.5
6	4	5	5	8	7.5	8	7	7.5	7.5	5	5	6	6.5	7	7.5	7	6.5	6.5
Mean	4.5	4.66	4.83	7.16	7.41	7.91	6.75	7.25	7.33	5.25	5.58	5.83	6.25	6.25	6.16	6.83	6.83	7.83

TABLE II: Percentage protection of the rat tail flick test

Drug	Rats used per group	Rats showing positive analgesia	Percentage protection
Aspirin	6	5	83.33
Compound no 3	6	3	50.00
Compound no 6	6	0	00.00
Compound no 8	6	0	00.00
Compound no 9	6	2	33.33

TABLE III: Showing results of ulcerogenic potential test

	Grade of injury			
Rat no	**Control**	**Phenyl butazone**	**Compound no 3**	**Compound no 9**
1	0	+++	++	0
2	0	++	+	0
3	0	+++	++	+
4	0	++	0	0
5	0	+++	++	++
6	0	+++	+	+

Discussion

Compounds 3, 6, 8 and 9 were chosen for evaluation of analgesic activity employing rat tail flick test. Compound no 3 showed maximum protection of 50%.This compound has phenyl substitution at position 3 of sydnone ring and P-bromophenyl on thiourea moiety. Compound 9 has exhibited 33.33% protection, having chlorophenyl group at position 3 of the sydnone ring and P-bromophenyl on thiourea. Compound 6 and 8 have shown zero analgesic activity, having P-methyl phenyl and P-chlorophenyl at position 3 of the sydnone ring respectively. P-chlorophenyl is substituted on thiourea moiety in both compounds.

From the above results –apparently bromine substitution on phenyl group attached to thiourea seems to enhance analgesic activity(compound 3 with 50% protection).Additional substitution of chlorine on phenyl ring attached to position 3 of sydnone ring reduces this activity to 33.33%(compound 9). Other substitutions as evidenced in compounds 6 and 8 had no analgesic activity. As bromine substitutions at the above stated positions enhances analgesic activity it would be interesting to check the analgesic activity of the compound with bromine substitutions at both positions.i.e., on phenyl ring attached to position 3 of sydnone and on thiourea moiety.

Summary and conclusion

The present study was undertaken to find out whether newly synthesized mesoionic compounds substituted thiazolyl thiourea sydnones possessed analgesic activity. The analgesic activity was looked for in compound 3, 6, 8 and 9.The gastric mucosal damage produced by these compounds has been evaluated in comparison with the standard drug phenylbutazone. Compound no 3 and 9 which showed marked analgesic activity showed far lesser gastric damage compared to standard drug phenylbutazone in acute models. A significant degree of analgesic activity has been demonstrated with these substituted thiazolyl thiourea sydnones at the dose of 100mg/kg body weight by oral route. The possibility of making further minor changes on these molecules with the yield of higher analgesic activity is an exciting possibility to be explored

References

1. Cotton RS ,Kumar V and Robbins Sl. Robbins pathologic basis of disease.5th edn Philadelphia B Sauderers company ,1994.p.53-4.
2. Ritchie A C. Boyd's textbook of pathology, Canada, edn 9, USA; lea and feberger, 1990:p.60-88, 1044.
3. Dey NC, Debashish S, Pey PK edn. Textbook of pathology; New central book agency ltd,19995:p.6.1-6.59.
4. Satoskar RS, Bhandarkar SD, Ainapur SS, Angiotensin, Kinins, Leukotrienes prostaglandins and cutokiones, 18th edn. Bombay India; Popular Prakashan pvt. Ltd. 2003 p 333-9.
5. Tripathi KD. Essentials of medical pharmacology, 5th edn .prostaglandin Leukotrienes, jaypee pub Pvt Ltd, 2004 P 156-166.
6. IUPAC Compendium of Chemical Terminology 2nd Edition, 1995, 67, 1370.
7. K Satyanarayana; SR Deshpande; B Subbarao; MNA Rao, Indian J. Pharm. Sci. 2004,(66),679.
8. CS Dunkley; CJ Thoman, Synthesis and Biological Bioorg. Med. Chem. Lett. 2003,(13),2899.
9. Baker W, Ollis WD, Poole VD. Cyclic meso-ionic compounds. Part 1, the structure of sydnones and related compounds. J. chem. soc.307,1949.
10. Wagner H, Hill JB. J Of Med.Chem.17:p1337; 1974.
11. M.-H. Shih and F.-Y.Ke, Bioorg. Med. Chem., 12, 4633 (2004).

12. C.V. Yelamaggad, M .Mathews, U.S. Hiremath, D.S.S. Rao And S.K. Prasad, Tetrahedron Lett., 46, 2623 ;2005.
13. Turner R A. Screening methods in pharmacology, Newyork and London. Academic press inc., 1965; p.323
14. Amour D, Smith DL. A method for determining the loss of pain sensation. J. pharmacol. Exp. Ther. 72 p.74-79; 1941.
15. Davies OL, Revantoo SJ, Walpole AL. A method for the evaluation of analgesic activity using rat. Br. J. pharm.1; p.255-264; 1946.
16. Seth UK, Dhadkar NK, Kamat UG. Selected topics in experimental pharmacology, Kothari book depot, Mumbai.1st edn p.156; 1972.
17. Walz DT, Dimartino MJ, Griffin CL, Misher A. Investigation of Carrageenan induced rat paw oedema assay and co-relation between anti-inflammatory activity and gastric hemorrhage production in the rat. Arch Ind Pharmacol. Dyn ,185, p.337-343;1970.