FERTILITY AND STERILITY
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Vol. 38, No. 8. December 1982
Printed in U.S.A.

Pharmacokinetic studies on quinacrine following intrauterine administration to cynomolgus monkeys*

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Recent efforts have been made to develop a chemical oviductal occluding agent. Intrauterine quinacrine has been used in certain areas of the world with moderate success in effected tubal closure. This report presents the pharmacokinetics of a quinacrine solution (30 mg) as administered to cynomolgus monkeys via the intrauterine route, compared with intravascular injection. The data show rapid transfer of the drug from the uterine to the vascular compartment and uptake by almost all tissues examined. Although plasma concentrations disappear within 24 hours, levels can be detected in most tissues for at least 1 week following intrauterine injection. After 28 days, however, tissue levels of the drug are absent or near the limit of detection. Fertil Steril 38:735, 1982

In recent years there have been efforts toward development of chemical methods of tubal occlusion.[1] One such method, that of intrauterine quinacrine administration, has been pioneered by Zipper et al.,[2] and, depending upon the precise mode of administration, has had an acceptable degree of efficacy.[3] While the procedure is used widely in certain parts of the world today (e.g., Chile), the lack of basic pharmacologic and toxicologic studies prevents use of the drug in the United States. This report presents the pharmacokinetics of the drug as administered by the intrauterine route, compared with intravascular administration, as performed in cynomolgus monkeys.

Received May 10, 1982; revised and accepted July 27, 1982.
*Supported by the International Fertility Research Program. Subcontracts 601 and 603.
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MATERIALS AND METHODS

EXPERIMENTAL APPROACH

Adult female cynomolgus monkeys were purchased from Primate Imports, Port Washington, NY. They were housed in individual cages and fed Charles River Primate Formula (Agway Company, Waverly, NY) supplemented with fresh fruit. Water was available ad libitum. The monkeys were followed daily for signs of vaginal bleeding by the use of cotton swabs.

Fifteen monkeys were studied. Three received intravascular (saphenous vein) injection of 30 mg quinacrine in a l-ml volume of sterile water. The injection solution was freshly prepared on the day of injection. Nine monkeys received intrauterine injection of a solution of 30 mg quinacrine in 1 ml sterile water. The injection procedure has been previously described in detail.[4] The dose was determined by the solubility of quinacrine and the volume capacity of the uterus. Three monkeys received intrauterine injection in 1 ml normal saline. We chose saline, rather than water, to eliminate the possible effects of a hypotonic- solution. Before intrauterine injection was made, daily vaginal swabs were made of each monkey to determine cyclic bleeding, and injections were made within 14 days of the first day of bleeding (proliferative stage of cycle). Within 24 hours prior to treatment, blood samples were obtained from the femoral vein for baseline quinacrine levels. Blood was also taken for chemical and hematologic studies (results of which are described in an accompanying paper[5]). Food was withdrawn 24 hours prior to treatment, but water was still available. The animals -were anesthetized with ketamine hydrochloride, and an indwelling catheter (Intracath, Deseret Company, Sandy, UT) was inserted into the saphenous vein. In those that received quinacrine intravenously, it was inserted in the vein contralateral to the injection. Lactated Ringer's solution (Cutter Injection USP, Berkeley, CA) was infused at approximately 0.25 ml/min with a Holter model 911 peristaltic pump (Extracorporeal Medical Specialties, Inc., King of Prussia, PA). Blood samples were obtained periodically with the use of a three-way stopcock. The bladder was also catheterized for continuous urine collection. The animal was restrained in a specially-designed chair for a 4-hour period so that blood and urine samples could be obtained. Following this procedure, the animal was returned to its cage and observed daily. Blood samples were obtained on days 1, 2, 3, 7, and 28 after treatment or until autopsy.

The three saline-treated monkeys and three of the monkeys receiving intravascular or intrauterine quinacrine were autopsied 24 hours after injection. Three other monkeys receiving intrauterine quinacrine were autopsied at 7 days and three others at 28 days after treatment.

