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Evaluation of Slow-Release Quinacrine Pellets in the Pig

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A number of studies have established that the uterine instillation of quinacrine in women causes the selective obstruction of the uterotubal junction (UTJ). So far, the procedure has been approximately 90% effective. However, most of the clinical studies performed have employed quinacrine in a suspension or slurry. A number of side-effects have been reported with the use of quinacrine in slurry, including oligomenorrhea, hypomenorrhea, pelvic inflammatory disease, abdominal pain, nervousness, distress, central nervous system excitation, abdominal distention, fever, and chemical vaginitis. Zipper presented evidence that most of these problems can be avoided if the transcervical uterine instillation of the quinacrine is done by a controlled release pellet rather than the injection of fluid. Based on this observation, PARFR contracted the Southern Research Institute to prepare slow-release pellets that liberated quinacrine over a 7-day period (see Chapter 14).

The objective of the present study was to evaluate the efficacy of these slow-release pellets in causing the obstruction of the UTJ and to determine the tissue levels of the quinacrine after it was released from the pellets. The pig was used as the animal model because the pig oviduct is structurally similar to that of the human fallopian tube. Additionally, the pig has two uteri, so one uterus can be used as control and the other uterus as the test.

EXPERIMENTAL PROTOCOL

Pellets were constructed by the Southern Research Institute under the direction of R. L. Dunn (see Chapter 14). Each pellet was tube-shaped and measured 5.0 mm in length and 3.8 mm in diameter. The core of the pellet consisted of 65% quinacrine dihydrochloride in polycaprolactone and was surrounded by a sheath consisting of polycaprolactone. The core contained a central hole that was 0.54 mm in diameter. *In vitro* the pellet had been shown to release

The authors gratefully acknowledge the aid of Dr. R. Richart, Columbia University, New York, in the histologic studies; Dr. N. Dubin and Ms. M. DiBlasi, of Johns Hopkins University, Baltimore, in the quinacrine assays; and Mr. R. L. Dunn, Southern Research Institute, Birmingham, Alabama, in the preparation of the pellets. The project was supported by a contract from the Program for Applied Research on Fertility Regulation.

all the quinacrine in 7 days. Three of the pellets were held together by 4-O prolene suture that passed through the central hole in the core of the pellets. The average amount of quinacrine in the pellets was 121.0 ± 0.5 mg. Blank (control) pellets were identical to these pellets, except that they did not contain quinacrine.

Ten mature, female pigs of mixed breed were purchased and isolated for 1 month to ensure that they were free of diseases. Before surgery, they were fasted for 12 hours, although water was allowed. The animals were premedicated with atropine (0.05 mg/kg, IM) and xylazine (Rompun) (2.0 mg/kg, IM). The latter is a nonnarcotic drug that produces sedation, analgesia, and muscle relaxation. Anesthesia was induced with thiamylal sodium (2.5%, IV to effect). The animals were intubated with a tracheal tube and maintained on 1.5% to 2.5% halothane with N_2O and O_2 in a 1:2 ratio. During the anesthesia, the animals were monitored electrocardiographically and with an esophageal stethoscope.

With one exception, none of the animals showed any complications during the surgery and recovery was normal. One animal began to show signs of malignant hyperthermia after the administration of halothane, and the anesthesia was immediately discontinued. At a later date, the pig was treated prophylactically with dantrolene (8 mg/kg), 4 hours before induction of the anesthesia. The surgical procedure and postoperative recovery were uneventful.

At the time of surgery, a midline laparotomy was made and the uteri were exposed. The junction between the uterus and the oviduct was isolated, and an incision was made in the uterus, approximately 2 cm long, starting 5 cm from the UTJ. A needle, to which the 4-O prolene suture with the three pellets was attached, was passed through the incision and threaded through the uterine lumen until it was approximately 0.5 cm from the uterotubal junction. The needle was then pushed through the uterine wall, and the pellets were pulled through the incision into the uterine lumen until the first pellet became situated immediately adjacent to the UTJ, with the other pellets directly adjoining each other. The needle was removed, and the two ends of the suture were tied to each other. The knot was pulled through the incision so that it became situated immediately in front of the last pellet. This prevented the pellets from moving away from the UTJ. The uterine incision was closed with 3-O Dexon.

