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A lifetime cancer bioassay of quinacrine administered into the uterine horns of female rats

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ABSTRACT

This study investigated if quinacrine can induce a tumorigenic response in rats when administered in a manner similar to the intended human use for female non-surgical sterilization. Young sexually mature female rats received two doses of quinacrine (or 1% methylcellulose control) into each uterine horn approximately 21 days apart, and were observed for 23 months after the second dose administration. Dose levels were 0/0, 0/0, 10/10, 70/70, and 70/250–350 mg/kg (first dose/second dose), which represent local doses in the uterus at approximate multiples of 1×, 8× and 40× the human dose (mg quinacrine/g uterine weight) used for female non-surgical sterilization. Rats were observed for viability, clinical signs of toxicity, and changes in body weight and food consumption. At necropsy, selected organs were weighed, macroscopic observations were recorded, and tissues were collected, fixed, processed, and examined for microscopic pathologic findings. Acute quinacrine toxicity was evident during the dosing period but did not affect long-term survival. Non-neoplastic findings were more common in treated animals than controls, providing evidence of the appropriateness of the bioassay. The incidence of uncommon tumors of the reproductive tract was similar to controls at doses of 10/10 mg/kg but increased with dose level and was significantly greater than controls at ⩾70/70 mg/kg. We conclude that two doses of quinacrine administered approximately 21 days apart into the uterus of young sexually mature rats at a local dose approximately 8 times the human dose used for non-surgical female sterilization increased the lifetime risk of tumor development in the reproductive tract.

1. Introduction

Much effort has been expended to develop a safe, effective, simple, and inexpensive method of female non-surgical sterilization that would offer advantages over surgical sterilization. One investigational non-surgical sterilization method is quinacrine sterilization (QS) (Zipper and Kessel, 2003). The most current method of administering quinacrine involves two transvaginal administrations (21 days apart) of seven quinacrine tablets (36 mg each, 252 mg total) directly into the uterine fundus. As the quinacrine tablets dissolve, the quinacrine enters the Fallopian tubes, where it induces tubal scarring leading to tubal closure (Lippes et al., 2003). QS has been shown to result in female infertility, with 1-, 5-, and 10-year cumulative pregnancy probabilities of 3.3%, 10.0%, and 12.1%, respectively (Sokal et al., 2008a) and has been promoted as a simple and inexpensive method of female sterilization (Lippes, 2002). However, to date, quinacrine has not received approval from regulatory authorities for use as a non-surgical female sterilization method in any country (Benagiano, 2001, 2003).

The mechanism by which quinacrine induces tubal closure is believed to be similar to the mechanism of internal tissue repair (Holmdahl and Ivarsoon, 1999). The primary effect of quinacrine in tubal closure occurs through tissue damage, due to its cytotoxic properties, leading to inflammation and tissue repair. Tissue repair, resulting in adhesion formation, is a multifactorial process influenced by the extent of tissue damage (a function of level and duration of exposure to the cytotoxic agent), the ability of the epithelium to regenerate, the relative closeness of opposing surfaces, and the kinetics of the fibrinolytic pathway. If sufficient tissue damage occurs, preventing regeneration of the epithelium, fibroblasts are able to proliferate and fibrin deposition occurs in the damaged area. The narrow lumen of the Fallopian tube allows for the opposing surfaces to be joined, through the fibrotic

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response, leading to a functional tubal closure (Eddy and Pauerstein, 1983; el-Kady et al., 1991).

Quinacrine is a DNA-intercalating agent (Davidson et al., 1978) and has been shown to be mutagenic and clastogenic *in vitro* (reviewed by Blake et al., 1983). A more recent report confirmed that quinacrine was positive for mutagenic activity in an Ames assay and a mouse lymphoma assay, positive for clastogenic potential in a chromosome aberration assay in Chinese hamster ovary cells (Clarke et al., 2001). In contrast, results of the *in vivo* mutagenic studies of quinacrine in the bone marrow micronucleus assay indicate that quinacrine is not clastogenic *in vivo* (Clarke et al., 2001).

The high local tissue concentrations of quinacrine in the reproductive tract tissue, the mechanism by which it induces tubal closure, and its mutagenic and clastogenic activity, as well as the identification of a provocative case of a rare uterine sarcoma in a cancer cluster analysis (Sokal et al., 1995), have raised concerns about the tumorigenic potential of quinacrine (Allen, 1998). A published 18-month chronic toxicity study in rats administered quinacrine orally in either a low- or a high-protein diet (Fitzhugh et al., 1945) did not address the potential tumorigenic effect of quinacrine administered by the intrauterine route. Quinacrine showed equivocal tumorigenic activity in a 52-week neonatal mouse carcinogenicity study (Cancel et al., 2006), in which mice received intraperitoneal doses of quinacrine on postpartum Days 8 and 15. This study found that two doses of quinacrine administered early in life at $\geqslant$50 mg/kg led to an increase in the incidence of uterine endometrial hyperplasia and of benign endometrial stromal polyps, both of which are common background lesions in mice.

