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Review

# Quinacrine-induced occlusive fibrosis in the human fallopian tube is due to a unique inflammatory response and modification of repair mechanisms

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ABSTRACT

Quinacrine has been widely used in treatment of parasitic diseases such as malaria and giardiasis, and in the treatment of autoimmune diseases. Quinacrine has also been used as an effective substitute for surgical contraception by causing occlusion of the fallopian tube. This minimally invasive treatment protocol involves intrauterine insertion of the drug in the form of pellets and has been studied in humans in a number of countries, including the United States. Despite its development in the 1970s, the cellular and molecular events induced by quinacrine in the human fallopian tube have not been described. Here we describe a plausible mechanism for quinacrine action in the fallopian tube. This is manifested as an acute pro-inflammatory response in the uterus and fallopian tube, characterized by loss of epithelial cell adhesion. This response relies on properties of gated channels found on the surface of epithelial cells in the reproductive tract. While the uterus returns to normal, the inflammatory response affects the uterotubal junction and transmural segment of the human fallopian tube, and initiates formation of mature collagen in the lumen of the fallopian tube, resulting in its permanent occlusion. The response within the fallopian tube appears similar to the protective mechanisms that have evolved in women to minimize the likelihood of systemic infection from *Neisseria gonorrhoeae*, and to some extent from *Chlamydia trachomatis*. This review could assist in development of experimental models used in investigating the mechanisms of fibrotic responses in humans as well as development of techniques for permanent non-surgical female contraception.


## 1. Introduction

Quinacrine hydrochloride (quinacrine, QC), when formulated into pellets and inserted into the uterus of women, causes fibrotic occlusion of the fallopian tubes and can be used as a substitute for surgical contraception. The currently recommended clinical protocol for this permanent female contraceptive method is two insertions, one month apart, of 252 mg of quinacrine hydrochloride in the form of seven pellets. Except for occlusion of the fallopian tubes, the reproductive organs return to normal (Merchant et al., 1995). Efficacy after one year is >99% and compares favorably to other reported efficacy rates for approved contraceptive methods (Hatcher et al., 2012). By 1996, over 100 000 women globally had undergone this procedure (Kessel, 1996) and its use has continued in many countries. Long-term follow-up studies suggest that the method is

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safe (Sokal et al., 2008, 2010) and effective (Alpizar, 2003; Bilgrami and Shah, 2003). In over 150 000 women who have undergone the procedure, only two have reported a severe allergic response (both cases successfully treated) with no other serious adverse events reported. Ectopic pregnancies following quinacrine have been reported to range from 0.26 to 0.60 per 1000 individuals (Sokal et al., 2000; Hieu and Luong, 2003; Zipper and Trujillo, 2003), which is similar to the ectopic pregnancy rates among women who use intrauterine devices or tubal ligation for contraception. All three interventions result in ectopic pregnancy rates considerably lower than in women using no contraceptive method.

In addition to quinacrine (Zipper et al., 1973), a number of drugs have been screened for their potential use as a minimally invasive, nonsurgical procedure for tubal closure because of their effectiveness as a sclerosant. Studies in animal models with tetracycline (Dubin et al., 1984a,b), erythromycin (Fail et al., 2000), and polidocanol (Jensen et al., 2004) have shown promise. However, studies conducted with tetracycline and erythromycin have shown that the failure rate following intrauterine administration is high, exceeding 50% in the case of tetracycline (Mullick et al., 1987), and 36% for erythromycin (Bairagy and Mullick, 2004).

