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Teratologic and Mutagenic Studies With Intrauterine Quinacrine Hydrochloride

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TERATOLOGY

Since 1969, intrauterine quinacrine has been used clinically in various developing countries as a means of effecting tubal closure. The advantage of this procedure is that it is a nonsurgical method of accomplishing sterilization. Because of the possibility that some patients who receive quinacrine might be pregnant at the time of introduction of the drug into the uterus, it is necessary to consider the fetal outcome of such exposure. The following studies were directed toward determining the embryopathic effect and maternal toxicity of an intrauterine injection of quinacrine on pregnant monkeys and rats.

MONKEYS

Methods

Female cynomolgus monkeys were followed for cyclic bleeding and mated during a limited period (3 days) at Hazelton Texas Primate Center, Alice, Texas. If palpation on two occasions revealed that pregnancy had occurred, the monkeys were shipped to Baltimore at approximately 30 days of pregnancy and were placed in individual cages. Pregnancy was confirmed by our personnel by palpation, and treatment was instituted prior to 50 days of pregnancy. We gave a 1-ml intrauterine injection of either 0.9% saline, 3 mg

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quinacrine HCl, or 30 mg quinacrine HCl (Quinacrine HCl; No. Q 0250FD, F&D lot No. 7-3-2, Sigma Chemical Co., St. Louis, Missouri). While the animal was under ketamine anesthesia, injections were made using a 12-cm 18-gauge blunt-end stainless steel needle inserted through the cervical canal.

Prior to treatment, blood was obtained for blood chemistry and hematology. Blood chemistry determinations included serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), γ -glutamyl transpeptidase, alkaline phosphatase, lactic dehydrogenase (LDH), cholesterol, triglyceride, total protein, albumin, globulin, blood urea nitrogen, creatinine, direct and total bilirubin, uric acid, glucose, iron, magnesium, sodium, potassium, calcium, phosphorus, and chloride. Hematology analyses included leukocytes, neutrophils, lymphocytes, erythrocytes, hematocrit, and hemoglobin. Food was withdrawn 24 hours prior to treatment. Water was available at all times. Following the treatment, monkeys were returned to their cages and observed daily. Additional blood samples were obtained at 7 and 28 days after treatment for blood chemistry and hematology analyses. On day 28 after treatment, the monkey was repalpated to establish the progress of pregnancy. If pregnancy continued, at approximately 120 days' gestation, the fetus was removed surgically and examined.

Results and Discussion

All monkeys received from the Texas Primate Center appeared in good health, although they appeared thin. Also, after arrival they did not eat for several days, possibly due to the new environment. Pregnancy in all animals was confirmed by palpation. Blood chemistry values prior to treatment were compared with those values obtained from nonpregnant cynomolgus monkeys (obtained from Primate Imports, Port Washington, NY). It was noticed that some values were significantly different. For example, the pregnant group of monkeys had higher SGOT (113.7 ± 20.7 vs. 50.6 ± 3.7 IU/liter, $p < 0.01$), LDH (857 ± 136 vs. 404 ± 34 IU/liter, $p < 0.001$), and γ -glutamyl transferase (42.8 ± 4.7 vs. 26.1 ± 2.0 IU/liter, $p < 0.001$), possibly resulting from the stress of the shipping procedure or from pregnancy.

Following injection of intrauterine physiologic saline, all three animals lost their pregnancies either by spontaneous abortion (i.e., passed fetal tissue; No. 191N and No. 17 1 N) or resorption of the fetus (No. 158N), as indicated by failure of the uterus to grow normally when palpated on day 28 after injection (Table 7-1). This was unexpected, because previous studies indicated that intrauterine physiologic saline injection resulted in a high percentage of normal pregnancies in cynomolgus monkeys (9/13, 70%).^{10,11}

All monkeys receiving 3 mg quinacrine resorbed their pregnancies, as indicated by palpation 28 days after injection (see Table 7-1). Following an injection of 30 mg quinacrine one monkey (No. 161N) was found dead in the cage 2 days later. An autopsy disclosed focal peritoneal necrosis and peritonitis, which possibly could have been the result of quinacrine injection but were not believed to be extensive enough to cause death. The possibility that the uterus was perforated and injection made directly into the peritoneal cavity was considered. Because of this possibility, a separate study was instituted to examine the effect of intraperitoneal injection of quinacrine (see Chapter 6). The fetus and placenta showed signs of autolysis indicating that

TABLE 7-1. Summary of Treatment of Each Monkey and Pregnancy Outcome

| ANIMAL NUMBER | INJECTION DOSE | PREGNANCY DAY OF INJECTION | OBSERVATION AT 28 DAYS POST INJECTION | FOLLOW-UP |
|---------------|----------------|----------------------------|---------------------------------------|--|
| 191N | Control | 40 | Uterus enlarged but hard | Passed tissue 30 days after injection |
| 171N | Control | 35 | Aborted on day 27 | High leukocyte count before and after abortion |
| 158N | Control | 45 | Not pregnant | |
| 170N | 3 mg QHCI | 39 | Not pregnant | |
| 200N | 3 mg QHCI | 36 | Not pregnant | |
| 180N | 3 mg QHCI | 46 | Not pregnant | |
| 176N | 30 mg QHCI | 42 | Uterus growing but small | Deformed fetus at pregnancy day 115 obtained by hysterectomy |
| 173N | 30 mg QHCI | 44 | Not pregnant | |
| 161N | 30 mg QHCI | 34 | Day 2-mother dead | |

QHCI, quinacrine hydrochloride

death of the fetus preceded that of the mother. This was most likely a result of the quinacrine injection.

