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Reproductive Physiology

EFFECTS OF QUINACRINE ADMINISTRATION ON THE TESTICULAR FUNCTION OF BAT (*RHINOPOMA KINNEARI* WROUGHTON)

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1. Quinacrine administration (1·2 mg/day/animal for 30 days) caused widespread testicular necrosis. Spermatogonia, spermatocytes and Sertoli cells showed degenerative changes. The lumen of the epididymis were devoid of spermatozoa. 2. The RNA, protein and sialic acid contents in the testis and epididymis were reduced. Serum transaminase and acid phosphatase activity were moderately changed, whereas, haemoglobin, hematocrit, blood-sugar and blood-urea levels were in normal range. 3. Regressed Leydig cell tissue and decreased production of RNA and sialic acid in the testis could be due to low androgen titre produced by quinacrine treatment. 4. In conclusion, quinacrine administration produced an effective inhibition of spermatogenesis in male bats.

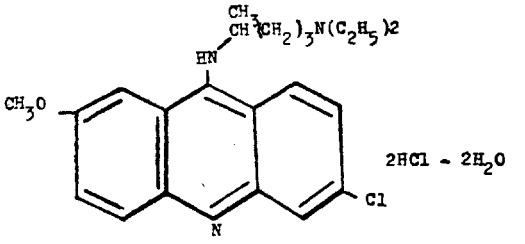
INTRODUCTION

Zipper *et al.* (1970) advocated the use of quinacrine for producing tubal occlusion as a potential method, thus substituting the various orthodox methods ranging from salpingectomy to methods using ligature and clips, etc. Malaviya *et al.* (1974) used quinacrine for vas occlusion in rhesus monkeys.

Quinacrine has not been used for its antispermatogenic activity in a hibernating mammal. It was thought worthwhile to study the long-term effects of quinacrine administration on the testicular function of a common rat tailed bat (*Rhinopoma kinneari* Wroughton). Quinacrine was selected by us for its anti-fertility properties in males since it has been injected with safety and without secondary effects into the peritoneal and pleural cavities of humans for the control of effusions resulting from metastatic lesions (Gellhorn, 1959; Rochlin, 1964).

MATERIALS AND METHODS

The bats (*Rhinopoma kinneari* Wroughton) were caught from the surroundings of Amber Fort in the month of June-July, 1977. Healthy adult sexually mature males were housed in wire cages in groups of 20 each. They were acclimatized in the laboratory for at least 7 days at $23 \pm 1°$ C.



Quinacrine hydrochloride

Quinacrine was supplied as hydrochloride salt in sterile ampoules by Winthrop-Sterling Ltd., U.S.A. The drug was disssolved in sterile physiologic saline solution. The male bats were injected intraperitoneally with 1·2 mg quinacrine in 0·2 ml solution/day/animal for a period of 30 days. The control animals received an equal amount of vehicle alone.

All animals were killed by rapid decapitation 24 hr after the last injection. Final body weight was recorded. Testes, epididymis, seminal-vesicles, thyroid and adrenal glands were dissected free of fat and weighed on a torsion balance. Right testes and epididymis were fixed in Bouin's fluid. Six μm paraffin sections were prepared and stained with haematoxylin and eosin. Left testes and epididymis were frozen and total protein, RNA, sialic acid, alkaline/acid phosphatase activity were later determined (Lowry *et al.*, 1951; Munro & Fleck, 1966; Warren, 1959; Fiske & Subbarow, 1925).

Plasma was separated from the blood obtained directly from the heart. Hepatic function was followed with determination of serum-glutamic-oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) (Mohun & Cook, 1957). Haemoglobin content per 100 ml of blood was determined with the help of Fisher's Haemophotometer model 55, by the Cyanmethaemoglobin method (*see* Varley, 1969). Haematocrit values were determined by using small capillary tubes. Blood-urea was determined by Urease Nesslerization method (*see* Varley, 1969). Blood-sugar levels were also determined by the method of Astoor and King (1954).