Laboratory studies to identify sclerosing agents that produce tubal occlusion have been limited by lack of experimental models that clearly mimic the response that occurs in humans, as suggested by studies in rats, pigs and monkeys (King et al., 1983; Dubin et al., 1982; Zaneveld and Goldsmith, 1984; Fail et al., 2000; Jensen et al., 2004; Cancel et al., 2010). Many of these sclerosing agents produce histological changes but do not produce tubal occlusion. In Cynomolgus monkeys, intrauterine administration of tetracycline or doxycycline, like quinacrine, produces morphologic damage of the uterine lining and intramural section of the tube, including necrosis, inflammation and fibrosis (Dubin et al., 1982, 1984b). The latter is consistent with the type of damage which may lead to tubal closure, but does not necessarily mean that closure will occur. In a recent 2-year rat bioassay where rats received intrauterine quinacrine administration (Cancel et al., 2010), uterine horns were specifically examined for fibrosis and lumen closure, and no dose-related increase in fibrosis was found nor lumen closure reported; the oviduct appeared unaffected.

While the mechanisms of action of several uses of quinacrine have recently been reported (Gurova, 2009), and the extensive use of quinacrine has made it one of the most widely studied drugs in humans (Ehsanian et al., 2011), little is known regarding its mechanism of action in the fallopian tube. Recent cellular and molecular studies have provided insights into this process. This review, which summarizes our current understanding of the mechanism of action of quinacrine in the human fallopian tube, may help resolve some of the questions raised during the development of this drug for nonsurgical sterilization. In brief, the composite studies suggest that quinacrine induces a species- and tissue-specific response in the uterotubal junction and transmural segment of the human fallopian tube, causing disruption of cell adhesion and an acute pro-inflammatory and pro-fibrotic cytokine cascade. This is followed by a higher order primate-specific and tissue-specific modification in the healing mechanisms that produces tubal occlusion by replacing the lumen with mature collagen, using the same mechanisms that have evolved in protecting women from systemic infection from *Neisseria gonorrhoeae* and, to some degree, *Chlamydia trachomatis*.

## 2. Quinacrine response in the human fallopian tube

In women, quinacrine is administered *via* the uterus (4 mg/kg body weight), and the target organ (fallopian tube) is exposed to only a small percentage of the administered dose (estimated at <5% of the total administered dose). The result is the formation of mature collagenous fibrotic material replacing the lumen and limited to the uterotubal junction and transmural segment of the fallopian tube (Fig. 1A). After one year, ≈90% of cases, a single application of ∼250 mg quinacrine hydrochloride pellets in the human uterus results in complete occlusion of both fallopian tubes and >99% following a second administration.

While acute inflammation is associated with this process in the uterus, the pathology is quickly resolved without any loss of organ function. The sequence of histopathological changes following intrauterine administration of quinacrine in women has been described in detail by el-Kady et al. (1991); within 10 days, necrosis of the epithelial lining of the fallopian tube occurs, along with an acute inflammatory reaction. This is followed by absorption of the inflammatory cellular exudate, progressive fibrosis with partial or almost complete occlusion of the lumen, and failure of regeneration of the epithelial lining. Occlusion is characterized by obliteration of the lumen by mature fibrous material and the absence of epithelium. The occlusion of the fallopian tube lumen with dense collagenous material is easily visualized by hysteroscopic video, as seen in the photograph taken of the uterotubal junction in Fig. 1B (Lu et al., 2003) and by ultra-sonography (Ferreira et al., 2003). Except for tubal occlusion, all other macro and microscopic effects are reversed.

The changes following exposure of the fallopian tube to quinacrine show similarities to observations reported in women who underwent surgical procedures for infertility and were found to have uterotubal obstruction (Fortier and Haney, 1985). Of 42 women studied, the most frequent lesion encountered was obliterative fibrosis (38.1%), characterized by complete obliteration of the tubal lumen with no evidence of other pathology. The lumen was replaced by densely collagenous connective tissue with no epithelium visible. Fibrosis was medial to the inner longitudinal muscle layer with minimal involvement of the muscle directly. The obstruction involved the entire transmural segment uniformly (including the uterotubal junction). Fortier and Haney further observed that in almost all instances the obstructing lesion began within the transmural portion of the oviduct and extended a variable distance into the isthmic segment but not beyond the ampullary–isthmic junction. These findings suggested that the process is initiated in the uterus, and fibrosis represents a response to injury of the transmural and isthmic segments of the tube.

