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## Discussion: Research and Clinical Experience With Quinacrine

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### **ARE THERE ANY HISTOLOGIC CLUES AS TO THE MECHANISM OF ACTION OF QUINACRINE?**

The mechanism of action of quinacrine in producing tubal occlusion is unknown, and the only information presently available has been gained from what can be seen at the histologic level. The term *autolysis* has been used, suggesting a self-dissolving mechanism, and some histologic studies have shown damage in which the neutrophils migrated in from the periphery, as in a classic infarct.

In the limited studies described, the electron microscope was not used, and in only a few cases was the time of injury definitely known to be within 24 hours, so as to provide information on the initial result of injury. The studies suggested that the primary damage was to the cytoplasm and cell wall, rather than to the nucleus. In the early lesions, some nuclei looked reasonably intact; the external structure of the cell was gone, and fragments of nuclei were left floating around in a "soup" that contained no cell membranes.

If a cell wall mechanism were involved, however, then in the *in vitro* tests, at least in the tissue culture systems, one would expect a direct cytotoxic effect, but this has not been seen.

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DO THE "SKIP" LESIONS SUGGEST THAT THE QUINACRINE DOES NOT ACT DIRECTLY ON THE EPITHELIUM DURING INTRAUTERINE ADMINISTRATION BUT AS A LOCAL VASCULAR TRANSPORT MECHANISM, FINDING ITS WAY THROUGH THE VARIOUS LAYERS OF THE TUBE TO THE EPITHELIUM?

Not much information is available on the "skip" lesions, except that if one looks carefully at the lesions the damage does not really skip. The histologic findings suggest a direct although superficial effect of the drug. In some of the endometria, the affected area runs down the gland, along the epithelium, all the way to the basalis on the epithelial surface, then up the other side of the gland and down into the adjacent gland, leaving a column of stroma untouched in between. There is no evidence that the drug leaves untouched any areas with which it comes in contact.

The only exceptions are sites exposed to a high concentration of the drug. When quinacrine solution is put into the uterus, it runs out the fimbriated ends of the fallopian tubes; however, invariably, the damage is confined to the interstitial portion and no damage is seen in the fimbrial portion of the tube. Also, no damage is seen on the ovarian surface or peritoneum. These are not skip areas, however. Possibly, in the expansion that takes place, as the drug gets through the isthmus and tube, the concentration decreases dramatically and local anatomical factors, not the tissue type or drug effect, have played the significant role.

HAVE ATTEMPTS BEEN MADE TO RADIOLABEL THE QUINACRINE TO DETERMINE IF ITS EFFECT IS A SURFACE PHENOMENON, AND WHETHER THERE IS LOCAL MICROCIRCULATION OR INTRINSIC VASCULAR REGULATION?

Investigations with fluorescence have provided usable pictures, showing a direct diffusion from the surface in contact with the drug into the subepithelial tissue in a decreasing manner, suggesting surface contact and then diffusion. Tritiated studies with related compounds of quinacrine (not quinacrine itself) show the same type of distribution. A large amount of the compound stayed in the endometrial tissues; some diffused into the myometrium; lesser amounts went down into the cervix and the fallopian tubes; and a small amount went into the ovaries.

WILL INCREASING THE LENGTH OF THE QUINACRINE LESION IMPROVE THE OCCLUSION RATE?

No studies to answer this question have been done. In general, the lesion produced by the quinacrine instillation is small, 2 cm or 3 cm in length, in the intramural portion of the tube. As yet, no investigator has serial-sectioned enough quinacrine-treated tubes to say that a more extensive injury would be more effective. The lesion seems invariably to be confined to the portion of the interstitial tube small enough for wall-to-wall apposition to occur, and

that determines the length of the lesion. As some investigators have shown, there is considerable variation in the path that the quinacrine takes, and the size of the lesion varies accordingly.

Eight years ago, clinical trials in Mexico with quinacrine instillation were discontinued because a high percentage of the patients were found to have patent tubes; the quinacrine had been kept in the tube for only about 30 seconds, which is obviously not a sufficient duration of exposure to the drug, even with increased concentrations of the quinacrine solution.

One possible means of improving effectiveness is to place a pellet directly into the tube, instead of near it, since the epithelial damage occurs only in the small narrow area near the tubal orifice. The problem is to keep the pellet in the tube and to know the proper duration of time to produce sufficient epithelial damage. Theoretically, because the quinacrine would be in direct contact with the epithelium, it would not have to be left in place very long and the side-effects would be minimized.

A difficulty with this approach is that very few operators have the necessary skill and expertise with the hysteroscope to place the pellets correctly.