When palpated on day 28 after injection, the fetus of the third monkey receiving 30 mg quinacrine (No. 176N) was "growing but smaller than expected" as described by the primate technician. The fetus was removed on pregnancy day 115 and examined. It was found to have multiple congenital deformities, including craniorhachischisis, scoliosis, epitheliogenesis imperfecta, atelectasis, ankylosis of the left tarsus, multiple skeletal abnormalities in the digits of paws and feet, and patent foramen ovale. It should be noted that the neural tube completes its closure by day 29 of development in monkeys and since the drug was administered on day 42, it is very unlikely that it could have been the causative factor. Defects such as ankylosis can occur at any stage of development; however, lower limb defects are often associated with neural tube defects in humans and thus there is a reasonable chance that the other defects observed were part of a spontaneous multiple congenital defect syndrome. The incidence of spontaneously occurring grossly observable abnormalities in newborn rhesus monkeys is approximately 1%.²⁸ Comparable data for cynomolgus monkeys are not available.

Some hematologic changes occurred following treatment. Since there were few animals in any one group, all pretreatment data were pooled, regardless of the treatment group to which the monkeys were assigned. This value was compared with all other data points by a t-test. The only differences that could be attributed to quinacrine were a decline in erythrocytes on day 7 for animals receiving 3 mg ($4\ 120 \pm 100 \times 10^3$ cells/cu mm) or 30 mg ($4575 \pm 195 \times 10^3$ cells/cu mm) compared with the initial value ($5688 \pm 337 \times 10^3$ cells/cu mm). Compared with pretreatment levels, there was a significantly lower hematocrit (22.8 ± 1.2 vs. $36.8 \pm 1.8\%$, $p < 0.001$) and hemoglobin concentration (6.4 ± 0.7 vs. 10.2 ± 0.5 gm/dl, $p < 0.01$) in monkeys 7 days

after treatment with 3 mg quinacrine, but levels were normal 28 days after treatment. None of these effects was dose related.

Similar analyses were made with blood chemistries. Total protein was significantly less in monkeys treated with 3 mg quinacrine, compared with pre-treatment concentrations (6.4 ± 0.2 vs. 7.3 ± 0.1 gm/dl, $p < 0.01$). By day 28, this value had returned to normal.

There was a slight, but significant ($p < 0.05$) elevation in SGPT at day 7 in monkeys receiving either dose level of quinacrine.

RATS

Methods

Virgin female Sprague-Dawley rats were bred by Charles River Breeding Labs, Wilmington, Massachusetts, or Harlan-Sprague Dawley of Indianapolis, Indiana. In each of the seven treatment groups, approximately 70% of the rats were obtained from Harlan-Sprague Dawley. The day on which a sperm-positive specimen was obtained was designated as day 0 of gestation. The animals were housed in a constant temperature and humidity room and were fed Charles River RMH-1000 formula. Water was provided *ad libitum* from an automatic system. Cages were lined with "Sani-Chips" bedding. The pregnant dams were weighed on arrival at our facility (day 6 of gestation), on the day of treatment, on gestation day 15, and on the day of necropsy (day 19).

Quinacrine hydrochloride (QHCl) was obtained from the same lot used in the study with monkeys. Quinacrine was dissolved in 0.2M sodium phosphate buffer, pH 7.4. Because of limited solubility, the highest dose given in the maximum injectable 0.1-ml volume was 4.0 mg. The low dose was 0.4 mg in a 0.1-ml volume.

Laparotomy was performed with the rat under ether anesthesia. The number of visible implantations in each horn was recorded. Intrauterine injections were made with a Hamilton syringe (0.1-ml capacity). In all treatment groups, one horn of each animal served as a control, receiving only the buffer vehicle. Those rats treated early in gestation (day 8) received a single 0.1-ml injection of quinacrine at the cervical end of the uterine horn. The contralateral horn received a single 0.1-ml injection of the buffer. Those rats treated later in gestation (day 12) received the drug in two 0.05-ml injections, one at the cervical end of the horn and the other at the ovarian end. The contralateral horn was treated similarly with buffer. The incision was sutured and the skin was closed with "autoclips." A buffer-sham treatment group had one horn treated with the buffer (0.1 ml) and the contralateral horn subjected to sham injections (insertion of the needle only).

To provide a positive control treatment group, pregnant rats were treated with 4-nitroquinoline-N-oxide (4NQNO; K&K Labs, Plainview, NY) at a dosage of 50 mg/kg by intrauterine injection on day 12 of gestation. The contralateral horn received 0.1 ml of the vehicle dimethyl sulfoxide (DMSO; Fisher Scientific Co, Silver Spring, MD).

All animals were killed by decapitation on day 19 of gestation. The fetuses were delivered by laparotomy/hysterotomy, measured (crown to rump), weighed, and examined for external anomalies. Approximately one of every three fetuses was fixed in Bouin's fluid (Allen's Modification B- 15) for visceral

examination by Wilson's technique. The remaining fetuses were cleared by glycerin-potassium hydroxide and stained with alizarin red S for skeletal study. Uteri were examined for resorption sites. Ovaries were examined for number of corpora lutea. Each uterine horn with attached ovary was fixed in 10% neutral buffered formalin.

The occurrence of resorptions, fetal death, and skeletal and visceral anomalies in specific treatment groups was examined by χ^2 analysis using the Yates correction.

Results and Discussion

Resorption. QHCl caused dose-related resorptions when given as a direct intrauterine injection on either day 8 or day 12 of gestation (Tables 7-2 and 7-3). Because there was an unequal number of implantations in the treated