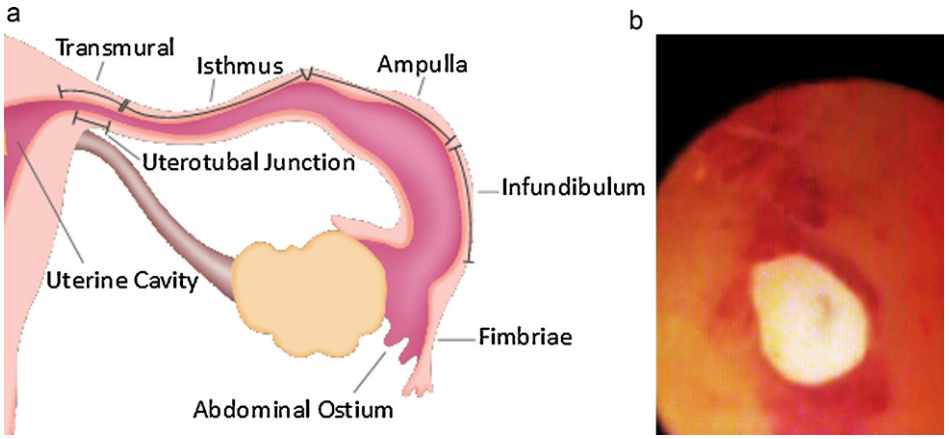


**Fig. 1.** (A) Fallopian tube. (B) Still photo of an occluded uterotubal junction taken from the perspective of the uterine cavity using a video camera attached to a hysteroscope.
Reprinted from Lu et al. (2003), Copyright 2003; with permission from Elsevier.

Merchant et al. (1995) studied 33 women awaiting hysterectomy for nonmalignant conditions who had received intrauterine quinacrine at doses of either 252 mg ($n = 10$) or 324 mg ($n = 23$), at least 6 weeks prior to surgery but not longer than 20 weeks prior. The key findings were that tubal closures were most directly related to quinacrine dose and to a lesser extent the time interval between insertion and hysterectomy. Only 55% of intramural tubal segments showed evidence of closure at the low dose, compared to 100% at the high dose. Histological changes were confined to the fallopian tube and sections through the regenerated endometrium, cervix and myometrium of the uterus were normal, confirming that the chemical has no lasting effect on these structures. No adhesions or other abnormal changes (including fibrosis) were detected in the uterus or cervix.

## 3. The fibrotic response within the fallopian tube

Wound healing is a dynamic process consisting of hemostasis, inflammation, proliferation, and remodeling. Remodeling of damaged tissues, as occurs with quinacrine, requires that each phase must occur in a precise and regulated manner. The events involved in tubal closure by quinacrine are initially associated with epithelial cell damage and an acute inflammatory response in the specialized tissue of the transmural portion of the fallopian tube. These processes damage the epithelium as well as the underlying stromal tissue. Over approximately three months, this early reaction undergoes a tissue remodeling and repair process resulting in a collagenous connective tissue plug that fills the lumen, and permanently occludes the fallopian tube (Merchant et al., 1995; el-Kady et al., 1991). Successful occlusion of the tube has been predicted to depend on the nature and duration of exposure to the causative agent, on the extent of tissue destruction, and on the type of tissue injured (Eddy and Pauerstein, 1983). Although usually beneficial, the healing process can become pathogenic if it continues unchecked, resulting in substantial remodeling of the extracellular matrix and formation of permanent scar tissue. Chronic fibrosis is associated with several diseases (Wynn, 2007). However, instead of a poorly regulated wound healing response or an incomplete resolution of the healing process, quinacrine produces a self-confined fibrotic response within the fallopian tube.

