

PHARMACOLOGICAL AGENTS THAT POTENTIATE  
OR INHIBIT THE OCCLUSIVE ACTION OF  
QUINACRINE IN THE RABBIT TUBE AND  
RAT UTERUS

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Earlier studies in the rat showed that intrauterine instillation of a variety of chemical agents produces significant morphological changes which may prove to be of functional significance in human fertility control. Epithelial tissue was gradually replaced by fibrous and granulomatous tissue, resulting in a gross obstruction of the uterine cavity. This obstructive lesion was related to the loss of fertility in the treated horn, since implantations on the control horn were normal. Some of these agents, such as cadmium and thio-TEPA, did not produce morphological changes, but prevented blastocyst implantation in the presence of ovulation for prolonged periods of time (1).

One of these compounds, quinacrine, shows a good potential for human use and has therefore been studied in detail. A suspension of 25 mg/ml quinacrine in distilled water produced only slight functional changes when locally applied to the uterine lumen of one horn; 50 mg/ml produced intense functional alterations that were manifested by a refractory response of the endometrium to blastocyst implantation that was normalized two months after instillation; 100 mg/ml produced obstructive morphological changes in the uterine lumen that lasted for a period of three months, after which morphological and functional normality was reestablished; and 200 mg/ml produced apparently irreversible changes in uterine structure in animals that were followed for periods of more than four months. It was also demonstrated that a single subcutaneous injection of 5 µg estradiol benzoate or 500 µg progesterone prevented the obstruction induced by quinacrine instillation or accelerated the process of recovery if the obstruction was already present (2).

IV-1 24

Similar attempts to induce morphological changes in the rabbit tube by quinacrine instillation demonstrated that this organ was almost completely insensitive, even to doses of 200 mg/ml. No histological changes persisted until the second week after instillation. This experiment indicated a related affinity for the quinacrine, which we have speculated might be related to the capacity of the quinacrine molecule to bind to the DNA of one or other species. The genital tract of the rabbit has a high zinc concentration, and as our initial hypothesis to explain the lack of sensitivity to quinacrine of this species, we suggested that the presence of this cation might hinder its binding to DNA. In order to verify this hypothesis, we investigated both the action of chelating agents that can bind Zn and the use of Cu as a specific cation to antagonize Zn by replacing it in different enzymatic systems (3).

We continued to use the rat in trying to verify the hypothesis of cationic antagonism because of its responsiveness to quinacrine. However, Zn was used to antagonize the effect of quinacrine. The role of Na and K cations was also investigated, both because of their important function at the cellular membrane level and, more specifically, because K is found in high concentrations in the uterine lumen during the estrous and proestrous periods in the rat (4).

We also investigated substances that have a relevant action in uterine physiology (5,6), such as epinephrine, metaproterenol (Alupent) and norepinephrine; the first two were used because of their stimulatory action on beta receptors, while the last was used because of its stimulatory action on alpha receptors. The local or topical effect of two steroid hormones, estradiol and testosterone, were also studied. Finally, we used Xylocaine, an anesthetic with topical anesthetic properties. Results will be described for the ability of these different chemical agents to act as inhibitory or potentiating agents of quinacrine action. The rabbit was used primarily to investigate potentiating agents of this drug, while the rat was used principally to investigate inhibitory agents.

The possible extension of these results to women will also be discussed, with the aim of obtaining an improved nonsurgical technique of tubal occlusion.

#### Material and Methods

##### 1. Experiments in the rabbit

Rabbits weighing 2500 to 3000 g were used. The right tube was instilled directly, under Nembutal anesthesia. The drug under investigation was administered in a volume of about 0.5 ml, and was kept inside the tubal lumen for a period of 15 min. The utero-tubal region was lightly clamped to avoid reflux into the uterine lumen.

Substances investigated

Quinacrine was always used in a concentration of 200 mg/ml. All other chemical compounds studied were used in concentrations of 10<sup>-1</sup> M, either alone or added to the quinacrine suspension.

Each group consisted of 6 animals. Animals were sacrificed 21 days after tubal instillation. The instilled and control horns were dissected and their macroscopic appearance observed, and representative sections were taken for histological study. Sections were fixed in 10 percent formalin and stained with Eosine-Hematoxylin, and serial histological examination was made for sections of both tubes. The following groups were studied.

- a) Quinacrine alone
- b) Quinacrine plus cysteine
- c) Cysteine alone
- d) Quinacrine plus BAL
- e) BAL alone
- f) Quinacrine plus penicillamine
- g) Penicillamine alone
- h) Quinacrine plus tetracycline HCl
- i) Tetracycline HCl alone
- j) Quinacrine plus versenate
- k) Versenate alone
- l) Quinacrine plus copper acetate
- m) Copper acetate alone
- n) Quinacrine plus zinc acetate

##### 2. Experiments in rat

Albino rats of a non-isogenic strain were bred in the Department of Physiology, and weighed about 200 g at the time of the experiment. Under tribromoethanol (Avertin) anesthesia, 0.2 ml of the quinacrine suspension was instilled into the uterine lumen and maintained there for 15 minutes. With the exception of groups t, u, and v, in which 50 mg/ml were used, the quinacrine concentration was 100 mg/ml. Animals were sacrificed 21 days after treatment. The macroscopic appearance of each uterus was observed and serial histological examinations of uterine sections were made. Tissues were fixed in 10 percent formalin and stained with Eosine-Hematoxylin. No fewer than 5 animals were studied in each group.