TABLE 7-2. Teratogenicity Evaluation of Quinacrine Hydrochloride (QHCl) Following Intrauterine Injection on Day 8 in Rats

| | TREATMENT | | | | | |
|-----------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| | BUFFER | SHAM | BUFFER | QHCl 0.4 MG | BUFFER | QHCl 4 MG |
| Number of uterine horns evaluated | 20 | 20 | 20 | 20 | 20 | 20 |
| Implantations | | | | | | |
| Total number | 126 | 106 | 108 | 114 | 103 | 137 |
| Mean (\pm SE)/horn | 6.3 \pm 0.3 | 5.3 \pm 0.4 | 5.4 \pm 0.4 | 5.7 \pm 0.4 | 5.2 \pm 0.2 | 6.9 \pm 0.5 |
| | (p < 0.05) | | | | (p < 0.01) | |
| Resorptions | | | | | | |
| Total number | 19 | 14 | 20 | 35 | 19 | 71 |
| Mean (\pm SE)/horn | 1.0 \pm 0.2 | 0.7 \pm 0.2 | 1.0 \pm 1.9 | 1.8 \pm 0.3 | 1.0 \pm 0.2 | 3.55 \pm 0.4 |
| Percent/horn† | 15.0 \pm 2.4 | 12.6 \pm 3.8 | 18.4 \pm 3.3 | 31.2 \pm 5.3 | 18.4 \pm 3.9 | 51.8 \pm 5.2 |
| | | | (p < 0.05) | | (p < 0.001) | |
| Viable fetuses | | | | | | |
| Total number | 107 | 92 | 87 | 78 | 84 | 65 |
| Mean (\pm SE)/horn | 5.4 \pm 0.3 | 4.6 \pm 0.4 | 4.4 \pm 0.4 | 3.9 \pm 0.4 | 4.2 \pm 0.3 | 3.3 \pm 0.4 |
| Percent/horn* | 85.0 \pm 2.4 | 87.4 \pm 3.8 | 80.6 \pm 5.2 | 68.0 \pm 5.2 | 81.6 \pm 3.9 | 47.6 \pm 5.2 |
| | | | | | (p < 0.001) | |
| Malformations | | | | | | |
| Total number of malformed fetuses | 6 | 5 | 8 | 6 | 1 | 2 |
| Percent malformed/horn§ | 6.0 \pm 3.3 | 6.3 \pm 2.9 | 10.3 \pm 4.5 | 5.9 \pm 2.8 | 1.0 \pm 1.0 | 6.0 \pm 5.0 |

*Injections made directly into uteri of anesthetized rats. For each rat, one uterine horn was treated with buffer and the other with buffer plus QHCl. Sham treatment involved insertion of the needle but no injection.

†Percent resorptions = number of resorptions/number of implantations x 100.

‡Percent viable = number of living/number of implantations x 100.

§Percent malformed = number of fetuses malformed/number viable x 100.

TABLE 7-3. Teratogenicity Evaluation of Quinacrine Hydrochloride (QHCl) Following Intrauterine Injection on Day 12 in Rats

| | TREATMENT* | | | | | | | |
|-----------------------------------|------------|------------|------------|----------------|-------------|-------------|-------------|------------------|
| | BUFFER | SHAM | BUFFER | QHCl 0.4 MG | BUFFER | QHCl 4MG | DMSO | 4NQNO 1.25 MG |
| Number of uterine horns evaluated | 19 | 19 | 18 | 18 | 19 | 19 | 19 | 19 |
| Implantations | | | | | | | | |
| Total number | 127 | 97 | 87 | 120 | 93 | 125 | 97 | 114 |
| Mean (± SE)/horn | 6.7 ± 0.3 | 5.1 ± 0.4 | 4.8 ± 0.4 | 6.7 ± 0.3 | 4.9 ± 0.4 | 6.6 ± 0.4 | 5.1 ± 0.5 | 6.0 ± 0.4 |
| | (p < 0.01) | | (p < 0.01) | | (p < 0.01) | | | |
| Resorptions | | | | | | | | |
| Total number | 18 | 22 | 18 | 34 | 28 | 86 | 48 | 92 |
| Mean (± SE)/horn | 1.0 ± 0.2 | 1.2 ± 0.3 | 1.0 ± 0.3 | 1.9 ± 0.4 | 1.5 ± 0.3 | 4.5 ± 0.7 | 2.5 ± 0.5 | 4.8 ± 0.3 |
| | | | | | (p < 0.001) | | (p < 0.001) | |
| Percent/horn† | 14.9 ± 3.3 | 22.5 ± 6.0 | 23.3 ± 6.0 | 27.0 ± 0.5 | 27.6 ± 4.4 | 65.5 ± 8.3 | 45.0 ± 7.9 | 85.0 ± 4.9 |
| | | | | | (p < 0.001) | | (p < 0.001) | |
| Viable fetuses | | | | | | | | |
| Total number | 109 | 75 | 69 | 86 | 64 | 39 | 49 | 22 |
| Mean (± SE)/horn | 5.7 ± 0.4 | 3.9 ± 0.5 | 3.8 ± 0.5 | 4.8 ± 0.3 | 3.4 ± 0.3 | 2.1 ± 0.5 | 2.6 ± 0.4 | 1.2 ± 0.4 |
| | (p < 0.01) | | | | (p < 0.05) | | (p < 0.05) | |
| Percent/horn+ | 85.1 ± 3.3 | 77.5 ± 6.0 | 76.7 ± 6.0 | 73.0 ± 5.0 | 71.3 ± 4.4 | 34.4 ± 8.3 | 55.0 ± 7.9 | 15.0 ± 4.9 |
| | | | | | (p < 0.001) | | (p < 0.001) | |
| Malformations | | | | | | | | |
| Total number of malformed fetuses | 10 | 7 | 4 | 9 | 2 | 1 | 1 | 4 |
| Percent malformed/horn§ | 9.9 ± 4.1 | 6.8 ± 3.3 | 5.5 ± 3.0 | 9.1 ± 3.3 | 2.6 ± 1.8 | 1.3 ± 1.3 | 5.3 ± 5.3 | 10.0 ± 5.8 |

*Injections made directly into uteri of anesthetized rats. For each rat, one uterine horn was treated with buffer and the other with buffer plus QHCl or sham; except for 4NQNO, which was dissolved in DMSO.

†Percent resorptions = number of resorptions/number of implantations x 100.

#Percent viable = number of living/number of implantations x 100.

§Percent malformed = number of fetuses malformed/number viable x 100.

