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An alternative interpretation of, "A lifetime cancer bioassay of quinacrine administered into the uterine horns of female rats"

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ABSTRACT

This companion article offers an alternative interpretation for the quinacrine-induced uterine tumors observed in a 2-year bioassay in rats (CaBio, Cancel et al., 2010), and provides additional data from two new experiments that support a different interpretation and analysis. Our major premise is that the design of the Cancel et al. bioassay was flawed, particularly regarding dose selection that allowed for misinterpretation of carcinogenic activity. We feel the totality of the information provided herein dictates that the doses (70/70, 70/250 and 70/350 mg/kg quinacrine) causing uterine tumors in their study clearly exceeded the maximum tolerated dose (MTD) typically administered in chronic cancer studies. Our new data support this conclusion and serve to explain the development of lesions, especially the uterine tumors, they have reported. We argue that the rat uterus is not a valid surrogate for the human fallopian tube. Further, we maintain that quinacrine is not genotoxic *in vivo*, as suggested in their paper. In summary, we believe that quinacrine is not carcinogenic in rats at doses that do not exceed the MTD.

1. Introduction

The purpose of this paper is to offer an alternative interpretation of "A Lifetime Cancer Bioassay of Quinacrine Administered into the Uterine Horns of Female Rats" (Cancel et al., 2010) and provide new data to buttress this opinion. Cancel et al. (2010) report that two doses of quinacrine in methylcellulose (MC), introduced into the uterine horn of rats approximately 21 days apart, resulted in the development of uterine tumors after 2 years at doses of 70/70 and 70/250–350 mg/kg quinacrine, but not at 10/10 mg/kg. We believe that the exposures causing the uterine tumors in their research exceeded the maximum tolerated dose. Our opinion is supported by additional studies and commentaries presented herein.

One of the most important issues in the design of a 2-year rodent bioassay for determining the carcinogenic potential of a chemical is the selection of the doses to be used in the study. A fundamental tenet in this process is to use a dose that meets, but does not excessively exceed, what is accepted, understood and referred to as the maximum tolerated dose (MTD). This is so critical because, when exceeded, the MTD can result in marked tissue and cellular destruction that lead to neoplastic cell growth, therefore confounding the interpretation of the carcinogenic potential of the xenobiotic in the test species and humans.

2. Background

In January of 2007, the 2-year rat carcinogenicity study of quinacrine conducted by Family Health International (FHI), as reported by Cancel et al. (2010), came to our attention. The reported tumors were unusual and rare, including primitive types of both epithelial (mixed Mullerian tumor, carcinosarcoma, squamous cell carcinoma and yolk sac carcinoma) and mesenchymal (granular cell tumor, hemangioma and hemangiosarcoma) origin. These are unexpected and rare observations in a standard 2-year cancer bioassay (CaBio) of a chemical. Another unusual feature is that only a single example of each of these rare tumors was found in the study. In a typical CaBio, an increased incidence of tumors that normally occur from a uterine carcinogen include endometrial adenomas and carcinomas, endometrial stromal polyps and sarcomas, leiomyomas and leiomyosarcomas and schwannomas (Leininger and Jokinen, 1990). In our opinion, the occurrence of the unusual tumors observed in Cancel et al. (2010) would likely result from se-

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vere cell damage to the endometrium and myometrium in which only a few stem cells survived.

In order to reconstruct what may have happened in the rat uterus as a result of exposure to quinacrine, we asked and received permission from FHI to examine the histopathologic slides from a prechronic multidose study (Cancel et al., 2010) involving the same 2-dose procedure employed in the 2-year study and used to help establish doses for the CaBio.

Briefly, FHI's range-finding study #2 (Cancel et al., 2010) used quinacrine doses of 0.7/0.7, 7/7, 14/14, 35/35 and 70/70 mg/kg. The rats were exposed twice (21 days apart) with quinacrine in an MC slurry via transcervical instillation, and were sacrificed 21 days after the second exposure with no interim sacrifices. For example, the 70/70 mg/kg dose represents a dose of 35 mg/kg into each uterine horn and a second dose of 35 mg/kg into each uterine horn 21 days later. Examination of the histopathologic slides from this study by one of us (EEM) confirmed the severe pathology reported by Cancel et al. (2010) at doses of 14/14 mg/kg quinacrine and above, and the absence of lesions at 7/7 mg/kg or 0.7/0.7 mg/kg quinacrine. Moreover, in addition to uterine dilation reported by them, there was chronic purulent inflammation in several animals and complete occlusion of one horn in one rat exposed to 14/14 mg/kg quinacrine. It is probable that other uterine horns were also obstructed because of the high incidence of dilation, but the tissue sampling approach used in the study (described by Fail et al., 2000) would have precluded finding most of the occlusions because they selected three consistent cross-sections (proximal, middle and near the cervix) with no attempt at opening the remaining uterus in search of lesions.