QUINACRINE TEST MATERIAL

Quinacrine hydrochloride was obtained from Sigma Chemical Company, St. Louis, MO, and the lot number of the material used was 7-3-2. We also obtained a standard preparation from the United States Pharmacopeia. Two separate stock solutions were prepared for each standard (USP and Sigma), and a standard solution of various dilutions was prepared from each stock solution. The excitation and emission spectra for both USP and Sigma standards (1000 ng) are shown in Figure 1. The two curves show maximum excitation and emission wavelengths equivalent to each other and in agreement with reported values. Mass spectra for both standards were found to be identical by the Mass Spectrometry Center, The Johns Hopkins University.

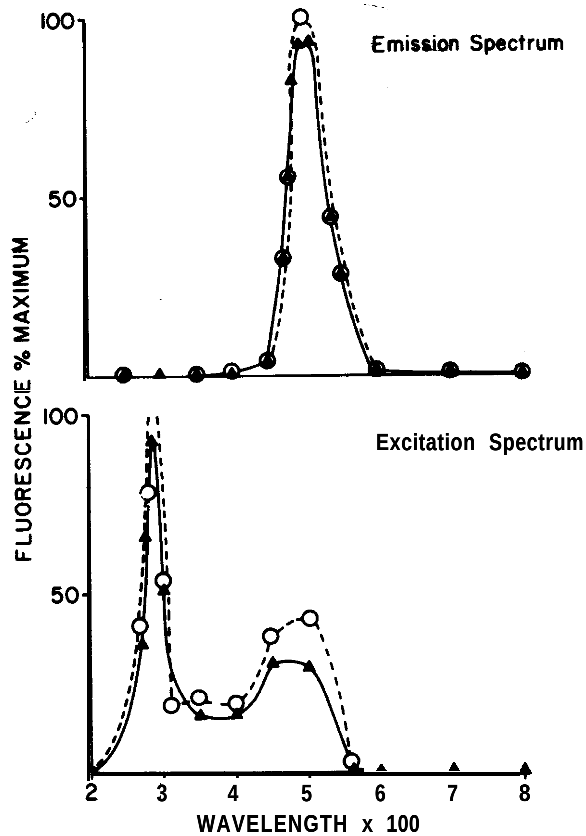

Figure 1
Emission and excitation fluorescence spectra of Sigma (O– – –O) and USP (▲——▲) standards. Emission spectra were scanned with an excitation wavelength of 285 nm. The excitation spectra-were scanned with an emission wavelength of 500 nm.

Thin-layer chromatography of these standards was performed on silica gel plates. Both Sigma and USP standards were spotted (approximately 1 μg) and chromatographed in 30% NH_4OH: MeOH (1.5:100).[6] Both quinacrine samples ran with the same Rf value and could be visualized as a yellow spot. Under an ultraviolet lamp, two other prominent spots could be visualized for both preparations. In one experiment, 5 μg of Sigma quinacrine was chromatographed, and 0.5cm sections from the origin to the front were scraped into test tubes and eluted with solvent. The tubes were centrifuged, and supernatant fractions were dried under nitrogen. Lactic acid solution was added, and each tube was assayed for fluorescence at an excitation wavelength of 285 nm and an