DMSO, dimethyl sulfoxide; 4NQNO, 4-nitroquinoline-N-oxide.

and control horns in most treatment groups, it was necessary to analyze these data as absolute values and also after normalizing the data by expressing as a percentage of implantations observed at the time of the treatment. A significant effect was observed in the high-dose groups when calculated either way. Susceptibility to QHCl-induced resorption appeared to be somewhat greater in early pregnancy since on day 8, the low dose (0.4 mg) caused a significant effect, whereas on day 12 this dose was ineffective. The magnitude of the resorption effect caused by the high dose of QHCl (4 mg) was similar on day 8 and day 12, taking into account the differences in control (buffer-induced) resorption rates at these gestational ages; the effect attributable to the drug was approximately 34% and 38% for day 8 and day 12 treatments, respectively. Injection of the buffer vehicle had no significant effect, since the resorption rate was not different between buffer and sham-treated (i.e., insertion of needle without delivery of any liquid) groups at either stage of gestation.

Because 4NQN0 is known to be a direct-acting mutagen (as is quinacrine), it was used as a positive control.¹⁹ An intrauterine dose of 1.25 mg given on gestation day 12 had a marked resorption effect (see Table 7-3). DMSO was the vehicle used for 4NQN0, and it also appeared to have a significant resorption effect, although not enough to account for the entire effect observed with DMSO and 4NQN0.

Malformations. A low rate of malformation, less than 10% of viable fetuses, was observed in control (buffer, DMSO, sham) groups (Tables 7-2 and 7-3). There was no observed increase in this overall malformation rate in any of the groups treated with QHCl or 4NQN0.

In order to be certain that a teratogenic effect of a specific type had not been obscured by grouping all malformed fetuses, we also analyzed the results according to separate classification by type of malformation (skeletal, Table 7-4; visceral, Table 7-5). No evidence of treatment-related malformations emerged from this analysis. Some malformations are not listed in these tabulations because they were observed only in control groups, perhaps because of their greater number of fetuses.

The malformation data are presented in a manner appropriate for evaluating the possible occurrence of multiple malformation syndromes in treatment groups in Table 7-6. This analysis also failed to identify a teratogenic effect of either quinacrine or 4NQN0.

This study has demonstrated that intrauterine administration of solutions of QHCl to pregnant rats during the period of embryogenesis results in a marked and dose-related resorption effect. The potency (i.e., dose required to produce a significant effect) of quinacrine for inducing resorptions appeared to be greater in early embryogenesis, whereas the efficacy of the drug (magnitude of effect at a maximal dose) was similar in early and late embryogenesis. There was no evidence of any malformations associated with quinacrine treatment. All viable fetuses were carefully examined for gross evidence of malformations. Skeletal and visceral examinations were performed on two thirds and one third, respectively. Therefore, the drug appears to have no teratogenic (i.e., malformation-inducing) effect. Likewise, the positive control, 4NQN0, did not have a teratogenic effect, although like quinacrine, it did induce resorptions.

TABLE 7-4. Results of Skeletal Examinations: Specific Malformations

| TREATMENT | NUMBER OF VIABLE FETUSES EXAMINED | NO STERNABRAE | INCOMPLETE SKULL BONES | METATARSALS NOT OSSIFIED | METACARPALS NOT OSSIFIED | 14 PAIRS OF RIBS | ADACTYLE |
|--------------|--|------------------|------------------------------|-----------------------------|-----------------------------|---------------------|----------|
| | | | | | | | |
| Control | | | | | | | |
| Sham | 112 | 7 (6.3%)* | 1 (0.9%) | 2 (0.6%) | 1 (0.3%) | 1 (0.3%) | 1 (0.9%) |
| Buffer | 353 | 25 (7.1%) | 4 (1.1%) | 2 (0.6%) | 1 (0.3%) | 1 (0.3%) | 1 (0.3%) |
| Total | 465 | 32 (6.9%) | 5 (1.1%) | 2 (0.4%) | 1 (0.2%) | 2 (0.4%) | 2 (0.4%) |
| QHCl | | | | | | | |
| Day 8 | | | | | | | |
| 0.4 mg | 50 | 5 (10.0%) | 2 (4.0%) | 1 (2.0%) | 1 (2.0%) | 1 (2.0%) | 1 (2.0%) |
| 4.0 mg | 44 | 1 (2.3%) | | | | | |
| Total day 8 | 94 | 6 (6.4%) | 2 (2.1%) | 1 (1.1%) | 1 (1.1%) | 1 (1.1%) | 1 (1.1%) |
| Day 12 | | | | | | | |
| 0.4 mg | 58 | 4 (6.9%) | 1 (1.7%) | 1 (1.7%) | 1 (1.7%) | 1 (1.7%) | 1 (1.7%) |
| 4.0 mg | 26 | 1 (3.9%) | | | | | |
| Total day 12 | 84 | 5 (6.0%) | 1 (1.2%) | 1 (1.2%) | 1 (1.2%) | 1 (1.2%) | 1 (1.2%) |
| Total | 178 | 11 (6.2%) | 3 (1.7%) | 2 (1.1%) | 1 (0.6%) | 2 (1.1%) | 2 (1.1%) |
| DMSO control | 32 | 1 (3.1%) | 1 (3.1%) | 1 (3.1%) | | | |
| 4NQNO | 16 | 2 (12.5%) | 1 (6.3%) | 1 (6.3%) | 1 (6.3%) | 1 (6.3%) | 1 (6.3%) |

*Numbers in parentheses are the percentage of number examined with abnormality.

