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Anti-Inflammatory Activity of Hydroxytriazenes and their Vanadium Complexes

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Abstract: The anti-inflammatory activity of hydroxytriazenes as well as their vanadium complexes has been studied using carrageenan induced hind paw oedema method in albino rats (Wister strain). Hydroxytriazenes *viz.*, 3-hydroxy-1,3-diphenyltriazene (HT-1), 3-hydroxy-3-phenyl-1-(4-sulfonamido)phenyl triazene (HT-2), 3-hydroxy-3-*p*-chlorophenyl-1-(4-sulfonamido) phenyltriazene (HT-3), 3-hydroxy-3-*m*-chlorophenyl-1-(4-sulfonamido)phenyltriazene (HT-4) and their respective vanadium complexes C-1, C-2, C-3 and C-4 have been synthesized using standard methods, purified, characterized and used for studying their anti-inflammatory activities. The hind paw oedema was produced by subplanter injection of carrageenan and the paw volume was measured plethysmographically after 0.5, 1, 2, 3 and 5h. The animals were given HT-1, HT-2, HT-3 and HT-4 and also vanadium complexes C-1, C-2, C-3 and C-4 dissolved in DMSO (at dose 5 mg/Kg body weight). Diclofenac sodium (5 mg/kg) was used as a standard drug. The standard drug shows maximum inibition up to 1h as 81.73% which goes on increasing up to 3h (88.94%) but further reduces to 74.93% at the end of 5h. The test compounds *i.e.* both ligands as well as their metal complexes show maximum percent inhibition only up to 1h. after which the efficacy reduces. Thus it can be said that both ligands as well as their vanadium complexes show very significant anti-inflammatory activity up to 1h which is comparable to standard drug.

Keywords: Anti-inflammatory activity, Hydroxytriazenes, Vanadium complexes.

Introduction

Hydroxytriazenes are a useful group of chelating agents. Their analytical utility in the spectrophotometric determination of both transition and non-transition metal ions is well established, as revealed by appearance of eight reviews[1-8] during last few years. Apart from the reference of Gublar[9], some attempts have been made to study biological activity of

hydroxytriazenes in our laboratory[10]. In the present investigation four hydroxytriazenes as well as their respective vanadium complexes have been synthesized and screened for their anti-inflammatory activity on the basis of PASS (Prediction of biological activity spectra for substances) which indicated good theoretical possibility of anti-inflammatory activity in these compounds.

Experimental

Synthesis of hydroxytriazenes

All the four hydroxytriazenes were synthesized as per standard method[11]. The general method is described below. Except for the difference in substituent, synthesis incorporated the same experimental conditions. The synthesis was done in three steps.

1. ***Preparation of arylhydroxylamine:*** In the preparation of aryl hydroxylamine, 0.2 moles of nitro aryl compound, 30 g of NH_4Cl and 250 mL of water were mixed and stirred mechanically at 40°C and then 40 g of Zn dust was added in the small lots such that the temperature of the reaction remained between 45-60°C. The reaction mixture was filtered, washed with ice cold water and the solution obtained was kept in refrigerator at about 0°C which was used for coupling.
2. ***Preparation of aryldiazonium salts***: Aryl amine (0.2 moles) was dissolved in mixture containing 50 mL of HCl and 50 mL of water. In other beaker 0.2 moles sodium nitrite was dissolved in minimum quantity of water. The temperature of the aryl amine hydrochloride solution was maintained between 0-5°C. To this solution, sodium nitrite solution was added drop by drop with stirring. The diazotized product so obtained was directly used for coupling.
3. ***Coupling***: The temperature of aryl hydroxylamine prepared in step-1 and diazotised product obtained from step-2 was maintained between 0-5°C. Step-2 solution was added drop-by-drop to solution obtained in step 1 and pH of solution was maintained between 5 and 6 by adding sodium acetate buffer. The resultant product was filtered, washed with cold water and dried. The crude compounds were purified and recrystallized. The purity of each hydroxytriazene was checked by I.R. studies and physical characteristics such as M.P. crystal shape *etc*. Their compositions were verified by elemental analysis. All these data have been given in Table 1.
4. ***Preparation of vanadium complexes*** : On the basis of solution and molar composition studies it was found that vanadium forms 1:1 complex with hydroxytriazenes in the pH range 5.8 - 8.5. Thus all the complexes were prepared using appropriate ratios and reaction conditions. The complexes were duly characterized by CHN analysis and IR spectral studies before screening for anti-inflammatory activity. The physical characteristics have been incorporated in table-1 along with CHN data.