With the above information in mind, two studies were conducted under the auspices of the International Services Assistance Fund (ISAF). The first was designed in an attempt to explain the dilation of the uterine horns. The second, a multidose study, was initiated to better define the early lesions and establish whether the dose levels met or exceeded the definition of MTD and, thus, might not be relevant to the human experience.

3. ISAF study of the effect of ligating a single uterine horn in the rat

To understand the marked uterine dilation noted in the FHI prechronic study, a hypothesis was developed: Could occlusion of a uterine horn, in and of itself, result in dilation and observed pathology as a result of mechanical pressure from the obstruction? To test this hypothesis, one of us (JL) conducted a study at the University of Buffalo, Buffalo, NY. A brief discussion of this research follows.

3.1. Study protocol

The following study was conducted in accordance with the Animal Welfare Act and was approved by the Institutional Animal Care Use Committee. Ten female Sprague Dawley rats, at 10 weeks of age and each weighing about 250 g, were selected for this investigation. Before surgery, the rats were housed in multi-cage units and were fed Harlan 2018 rat diet. Prior to surgery, incision(s) site(s) were shaved, scrubbed with antiseptic soap, wiped with alcohol, and followed by antiseptic paint.

Bupremorphine was injected for analgesia subcutaneously before anesthesia and then post-operatively as oral tablets on the next day at 0.05 mg/kg, and as needed thereafter. Rats were anesthetized with isofluorine, 4% for induction and 2% for maintenance. At 10-min intervals, corneal reflexes, positive toe pinch and the color of mucous membranes were assessed to be sure that the animals were pain free. A midline incision was made to expose the reproductive organs. The left uterine horn was ligated at both the cervical end and the utero-oviductal junction. Great care was taken to place needles with 4–0 Vicryl close to the serosa, avoiding any diminution of the blood supply to the rat uterine horn, as described previously by Lippes et al. (1972). The abdominal incision was closed in anatomical layers using interrupted sutures of 4–0 Vicryl. The skin was closed with a subcuticular running stitch of 4–0 Vicryl. Time elapsed for this surgery was approximately 1 h.

Following surgery, rats were housed individually in open-top polycarbonate caging with aspen bedding. After 21 days, animals were euthanized in a carbon dioxide chamber. Death was assured by cutting the chest open. The uteri were photographed *in situ*, removed, and placed unopened in 10% neutral buffered formalin. A single (5 μm) cross-section from the mid-portion of each uterine horn was made and stained with hematoxylin and eosin. The sections were examined microscopically by one of us (EEM) to determine the extent of damage, if any, from the ligation. The unligated horn served as the control.

3.2. Results

One rat died early in the study (day 7). The ligated horns of all of the surviving rats showed marked dilation (Fig. 1). Most of the uterine glands were not present due to pressure atrophy. Interestingly, the bursa of the ovary was also dilated (Fig. 2). No uterine lesions, especially purulent inflammatory material as seen in the Cancel et al. (2010), were observed in these rats.

3.3. Interpretation

This research demonstrates that ligation (occlusion) alone can result in uterine horn dilation of the severity that was also observed in the CaBio's dose range-finding study #2 and in Fail et al. (2000). And, that such dilation can occur within 21 days of the ligation. But we emphasize there was no evidence of uterine inflammation or cell damage other than atrophy in any of the animals whose uterine horns were ligated.

We believe the explanation for this relates to the anatomy of the rat reproductive tract. Unlike the fimbria of the human fallopian tube which is open to the ovary, and indirectly to the abdominal cavity, the rat ovary is completely encased in a bursa that is an extension of the fallopian tube. Therefore, increased pressure from occlusion would not be released into the abdominal cavity as

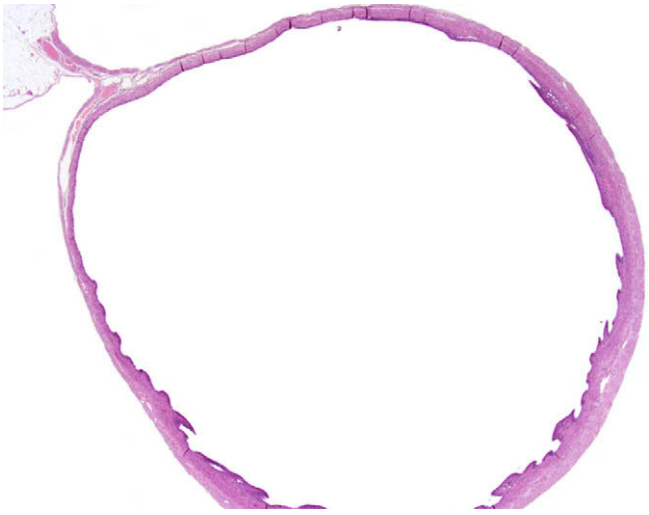

Fig. 1. Marked dilation of a ligated uterine horn. H&E stain, Original magnification 20×.