### **DOES QUINACRINE PRODUCE A LESION THAT IS HISTOLOGICALLY UNIQUE?**

Quinacrine-induced changes are not characteristic of any of the routine disease states known to occur in the human female, so in looking at a quinacrine lesion, one would certainly suspect that some sort of trauma had occurred, but whether the lesion is unique to quinacrine is not certain; in fact, other agents are known to have similar effects.

Most observations of the quinacrine lesions are more a reflection of a repair process than of any intrinsic initial effect of the drug.

### **DOES THE METHOD OF QUINACRINE INSTILLATION AFFECT THE RATE OF ABSORPTION?**

No data are available on this, but one would expect that with the quinacrine in solution, the rate of absorption might be increased.

### **HAVE SPECIES DIFFERENCES IN THE EFFECTS OF QUINACRINE BEEN CONFIRMED, AND IF SO, DO THEY PROVIDE ANY CLUES AS TO THE MECHANISM OF ACTION?**

Unlike all the other chemical agents that have been used in the fallopian tubes, which seem to have similar effects in all species, quinacrine apparently acts differently in some species than in others, although there is no evidence that any species has an epithelium that is particularly resistant to the effects of quinacrine.

The variability in species response may be related to the nature of the isthmus. What probably happens is that in some species the interstitial portion

of the fallopian tube is sufficiently small in diameter that adherence occurs during the healing process and occlusion is effective. If the interstitial portion is large, or the drug delivery system produces a hole so big that reepithelialization takes place before adherence takes place, the drug is ineffective.

This may explain why quinacrine has not worked well in the pig, an animal in which the junction between the endometrial cavity and the tube is relatively large. In pig studies, even when the drug was sutured directly against the cornua and appeared to completely destroy it, some days later the cornua appeared undamaged and healthy.

### **HAVE ANY STUDIES CONFIRMED THAT ESTROGEN CAN AFFECT RECANALIZATION OF QUINACRINE-INDUCED LESIONS?**

There is very little information on the role of estrogen in recanalization; since the effectiveness of quinacrine seems to result from the outcome of a race between estrogen and the proliferation of the fibroblasts, it would be interesting to do studies in women in a natural hypoestrogenic phase—for example, during early lactation or following danazol treatment—thereby possibly minimizing the effect of endogenously produced estrogen.

Zipper has provided the only report of work in this area, but in his studies women were excluded who had had instillations 40 to 50 days postpartum.

### **HAVE ANY SERIOUS SIDE-EFFECTS OCCURRED WITH THE QUINACRINE IUD, AND CAN THE CLOSURE RATES BE CONSIDERED RELIABLE?**

None of the 72 patients who have worn quinacrine IUDs have had any serious adverse effects. The worst event that occurred in these women was a scanty yellow discharge, which usually lasted 4 to 6 hours and was gone by the next day.

The IUD vector seemed at first to be an ingenious solution to one of the main problems with quinacrine and other chemical agents—the complexity of the delivery system. It provided close proximity for diffusion of the drug and dissociated the delivery from the effectivity. It also provided intermediate protection through its IUD mechanism.

In a significant number of fallopian tubes, however, no histologically demonstrable effect is produced by the quinacrine-laden IUD. The presumed closure rate is based on projections from histologic reminiscences, and although the closure rate in monkeys is 80% to 92%, depending on what denominator is used, these figures should not be considered real rates, because in many cases, although a significant amount of tubal damage was produced at first, epithelialization was extraordinarily efficient. The tube seems to be programmed to stay open, and it does so extremely well.

The problem of how to enhance the activity of the quinacrine is an important one. A promising lead that should be pursued is the concept of prolonged progestational therapy following instillation of quinacrine, with the hope of suppressing epithelial proliferation. Investigators are also looking

at delayed-release pellets, and various drug mixtures on the IUD, but these studies are only just being initiated.

**IS THE HYSTEOSALPINGOGRAM (HSG) JUSTIFIED AS A PART OF THE STERILIZATION PROTOCOL, CONSIDERING THE VARIABLE RESULTS IN PREDICTING TUBAL CLOSURE, THE RISKS WITH THE METHOD, AND THE POSSIBILITY THAT INJECTING THE DYE MIGHT ITSELF OPEN SOME TUBES?**

The HSG has been used strictly as a research procedure in quinacrine studies, to provide comparative data between the quinacrine and methyl cyanoacrylate results. The HSG was never intended to be used in a clinical setting.

