

Action of MER-25 and of Clomiphene on the Human Ovary (1963)

Between 1958 and 1962, physicians Olive W. Smith, George V. Smith, and Robert W. Kistner performed experiments that demonstrated the effects of the drugs MER-25 and clomiphene citrate on the female human body. MER-25 and clomiphene citrate are drugs that affect estrogen production in women. At the time of the experiment, researchers did not know which organ or organs the drugs affected, the ovaries and/or the anterior pituitary gland. To determine that, the physicians reviewed nine of their own previous studies in women in which they measured the urinary output of hormones after the administration of either drug. Based on their examination, the researchers concluded that MER-25 appeared to influence the anterior pituitary gland in the brain to produce a response in the ovaries and that clomiphene citrate appeared to act on the ovaries. Their results provided early evidence about the mechanisms of both drugs. Later, clomiphene citrate became a common fertility drug.

The researchers all worked in the field of gynecology. Throughout the 1900s, spouses Smith and Smith studied the effects of sex hormones on pregnancy and different types of cancers. Kistner primarily studied endometriosis and infertility. Endometriosis results in uterine tissue becoming relocated outside the uterus, which causes pain and, oftentimes, infertility. Given their study of sex hormones and female reproduction, all three physicians were familiar with the fertility drugs MER-25 and clomiphene citrate.

Throughout the mid-twentieth century, both MER-25 and clomiphene citrate were used frequently in medical care. During the 1950s, researchers had conducted clinical trials using MER-25 to treat breast and endometrial cancers while clomiphene citrate was used as a fertility drug in animals. By the 1960s, researchers had only administered MER-25 in clinical trials to treat certain cancers, and they knew little about the drug's side effects. Researchers knew that both drugs affected estrogen production in women. However, researchers were uncertain how the drugs affected estrogen production, as they did not know which organs the drugs affected. Because of the chemical similarity of the drugs, most scientists hypothesized that both drugs affected the same organ, either the ovary or the anterior pituitary gland. The anterior pituitary gland, located in the brain, produces sex hormones that are related to reproduction, development, and stress responses. The ovaries secrete similar sex hormones that control the menstrual cycle in women. Those sex hormones include estrogen, progesterone, and testosterone. Smith, Smith, and Kistner sought to determine which organ the drugs affected by looking at and reviewing several previously reported experiments.

Each of the experiments the researchers reported on investigated the hormone content of urine. The physicians were able to detect the quantity of hormones in the urine. During each experiment, the physicians had administered either MER-25 or clomiphene citrate to a female patient with a particular ailment. All three researchers had measured urinary hormones in their research prior to the experiments, and it was one of the very few methods of hormone collection known at the time. Smith, Smith, and Kistner measured both estrogen and gonadotropin levels in the urine outputs of each woman before, during, and after each trial. Gonadotropins are a group of hormones secreted by the pituitary gland to stimulate the gonads, a term that refers to the ovaries or the testes. Humans produce two primary gonadotropins, luteinizing hormone and follicle-stimulating hormone. In women, those two gonadotropins help to control the different phases of the menstrual cycle by acting on the ovaries and changing their production of estrogen. Estrogen is only produced by the ovaries. The absence or presence of those hormones in the urine can help researchers determine the action site of the drugs.

Smith, Smith, and Kistner conducted nine experiments to determine the drugs' effects. In their experiments, the researchers administered MER-25 to three women, clomiphene citrate to four women, while one woman received each drug singularly in two separate trials. That woman served as a control in the experiment because she did not have ovaries, meaning the researchers could see if the drugs still affected the woman in the absence of her ovaries. Also, some of the women who were given clomiphene had their ovaries removed to test the hormonal contents of the fluids inside. Before their ovaries were removed, the drugs were administered and urinary hormone levels were collected. Then, the ovaries were removed for various reasons for each patient, and the same tests were performed again.