The same procedure was repeated on the other uterine horn. One of the uterine horns received the quinacrine pellets and the other received the blank (control) pellets. After the abdomen and skin were closed, the pig was allowed to recover and then was returned to her pen.

The pigs were euthanized after the pellet had been inserted for either 2, 4, 6, 8, or 10 weeks. Two pigs were euthanized at each time period. Immediately on euthanasia, the cervix, uteri, and oviducts were excised. These were divided into several sections to be studied either for histologic changes or for quantitation of quinacrine.

The tissues collected for histology were as follows: 1) the UTJ area, consisting of 5 cm of uterus attached to 2 cm of oviduct; 2) a 3-cm portion of the more cranial uterus, excised 12 cm from the UTJ; 3) a 2-cm portion of the isthmus, excised 9 cm from the UTJ; and 4) a portion of the ovary. These

tissues were fixed in phosphate-buffered formaldehyde and shipped to New York, where they were studied by Dr. R. Richart at Columbia University.

For quinacrine quantitation, the following tissues were collected: 1) a 7-cm strip of endometrium, starting 5 cm from the UTJ ("cranial endometrium"); 2) a 7-cm strip of myometrium ("cranial myometrium"), underlying the cranial endometrium; 3) a 7-cm strip of endometrium obtained from the middle of the uterus ("middle endometrium"); 4) a 7-cm strip of myometrium ("middle myometrium"), underlying the middle endometrium; 5) a 7-cm portion of the isthmus, starting 2 cm from the UTJ; 6) a 7-cm portion of the ampulla; 7) a portion of the ovary; and 8) the cervix. Additionally, pieces of the liver, kidney, and the adrenal glands were collected for quinacrine measurements. The tissues were frozen at -20°C and shipped on dry ice to Dr. N. Dubin at Johns Hopkins University.

After the pellets were removed, they were rinsed to cleanse them of blood and sent to R. L. Dunn, at the Southern Research Institute, for quinacrine analyses.

RESULTS

None of the animals showed any side-effects from the surgery or the quinacrine. They continued to gain weight and appeared healthy at the time of euthanasia. At autopsy, none of the animals showed any visible lesions, except that occasional adhesions were present among some of the uterine loops. These occurred with equal frequency on the control and test sides, so they are most likely due to the handling of the uteri during pellet implantation. The incisions in the uteri were completely healed and showed some small scars. In one uterus containing quinacrine pellets, some granulomatous material was detected on the outside wall of the uterus where the incision was made. The suture material was in place in each case and did not cause rupture or strangulation of the uterine wall.

When the uterus was incised, the pellets were found to be still in place, that is, the first one was adjacent to the UTJ. No pellet was visibly altered. In both pigs in which pellets had been implanted for 2 weeks, the endometrium showed yellowish discoloration, starting at the UTJ and extending for 10 cm to 15 cm toward the cervix. None of the pigs with implantations of longer duration showed this, with the exception of one pig with implantations for 4 weeks (No. 801) and one pig implanted for 6 weeks (No. 796). In these animals, some yellowish discoloration was seen at the UTJ. No visible lesions were detected in any of the uteri.

The residual quinacrine in the pellet is shown in Table 13-1. Essentially all of the quinacrine was released from the pellets in all of the animals, even the ones with implantations for only 2 weeks. Therefore, the *in vivo* (uterine) release rate of the pellet is less than 14 days, corroborating the 7-day release rate found *in vitro*.