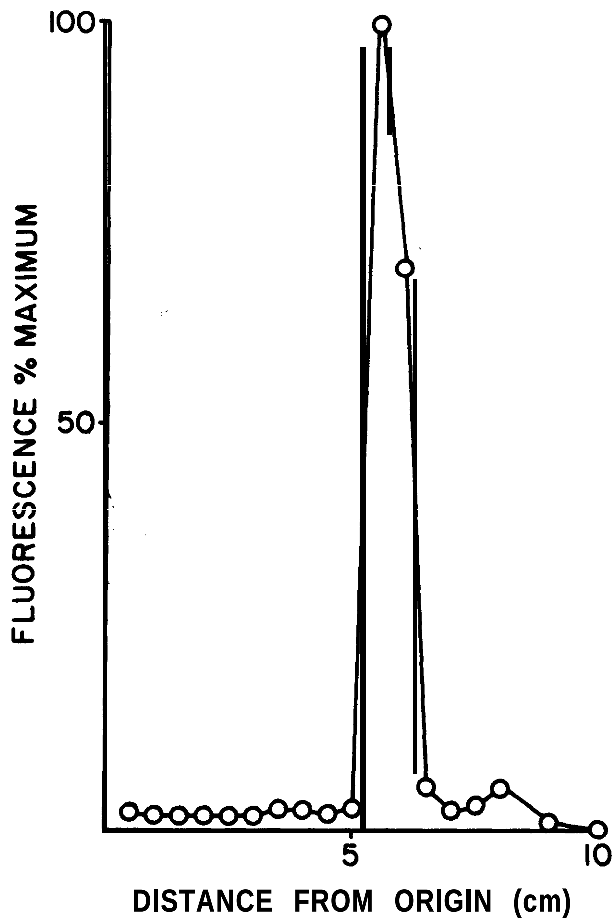

Figure 2
Relative fluorescence of the quinacrine standard as detected in eluted samples of 0.5-cm sections scraped from a chromatographic plate following development in NH_4OH:MeOH (1.5100).

emission wavelength of 500 nm. In the peak corresponding to the quinacrine standard (Fig. 2), 98.5% of the total fluorescence in the chromatogram was found.

QU INACRINE ASSAY

Quinacrine was quantified by a fluorometric method based upon the procedure by Brodie and Udenfriend.[7] Blood was collected in 5-ML tubes containing 10 mg potassium oxalate and centrifuged at 2000 RPM for 10 minutes at 4” C, and plasma was separated. We added 0.3 ml 0.2 M Na_2HPO_4 to 1 ml plasma and 3 ml ethylene dichloride. The solvent extract was then shaken with 0.1 ml distilled water and 0.9 ml 85% lactic acid for 15 minutes and centrifuged. The aqueous layer was transferred to a quartz cuvette and read fluorometrically in an Aminco-Bowman spectrophotofluorometer (American Instrument Company, Silver Spring, MD) at an excitation wavelength of 285 nm and an emission wavelength of 500 nm. We then interpolated the fluorometric readings from a quinacrine standard curve to obtain quinacrine concentrations. Weighed tissues were prepared by homogenization with a Polytron PT 10-35 (Brinkmann, Westbury, NY) equipped with a PT 105T probe at 4” C in a minimum amount of distilled water. The concentration of homogenized tissue was diluted with distilled water to bring the final concentration to 100 mg tissue/ml. For urine and tissues, the ethylene dichloride layer was subjected to an additional wash with 2.5 N NaOH prior to addition of lactic acid. Background fluorescence was detected in body fluid and tissues of control monkeys, and these levels were used as the appropriate blank, which was subtracted from those values obtained from treated animals.

Standard solutions of both Sigma and USP quinacrine were prepared in 85% lactic acid solution. Figure 3 shows the relative fluorescence intensity of various concentrations of quinacrine. There was a linear relationship between quinacrine concentration and relative fluorescence intensity over a long range of concentrations. The linearity of the curve extends to at least 1000 μg/ml. The higher concentrations are not shown in the figure. The sensitivity of the assay was 2.5 ng. Known concentrations of quinacrine added to plasma, which were then assayed, yielded a recovery of > 85%.

A chromatographic analysis of plasma from a quinacrine-treated monkey was also made (Fig.