One hundred seminiferous tubules appearing circular in section were traced with camera lucida at × 80. Two perpendicular diameters of each tracing were measured, averaged and expressed in terms of mean tubular diameter. Student's 't' test was applied in comparing means. The measurements of the diameter of the 100 Leydig cell nuclei were carried out on four sections from each testicle with camera lucida drawings at × 800.

Observations

In male bats, the testes are permanently intra-abdominal and a scrotum is absent. The spermatogenic cycle is completed in the intra-abdominal testes.

TABLE I

Changes in the body weight and the weights of testes, epididymis, seminal vesicle, adrenal and thyroid glands together with changes in the seminiferous tubule and Leydig cell nuclear diameter of Rhinopoma kinneari *treated with quinacrine hydrochloride*

Treatment		Body wt. (g)	Testes	Epid.	Seminal vesicle	Adrenal	Thyroid	Seminiferous tubule diam. (μm)	Leydig cell nuclear diam. (μm)
			mg/100 g body weight						
Control	(20)	32± 5	183± 13	163± 17	65± 9	16·5± 3·7	14·6± 2·5	175±7	10·5±0·2
Quinacrine (1·2 mg/bat/day for 30 days)	(20)	23± 7	95·3±* 17	80·6±* 19	26·5±* 11·5	15·6± 2·5	15·6± 1·9	134±5*	7·8±0·3*

* $P < 0·01$ compared with controls. Figures in parentheses represent the number of animals examined. All figures ± S.E.M.

TABLE II

Biochemical changes in the testes, epididymis, blood/serum of bat treated with quinacrine hydrochloride

Treatment	Protein μg/mg tissue						Phosphatase enzyme activity of testis		SGOT / SGPT		Blood sugar (mg/100 ml)	Blood urea (mg/100 ml)
			R N A		Sialic acid							
	Testis	Epid.	Testis	Epid.	Testis	Epid.	Alkaline	Acid†				
Control	177± 19	153± 23	2·7± 0·2	2·1± 0·1±	6·6± 0·9	7·5± 0·3	7·8± 0·3	2·3± 0·1	73±11	35±7	62·5± 5·2	35·6± 3·5
Quinacrine (1·2 mg bat/day for 30 days)	109±* 17	81±* 12	1·5±* 0·2±	1·1± 0·2	4·2±* 0·3	5·2±£ 0·4	7·2± 0·7±	1·3±* 0·3	128±8	21±5	57·5± 3·4	33·7± 2·3

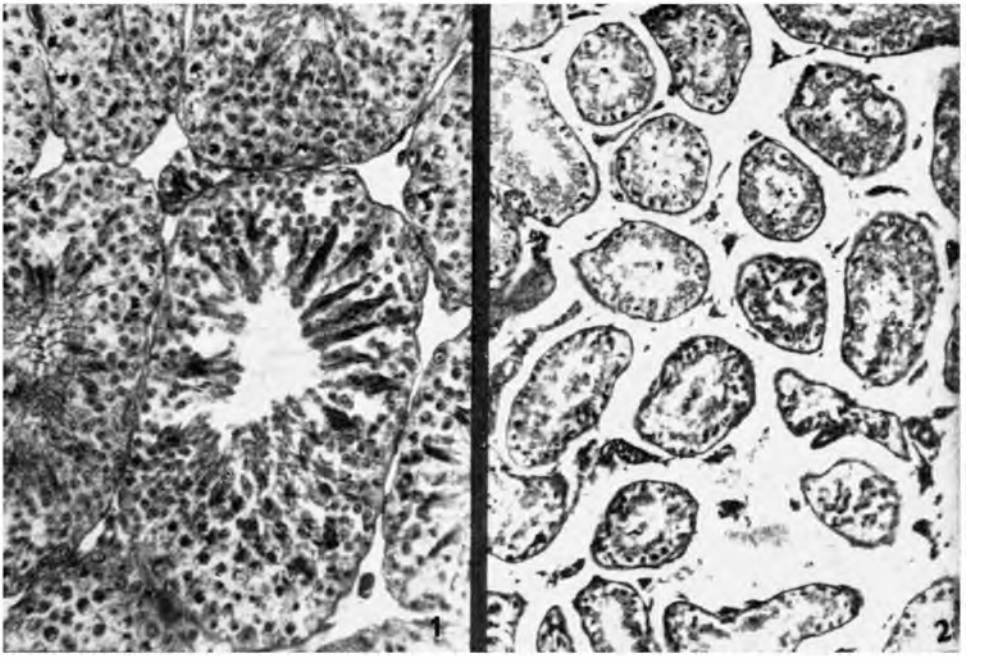
* $P < 0·01$ compared with controls. All figures ± S.E.M.