## 4. The immune system and the fallopian tube

The human fallopian tube is lined by columnar ciliated cells and secretory cells with microvilli which function as a channel and storage organ for spermatozoa; a collecting vessel for oocytes released from the ovaries; the site of sperm capacitation, fertilization, and zygote formation; and as a means for transporting the early embryo to the uterus. The female reproductive system has evolved to address various threats to survival by developing site-specific immunoregulatory mechanisms. For example, the upper female genital tract has a powerful innate inflammatory response that can rapidly protect the reproductive organs from pathogenic challenge, and prevent a pathogen from ascending the fallopian tube to infect the ovary and the peritoneum while maintaining tissue function and integrity (Quayle, 2002).

Epithelial cells and stromal fibroblasts are the primary sources of inflammatory mediators in the human fallopian tube and have evolved unique, site-specific mechanisms for recognizing viral and bacterial infection. In this respect, each segment, *i.e.* the isthmus, isthmic–ampullary junction (or middle section), ampulla and fimbriae, could be considered anatomically and immunologically distinct (Ochiel et al., 2008). Studies of the combined morphological and ultrastructural features of the epithelial lining along the length of the fallopian tube substantiate the concept of functional differentiation among these segments (Crow et al., 1994).

Human fallopian tube epithelial cells secrete a broad spectrum and unique pattern of proinflammatory cytokines and chemokines from the apical and basolateral compartments. These include, among others, interleukin (IL)-8, IL-6, monocyte chemoattractant protein-1 (MCP-1), granulocyte–macrophage colony-stimulating factor (GM-CSF), TNF-$\alpha$ and macrophage inflammatory peptide-1$\beta$

(MIP-1β) (Fahey et al., 2005). IL-1 is also expressed by epithelial cells in the human fallopian tube (Hess et al., 2009). Toll-like receptors (TLRs) and their attendant cooperating receptors, which initiate proinflammatory responses to pathogen-associated molecular patterns (PAMPs) and other agonists, are readily expressed in the reproductive tract (Parker et al., 2007; Fichorova et al., 2002).

In tissues of the human female reproductive tract, quantitative analysis of TLR2 mRNA levels revealed that the highest expression occurs in fallopian tube and cervical tissues, followed by endometrium and ectocervix (Pioli et al., 2004). In contrast to TLR2, TLR4 expression declined progressively along the tract, with highest expression in the upper tissues (fallopian tubes and endometrium), followed by the cervix and ectocervix. In addition to mRNA, protein expression of TLR2 and TLR4 was also documented in these tissues (Pioli et al., 2004). Taken together, these data suggest that TLRs are differentially expressed in distinct segments of the female reproductive tract and that inflammation within the tract is highly regulated. Proinflammatory cytokines are also important for initiating a fibrotic response. For instance, there are many examples showing that TNF-α induces fibroblast proliferation and collagen production, much of which occurs through production of plasminogen activator inhibitor (PAI-1). IL-1 has also been shown to be profibrotic by stimulating TGF-α and fibroblast growth factor (FGF) synthesis production of collagen and fibronectin (Mutsaers et al., 2004).

## 5. Quinacrine and proinflammatory cytokine secretion

Levels of the proinflammatory cytokines, including TNF-α and IL-1β, increase after quinacrine instillation into the pleural cavity (Agrenius et al., 1994). Within 4 h after a single administration of 150 mg of quinacrine, injected interstitially into C6 glioma cells implanted in the subcutaneous tissue of Wistar rats, a temporal increase in TNF-α can be detected in tumor cell homogenates. Similarly, an increase in proinflammatory cytokines is detected in cell homogenates 24 h after quinacrine injection, independent of cell toxicity (Sotelo et al., 2004). In human endothelial cells, chloroquine (150 μM) increases the production of IL-1α. While LPS (5 μg/ml), by itself, significantly increased the production of IL-1α, when it is combined with chloroquine, a synergistic increase is found. IL-1α production is associated with the direct loss of endothelial cells similar to that observed in the human fallopian tube. After 24 h of exposure, 150 μM of the drug causes significant IL-1α production, as well as the detachment of about 50% of the cells (Potvin et al., 1997).