In short term tumor inhibition models quinacrine has been shown to have anti-carcinogenic activity (NCI, 1994); but these studies do not address the long-term safety concerns of direct administration of quinacrine into the uterus that result in potentially high local quinacrine concentrations and local tissue damage. To examine the long-term safety concerns of quinacrine, we investigated the potential tumorigenic effect of quinacrine in the rat uterine horn model. This study reports the results of a lifetime cancer bioassay where quinacrine was administered into the uterine horns of young sexually mature rats in two doses, approximately 21 days apart, to mimic the intended human use of quinacrine. We used the rat uterine horn model because it is an accepted and routinely used animal model to evaluate efficacy of investigational drugs for female non-surgical sterilization (Fail et al., 2000; Jensen et al., 2004; Zipper et al., 1968, 1973), and has been used in previous studies to evaluate tumor induction after direct intrauterine administration of drugs (Kitamura et al., 1995; Takahashi et al., 1995; Tanaka and Mori, 1983).

2. Materials and methods

2.1. Animals and animal husbandry

Nulliparous and non-pregnant female Albino Rats (Outbred) VAF/Plus®, Crl:CD®(SD)IGS BR were obtained from Charles River Laboratories, Raleigh, North Carolina at approximately 43 days of age. More rats than required for the study were purchased and acclimated. Animals considered suitable for study, and determined to be in diestrus, were randomized daily into dose groups. Randomization was stratified by animal weight to limit weight variation among groups to <20%. A total of 60 females per group were allocated into each study group with an average weight per group of 190 g. Each rat was then identified with a metal ear tag bearing an assigned unique animal number.

A total of 245 females were placed on the main study and an additional 40 rats were included for health monitoring purposes. Animals were acclimated for at least 14 days prior to the study. All animals were examined during the acclimation period to confirm their suitability for entry into the study.

Animals were individually housed in elevated, stainless steel, wire mesh cages. A 12-h light/dark cycle controlled via an automatic timer was provided. Temperature was monitored and maintained between 20.2 °C and 26.1 °C. The relative humidity was monitored and maintained between 30% and 70%. A few excursions outside the specified humidity range occurred (range 18.3–74.7%) but were not considered to have affected the integrity of the study. Cage racks were rotated periodically to avoid bias from the microenvironment in a given area of the room. Certified Rodent Diet, No. 5002 (Meal) (PMI Nutrition International, St. Louis, Missouri) and water (Elizabethtown Water Company, Westfield, New Jersey) were available *ad libitum*. No known contaminants were identified in the feed or water that might be expected to interfere with the results of the study.

2.2. Test materials and dosing suspensions

Two lots of quinacrine dihydrochloride dihydrate were used in these studies. The first and second dose range-finding studies were conducted using quinacrine manufactured by Vipor Chemicals, India and supplied by SiPharm Sisseln AG, Switzerland. Quinacrine used for the third dose range-finding study and for the lifetime cancer bioassay was manufactured and supplied by Ricerca Biosciences (Ohio, USA) and was of 92.8% purity (excluding water as hydration). Quinacrine, in the form of quinacrine dihydrochloride dihydrate, a yellow powder, was stored in a sealed brown glass container in a controlled environment, at room temperature (10–30 °C).

Methylcellulose was obtained as a white powder, manufactured and supplied by Fisher Scientific (New Jersey, USA). The vehicle was prepared by suspending methylcellulose in 0.9% saline.

Quinacrine dosing suspensions were prepared under yellow fluorescent light by suspending quinacrine in 1% (w/v) methylcellulose in 0.9% saline. Dosing suspensions were stored refrigerated in sterile amber bottles with a stir bar. On days of dosing, dosing formulations were removed from the refrigerator and brought to room temperature (approximately 1 h) by continuous stirring. Dosing formulations were stirred at least 5 min prior to dosing and continuously during dosing.

All dosing formulations were analyzed for quinacrine concentration using a Huntingdon Life Sciences validated High Performance Liquid Chromatography–UV Detection Method for the determination of quinacrine in 1% methylcellulose solution. Samples were analyzed on the day prior to use by taking duplicate samples on the day of preparation. Measured quinacrine concentrations in dosing suspensions were within 95% and 106% of the nominal concentration.

Homogeneity and stability of quinacrine in dosing suspensions used in this study were established prior to initiation of the study, using test batches of the dosing suspensions. Refrigerated stability was conducted on Day 0 and Day 14 from the date of preparation. In addition, room temperature stability was established at time 0, 4, and 8 h following preparation. Quinacrine was uniformly distributed and stable in dosing suspensions under the conditions of storage and use during the study.

2.3. Dose administration

Doses were administered through the uterine cervix into each uterine horn during diestrus. Each rat was treated twice. Administration of the first dose was staggered over a period of approximately 2 weeks due to the large group sizes and particular dosing procedure. The second dose was given approximately