TABLE 7-5. Results of Visceral Examinations: Specific Malformations

| TREATMENT | NUMBER OF VIABLE FETUSES EXAMINED | CLEFT PALATE | CAVITY IN ADRENAL | MISSING ONE KIDNEY, OVARY, AND UTERINE HORN | HYDRO-NEPHROSIS | ANENCEPHALY |
|--------------|-----------------------------------|--------------|-------------------|---|-----------------|-------------|
| Control | | | | | | |
| Sham | 55 | | 1 (1.8%) | | 2 (3.6%) | |
| Buffer | 167 | | | | | |
| Total | 222 | | 1 (0.5%) | | 2 (0.9%) | |
| QHCl | | | | | | |
| Day 8 | | | | | | |
| 0.4 mg | 28 | | | | 1 (3.6%) | 1 (3.6%)* |
| 4.0 mg | 21 | | | | 2 (9.5%) | |
| Total day 8 | 49 | | | | 3 (6.1%) | 1 (2.0%) |
| Day 12 | | | | | | |
| 0.4 mg | 28 | | | 1 (3.6%) | 2 (7.1%) | |
| 4.0 mg | 13 | | | | | |
| Total day 12 | 41 | | | 1 (2.4%) | 2 (4.9%) | |
| Total | 90 | | | 1 (1.1%) | 5 (5.6%) | 1 (1.1%) |
| DMSO | 17 | | | | | |
| 4NQNO | 6 | | | 1 (16.7%) | | |

*Only fetus from the pregnancy, no corresponding control hom. (%), percent malformed/number examined.

TABLE 7-6. Results of Skeletal Examinations: All Malformations

| TREATMENT | NUMBER OF VIABLE FETUSES EXAMINED | NUMBER OF MALFORMATIONS | | | | |
|--------------|-----------------------------------|-------------------------|-----------|-----------|-----------|-----------|
| | | 1 OR MORE | 2 OR MORE | 3 OR MORE | 4 OR MORE | 5 OR MORE |
| Control | | | | | | |
| Sham | 112 | 9 (8.0%)* | 1 (0.9%) | | | |
| Buffer | 353 | 29 (8.2%) | 7 (2.0%) | 2 (0.6%) | 1 (0.3%) | |
| Total | 465 | 38 (8.2%) | 8 (1.7%) | 2 (0.4%) | 1 (0.2%) | |
| QHCl | | | | | | |
| Day 8 | | | | | | |
| 0.4 mg | 50 | 6 (12.0%) | 2 (4.0%) | 2 (4.0%) | 1 (2.0%) | |
| 4.0 mg | 44 | 1 (2.3%) | | | | |
| Total day 6 | 94 | 7 (7.5%) | 2 (2.1%) | 2 (2.1%) | 1 (1.1%) | |
| Day 12 | | | | | | |
| 0.4 mg | 58 | 6 (10.3%) | 1 (1.7%) | | | |
| 4.0 mg | 26 | 1 (3.9%) | | | | |
| Total day 12 | 84 | 7 (8.3%) | 1 (1.2%) | | | |
| Total | 178 | 14 (7.9%) | 3 (1.7%) | 2 (1.1%) | 1 (0.6%) | |
| DMSO | 32 | 1 (3.1%) | 1 (3.1%) | 1 (3.1%) | 1 (3.1%) | 1 (3.1%) |
| 4NQNO | 16 | 3 (18.8%) | 1 (6.3%) | 1 (6.3%) | 1 (6.3%) | |

*Numbers in parentheses are the percentage of number examined with abnormality.

MUTAGENICITY

BACTERIA

Materials and Methods

The following compounds (obtained from Sigma Chemical Co., St. Louis, MO) were tested in the *Salmonella*/mammalian-microsome mutagenicity assay: acridine, chloroquine diphosphate, primaquine diphosphate, and quinacrine hydrochloride. Five dose levels (1,000 μg , 100 μg , 10 μg , 1 μg , and 0.1 μg /plate) were assayed with and without exogenous metabolic activation. Higher doses of chloroquine (10,000 μg and 2,500 μg /plate) were also tested to achieve the maximum noninhibitory dose. All test compounds except chloroquine diphosphate were dissolved in DMSO (Fisher Scientific, Fair Lawn, NJ). Chloroquine was dissolved in double distilled water and then filter-sterilized using a 0.22 μm disposable unit (Millipore Corp, Bedford, MA). All dilutions of test drugs were prepared immediately prior to use. Purified sodium azide (NaN_3) (Fisher Scientific, Fair Lawn, NJ) and 4NQNO (K&K Labs, Plainview, NY) were used as direct-acting mutagens. Benzo(a)pyrene (BP; Aldrich Chemical Co., Milwaukee, WI) and 2-anthramine (Sigma Chemical Co., St. Louis, MO) were used as positive controls requiring metabolic activation.

The *Salmonella typhimurium* strains used (TA 98, TA 100, TA 1535, TA 1537, and TA 1538) were provided by Dr. Bruce N. Ames, University of California, Berkeley. On receipt of the strains, the characteristic markers of each were checked as outlined by Ames and associates.⁴ Overnight cultures

were prepared from frozen (-70°C) stocks by incubation with 2.5% Oxoid nutrient broth (KC Biologicals, Inc, Lenexa, Kansas) for 16 to 17 hours at 37°C .

Rat liver S9 was used as a source of exogenous metabolic activation. Livers were obtained aseptically from adult male Sprague-Dawley rats, 5 days after intraperitoneal treatment with Aroclor 1254 (Monsanto Chemical Co, St. Louis, MO) at a dosage of 250 mg/kg. The excised tissue was homogenized at 4°C with three volumes of 0.15M KCl using a Brinkmann Polytron. The homogenate was centrifuged at $9000 \times g$ for 15 minutes at 4°C . The supernatant fraction (S9) was stored in a -70°C Revco freezer. Protein content was determined by a modified Lowry procedure.²² The cofactor mixture contained per milliliter: MgCl_2 (8 μmole), KCl (33 μmole), glucose-6-phosphate (5 μmole), NADP (4 μmole), and sodium phosphate, *pH* 7.4 (100 μmole). The S9 was diluted with 0.15M KCl so that when added to the cofactor, the final concentration of S9 protein was 1.0 mg per milliliter of S9-cofactor mix.