A number of drugs and agents, including tetracycline, minocycline, *Corynebacterium parvum*, talc, bleomycin, nitrogen mustard, doxorubicin, radioactive colloid gold and quinacrine, have been used to decrease or prevent fluid accumulation in the pleural cavity for treatment of malignant pleural effusion. While each has demonstrated its own unique properties, like quinacrine, in all cases successful treatment entails a robust intrapleural inflammatory reaction, tissue remodeling and fibrin deposition, resulting in the adhesion of the visceral to the parietal pleura and pleurodesis (Kroegel and Antony, 1997). In fact, quinacrine has been used over the last four decades for pleurodesis in Scandinavian countries (Dikensoy and Light, 2005).

## 6. Similarities between protection from systemic *N. gonorrhoeae* infection and quinacrine administration

Worldwide, fibrotic obstruction of the fallopian tube is a leading cause of infertility in women, and can be attributed, to a large degree, to two pathogens: *N. gonorrhoeae*, and *C. trachomatis*. It has been suggested that to protect against the ascension of *N. gonorrhoeae* into the abdominal cavity and protect women from systemic infection, the fallopian tube undergoes rapid cell exfoliation (Muenzner et al., 2010) and the induction of an acute pro-inflammatory and pro-fibrotic response culminates in tubal occlusion (Stephens, 2003; McGee et al., 1999). This protective response has similar biological processes to the action of quinacrine.

The response to gonorrhea may have disseminated through positive selection across human populations, and studies indicate that the point in the phylogenic tree at which susceptibility to gonococcal infection commences is between baboons and chimpanzees (or between monkeys and apes) (McGee et al., 1990). Thus, while humans and higher order non-human primates are susceptible to experimental mucosal infection, these pathogens attach very infrequently or not at all to the mucosa of rabbit, porcine, or bovine oviduct, and no histological damage occurs. Taken together, host specificity appears to be determined, at least in part, by the species differences in the ability of the organisms to attach to and damage the genital mucosa (Johnson et al., 1977).

The occlusion of the fallopian tube is the final stage of an elaborate progression of organ-specific pathological and repair mechanisms in protection from systemic infection in the upper reproductive tract. The human fallopian tube opens into the abdominal cavity, making it the most available and susceptible pathway for dissemination of pathogens directly into the peritoneum. Occlusion of the fallopian tube may confer a distinct survival advantage, as fallopian tube occlusion can prevent pathogens from ascending the tube into the peritoneum, thus preventing systemic infection or death. The downside, by comparison, is minimal. Redundancy allows the possibility of the uninfected fallopian tube to maintain fertility. In the worst case, where both fallopian tubes are occluded, mothers can continue to nurture and raise their children to reproductive age.

## 7. Mechanistic basis for occlusion by *N. gonorrhoeae* and *C. trachomatis*

*N. gonorrhoeae* and *C. trachomatis* also have developed an extensive repertoire of pathogenic mechanisms by which they resist the immune mechanisms of the human reproductive tract. Rapid epithelial cell exfoliation (cell sloughing) is a survival mechanism used in several organs to effectively clear infected cells and

Gram-negative bacteria from the tissue (Mulvey et al., 2000). In the fallopian tube, *N. gonorrhoeae* blocks the shedding of infected epithelial cells by stimulating integrin activation (Muenzner et al., 2010). By controlling cell adhesion processes, pathogen-infected cells cannot be shed and pathogen colonization is assured. During infection with *N. gonorrhoeae* or *C. trachomatis*, the human fallopian tube undergoes an inflammatory response prior to collagen depositing (Edwards and Apicella, 2004; Patton et al., 1989).