The tissue levels of quinacrine are shown in Table 13-2. In regard to the test sides, very high levels were present in the cranial endometrium of the animals implanted for 2 weeks. High cranial endometrial levels were also found in one of the 4-week animals (No. 801) and one of the 6-week animals

TABLE 13-I. Residual Quinacrine in Pellets Recovered From Pigs

PIG NO.	WEEKS IMPLANTED WITH PELLETS	QUINACRINE CONTENT OF IMPLANTED PELLETS (MG)	RESIDUAL QUINACRINE	
			IN RECOVERED PELLET AFTER EUTHANASIA (MG)	% OF QUINACRINE RELEASED
797	2	120.8	0.074	99.94
805	2	120.9	0.133	99.89
798	4	120.8	0.037	99.97
801	4	120.9	0.059	99.95
796	6	121.9	0.046	99.96
799	6	120.6	0.042	99.96
802	8	120.8	0.045	99.96
800	8	122.2	0.088	99.93
804	10	120.7	0.040	99.97
803	10	120.7	0.146	99.88

Measurements of quinacrine performed by R. L. Dunn, Southern Research Institute, Birmingham, Alabama.

(No. 796), corresponding to the visual presence of yellowish material (see above). Much less quinacrine was present in the cranial endometria of the other 4- and 6-week implanted animals. Significant amounts were still present in the cranial endometrium of the 10-week implanted pigs. Fairly high amounts of quinacrine could also be found in the cranial myometrium and middle endometrium of the pigs implanted for 2 weeks, the middle endometrium of one of the pigs implanted for 4 weeks (No. 798), and the ampulla and ovary of one of the pigs implanted for 2 weeks (No. 797). Significant amounts of quinacrine were also present in the cervix and isthmus of one of the 2-week implanted pigs, and in the isthmus and middle myometrium of one of the 10-week implanted pigs (No. 804). The ovaries of most pigs tended to possess quinacrine in levels that were often higher than those of the isthmus. In all other cases, tissues showed a concentration of less than 10 ng/100 mg tissue, many of the levels being below detection (< 2.5 ng/100 mg tissue).

The control tracts only showed low levels of quinacrine. Interestingly, the levels tended to be the highest when the corresponding test side was also high in quinacrine (e.g., see the 2-week implanted animals). It was further notable that the ovaries of both the test and control sides possessed approximately the same levels of quinacrine.

Of the nonreproductive organs, only extremely low levels of quinacrine could be detected in the liver and kidney, with the exception of one of the 2-week implanted animals (No. 797). The adrenal gland tended to contain more quinacrine than the liver or kidney, but the levels were only higher than 10 ng/100 mg tissue in the 2-week implanted pigs.

Histologically, all the sections of the oviducts, uteri, and ovaries appeared within normal limits. No evidence of tubal or peritubal changes was noted. One exception was a fragment of granulation tissue in the uterine lumen in pig No. 801, but no further evidence of pathology was found. The UTJs were open in each case, on both the control and the test side.