Whenever the HSG is used, a diagnosis of bilateral occlusion should be made with caution, because some of the changes in the cornual region produced by the quinacrine are small but important, and an accurate interpretation of the HSG requires considerable skill.

**DO THE SAME BLOOD LEVELS OCCUR WITH PELLETS AS WITH OTHER DELIVERY VEHICLES?**

There are no good plasma level data with the pellets. However, in an early study, lysed blood samples and saliva measurements were obtained from volunteers who received dosages of 250 mg in pellets and the quinacrine levels in the blood were as high as 40 ng/ml; levels in saliva were less consistent and less reliable.

In another study, the kinetic picture of decay or elimination of quinacrine was shown to be multiphased, with a relatively rapid initial phase and then a much slower phase thereafter. In the initial phase, the ratio between tissue and plasma was 5000: 1; the body acted as a sponge, and the drug was greatly absorbed.

Even without calculations one can predict that slow entry of the drug into the circulation, with the pellets, will result in much lower plasma levels than will rapid entry, but whether this is compatible with efficacy will have to be determined in models with the blood levels being measured simultaneously with drug release.

**WOULD COMBINING A QUICK AND ACUTE INSULT OF THE TUBE WITH A LONG INSULT IMPROVE THE EFFECTIVENESS OF THE QUINACRINE?**

Many investigators believe that chronic exposure by means of a prolonged release rate is critical. What seems to be happening is a race between the epithelium reestablishing the lumen and the fibroblasts obliterating the lumen. The epithelial cells must be kept down while the fibroblasts come in. In most responses, the fibroblasts are relatively resistant to most of the chemicals applied to them, whereas the epithelial cells are highly vulnerable. Thus,

a toxic chemical with a slow low-level release rate will probably suppress epithelial regeneration while the scar tissue can form and the tubal occlusion will be effective. Without the scar tissue, recanalization is likely to occur.

#### **WHAT IS KNOWN ABOUT THE QUINACRINE DOSE AND DURATION OF EXPOSURE REQUIRED FOR TUBAL OCCLUSION?**

The problem related to the quinacrine lesion in the intramural portion is a matter of time and drug concentration. The time required for dissolution of the pellet is about 10 minutes; just what the actual concentration is in the tube is not known, because the amount of material extruded through the cervical os varies in different patients. Studies in rats indicated that time and concentration are important; a slow-release pellet is required, but a suitable concentration to produce damage is not known and the amount of material inside the uterine cavity apparently must be increased. With a pellet that will last 1 hour, the concentration of the small 10-minute pellets would need to be increased to six times the present size and that would be impractical.

It is doubtful that the slow-release approach alone will produce an adequate effect. A pellet will be required that will give a maximum of 30 minutes instead of 10 minutes. Possibly one can duplicate the size of the pellet, so as to have the same concentration in the blood, but the unstable and higher concentration will be in the uterus. It may be useful to combine the quinacrine with another substance, such as tetracycline or perhaps a chelating agent. It would also be desirable to be able to use a single pellet. Investigators are looking at these possibilities.

#### **HAVE TOXICOLOGY TESTS BEEN DONE WITH THE QUINACRINE ANALOGS?**

The US Army has done some toxicology studies of the analogs, but very little of the testing has been done in humans, and none has been done with intra-uterine administration.

#### **DO QUINACRINE ANALOGS INTERCALATE WITH DNA?**

Whether the analogs molecularly insert themselves between DNA strands is not known. If they do, intercalation is probably not done easily; the analogs have far less avidity for tissue and for DNA than quinacrine does.

#### **WAS THE HIGH BACKGROUND LEVEL OF MALFORMATIONS IN THE SHAM GROUP (6% TO 7%) INFLUENCED BY SOME SPECIAL FACTOR, SUCH AS THE STRAIN OF RATS, DIET, SEASONAL VARIATIONS, OR THE DEFINITION USED TO IDENTIFY A MALFORMATION?**

Sprague-Dawley rats, which were used in the study presented, have a higher spontaneous malformation rate than do Long-Evans rats, but this factor alone could not account for the high malformation rate. The definitions used could

also make a difference in the malformation rate; many of the malformations were not gross or unusual and might be considered by some investigators to be retarded growth rather than malformations. All malformed fetuses were grouped together, regardless of malformation or number of malformations per fetus. Subsequent analysis of the data by malformation and by syndrome, in terms of combinations of malformations, indicated a dose-related effect.

In a similar study by another group, using Long-Evans rats, the background level was much lower, although serious malformations, including meningomyeloceles and ophthalmia, and minor ones, such as loss of the xyphoid process, were found.