Animals

Experiments were performed on albino rats of either sex (Wister strain) weighing (150-175 g). They were given standard laboratory diet and water ad *Libitum*.

Anti-inflammatory activity

Carrageenan induced paw oedema: Anti-inflammatory activity was determined by paw oedema method in rats described[12] by Winter *et al*., (1962). The rats were divided into ten group (n=6). Group I was served as control (saline). Group II was given diclofenac sodium orally (5 mg/kg) as a standard drug. Groups III-VI were administered hydroxytriazene compounds (HT-1, HT-2, HT-3, HT-4) and groups VII-X were administered their respective vanadium complexes (C-1, C-2, C-3, C-4), 5 mg/kg orally.

Table 1. Physical characteristics data of the test compounds.

Compound	Name	Elemental analysis				M.P. °C	Characteristic I.R. bands, cm^{-1}
			% C	% H	% N		
HT-1	3-hydroxy-1,3-diphenyltriazene	Th	67.60	5.16	19.72	119	ν OH = 3480
		Ex	67.60	5.18	18.70		ν NH - 3190
HT-2	3-hydroxy-3-phenyl-1-(4-sulfonamido)phenyltriazene	Th	49.28	4.13	19.17	170	ν OH = 3415 (s)
		Ex	47.29	5.34	19.63		ν NH = 3250 (b)
HT-3	3-hydroxy-3-*m*-chlorophenyl-1-(4-sulfonamido)phenyl-triazene	Th	44.10	3.36	17.15	150	ν OH = 3580
		Ex	44.98	3.30	16.30		ν NH = 3230
HT-4	3-hydroxy-3-p-chlorophenyl-1-(4-sulfonamido) phenyltriazene	Th.	44.10	3.36	17.15	161	ν OH = 3588
		Ex	42.88	3.00	16.36		ν NH - 3290
C-1	$VO(C_{12}H_{11}N_3O)_2$	Th	58.66	4.10	17.10	**	*
		Ex	58.62	4.09	17.00		
C-2	$VO(C_{12}H_{11}N_4O_3S)_2$	Th	44.28	3.68	17.20	**	*
		Ex	43.68	3.14	16.23		
C-3	$VO(C_{12}H_{11}N_4O_3SCl)_2$	Th	40.00	3.05	15.55	**	*
		Ex	40.11	3.82	14.56		
C-4	$VO(C_{12}H_{11}N_4O_3SCl)_2$	Th.	40.00	3.05	15.55	**	*
		Ex	39.00	14.06	14.98		

**The characteristic peaks in complex disappeared at the desired positions showing complex formation. ** No sharp melting points.*

The paw volume was measured by plethysmographic method at (0.5, 1, 2, 3, 5h), after the sub planter injection of 0.1 mL of 1% freshly prepared suspension of carrageenan (Sigma Chemical Co.). Drug pretreatment was given 1h before the injection of carrageenan. Mean increase in paw volume was measured, expressed as mean $\pm$SEM and % inhibition was calculated. The values have been incorporated in Table 2.

Results and Discussion

Perusal of Table 2 shows that all the four hydroxytriazenes and their vanadium complexes efficiently act to inhibit inflammation. The results are comparable to the standard drug diclofenac sodium. The distinct feature of diclofenac sodium is that effect from 0.5 to 5h has regular trend and value decreases to 74.93% at 5h, ranging from 75 to 89%. Thus average efficiency of the standard drug is more than 75%. The test compounds *i.e.* hydroxytriazenes in general show maximum inhibition percentage at about 1h (except HT-2 which shows maximum value at 0.5h) and C-4 which also show maximum value at 0.5h). With time after 1h it goes on reducing and minimum at about 5h.

Basically inflammation is a protective response to cell injuries in animals. It is manifested in the form of common clinical signs such as erythema, oedema, hyperalgesia, pain and loss of function at macroscopic level. To study the anti-inflammatory activity of any compound the suppression of these signs is observed in laboratory animals. This includes

using three models *viz*. acute, sub-acute and chronic. In the present study carageenan induced acute inflammation model has been used for studies. The results have been compared with standard drug diclofenac sodium which is categorized as a NSAID (Non-steroid anti-inflammatory drug) this category of drug acts at the periphery and not at CNS. Acting at the site of tissue injury these drugs block the synthesis of ecosanoids, finally blocking the cycloxygenase (COX) pathway.