## Substances investigated

- a) Quinacrine plus  $10^{-1}$  M NaCl, 6 animals
- b)  $10^{-1}$  NaCl, 6 animals
- c) Quinacrine plus  $10^{-1}$  M KCl, 6 animals
- d)  $10^{-1}$  M KCl, 6 animals
- e) Quinacrine plus  $10^{-1}$  M copper acetate, 13 animals
- f)  $10^{-1}$  M copper acetate, 6 animals
- g) Quinacrine plus  $10^{-1}$  M zinc acetate, 12 animals
- h)  $10^{-1}$  M zinc acetate, 6 animals
- i) Quinacrine plus 100  $\mu\text{g/ml}$  epinephrine, 5 animals
- j) 100  $\mu\text{g/ml}$  epinephrine, 5 animals
- k) Quinacrine plus 100  $\mu\text{g/ml}$  norepinephrine, 5 animals
- l) 100  $\mu\text{g/ml}$  norepinephrine, 5 animals
- m) Quinacrine plus 100  $\mu\text{g/ml}$  alupent
- n) 100  $\mu\text{g/ml}$  alupent
- o) Quinacrine plus 2% Xylocaine, 6 animals
- p) Quinacrine plus 5% Xylocaine, 6 animals
- q) 5% Xylocaine, 6 animals
- r) Quinacrine plus 100  $\mu\text{g/ml}$  estradiol benzoate, 6 animals
- s) Quinacrine plus 100  $\mu\text{g/ml}$  testosterone, 6 animals
- t) 50 mg/ml quinacrine plus 100 mg/ml epinephrine, 6 animals
- u) 50 mg/ml quinacrine plus 100 mg/ml norepinephrine, 6 animals
- v) 50 mg/ml quinacrine plus 2% Xylocaine, 6 animals

## Results

## Experiments in the rabbit

- a) The effects obtained with quinacrine alone or chelating agents alone are shown in Figures 1-6. No effect of these agents on either gross or microscopic examination can be seen 21 days after the instillation. Similarly, no effects are observed in animals instilled with copper acetate alone. The effects obtained with quinacrine plus zinc acetate are shown in Figure 7.
- b) The effects produced by combining quinacrine with chelating agents or copper acetate are shown in Figures 8-11. Intense changes are macroscopically and histologically observed in the tubal structure, with complete obstruction of the tubal lumen in most of the groups studied. The intensity or grade of obstruction observed through the detailed study of the serial sections permitted us to rank the agents combined with quinacrine in a decreasing order of potentiation as follows: cysteine, copper, BAL, penicillamine, tetracycline and versenate.

## Experiments in the rat

The alterations produced by a 100 mg/ml quinacrine suspension can be seen in Figure 12. Figures 13-15 show the histological changes brought about by the potentiation of quinacrine with potassium and copper cations and the sympathomimetic amines epinephrine and norepinephrine. Detailed study of the histological preparations indicated that the action of the latter amine is somewhat less intense than that of the first. These potentiators of quinacrine do not produce obstructive action of the uterine lumen when instilled alone. Metaproterenol, 2 percent Xylocaine, 5 percent Xylocaine, sodium, and zinc cations antagonize the obstructive effects of quinacrine treatment. The effect is surprising in the case of the Xylocaine quinacrine association (Figures 16-18); both uteri present an estrogenic appearance, particularly the treated one.

Results with esteroïdal hormones associated with quinacrine

Estrogen and testosterone have an intense inhibitory effect on quinacrine.

## Comments

The precise mechanism of action of quinacrine as an occlusive agent in the intramural region of the human Fallopian tube (7) and the rat uterus is unknown, as is the reason for its lack of occlusive effect in the rabbit tube. As a working hypothesis, we have postulated that "binding" of quinacrine to DNA is the essential factor in this process and that this binding is dependent upon the zinc concentration in the endometrium. The findings with Zn and Cu are in total agreement with the initial hypothesis, as are the experiments with chelating agents in the rabbit tube. The diverse actions of sodium and potassium cations is difficult to explain as are the estrogenic effects induced by Xylocaine when it is combined with quinacrine in the rat.

Epinephrine and metaproterenol are adrenergic beta agents that inhibit uterine motility, which is associated with an increase in cyclic adenosine 3, 5-monophosphate and an increased glucose production from glycogen (8). Norepinephrine activates alpha receptors, but its catabolic effects on glycogen are slight. Our observation that both drugs act in a very similar way, although epinephrine is somewhat less effective, could be regarded as a problem exclusively of dose. The fact that metaproterenol acts differently than epinephrine in the rat is difficult to explain in this stage of investigation, but preliminary studies in the rabbit indicate that in this species they both potentiate the action of quinacrine.

Possible clinical applications of these findings

Clinical experiments indicate that quinacrine acts as a powerful obstructive agent on the epithelium of the intramural region of the human tube, without altering the histology of the endometrium. Studies by Hagenfeldt et al (9) indicate that this epithelium is rich in Zn, which might explain this clinical finding. The principal limitation of this nonsurgical sterilization technique is the fact that 2 instillations are required in order to obtain 90 percent tubal obstruction. Four years of clinical experience with **quinacrine** (10) indicate that pregnancy and deobstruction rates are very low after tubal obstruction has been successful. This means that this clinical problem could be solved by obtaining 100 percent obstruction with a single instillation. To achieve this goal we would require a) finding **specific** potentiating agents for the human species, which might be different than the potentiators in other animal species; b) the use of utero-relaxing agents to prevent the inevitable tubal spasm that is produced by the stimulation of the cervix when the instillation cannula is introduced.

Theoretically, with the evidence presented in this work, we think that both of these required properties — the **potentiation** of quinacrine action and the uterine relaxation or beta receptor stimulation — might be associated in the same molecule.

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