The assays were performed as suggested by Ames and associates using Vogel-Bonner Medium E-minimal glucose agar plates." Briefly, 0.1 ml of the overnight broth culture, containing approximately 10^8 organisms, was added to 2 ml molten top agar supplemented with 0.5% NaCl, 15 μg histidine, and 22 μg biotin. The appropriate dose of test compound, solvent, or positive control in a 0.1 ml volume was then added. For metabolic activation assays, the molten agar received 0.5 ml of the rat liver S9-cofactor mix. This molten agar overlay was vortex-mixed after each addition and then poured over the base plate. Hardened plates were inverted and incubated at 37°C for 48 to 60 hours. Colonies were counted by direct inspection using a colony counter (Fisher Scientific, Fair Lawn, NJ). Results are expressed as the ratio of the number of revertant colonies in the presence of test compound to the average number of revertant colonies in the absence of test compound (spontaneous revertants).

Results and Discussion

The results of *Salmonella* mutagenicity tests with acridine, chloroquine, primaquine, and quinacrine are shown in Table 7-7. Acridine, chloroquine, and primaquine had no detectable mutagenic activity with or without a rat liver metabolizing system. Quinacrine was clearly mutagenic to TA 1537, a frameshift test strain; the mutagenic activity appeared to be slightly enhanced by rat liver activation. Acridine, chloroquine, and primaquine are chemically related to quinacrine, and the latter two compounds are commonly used antimalarial agents. A review of published studies on the mutagenic activity of quinacrine is presented in Table 7-8. These results provide evidence of significant genetic toxicity of quinacrine, particularly in bacterial systems. Quinacrine is known to intercalate in deoxyribonucleic acid, and this property is utilized in many karyotyping laboratories for banding of chromosomes. Mammalian mutagenicity tests did not show a high level of activity, and conflicting results appear in the literature. Our unpublished study of karyotypes of lymphocytes from quinacrine-treated monkeys failed to demonstrate any chromosomal abnormalities (see below).

In summary, quinacrine is a direct-acting frameshift mutagen in bacterial systems. Its genotoxic potential in mammalian cells is equivocal. The lack of

TABLE 7-7. *Salmonella typhimurium* Mutagenicity Tests: Ratio of Revertants With Test Compound to Spontaneous Revertants

| COMPOUND | µG/ PLATE | TA98 | | TA 100 | | TA 1535 | | TA 1537 | | TA 1538 | |
|--------------|--------------|---------------|---------------|---------------|---------------|--------------|--------------|---------------|---------------|---------------|---------------|
| | | NA* | S9† | NA | S9 | NA | S9 | NA | S9 | NA | S9 |
| Acridine | 1,000 | Toxic | Toxic | Toxic | Toxic | Toxic | Toxic | Toxic | Toxic | Toxic | Toxic |
| | 100 | 0.38 | 0.55 | 0.53 | 0.69 | 1.23 | 0.89 | 1.26 | 1.00 | 1.00 | 1.14 |
| | 10 | 0.57 | 0.83 | 0.92 | 0.79 | 1.23 | 0.89 | 0.85 | 1.00 | 0.71 | 1.00 |
| | 1 | 0.74 | 0.83 | 1.05 | 0.91 | 0.82 | 1.04 | 1.00 | 1.00 | 0.81 | 0.71 |
| | 0.1 | 0.74 | 0.62 | 1.05 | 0.82 | 0.86 | 1.14 | 0.74 | 1.00 | 0.62 | 1.07 |
| Chloroquine | 10,000 | 0.29 | 0.48 | 0.34 | 0.66 | 0.52 | 0.65 | Toxic | 1.18 | Toxic | 0.61 |
| | 2,500 | 0.82 | 0.66 | 0.96 | 0.96 | 1.16 | 0.59 | 0.64 | 1.12 | 0.78 | 1.04 |
| | 100 | 0.92 | 0.83 | 0.88 | 0.94 | ND | ND | 1.50 | 1.00 | 0.85 | 1.00 |
| | 10 | 1.04 | 0.93 | 0.85 | 0.94 | ND | ND | 0.50 | 0.67 | 1.08 | 1.13 |
| | 1 | 0.88 | 1.10 | 1.02 | 0.91 | ND | ND | 0.63 | 0.58 | 0.92 | 0.93 |
| Primaquine | 0.1 | 0.92 | 0.90 | 1.00 | 0.99 | ND | ND | 1.00 | 0.75 | 0.92 | 1.13 |
| | 1,000 | Toxic | 0.40 | Toxic | 0.41 | Toxic | 0.68 | Toxic | 1.18 | Toxic | Toxic |
| | 100 | 1.14 | 0.86 | 0.98 | 1.10 | 0.64 | 0.89 | 1.00 | 0.73 | 0.71 | 1.21 |
| | 10 | 1.14 | 0.74 | 0.76 | 0.88 | 0.82 | 0.71 | 0.67 | 0.91 | 1.00 | 0.86 |
| | 1 | 0.95 | 0.64 | 0.76 | 1.06 | 1.00 | 1.29 | 0.44 | 0.64 | 1.29 | 1.21 |
| Quinacrine | 0.1 | 0.91 | 0.74 | 0.66 | 1.03 | 1.50 | 0.71 | 0.56 | 0.55 | 0.64 | 1.07 |
| | 1,000 | Toxic | 0.86 | Toxic | 1.07 | Toxic | Toxic | TNTC | Toxic | 0.71 | 1.74 |
| | 100 | 0.83 | 0.84 | 0.76 | 1.07 | 1.27 | 0.82 | 6.48† | 11.42† | 1.29 | 0.61 |
| | 10 | 0.57 | 1.06 | 0.90 | 0.93 | 1.23 | 0.96 | 0.67 | 1.00 | 0.67 | 1.03 |
| | 1 | 0.38 | 0.86 | 0.88 | 0.87 | 1.27 | 0.82 | 0.67 | 0.74 | 0.81 | 1.03 |
| 4NQNO | 0.1 | 0.38 | 0.83 | 0.84 | 0.83 | 1.00 | 0.64 | 0.67 | 0.89 | 0.67 | 0.97 |
| | 0.5 | 13.17† | | 17.95† | | ND | | ND | | 23.67† | |
| | 100 | ND | | ND | | ND | | 11.80† | | ND | |
| | 1 | ND | | ND | | ND | | ND | | ND | |
| | BP | 5 | | 5.96† | | 6.72† | | ND | | 4.22† | |
| 2-anthramine | 2.5 | | 67.50† | | 28.60† | | 2.21† | | 26.21† | | 63.29† |
| | 1.0 | | ND | | ND | | 6.83† | | ND | | ND |

*NA, no addition

†S9 = 9000 x g supernatant fraction of rat liver homogenates (Aroclor pretreated).