The epithelial layer and the mucosal surface act as physical and biological barriers against microbial invasion which is maintained by tight cell–cell junctions (TJ) and the basement membrane. The pathogen circumvents this barrier by expressing lipooligosaccharide (LOS) which induces epithelial cells to express tumor necrosis factor (TNF)-α that, in turn, causes impaired TJ function in a number of epithelial and endothelial cell lines (Capaldo and Nusrat, 2009). The early response involves cell detachment and a transient but robust inflammatory response characterized by pro-inflammatory cytokine secretion, such as TNF-α and IL-1 (McGee et al., 1999). In organ explant cultures, the levels of TNF-α are directly proportional to the loss of ciliated cells from the epithelium, which closely mimics the progression of gonococcal infection observed *in vivo* (McGee et al., 1999).

Because *C. trachomatis*, *N. gonorrhoeae* and quinacrine can each cause occlusion in the human fallopian tube, a comparison of *C. trachomatis* with *N. gonorrhoeae* is relevant to the mechanism of action of quinacrine proposed here. The differences are significant and instructive. Like *N. gonorrhoeae* (and quinacrine) the tissue damage induced by *C. trachomatis* infection occurs as an immune response to infection. In the case of *C. trachomatis*, the cytokine IL-1, induced by the presence of *C. trachomatis* has been shown to be the initiator of tissue damage and inflammation as well as the induction of the secondary cytokine IL-8, a potent neutrophil chemoattractant (Hvid et al., 2007). In *N. gonorrhoeae* both TNF-α and IL-1 are active initiators of tissue damage. Multiple infections of *C. trachomatis* are required for tubal scarring to occur (Paavonen and Eggert-Kruse, 1999). In *N. gonorrhoeae* a single untreated infection is sufficient. One study of *C. trachomatis* involving 1844 women concluded that, following primary infection, each reinfection of *C. trachomatis* roughly doubled the risk of tubal occlusion (Haggerty et al., 2010). *C. trachomatis* causes cell–cell attachment disruption of epithelial cells but not desquamation (Prozialeck et al., 2002). The response to *N. gonorrhoeae* is complete disruption of cell attachment and desquamation of the epithelium. *C. trachomatis* infection of the oviduct has been successfully studied in several species susceptible to the pathogen including pigs (Vanrompay et al., 2006), guinea pigs, mice (Su et al., 1997), and macaques (Patton et al., 1989, 1990, 1997). Conversely, like *Haemophilus influenzae*, *Moraxella catarrhalis*, and *N. meningitidis*, *N. gonorrhoeae* is a human-specific pathogen (Schmitter et al., 2004; Muenzner et al., 2010) and the chimpanzee (*Pan troglodytes*) is the only animal species other than humans in which localized urethral infections of 3–6 weeks in duration have been established (Arko, 1989).

## 8. Quinacrine disrupts cell adhesion

In most tissues, cell disruption with loss of adhesion properties occurs in response to cytotoxic chemicals or inflammation. In the bladder urothelium, procedures that efficiently induce cell removal are associated with an inflammatory response and prolonged cell desquamation, resulting in epithelial hyperplasia (Veranic et al., 2009). In the model for tubal closure by quinacrine proposed here, one of the earliest events following intrauterine quinacrine administration is sloughing of surface epithelial cells. *In vitro* studies with quinacrine in different cell models have shown that the drug commonly causes disruption of cell adhesion. For example, culturing rat hepatocytes with quinacrine at concentrations as low as 50 μM rapidly results in cell detachment (Leduc et al., 1981). Quinacrine at 10 μM also causes cell rounding, reduced cell size, blebbing and detachment in head and neck squamous cell carcinoma cells (Friedman et al., 2007). Quinacrine also induces cell detachment in porcine aortic endothelial cells. Prior to detachment, quinacrine causes the loss of monolayer integrity and the formation of intercellular gaps (Stuhlmeier, 2000).