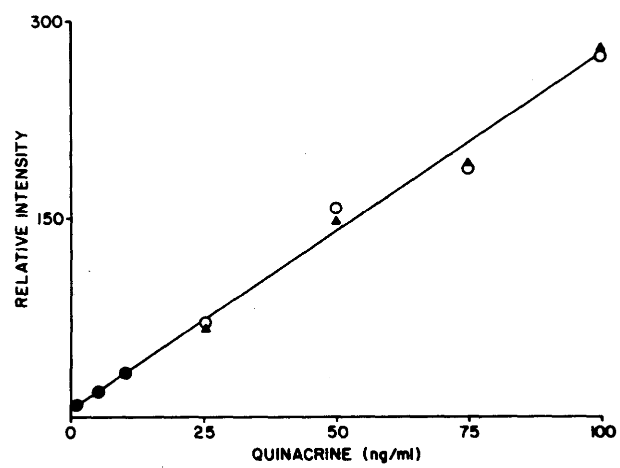

Figure 3
Standard **curve** of both Sigma (○) and USP (▲) **quinacrine samples.**

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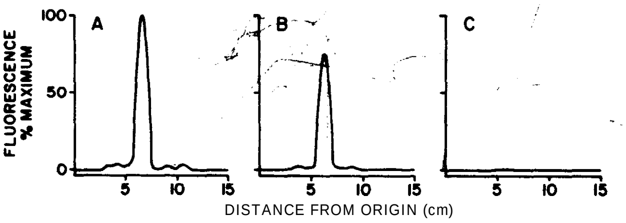

Figure 4
Chromatography of fluorescent material from (A) quinacrine standard; (B) extracted plasma of quinacrine-treated monkey plasma; (C) extracted plasma from a nontreated monkey.

4). Following extraction of plasma, thin-layer chromatography was performed as described. The developed chromatogram was scanned for fluorescence with the use of an Aminco-Bowman Thin-Film scanner (American Instrument Company, J4-3247) and recorded on a Harvard apparatus IO-speed chart mover. One major peak corresponding to that of authentic quinacrine was seen. Thus, it appears that the substance measured by the fluorescence assay is indeed quinacrine and not a metabolic product.

RESULTS

Following intravascular injection of 30 mg quinacrine, two of three monkeys vomited or retched within 3 minutes of the injection. This did not occur in monkeys receiving intrauterine injections of quinacrine or saline.

Following intravascular injection, the range of plasma quinacrine was 99 to 234 ng/ml at 30 minutes. All animals receiving quinacrine by the intrauterine route had significant amounts of the drug in the plasma by the time the first post-treatment blood collection was made (30 minutes). These concentrations were below the range found in animals receiving intravascular injections of quinacrine, except for one, which had a plasma concentration of 291 ng/ml. It is possible that this one intrauterine injection resulted in an intravascular spill. Excluding the values from this one animal, which greatly skewed the data, the average plasma levels over the 4-hour collection period for both treatment routes of delivery are shown in Figure 5. The disappearance half-life for quinacrine in plasma during the 4-hour period of observation was approximately 2 hours.

A total of 248 ± 32 μg quinacrine was found in a 4-hour posttreatment urine collection in those animals receiving intravascular injection, accounting for 0.8% of the original injection. In those receiving an intrauterine injection, 128 ± 34 μg was found. The plasma concentration of quinacrine was 23.5 ± 8.5 ng/ml 24 hours after intravascular administration of the drug. In monkeys receiving intrauterine injections, plasma quinacrine was not detected 24 hours after administration.

Figure 6 represents the tissue levels of quinacrine in various treatment groups. One day after intravascular injection of 30 mg quinacrine, the highest levels were observed in lung (40.2 ± 11.45 ng/mg, mean ± standard error of the mean [SEMI). All other organs studied had concentrations of quinacrine, the least being 1.6 ± 0.8 ng/mg in skeletal muscle. Even this tissue had a tissue:plasma ratio of 68:1 1 day after injection. Twenty-four hours following intrauterine injection of 30 mg quinacrine, the highest concentrations were found in the isthmus (33.9 ± 6.0 ng/mg) and the endometrium (23.5 ± 11.5 ng/mg). High levels (> 10 ng/mg) were found also in the cervix and ampulla. Of the nonreproductive organs, the adrenal had the highest concentration

