EFFECTS OF ORGANOANTIMONY(III) COMPOUNDS OF STERICALLY HINDERED BIFUNCTIONAL TETRADENTATE LIGANDS ON THE REPRODUCTIVE SYSTEM OF MALE RATS

R.K. Sharma[1], M.P. Dobhal[1], Y.P. Singh*[1], A.K.Rai[1], R. Sharma[2], R. Chaudhary[2], R. Yadav[2] and R.S. Gupta[2]

[1] Department of Chemistry, University of Rajasthan, Jaipur (India) 302 004
[2] Department of Zoology, University of Rajasthan, Jaipur (India) 302 004

Abstract

The antifertility activity of organoantimony(III) complexes $PhSb[RC(NC_6H_4S)CH_2(NC_6H_4S)CR']$ {R = R' = CH_3 (R_1) and R = R' = CF_3 (R_2)} derived from corresponding sterically hindered bifunctional tetradentate ligands in the male rats was determined. The administration of compounds R_1 and R_2 at the dose level of 20 mg/kg. b. wt. significantly reduced the weights of testes and epididymides. Auxiliary glands showed a significant reduction after the treatment of compound R_1 only. Treated animals showed a notable depression of spermatogenesis. The preleptotene spermatocytes were decreased by 76.19 and 47.06; the secondary spermatocytes by 87.4% and 54.8% and the step-19 spermatids by 72.9 and 46.77% respectively, following the compound R_1 and R_2 treatment. Reduced sperm count and motility resulted in 100% negative fertility in both the treated groups. A significant fall in the content of various biochemical parameters of reproductive tissues was observed after R_1 and R_2 treatment in comparison to controls.

Introduction

A large number of antimony(III) compounds have been tested as bactericides[1] and fungicides[2]. The pharmacological activity of antimony compounds has developed ever since the advent of rational chemotherapy [3,4]. A number of antimony compounds have been found most effective against various diseases [5]. Phenothiazines and related compounds with the $-SC_6H_4N-$ moiety are well known to affect the hypothalamous pituitary gonadal axis and thus resulting in a delay in ovulation and menstruation in women [6]. Such type of effects were also observed in rates and dogs [7,8]. The rate of implantation was lowered and reduction in litter size have been reported by some phenothiazine derivatives [9,10]. The survey of the literature revealed that no attention has been paid to the activity of these compounds on the reproductive system of male rats. In the present investigation we are reporting the antifertility activity of organoantimony(III) complexes of sterically hindered bifunctional tetradentate ligands on the male rats.

Material and Methods

The organoantimony(III) compounds $Ph_3Sb[RC(NC_6H_4S)CH_2(NC_6H_4S)CR']$ where R = R' = CH_3 (R_1) and R = R' = CF_3 (R_2) were synthesised by the reaction of Ph_3Sb with the corresponding ligand in 1:1 molar ratio in refluxing benzene. The structure of these compounds have been reported earlier [11]. The organic precursors used for the preparation of these complexes have been synthesised by the condensation of 2-aminothiophenol with corresponding β-diketones ($RCOCH_2COR$).

Wistar rats, weighing 150-180g, obtained from Jamia Hamdard, Hamdard University, New Delhi, were used. Animals were housed in steal cages and maintained under standard conditions (12h light/12 h dark cycle; 25 ± 3°C; 35-60% relative humidity). Rat feed (Hindustan Lever Ltd.) and tap water were provided *ad libitum*.

The route and regimen of treatment were as out lined in table I. On day 61 testes, epididymides, seminal vesicles, ventral prostate, heart, liver and adrenal were removed. The total protein, glycogen and sailic acid were measured [12-14]. Tissues were fixed in Bouin's fluid. Paraffin sections were made and stained with hematoxylin and eosin. The evaluation of cell population dynamics was based on the calculations made for each cell type per cross tubular sections. Various cell componenets were quantitatively analysed. The mating exposure tests of R_1 and R_2 treated male groups were performed from day 55 to day 60. The mated females were separated to note the implantation sites on day 16th of pregnancy through leparotomy. The results were analysed using student "t" test.