Biochemical estimations : means of six determinations. †Alkaline/acid phosphorus/hr/mg of tissue.

Adult males receiving, 1.2 mg of quinacrine for a period of 30 days showed a marked reduction in the weights of testes, epididymis and seminal vesicles. The testis is small, flaccid and somewhat edematous.

Histological preparation showed a widespread necrosis, leucocytic infiltration of the epithelial cells. Spermatogonia, spermatocytes and Sertoli cells showed degenerating changes (Figs. 1 and 2). Cytolysis and chromatolysis were conspicuous. Shrinkage of the seminiferous tubule and Leydig cell nuclear diameter was noticed (Table I).

The epididymal canals were devoid of spermatozoa. There were no apparent histological changes in the luminal epithelial cells.



FIGS. 1-2, Testis of a control bat showing full spermatogenesis. × 200 HE; 2, Testis of a bat after quinacrine hydrochloride treatment (1·2 mg/day/animal for 30 days). Note the severe damage caused to the spermatogenic cells. × 100 HE.

Biochemical Changes

The total RNA and protein contents of testes and epididymis were significantly reduced in quinacrine treated bats as compared with that of controls ($P < 0.01$, Table II).

Sialic acid: Sialic acid levels were low in the testes and epididymis (Table II).

Alkaline/Acid phosphatase enzyme activity: Alkaline phosphatase enzyme activity did not change, whereas the acid phosphatase enzyme activity was low (Table II).

Serum enzyme activities: Serum glutamic oxalo-acetic transaminase (SGOT) activity was moderately increased ($P < 0{\cdot}02$, Table II), whereas the serum glutamic pyruvic transaminase (SGPT) activity was in normal range (Table II).

Hematological Studies

Red cells — $5{\cdot}23 \times 10^6/mm^3$; haemoglobin — 9·5 g/100ml ; packed cell volume (PCV : hematocrit value) — 29·6 % and Leucocytes — $11050/mm^3$ were in normal range. The histopathological examination of liver taken at biopsy did not show any damage. The architecture was microscopically normal.

Blood-Sugar and Blood-Urea

Blood-sugar and blood-urea levels were at the control levels (Table II).

Discussion

Intra-peritoneal administration of quinacrine (1·2 mg/day/animal for a period of 30 days) caused testicular injury. The mechanism by which quinacrine produces such damage is not clear.

The effects of quinacrine at the cellular level may be due in part to its interference with nucleic acid metabolism (Ciak & Hahn, 1967). A direct cytotoxic effect of quinacrine on the germ cells as well as on the Sertoli cells is certainly a possibility. The Sertoli cells showed varying degrees of vacuolation of the cytoplasm.

Quinacrine arrested spermatogenesis at the level of primary spermatocytes. Levels of RNA, protein and sialic acid were low in the testes and epididymis. This is probably due to inhibition of spermatogenesis in the testes. Peyre and Laporte (1966) reported a fall in sialic acid content in cryptorchid testes.

Androgen-dependent growth and secretory activity of the epididymis and seminal vesicles were low in quinacrine treated bats. A depression of testosterone production (also a Leydig cell function, Hall *et al.*, 1969) is supported by regressed Leydig cell tissue and decreased production of RNA and sialic acid in the testis (Peacocke & Skerrett, 1956 ; Pyre & Laporte, 1966).

Decreased acid phosphatase enzyme activity was correlated with an inhibition of spermatogenesis. It has been claimed that the function of an endocrine gland in an organism would depend on their regulatory effect on enzymatic reactions (Pulkkinen & Willman, 1967).

Our data weigh in favour of normal functioning of liver as revealed by clinical and histopathological examination.

Acknowledgements

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