TABLE 13-2. Tissue Levels of Quinacrine*

WEEKS PIG NO. IMPLANTED	TEST/ CONTROL SIDE	CRANIAL		MIDDLE		MIDDLE		CERVIX	ISTHMUS	AMPULLA	OVARY	ADRENAL		LIVER	KIDNEY
		ENDO- METRIUM	MYO- METRIUM	ENDO- METRIUM	MYO- METRIUM	GLAND	KIDNEY								
797	2 Control	X	183	15.3	21.7	X	50.3	X	80.5	72.9	18.8	12.1	7.1		
	Test	5176.5	299.3	27.3	22.7		80.5		80.5	72.9		12.1	7.1		
805	2 Control	6.9	6.1	<2.5	12.0	5.6	9.6	8.9	25.9	19.6	25.9	<2.5	4.7		
	Test	6965.2	76.9	4.7	29.3		9.6		25.9	16.7		<2.5	4.7		
798	4 Control	<2.5	<2.5	<2.5	4.3	<2.5	X	3.4	<2.5	13.9	<2.5	3.0	<2.5		
	Test	156.6	45.7	3.0	4.3		X		<2.5	11.1		3.0	<2.5		
801	4 Control	2.8	<2.5	<2.5	<2.5	<2.5	X	3.0	3.0	8.5	9.9	<2.5	<2.5		
	Test	1280.4	<2.5	<2.5	<2.5		X		3.0	12.1		<2.5	<2.5		
796	6 Control	3.8	3.1	<2.5	X	2.5	X	8.9	9.4	8.9	9.4	c2.5	<2.5		
	Test	1259.5	4.4	4.0	3.1		X		9.4	8.7		c2.5	<2.5		
799	6 Control	<2.5	X	<2.5	<2.5	<2.5	<2.5	5.1	3.9	5.1	3.9	2.5	2.8		
	Test	104.6	<2.5	<2.5	<2.5		<2.5		3.9	3.1		2.5	2.8		
802	8 Control	<2.5	<2.5	11.0	<2.5	<2.5	X	3.4	<2.5	3.4	2.8	<2.5	<2.5		
	Test	<2.5	<2.5	2.6	<2.5		X		<2.5	2.9		<2.5	<2.5		
800	8 Control	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5		
	Test	4.1	<2.5	<2.5	<2.5		<2.5		<2.5	13.6		<2.5	<2.5		
804	10 Control	2.9	3.0	3.2	<2.5	3.0	2.6	3.4	6.5	3.4	6.5	3.1	2.8		
	Test	71.7	5.3	28.6	<2.5	12.4	4.4	7.5	6.5	3.4	6.5	3.1	2.8		
803	10 Control	4.7	2.5	2.5	2.7	<2.5	3.1	2.7	6.6	2.7	6.6	3.8	<2.5		
	Test	44.6	4.2	4.0	5.4		2.6		6.6	3.1	6.6	3.8	<2.5		

*Values are in ng/100 mg tissue. The tissues were analyzed for quinacrine by Dr. N. Dubin and Ms. M. DiBlasi of Johns Hopkins University, Baltimore, Maryland.

X, Not tested.

DISCUSSION

The data show that the slow release of quinacrine over a 7-day period at the UTJ of a pig does not result in the obstruction of the oviductal lumen. This is in direct contrast to the results obtained in women and primates with the quinacrine in slurry or with the 1-day release pellets. Quinacrine solutions were also shown to be active in rats but ineffective in rabbits. These results indicate that the female genital tract of the pig is less receptive to the action and migration of quinacrine than the genital tract of some other species, including the human and nonhuman primate.

Evidence that this hypothesis is correct can be obtained from a study that was performed by researchers by Johns Hopkins University (see Chapters 6 and 7). These investigators injected 30 mg quinacrine into the uteri of cynomolgus macaque monkeys. Tissue levels were measured 24 hours and 7 days after the injection of the quinacrine. Since our quinacrine pellets released their content in 7 days, quinacrine levels in the tissues of the pigs euthanized 2 weeks after insertion of the pellet are comparable to the levels in monkeys observed 7 days after the single instillation of the quinacrine. The endometrial tissue levels were much higher in the pigs than in the monkeys (an average of 3 129 ng/100 mg vs. 23 1 ng/100 mg tissue). By contrast, the levels of quinacrine in the isthmus of the pigs were much lower than those in the monkeys (26 ng/100 mg vs. 1190 ng/100 mg), as were the levels in the ampulla (45 ng/100 mg vs. 1868 ng/100 mg). This can be interpreted to mean that the quinacrine in the pig has a tendency to concentrate in the uterus rather than the UTJ or the oviduct, whereas the quinacrine in the primate concentrates in the oviduct, causing damage to the UTJ.

From a more positive standpoint, the small amount of quinacrine in the nonreproductive organs is very encouraging. In the primate study described above, much higher amounts of quinacrine were found in the adrenal glands, kidney, and liver 7 days after the administration of a solution of 30 mg quinacrine than was found in the pig (respectively, 285, 2 12, and 120 ng/100 mg vs. 22, 6, and 7 ng/100 mg). Unless species differences exist in this regard, the slow release of quinacrine appears to result in much lower nonreproductive tissue levels of quinacrine than the acute administration of quinacrine and should therefore be less toxic. Since high levels of quinacrine can be released from the slow-release pellets, it can be expected that, in the primate or the woman, the slow release of quinacrine from the pellets should effectively obstruct the UTJ, while causing minimal, if any, side-effects.