*Positive mutagenic response.

ND, not determined; TNTC, too numerous to count.

TABLE 7-8. Mutagenicity Tests With Quinacrine

| TEST SYSTEM | DRUG | METHOD | RESULTS | REFERENCE |
|--|--|--|---|-----------|
| Bacterial tests | | | | |
| <i>Salmonella typhimurium</i> C207 (frameshift) | Quinacrine 5µg/plate | Ames test | "Weak mutagen" (no data provided) | 3 |
| <i>S. typhimurium</i> TA 1537 (frameshift) | Quinacrine 1 00µg/plate± S9 | Ames test | control 12; control + S9 12 drug 1000; drug + S9 1000 | 19 |
| <i>S. typhimurium</i> his G46 TA 1530, 1531, 1532, 1534 | Quinacrine diHCl | Ames test | positive in TA 1532 only | 28 |
| <i>Bacillus subtilis</i> his- his 8103, B201, 8204 | Quinacrine HCl crystal or solution spot test | Reversion to his+ | Positive with his 8103 | 13 |
| <i>Escherichia coli</i> K-12 strain ND 160 lac ⁻ | Quinacrine HCl as positive control 1 0µg/ml medium | Score lac ⁺ revertants | Positive | 9 |
| <i>E. coli</i> B/r strain ± deficient repair, trp ⁻ | Quinacrine HCl 1 0µg/ml medium 20µg/ml medium | Drug incorporated in postirradiation medium score str ^r mutants, score trp ⁺ mutants | Positive enhancement of U-V induced str ^r mutational yield in strains with intact repair similar results with trp ⁺ yield | 8 |
| <i>S. typhimurium</i> TA 1535, 1537, 98, 100 ± S9 | Quinacrine diHCl 4 conc. but ams not listed | Ames test | Positive, but not listed which strain, concentration, etc. | 15 |
| <i>S. typhimurium</i> gal E 503 | Quinacrine diHCl | Screen for mutagens that cause deletions | Incapable of generating such deletions | 2 |
| <i>S. typhimurium</i> G46 mouse host BP substitution | Quinacrine | <i>In vitro</i> spot test for his ⁺ revertants <i>In vivo</i> host mediated assay plate peritoneal fluid | Negative in both tests | 20 |

TABLE 7-8. Mutagenicity Tests With Quinacrine (Continued)

| TEST SYSTEM | DRUG | METHOD | RESULTS | REFERENCE |
|--|---|--|---|-----------|
| <i>S. typhimurium</i> G46 base pair C3076 frameshift | Quinacrine | Spot test with saturated disc | Positive with frameshift Negative with base pair | 6 |
| Mammalian (nonhuman) Rat | Quinacrine diHCl 160 mg/ kg | Dominant lethal test | Equivocal, too few animals | 12 |
| Mouse sperm | Quinacrine diHCl | Score sperm head abnormalities | Negative | 15 |
| Mouse polychromatic erythrocytes of bone marrow | Quinacrine diHCl | Micronucleus assay for chromosome breakage | Negative? | 15 |
| Mouse bone marrow erythrocytes | Quinacrine diHCl 0.28 mg/ kgb.wt. 1P | Micronuclei | Positive: increase of micronucleated erythrocytes (up to 65% increase) | 17 |
| Mouse bone marrow erythrocytes | Quinacrine diHCl 35, 70, 95, 142 mg/kg | Micronuclei | Negative: disputes reference 17 | 21 |
| Chinese hamster ovary cells | Quinacrine diHCl 5µg/ml medium | Determining cell survival after preirradiation and postirradiation to drug | Sensitized cells to killing by x-rays; greater effect with preirradiation exposure to drug may inhibit DNA repair | 26 |
| Chinese hamster ovary cells | Quinacrine diHCl 0.2µM, 2µM, 20µM | Score chromosome breaks in metaphase Score abnormal anaphases | Frequency of chromosome breakage 3 to 5 times that of control (not considered high) | 16 |

| | | | | |
|---------------------------------------|---|--|--|----|
| Mouse leukemic cells L 1210 | Quinacrine HCl 0.2μg/ml medium | Score ara C ^s → ara C ^r | Frequency reduced 5- to 16-fold "Antimutagenic" | 5 |
| Rat sarcoma cells (Jensen) asparagine | Quinacrine HCl 0.01 μg, 0.1 μg, 1.0μg, 5.0μg/ml | Score asp- → asp+ cells | Ineffective in increasing or decreasing mutation frequency | 24 |
| Miscellaneous systems | | | | |
| Silkworm pupae | Quinacrine HCl 0.25 mg/capita 0.50 mg | Score mutations by specific locus method | 0.5-mg dose increased number of mosaic type mutations in females only | 23 |
| <i>Saccharomyces cerevisiae</i> | Quinacrine | Auxotrophic → prototrophic revertants drug-saturated disc placed in center of plate, score ring of mutants | Negative with both strains | 6 |
| Human cell tests | | | | |
| Human sternal marrow cells (D-98) | Quinacrine HCl 0.06μg/ml | Score azaguanine resistant mutants | Cultures with drug contained 1/2-1/3 the mean of mutants found in control cultures | 18 |
| Human peripheral leukocytes | Quinacrine HCl 0.3 g for 14 days | Treated for protozoan infection karyotyped three times pretreatment and posttreatment | No abnormalities prior to treatment After treatment modal no. of 48 chromosomes, abnormal marker chromosomes | |