Results and discussion

The administration of R_1 at the dose level of 20 mg / kg b. wt. caused the significant reduction in the body weights of treated rats. However, R_2 did not cause any significant change in body weights. The weights of testes ($P < 0.001$), epididymides ($p < 0.001$), seminal vesicle ($p < 0.01$) and ventral prostate ($p < 0.001$) were reduced significantly following the R_1 treatment. Whereas R_2 treatment only reduced the weight of testes and epididymides. The number of step-19 spermatids were decreased by 72.9 and 46.77% following R_1 and R_2 administration, respectively. The population of preleptotene spermatocytes were decreased by 76.19

and 47.06 in R_1 and R_2 treated rats. The secondary spermatocytes were decreased by 87.4% and 54.8%, respectively (table-II).

Table-I. Effects of R_1 and R_2 on the body wt. and the organ weights.

Treatment	Body Weight(g)	Organ Weight (mg/100 g b.wt.)			
		Testes	Epididymides	Seminal Vesicle	Ventral Prostate
Gr.1	240±1.4	1345±4.7	529.5±11.2	605.7±9.5	308.5±2.02
Gr.2	165**±7	1031.53**±45	293.61**±9.14	566.8*±7.0	123.82**±11.01
Gr.3	218±10^{ns}	1065±88*	369**±13.4	607±25^{ns}	336.72±14.0^{ns}

ns : non significant, All figures ± SEM, Levels of significance : *P < 0.01, **P < 0.001
Gr.1 Rat receiving vehicle (olive oil 0.2 ml/day) i.p. for 60 days
Gr.2 Rat treated with R_1 (20 mg/kg b.wt.) i.p. for 60 days
Gr.3 Rat treated with R_2 (20 mg/kg b.wt.) i.p. for 60 days

Table-II. Testicular cell population dynamics following R_1 and R_2 Treatment

	Testicular cell counts (number / 10 cross section)					
	Sertoli cell	Spermatogonia	Preleptotene	Pachytene	Sec.spermat ocytes	Step-19 spermatids
Gr.1	2.81 ±0.02	6.87±0.65	19.95±1.9	29.29±0.73	48.1±3.8	29.8±2.8
Gr.2 Percent deviation	1.75 ±0.07**	2.27±0.1** (-) 66.95	4.75±0.45** (-)76.19	15.65±1.2** (-)46.56	6.06±1.9** (-)87.40	8.075±1.1** (-)72.9
Gr.3 Percent deviation	2.6 ±0.15^{ns}	3.8±0.4* (-)44.68	10.56±1.2* (-)47.06	25.04±1.1* (-)14.51	21.74±2.5* (-)54.8	15.86±1.7* (-)46.77

ns : non significant, All figures ± S.E.M.,
Levels of significance: *P < 0.01 Compared with Control, **P < 0.001 Compared with controls

As shown in table III the protein content of testes, epididymides and ventral prostate were reduced significantly ($p < 0.001$) following R_1 treatment at the dose level of 20 mg / kg b. wt. as compared with control. R_2 treatment bring about the reduction of protein contents of cauda epididymides, sailic acid contents of the testes, epididymides and auxiliary glands (seminal vesicle and ventral prostate) were depleted in both the treated groups. Glycogen contents of testes were decreased significantly ($p < 0.001$) in comparison with controls. Fructose in seminal vesicle was also declined.

R_2 as shown in table IV the R_1 and R_2 treated rats showed significant ($p < 0.001$) reduction in the sperm concentration of testes and epididymides. The motility of the cauda epididymal sperm was also reduced significantly ($p < 0.001$). Both the treatments reduced the fertility of male rats by 100%.

Administration of R_1 and R_2 for 60 days to male rats brought about a significant loss in testes weights, which is mostly related to the nubmer of spermatids and spermatozoa present in the tissue. The reduced testicular weights also indicative of wide spread damage [15]. Low cauda epididymal sperm count, presence of non-motile spermatozoa and significant reduction in epididymal weights imply that these compounds induced infertility might be caused by several factors [16] including the oxidative phosphorylation uncoupling [17].