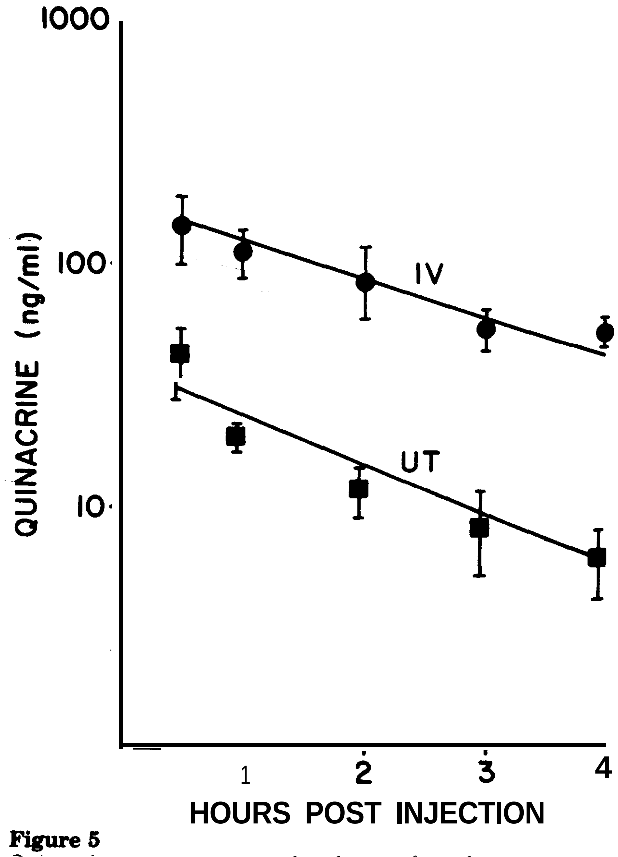

Figure 5
Quinacrine concentration in the plasma of monkeys at various times after injection of 30 mg of quinacrine by an intravascular (IV, $n = 3$) route or an intrauterine (UT, $n = 8$) route.

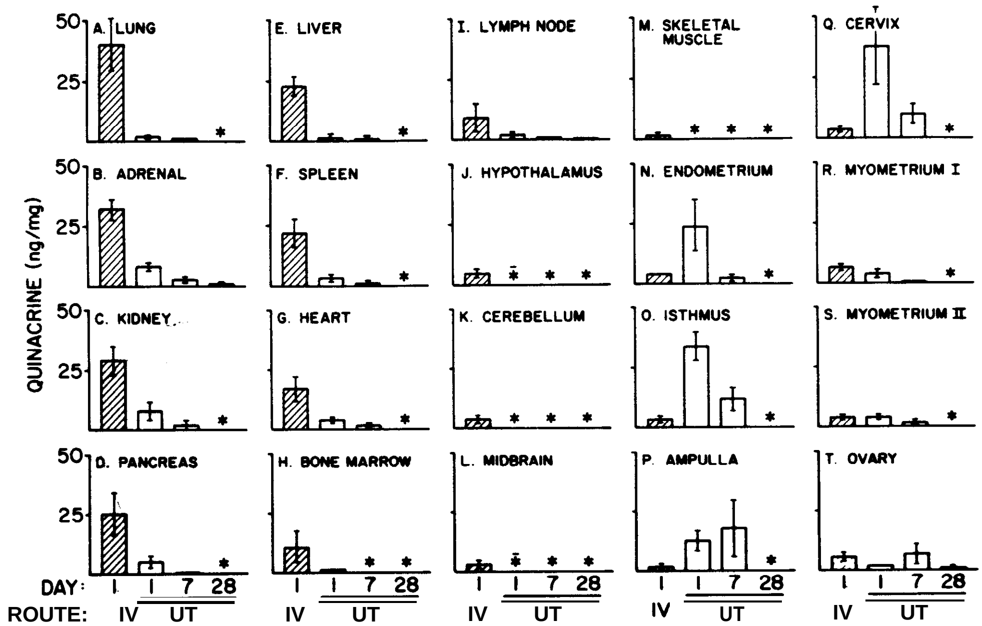

Figure 6
Tissue concentrations of quinacrine after either IV administration (hatched bars) or **UT administration (solid bars) of 30 mg quinawine.** ***Vertical lines*** **indicate ± SEM (n = 3). For** UT injection concentrations are shown at 1, 7, and 28 **days** after injection. The asterisk indicates that levels were not significantly different from the background fluorescence of the control animals given injections of saline. The asterisk with the bar above it indicates that levels were less than ***0.5*** ng/mg but were significantly higher ($P < 0.01$) than the corresponding control levels.

of quinacrine (8.4 ± 2.9 ng/mg) **1** day after intrauterine injection of the drug. Quinacrine was not detectable, or near the limit of detection, in all. tissues at 28 days following intrauterine injection.