The mechanism by which quinacrine disrupts cell adhesion may rely on properties of gated channels found on the cell surface of epithelial cells in the reproductive tract. In addition to neurons (Gotti and Clementi, 2004; Arias et al., 2006), acetylcholine receptors (AChRs), members of the Cys-loop superfamily of ligand-gated ion channels, are also expressed on the surface of endothelial, epithelial and immune cells (Wessler and Kirkpatrick, 2008). One of the important biological functions of non-neuronal AChRs is to allow for cell adhesion. The downstream targets of these AChRs include intercellular adhesion molecules, such as classical and desmosomal cadherins, and integrins, mediating keratinocyte adhesion to a substrate (Grando, 2006). Quinacrine can act as a noncompetitive inhibitor (NCI) for both the muscarinic acetylcholine receptor (mAChR) and the nicotinic acetylcholine receptor (nAChR). Yu et al. (2003) mapped the binding site for quinacrine in the open channel of the non-neuronal ACh receptor in cysteine-substituted mutants of the α subunit expressed with wild-type β, γ, and δ subunits. In an analysis of the mechanistic basis for the noncompetitive action of quinacrine and the kinetic changes in nAChRs at both the single-channel and macroscopic current levels, the main effect of quinacrine was a profound concentration-dependent decrease in both the frequency of opening events and the duration of clusters elicited by high acetylcholine concentrations (Spitzmaul et al., 2001).

Results from studies with smooth muscle cells isolated from guinea pig vas deferens and urinary bladder showed that quinacrine is also a potent $Ca^{2+}$ channel blocker (Nagano et al., 1996), which regulates cell adhesion as well. The $IC_{50}$ for quinacrine is about 1.2 μM, apparently lower than that reported for the inhibition of high-threshold voltage-dependent $Ca^{2+}$ channel current in rat hippocampal neurons (30 μM), and slightly lower than that in guinea pig ventricular myocytes (6 μM). The inhibition of A-type $K^+$ current in melanotrophs and other membrane currents or channels in various cells requires higher concentrations