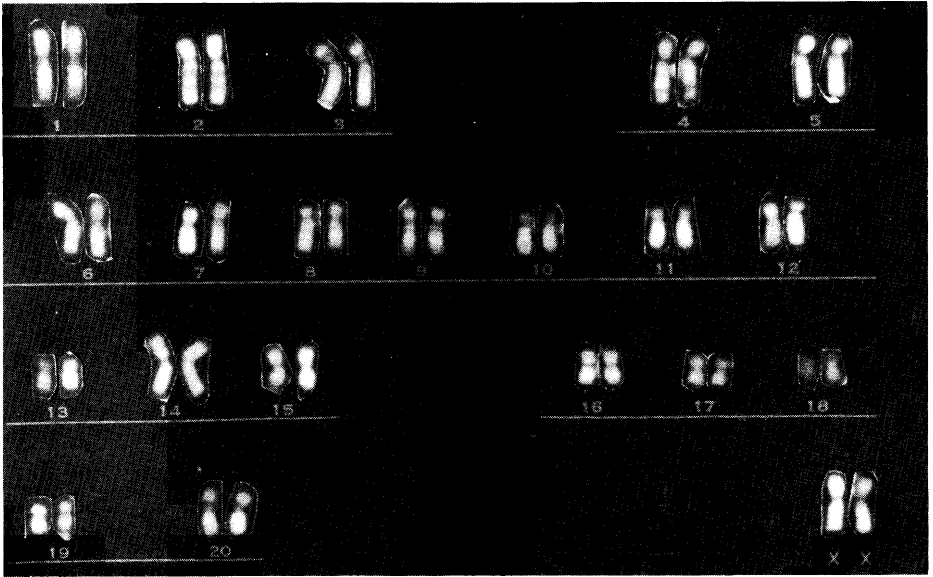


FIG. 7-1. Karyotype of *Macaca fascicularis* arranged according to standard nomenclature.

mutagenic activity (in a bacterial system) for two chemically related antimalarial drugs (chloroquine and primaquine) suggests that the tubal occlusive properties of nonmutagenic analogs of quinacrine may be a productive area for further research.

MONKEY CHROMOSOME ANALYSIS

Peripheral blood samples were taken from a total of 15 monkeys before treatment and at varying times thereafter, ranging from 24 hours to 28 days. Nine received intrauterine quinacrine HCl (30 mg), three received intravenous quinacrine HCl (30 mg), and three received intrauterine saline. Chromosomes were prepared from cultured lymphocytes according to standard human cytogenetic procedures,²⁵ and slides were prepared for fluorescent banding.⁷ For each analysis, 20 banded metaphase spreads were examined and a karyotype was prepared. In every case, the modal chromosome number was found to be 42. No apparent aneuploidy or structural rearrangements were observed for any monkey, regardless of treatment. Chromosome breaks and chromatid gaps were not seen. A typical karyotype for the female cynomolgus monkey is included (Fig. 7-1).

SUMMARY AND CONCLUSIONS

The purpose of these studies was to evaluate the embryopathic potential of quinacrine in the event that the drug is inadvertently administered to a pregnant patient. Such studies in laboratory animals suffer from two major limitations: (1) uncertain suitability as a model for the human and (2) statistical, particularly type II, error (i.e., concluding that no differences due to treat-

ment exist, when in fact they do). Such an error might easily occur in extrapolation of results from a relatively few animal subjects to a much larger population of human patients. We attempted to address these limitations by including two species, the monkey and the rat. We reasoned that although a statistically significant number of rats could be studied, it would be useful to include teratologic testing of a primate species even though the numbers of treated animals would be statistically insignificant. Moreover, although it was difficult to accomplish, we thought it was necessary to use the intrauterine route of treatment in monkeys as well as in rats in order for the study to be relevant to the human circumstance.

The results of the studies in rats clearly demonstrate the embryotoxic potential of quinacrine, manifested as dose-related resorptions. It is important to note, however, that quinacrine treatment did not cause malformations. Thus, strictly speaking, quinacrine is not a teratogen in rats (i.e., it does not cause malformed fetuses, although it is quite embryo-lethal). It is possible, of course, that a much larger study would have demonstrated a low incidence of quinacrine-induced malformations. The sample size (20 pregnant rats per treatment group) was chosen because it is the conventional size for such studies.

Interpretation of the monkey teratologic results is more difficult, primarily because of the small number of animals (three) per treatment group. This is further complicated by the unanticipated result with the control group, where two pregnancies aborted and one resorbed. Our previous experience with intrauterine injections of 0.9% saline in cynomolgus monkeys at this stage of pregnancy suggested a 70% pregnancy continuation rate. However, two of the three control fetuses were aborted (passed tissue) rather than resorbed. This is in contrast to the quinacrine-treated monkeys, in which fetal resorptions occurred but not abortions. Thus, the resorption effect of quinacrine observed in rats also appeared in monkeys. The grossly malformed fetus appeared to be related to factors other than quinacrine, since the type of malformations suggested that they occurred prior to the treatment.

The mutagenicity studies were performed primarily to corroborate what was already published for quinacrine (i.e., it is a direct-acting frameshift mutagen in bacterial systems). As noted in the review of the literature, quinacrine has much less mutagenic activity in eukaryotic cells and no clear mutagenic activity in mammalian systems. It is particularly noteworthy that no chromosomal abnormalities could be detected in the peripheral lymphocytes of cynomolgus monkeys that had received quinacrine by intrauterine or intravenous injection.

Within the limitations of such extrapolations, from these studies quinacrine would appear to have little potential for inducing fetal malformations when administered by intrauterine injection. The embryo-lethal effect is expected for direct-acting mutagens.

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