DISCUSSION

Pharmacokinetic information is available for quinacrine following administration via the oral route because of its widespread use as an antimalarial agent during World War II. However, no data are available for its absorption and distribution following intrauterine administration. The cynomolgus monkey was chosen for this particular study because its reproductive anatomy is similar to that of man, and its cervical canal, although not straight like that of a woman, can be penetrated by a blunt-end needle[4] so that quinacrine can be directly deposited into the uterine cavity. A solution of quinacrine was used, since this was part of a broader toxicology **study**[5] and the "worst case" of introduction of quinacrine into the circulatory system was desired. The dose given (30 mg/ml) is approximately 2 times the 250-mg dose (on a per body weight basis) that would be given to women via the uterus at any one time of administration.

Quinacrine entered the blood very rapidly following intrauterine injection. The highest levels were observed at 30 minutes, when the first sample was taken. In humans, quinacrine is reported to reach its peak from the gastrointestinal tract in 2 **hours.**[8]

It is estimated that the blood volume is 80 ml/kg body weight. The average weight of the three monkeys receiving intravascular injection was approximately 3 kg. Thus, the estimated blood volume is 240 ml. If 30 mg quinacrine were distributed in this volume, the expected concentration at time zero would be 125 **μg/ml.** The earliest plasma sample obtained 30 minutes after intravascular injection averaged 149 ± 42 ng/ml, thus indicating that quinacrine was removed extremely rapidly from the blood, probably being concentrated in tissue. The tissue level data suggest that this was indeed the case. This situation, that of enormous apparent volume of distribution and rapid binding to tissue, has been reported in humans following intravascular or enteral ab**sorption.**[8]

Following intravascular administration, quinacrine could be detected in all organs examined 24 hours later. Following intrauterine injection, levels at 24 hours were highest, as expected, in the endometrium, cervix, and portions of the tube. However, quinacrine could also be detected at this time in all tissues except cerebellum and skeletal muscles. The highest concentration of quinacrine in a nonreproductive organ was the adrenal, which was not unexpected because it has a large blood supply on a tissue mass basis.

Many tissues from animals which were injected with intrauterine quinacrine and autopsied 1 week later also contained significant amounts of quinacrine, although generally the levels were less than the 24-hour levels. By 28 days, all tissue levels of quinacrine were undetectable or near the limit of detection.

Urinary excretion was very low, as illustrated by the fact that less than 1% of the injected dose was eliminated in the urine after 4 hours. Similarly, low excretion rates have been reported for humans. Significant amounts of quinacrine have been detected in the urine up to 2 months after dosing.[9] Based upon the rate of achievement of a steady-state plasma level with constant oral dosing, the elimination half-life has been estimated to be approximately 7 days.[8]

In summary, the study described illustrates that intrauterine injection of quinacrine results in a rapid entry into the circulatory system, followed by rapid and tenacious uptake by body tissues. Quinacrine can be detected in the reproductive tissues and some of the nonreproductive tissues 7 days after intrauterine injection. However, by 1 month all tissues have undetectable levels of quinacrine or levels near the limit of detection. In our accompanying report,[5] we demonstrate the lack of pathologic changes in any of the nonreproductive organs examined at either 1, 7, or 28 days, despite the uptake and retention of quinacrine.

Acknowledgments. The authors-gratefully acknowledge Ralph Cox for his technical assistance, Doris Cummings for help in validation of the quinacrine assay, and Sue Scheper for preparation of the manuscript.

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