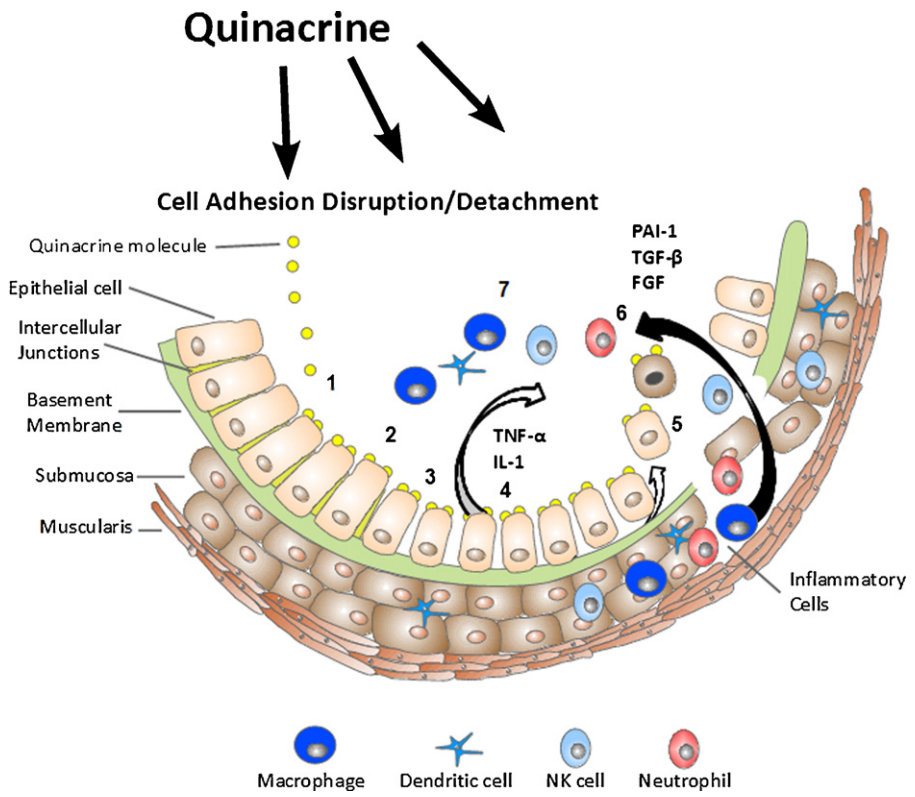


**Fig. 2.** Proposed mode of action for quinacrine in the fallopian tube. Cross-section of the fallopian tube and lumen showing the proposed mode of action of quinacrine-induced inflammatory response. (1) Dissolved, positively charged molecules of quinacrine bind to the extracellular domain and open channel of acetylcholine receptors on the surface of epithelial cells. (2) Quinacrine-bound acetylcholine receptors disrupt cell adhesion. (3) Epithelial cells shrink, round up and intercellular and cell matrix adhesion is disrupted. (4) Pro-inflammatory cytokines (TNF-α and IL-1) are expressed from the apical portion of the epithelial cells. (5) TNF-α and IL-1 induce epithelial cell sloughing and cell death through apoptosis. (6) Inflammatory cells and cytokines are released from the submucosa into the lumen, leading to the release of superoxides. (7) This response destroys mucosal cells, which leads to fibrosis and collagen formation. *Abbreviations*: PAI-1 = plasminogen activator inhibitor-1; TGF-β = transforming growth factor-β; FGF = fibroblast growth factors.

of quinacrine. The effect of quinacrine is apparently due to direct block of L-type $Ca^{2+}$ channels from the outside. Not only $Ca^{2+}$ channels, but also many other channels, including ligand-operated channels, are directly blocked by quinacrine, suggesting nonspecific effects of quinacrine on ion channels. Since internally applied quinacrine cannot reach the binding site in the $Ca^{2+}$ channel, only protonated quinacrine in external solution can bind to the site from the outside (Nagano et al., 1996).

## 9. Summary

As depicted in Fig. 2, the events invoked in tubal closure by quinacrine are postulated to occur through disruption of cell adhesion and detachment of epithelial cells in the reproductive tract which, in turn, may rely on the ability of quinacrine to affect gated channels found on the cell surface. This event leads to an acute inflammatory response in the specialized tissue of the transmural portion of the fallopian tube, which damages the epithelium as well as the underlying stromal tissue. Over approximately three months, the tissue undergoes a remodeling and repair process, forming a collagenous connective tissue plug that fills the lumen and permanently occludes the fallopian tube. Successful occlusion of the tube can depend on the nature and duration of exposure, on the extent of tissue destruction and on the type of tissue injured. This model is based on the human response that prevents sexually transmitted infections, such as *N. gonorrhoeae* and *C. trachomatis*, from becoming systemic. Available data suggest that the effect on the fallopian tube is both tissue-specific and species-specific, at least for humans and certain higher order primates. The identification of a species/tissue-specific immune/inflammatory mechanism of quinacrine-induced fibrotic occlusion of the human fallopian tube answers many questions posed by investigators and healthcare providers, and challenges the basis for the assumptions used to rationalize dose selection and animal/organ model choices in studies of quinacrine in non-human species.

The authors hope this review will assist in the development of experimental models used in investigating the mechanisms of fibrotic responses in the human body as well as the development of techniques for permanent non-surgical female contraception.

## Conflict of interest

The authors serve as scientific consultants for International Services Assistance Fund (ISAF). The authors have no financial or personal relationships with ISAF or other

organizations that inappropriately influenced (biased) their contribution to this work. The authors alone are responsible for the content and writing of this paper.

## Authors' contribution

RGG and MIL initiated and designed the review, and drafted and revised the manuscript. PAF and JL reviewed and edited the manuscript.

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