

# Toxic and antifertility effects of quinacrine hydrochloride in rats

A. A. JOSEPH  
FRED A. KINCL

*Brooklyn, New York*

***A single instillation of an aqueous suspension containing 40 mg. of quinacrine hydrochloride into the uterine horns of adult rats prevented implantation for at least 4 weeks. A suboptimal dose of 5 mg. could be potentiated by the addition of epinephrine or metaraminol. In some animals, the use of the quinacrine hydrochloride suspension resulted in atrophy and necrosis of the treated uterine horn, a constriction of the lower colon which blocked the propulsion of intestinal contents, and death.***

ZIPPER AND ASSOCIATES<sup>1</sup> have shown that an intrauterine instillation of an aqueous quinacrine hydrochloride suspension into the rat uterus induces the formation of fibrous and granulomatous tissue which may result in complete obstruction of the uterine lumen. The same group<sup>2</sup> and Davidson and Wilkins<sup>3</sup> have reported that in women intrauterine instillation of quinacrine hydrochloride results in tubal occlusion and infertility. Since a rapid, safe method of chemically occluding the oviducts could become an important method of regulating fertility, an animal model to test tubal occluding agents would be of importance. Therefore, we have re-examined the effect of quinacrine hydrochloride, and other agents, in rats.

## Methods

Adult female Holtzman strain rats were used. The animals were divided into several groups of 5 female rats each. At laparotomy,

***From the Laboratory for Reproduction, Brookdale Hospital Centers.***

***Received for publication October 1 I, 1973.***

***Revised December 14, 1973.***

***Accepted January 2, 1974.***

***Reprint requests: Dr. Fred A. Kincl, Lamar Research, 8 Oakwood Dr., Weston, Connecticut 06880.***

with ether anesthesia, 0.2 ml. of the test solution was injected into the left uterine horn, about 5 mm. below the tubouterine junction. The needle was left in place for approximately 30 seconds. The right horn served as a control. Two groups were handled as directed by Zipper and associates; injection flow was directed from the vaginal end while the inferior portion of the horn was tied off with silk thread for 15 minutes to avoid reflux.

To prepare the test suspension, quinacrine hydrochloride\* was milled and passed through a 100 mesh sieve. The final concentration was adjusted to contain the desired amount suspended in 0.2 ml. of water. In one preparation, polyvinylpyrrolidone (PVP), 2 per cent v/v, was added. In addition, the following combinations were used: quinacrine hydrochloride 5 and 20 mg. suspension, combined with 2 mg. of metaraminol bitartrate†; quinacrine hydrochloride suspension containing 2 mb. of lidocaine hydrochloride‡; and quinacrine hydrochloride suspension containing 0.1 ml. of

\*Sigma Chemical Co., 3500 DeKalb St., St. Louis, Missouri.

†Aramine bitartrate, Merck Sharp & Dohme, Div. Merck & Co., Inc., West Point, Pennsylvania 19486.

‡Xylocaine hydrochloride, Astra Pharm. Products, Inc., 7 Neponset St., Worcester, Massachusetts 01606.

Table I. Influence of quinacrine hydrochloride and other agents instilled into the left uterine horn on fertility in rats

Treatment	Quinacrine dose (mg.)	No. of rats surviving/fertile	Average No. of fetuses (range)	
			Treated horn	Control horn
Saline	0	5/5	6.4 (4-8)	5.8 (3-8)
Saline, pH 3.5	0	5/5	7.2 (5-10)	6.6 (5-8)
Quinacrine hydrochloride	10	7/6	4.4 (0-7)	6.7 (1-11)
	40	4/2	2.8 (0-7)	8.0 (7-9)
	40*	7/1	0.9 (0-7)	2.0 (0-10)
	40†	6/1	0.9 (0-5)	2.2 (0-7)
Quinacrine hydrochloride plus metaraminol bitartrate	5	5/4	6.4 (0-9)	5.4 (1-8)
Quinacrine hydrochloride plus lidocaine hydrochloride	20	1/0	0.0	0.0
Quinacrine hydrochloride plus epinephrine	5	4/3	4.8 (0-9)	6.8 (4-9)
Quinacrine hydrochloride plus epinephrine	5	3/3	0.0 (0-0)	7.0 (6-8)

\*Quinacrine hydrochloride suspension containing PVP.

†Method as directed by Zipper and associates.

epinephrine (1:1,000). Saline and saline acidified with hydrochloric acid to a pH of 3.5 were used to inject control animals.

The animals were maintained with food and water ad libitum. Three weeks after quinacrine instillation, the female rats were bred. Animals with positive sperm were identified and autopsied 2 weeks later. At autopsy, the number of live fetuses and resorbing implants was noted. For microscopic examination, the tissues were fixed in neutral formalin and embedded in paraffin; 6  $\mu$  sections were cut and stained with hematoxylin and eosin.

#### Results and comment

The results of the study are summarized in Table I. Table I indicates the material injected into the lumen of the left ("treated") horn, the number of animals surviving the experiment approximately 6 weeks), the number of animals with implants in the treated horn ("fertile" rats), and the average number of live fetuses found in the "treated" and "control" (not injected) horns.

The injection of saline or saline acidified to a pH of 3.5 (controls) into the left uterine horn had no effect on subsequent fertility. In contrast, instillation of a quinacrine hydrochloride aqueous suspension had a marked, dose-related effect. A dose of 10 mg. was only moderately active since im-

plantation failed in the treated horn in only 1 of 7 animals. Increasing the dose to 40 mg. produced sterility in more than one half of treated rats. It would appear that a preparation containing 2 per cent PVP to aid in the formation of a better suspension was more effective. Similarly, the incidence of sterile animals may be higher if the suspension is kept in the horn for 15 minutes as directed by Zipper and associates. In both groups implantation was also inhibited in the control horn, possibly secondary to toxic effects (see below). Both groups were relatively small, and additional studies would be required to confirm these observations.