### **WITH A SLOW-RELEASING DOSAGE, MIGHT THE RESORPTION EFFECT BE AVOIDED AND THEREFORE A HIGHER NUMBER OF MALFORMATIONS BE SEEN?**

This is an important point. With a dose system such as a bolus or aqueous type, which delivers the quinacrine rapidly, the number of resorptions increases, thereby precluding some of the malformations that might be noted with the quinacrine pellet, where administration is of an intermediate duration and occurs over many hours or maybe over several days.

### **WHEN QUINACRINE WAS USED AS AN ANTIMALARIAL DRUG, AND WAS TAKEN BY PREGNANT WOMEN, DID IT AFFECT FETAL GROWTH AND DEVELOPMENT?**

Investigators have looked for such reports. Some articles on quinacrine that predate the thalidomide episode claim that quinacrine does not produce malformations. However, a critical systematic evaluation of this problem, either retrospective or prospective, has yet to be done.

### **WHAT BLOOD LEVELS OF QUINACRINE ARE ASSOCIATED WITH CENTRAL NERVOUS SYSTEM EXCITATION, HEART BLOCK, AND OTHER SERIOUS COMPLICATIONS?**

Probably the only data in humans come from an early study by Engle and associates, who looked at the effect of quinacrine on electroencephalograms and psychosis-type phenomena in patients who never had malaria. The study showed that blood levels of about 30 ng/ml were associated with accelerations in the electroencephalographic frequencies. Higher quinacrine levels were associated with nervousness and other central nervous system phenomena, and levels of about 1200 ng/ml were associated with psychotic episodes and hallucinations.

In one quinacrine study, the investigators cannulated the jugular vein of two rats for injection of various doses of quinacrine and also cannulated the carotid artery and attached the cannula to a pressure transducer. They found that injected saline had no effect on the blood pressure, nor did 0.5 mg quinacrine injected directly into the blood. However, 1 mg of the drug began

to lower the blood pressure within about 6 seconds, and recovery of normal pressure took about 30 seconds. Higher doses produced lower levels of blood pressure and longer recovery times. With 2.5 mg quinacrine, the blood pressure dropped very low and the animals died.

#### HAVE ANY DEATHS BEEN ATTRIBUTED DIRECTLY TO QUINACRINE USE?

The two deaths mentioned in association with quinacrine instillation were not related to the quinacrine but to other drug effects.

#### WHAT IS THE DANGER OF ECTOPIC PREGNANCIES IN QUINACRINE-TREATED WOMEN, IF THE TUBES ARE NOT SUFFICIENTLY DAMAGED?

In Chile, the general ratio of normal to ectopic pregnancies is about 250: 1, but in the Chilean quinacrine studies no ectopic pregnancies have been seen in almost 250 pregnancies. The reason for this has not been explained.

#### WHAT TREATMENT WAS GIVEN TO THE CHILEAN PATIENTS EXPERIENCING CNS EFFECTS FOLLOWING QUINACRINE INSTILLATION? DID THESE PATIENTS EXPERIENCE OTHER SIDE-EFFECTS?

Three to 5 minutes following the instillation, several women became over-excited. They were given phenobarbital, and after resting for 1 or 2 hours they were able to leave the clinic and go home, where they continued to take phenobarbital for 2 days. The process was usually over in 12 to 24 hours. One woman was hospitalized for observation, but she, too, recovered after 24 hours. Her blood pressure was normal, but her urine was yellow, presumably as a result of a massive penetration of the quinacrine slurry into the bloodstream. No hematologic studies were performed on any of the women, but none of the women experienced any prolonged complications.

#### HAS ANY WORK BEEN DONE ON THE EFFECTS ON NEONATES OF THE PASSAGE OF QUINACRINE IN BREAST MILK?

Information on this question is not presently available, but if this method is to be promoted for developing countries, where a majority of women are either pregnant or lactating, and if it is to be promoted as a method preferable during the quiescent phase of the endometrium, the effects of giving this drug to a lactating woman must be evaluated.

**IF THE QUINACRINE IUDS BECOME WIDELY AVAILABLE IN DEVELOPING COUNTRIES, OR IN THE DEVELOPED WORLD, WHAT WILL THE CONSEQUENCES BE IF THE QUINACRINE DEVICE IS MISTAKEN FOR A REGULAR IUD AND ACCIDENTALLY INSERTED IN A WOMAN?**

This possibility points up the need to find a name for the quinacrine IUD that totally dissociates it from an IUD, which as a reversible method of contraception is significantly different in intent and effect from the quinacrine IUD.