Table 2. Effect of hydroxytriazenes and their vanadium complexes on carrageenan induced rat paw edema**.**

Groups (n=6)	Dose Unit/Kg	Edema volume after				
		0.5h	1h	2h	3h	5h
Control (Saline)	5 mL	2.85 ± 0.24	6.36 ± 0.96	7.30 ± 0.72	8.00 ± 0.84	7.36 ± 0.89
Control DMSO (Vehicle)	5 mL	2.83 ± 0.13 (0.70)	6.34 ± 0.41 (0.15)	7.36 ± 0.35 (0.82)	8.18 ± 0.39 (2.25)	7.38 ± 0.58 (0.27)
Diclofenac Sodium	5 mg	0.61 ± 0.20** (78.44)	1.16 ± 0.37** (81.73)	1.30 ± 0.40** (82.34)	0.90 ± 0.40** (88.94)	1.85 ± 0.46** (74.93)
HT-1	5 mg	1.28 ± 0.29** (54.77)	3.12 ± 0.38** (50.87)	6.26 ± 0.67^{NS} (14.95)	7.23 ± 0.49 ^{NS} (11.61)	7.26 ± 0.62 ^{NS} (0.02)
HT-2	5 mg	0.25 ± 0.14** (91.17)	0.51 ± 0.34** (91.95)	1.91 ± 0.34** (74.04)	2.71 ± 0.45** (66.87)	4.50 ± 0.48** (39.03)
HT-3	5 mg	1.60 ± 0.23** (43.46)	2.38 ± 0.39** (62.51)	2.98 ± 0.50** (59.51)	3.60 ± 0.43** (55.99)	3.83 ± 0.60 (38.34)
HT-4	5 mg	2.21 ± 0.20** (21.91)	2.71 ± 0.42** (57.32)	3.33 ± 0.34** (54.75)	4.48 ± 0.49** (45.23)	4.55 ± 0.51** (38.34)
C-1	5 mg	1.21 ± 0.42 (57.03)	2.52 ± 0.49 (60.22)	4.44 ± 0.59 (39.67)	6.03 ± 0.05 (26.24)	6.23 ± 1.16 (15.58)
C-2	5 mg	0.73 ± 0.28 (74.09)	1.35 ± 0.38 (78.74)	2.00 ± 0.46 (72.82)	2.50 ± 0.42 (69.43)	3.60 ± 0.45 (51.21)
C-3	5 mg	1.19 ± 0.15 (57.84)	2.19 ± 0.44 (65.46)	2.66 ± 0.52 (63.86)	3.16 ± 0.57 (61.30)	3.22 ± 0.16 (56.37)
C-4	5 mg	0.41 ± 0.23 (85.30)	1.35 ± 0.44 (78.74)	1.76 ± 0.34 (76.01)	2.20 ± 0.46 (73.10)	2.41 ± 0.48 (67.26)

*Each value is the mean ± SEM of 6 rats. Figure in parentheses indicates the % anti-inflammatory activity. *p < 0.05; **p < 0.001 compared to control. NS : Statistically not significant*

Both ligands as well as vanadium complexes are showing moderate to very good anti-inflammatory activity up to 1h, which goes on reducing with the time. The probable mechanism of action of carageenan induced edema is bi-phasic; the first phase is attributed to release of histamine -HT, kinins in the first hour while the second phase is attributed to

the release of prostaglandin like substance in 2-3h. The activity of hydroxytriazenes is structure dependent and is excellent in inhibiting carageenan induced edema.

DMSO has no anti-inflammatory activity. The parent compound HT-1 has little activity. The substitution in benzene ring at 1 position by sulphonomide group enhances anti-inflammatory activity significantly. It can be observed that HT-2 has better activity compared to standard drug at 0.5h. Further complex C-4 also has activity greater than the standard drug at 0.5h. Although structure activity relationship with such a small number of observations can not be established one thing is clear that both ligands as well as their vanadium complexes can be potential anti-inflammatory drugs if explored further.

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