Addition of lidocaine hydrochloride or epinephrine to a 5 mg. suboptimal dose resulted in a significant decrease in fertility. Addition of metaraminol bitartrate to a 20 mg. dose was toxic, and only one animal survived. Our data concerning the antifertility effect of quinacrine are in agreement with the material published by Zipper and associates.<sup>4,5</sup> The antifertility effect appears to be mediated by the acridine molecule since saline acidified to the same pH value as the suspension used produced no noticeable effect. We could also confirm that some agents potentiate the quinacrine effect by a mechanism of action yet to be elucidated.

We have found quinacrine to be toxic in rats. During the study 13 animals became weak and emaciated. Five died 2 to 4 weeks

**Table II.** Toxic effects on quinacrine hydrochloride in rats

Treatment	No. of rats used/ surviving	Deaths (%)	Remarks
Saline	10/10	0	No pathology
Quinacrine			
5 mg.	12/12	0	No pathology
10 mg.	8/7	12	Enteromegaly (1*)
20 mg.	5/1	80	Enteromegaly (5), uterine necrosis (4)
40 mg.	25/17	32	Enteromegaly (7), uterine necrosis (4), adhesions between uterus and bowel (3)

\*Indicates the number of animals involved.

following quinacrine instillation, and during the same period 8 rats were killed when they became too ill to survive. The over-all incidence of toxic reaction was almost 22 per cent in the treated groups, with none seen in the controls. At autopsy it was noted that the intestines were inflated with gases, the lower colon was constricted, and the propulsion of intestinal contents was blocked. This condition was apparently dose-dependent (Table II).

In Table II, the treatment groups receiving the same dose of the sclerosing agent were combined; detailed group distribution is found in Table I. The high incidence of enteromegaly seen in animals that received 20 mg. of quinacrine may be the result of a potentiating effect of 2 mg. of metaraminol bitartrate. The group was small, however, and additional studies will be needed to clarify this.

In 10 animals quinacrine-treated uterine horns were markedly enlarged and filled with bloody fluid; in 3 animals the control horn was also involved. Sections of enlarged uterine horns, the ovaries, and the kidneys were taken for microscopic examination from 3 animals.

The enlarged uterine horns were found to be mostly necrotic tissue with only a few apparent blood vessels and disintegrated

erythrocytes present in the lumen. Abnormalities indicative of degenerative changes in the tubules and glomerules with hemorrhage and necrosis in several loci were noted in kidneys. Frank leukocytic infiltration, suggestive of inflammation, was not noted. The ovaries presented a normal morphologic picture with corpora lutea and primary and secondary follicles present.

The extraordinary enlargement of the cecum, the appendix, and the ileum resembled the effects induced by Keeler and colleagues,<sup>6</sup> in 1966, by injecting intraperitoneally 10 mg. of quinacrine hydrochloride (0.5 per cent aqueous solution) daily for 5 days.

The Keeler group found that the treatment used produced toxic manifestations in all animals. They noted that quinacrine blocked both normal and acetylcholine-induced motility of the gut and speculated that the compound may directly affect intestinal motility by destroying parasympathetic ganglion nerve cells. The known high affinity of quinacrine for nucleic acids may well be the mechanism of action involved in the pathology.

It would appear that the enteromegaly and deaths seen in our studies were produced by small amounts of test suspension which leaked from the injection site into the peritoneal cavity, but we cannot be sure of this. Additional studies with graded doses of the sclerosing agent and groups of rats autopsied at different times after the treatment period are needed to clarify this point. If our results are confirmed, a much higher toxicity of quinacrine in rats than noted by Keeler and co-workers would be indicated.

Our studies were originally designed to develop an assay technique and to confirm the data of Zipper and his collaborators. To our concern, only 37 animals from a group of 50 survived treatment with the various quinacrine preparations. We cannot fully evaluate the toxic effects of quinacrine hydrochloride on intestinal movement and uterine necrosis at this time. Such effects could be specific to rats, as was suggested by Keeler and colleagues,<sup>6</sup> but available in-

formation is too meager to be sure of this. The high incidence of toxicity is of utmost concern and more comprehensive studies are needed to establish the premise that the deleterious effects are specific to rats and not to other mammalian species.

We wish to thank Dr. Z. Hrůza, School of Medicine, New York University, for the pathologic evaluation of the various tissues.

---

REFERENCES

1. Zipper, J., Medel, M., and Prager, R.: *AM. J. OBSTET. GYNECOL.* **101**: 971, 1968.
2. Zipper, J., Stachetti, E., and Medel, M.: *Fertil. Steril.* **21**: 581, 1970.
3. Davidson, O. W., and Wilkins, C.: *Contraception* **7**: 333, 1973.
4. Zipper, J., Insunza, S.: Pharmacologic agents that potentiate or inhibit the occlusive action of quinacrine on the rabbit tube and rat uterus, *in* Duncan, G., Galb, R. D., and Speidel, J., editors: *Female Sterilization*, New York, 1972, Academic Press, Inc.
5. Zipper, J., Prager, R., and Medel, M.: *Fertil. Steril.* 1973. In press.
6. Keeler, R., Richardson, H., and Watson, A. J.: *Lab. Invest.* **15**: 1253, 1966.