The reduction in sperm density and motility in cauda epididymides is of importance with regard to fertilization [18]. Reduction in number of sertoli cells following R_1 treatment adversely affects the cell cycle kinetics and influence both spermatogonia and praleptotene spermatocytes [19]. Depletion in protein, sailic acid contents of testes and epididymides and auxiliary glands and testicular glycogen and fructose in seminal vesicle reflects the antispermatogenic effects of the R_1 and R_2 compounds. The results demonstrate that the compound R_1 is more potent than compound R_2.

Spectral evidences for both the compounds suggest [11] the presence of a pentacoordinated antimony(III) atom in the pseudo-octahedral complexes, the geometry of which is due to the presence of lone pair of electrons is. Spectral evidences also suggest a distortion in the basal plane of the complexes.

Table-III. Effect of R_1 and R_2 Treatment on biochemical parameters of male rat.

	Protein(mg/gm)				Sialic acid (mg/gm)				Glyco-gen (mg/g)	Fruc-tose (mg/g)	Ascorbic acid (mg/gm)
	Testes	Cauda Epididy-mis	Seminal Vesicle	Ventral Prostate	Testes	Cauda Epidid-ymis	Seminal Vesicle	Ventral Prostate	Testes	Seminal Veside	Adrenal
Gr.1	173.88 ± 5.31	211.14 ±1.13	192.74 ± 2.94	171.12 ± 1.06	4.67 ± 0.01	6.27 ± 0.05	5.26 ± 0.04	4.82 ± 0.02	2.16 ± 0.4	4.66 ± 0.2	3.30 ± 0.2
Gr.1	155.53* ± 2.12	148.87** ± 3.85	182.2^{ns} ± 5.13	155.54** ± 3.8	3.57** ± 0.01	3.63** ± 0.05	3.39** ± 0.05	3.63** ± 0.01	1.48** ± 0.02	2.66 ± 0.1**	3.18^{ns} ± 0.04
Gr.3	168.86^{ns} ± 2.5	153.3** ± 5.8	191.09^{ns} ± 1.28	166.64^{ns} ± 1.28	3.66** ±0.05	3.66** ± 0.017	3.54** ± 0.034	3.66** ± 0.03	1.61** ± 0.02	3.99 ± 0.1*	3.44 ± 0.1^{ns}

ns : non significant, Values are mean ± S.E. of six determinations,
Levels of significance : *P < 0.01, **P < 0.001

Table-IV. Sperm motility, concentration and fertility after R_1 and R_2 treatment

Treatment	Sperm motility (Cauda epididymes)	Sperm density (million/ml)		Fertility (%)
		Testes	Cauda epididymides	
Gr.1	68.02 ± 2.78	4.66 ± 0.59	44.21 ± 2.3	100 (+ ve)
Gr.2	9.78 ± 2.64**	1.15 ± 0.4**	4.30 ± 0.17**	100 (-ve)
Gr.3	33.89 ± 4.0**	2.35 ± 0.2*	8.42 ± 1.6**	100 (-ve)

All figures ± SEM, Levels of signficance : *P < 0.01, **P < 0.001

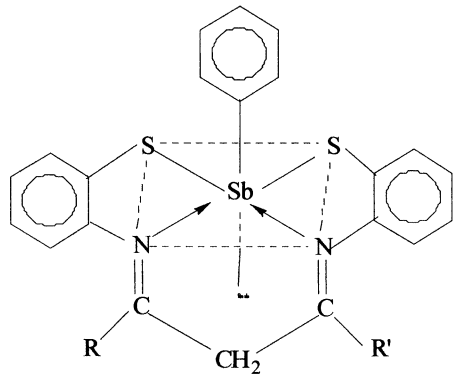

Fig. 1 Structure of $PhSb[RC(NC_6H_4S)CH_2(NC_6H_4S)CR']$

It is evident from the above disussion that the antifertility activity of the compound (R_1) is more than the compound R_2. It is a well known fact that the presence of halogen atom in the compound enhances its antifertility activity[20,21]. But in the present study the hexafluoro derivative R_2 was found to be less active than the non-fluorirated derivative. It might be possible that compound R_2 may have a positive effect on male reproductive system. This may be verified by carrying out the toxicological effect of both the compounds. The study is in progress and the results will be published in due course of time.

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