Every complication that one could imagine and perhaps others that have not even been thought of may occur if one uses this approach of delivering quinacrine; someone may perforate a uterus, someone may put an IUD into a pregnant uterus, and so forth. Still other problems come into question; for example, what will happen if the quinacrine IUD is put into a woman who has a uterine body distorted by myomas? Obviously, the drug will not be delivered uniformly to both sides. And other pelvic abnormalities obviously could contraindicate the use of such an approach.

At present, however, investigators are just promulgating this approach as a vector for delivering the drug more effectively to the tubal ostium; they are still trying to evolve the right device with the right dose and the right release rate. They have no intention at this point of beginning clinical trials of the device. Every case presented thus far has been a pre-hysterectomy case and will continue to be until the investigators feel they have enough experience to begin a very carefully controlled phase I trial.

If investigators do succeed in finding the right vector with the right dose, they may be able to make the IUD itself a biodegradable component of a whole delivery system, so at the end of 1 or 2 years perhaps, it would completely disappear. However, those possibilities are in the future. Meanwhile, it seems obvious that there is a desire and a need for chemical sterilization and this work will continue.

**WHAT IS THE MEXICAN EXPERIENCE WITH QUINACRINE?**

When clinical trials with quinacrine in Mexico ended about 8 years ago, some 60 patients had been treated with quinacrine suspension. The drug was instilled in two concentrations: (1) 25 mg in 1 ml of saline solution and (2) 50 mg in 1 ml of saline solution. No problems occurred with introducing the quinacrine, but 10% of the attempts failed. Patients were very carefully selected—only women with grossly normal uteri were accepted—and all procedures were performed with the patient in the proliferative phase of her cycle. In the preliminary report, 6 of 15 patients had both tubes occluded, as shown by a hysterosalpingogram (HSG) 3 months after instillation. The HSG was repeated every 3 months for a year.

The first instillations were done on hysterectomized uteri, to be sure that the quinacrine was being properly introduced into the tube. The procedure was then done in five patients who were waiting to be operated on for another reason. The pathology report on the tissues removed from a patient who was

operated on 1 month after the instillation showed an inflammatory reaction in the tubal lumen.

Another patient was operated on 1 year after instillation of quinacrine in one tube and electrocoagulation in the other. The electrocoagulated tube was found to be occluded, but the quinacrine-treated tube was completely normal. This indicated to the investigators that they needed to increase the length of time that the chemical agent was in contact with the tubal epithelium. It provides an impetus for investigating the possibility of a tubal block with a slow-releasing system that could be placed into the intramural portion of the tube.

All the patients had side-effects from the quinacrine, but none of them was serious or of the type described by other investigators. The type and severity of side-effects seemed to depend on the amount of quinacrine instilled. For example, those patients in whom a small amount of quinacrine was instilled prior to operation had no damage of the intraperitoneal structures and no problem with toxicity.

#### WHAT IS THE EXPERIENCE IN INDIA WITH THE QUINACRINE PELLETS?

If chemical sterilization can be made as effective as the surgical methods, women in developing countries as well as in the developed world would be likely to prefer it to surgical sterilization. However, since it is not presently as effective, its use in developing countries requires careful consideration.

At Baroda, India, investigators have introduced quinacrine pellets into about 85 women and have a follow-up of 2 years. The selection criteria were standard: women had to be in the proliferative stage of the cycle and in the reproductive age-group (average age was 31.4 years, parity was 4.1). Two patients conceived after completing the first instillation and dropped out of the program. Seventeen of 85 women complained of mild to moderate discomfort in the lower abdomen. Menstrual irregularities (delayed cycle and extended period) were experienced by 14% of the women, and pregnancy tests were done in women fearing pregnancy. In a majority of the women, normal cycles returned in about 4 months. Thus far, six pregnancies have occurred, none of them ectopic; one pregnancy occurred at 2 months, one occurred at 8 months, and one occurred at 16 months after a third instillation.

The study has shown that the pellets are more convenient to introduce than quinacrine suspension, which came out through the cervix shortly after instillation. The pellets are sustained *in utero* for a longer time. Pain in the first 48 hours could be prevented or reduced by giving analgesic drugs, then beginning the procedure. There is delay in the return of menstruation in about 10% of cases, and the women worry about possible pregnancy.

The Indian investigators have found that their initial experience with the quinacrine pellets is very encouraging, especially because friends of women in the study are inquiring about whether they, too, can have the treatment. Now the investigators are looking forward to the third generation of IUD quinacrine pellets, which promise even better results than have been seen so far. The 85 women who have had the treatment will be followed for longer than 2 years, in the hope of learning whether any further pregnancies will result.