The researchers made several assumptions in their experiments, which they described in an article they published in 1963. They assumed that higher estrogen levels in the women's urine after the administration of either drug indicated the inclusion of the ovary in the mechanism of a drug. Because the ovaries are the only organs capable of producing estrogen, elevated estrogen levels could only occur if the ovaries were somehow involved in the action of the drug. However, that would not have answered whether the pituitary gland was involved, because the hormones produced by the pituitary gland are capable of activating the ovaries and thus cause an increase in estrogen. To determine if the pituitary gland was involved in the action of the drug, the researchers measured the gonadotropin levels in the urine. They did so under the assumption that higher gonadotropin levels would indicate the inclusion of the pituitary gland in the mechanism of a drug.

In the first experiment, the researchers administered MER-25 to a woman who was age sixty-eight. The woman had breast cancer and was also postmenopausal, meaning she did not have a menstrual cycle anymore. Before giving the woman MER-25, the researchers measured her hormone levels and found that her urinary estrogen and gonadotropin levels did not correspond with typical levels in a postmenopausal woman and her estrogen. However, the researchers attributed that to hormonal fluctuations associated with breast cancer. The researchers remeasured her hormone levels when giving her MER-25 for ten days and found that the woman's estrogen levels and gonadotropin levels had doubled. The researchers conducted two more experiments using MER-25 before they concluded what those results indicated.

In the second experiment, the researchers gave MER-25 to a woman who was postmenopausal and who had endometrial cancer. The researchers performed two sub-trials with the woman. In the first sub-trial, the researchers measured the woman's levels of urinary hormones prior to giving her drugs, which they say were normal for a postmenopausal woman. Then they remeasured her urinary estrogen and gonadotropin levels after giving her the drug for ten days. The MER-25 caused estrogen and gonadotropin levels to rise significantly, just as they had in the woman in the first experiment. Then the researchers removed her ovaries, and began the second sub-trial. Three months following the removal of the woman's ovaries, the physicians gave the woman MER-25 again, which resulted in no significant rise in estrogen or gonadotropin levels. The researchers concluded that the ovaries are involved in the drug mechanism of MER-25 because estrogen levels had increased in response to the drug. However, because MER-25 was an antiestrogen and could not increase estrogen production on its own, and because the gonadotropin levels in the urine had increased, the researchers predicted that MER-25 affects the pituitary gland as a means of producing gonadotropins which, in turn, helps the ovary produce estrogen.

In the last MER-25 experiment, the researchers gave MER-25 to a third woman, age twenty-six, in an attempt to stimulate ovulation. Prior to the experiment, the twenty-six-year-old woman had not menstruated or ovulated except in response to hormonal therapy. The researchers then gave the woman two courses of MER-25 treatment, and the woman began ovulating. However, after observing the similar rise and fall of her urinary hormones, the researchers observed that the woman appeared to have exhibited the same mechanism found in the two postmenopausal women. The researchers concluded that MER-25 acts on the pituitary gland, which causes it to release hormones that then act on the ovary, which causes it to produce estrogen.

After conducting the three MER-25 experiments, the researchers ran four additional experiments in which they gave women clomiphene citrate. In the first clomiphene experiment, Smith, Smith, and Kistner gave clomiphene to a twenty-four-year-old woman who had not had a menstrual cycle in seven months to induce ovulation. Prior to the experiment, physicians had removed the woman's

right ovary due to the presence of what the woman described as a painful mass. The researchers observed that the woman was not ovulating, which was associated with low-levels of estrogen. By the end of the treatment with the drug, the researchers found that the woman's estrogen levels had increased from thirty-nine micrograms per liter to a very high 267 micrograms per liter. The woman's gonadotropin levels remained the same, which indicated to the researchers that the drug they were testing does not affect the gonadotropin levels. Therefore, the researchers predicted that clomiphene citrate does not affect the ovaries. The researchers conducted a set of three subsequent experiments to determine whether their predictions were correct.

In the final three clomiphene experiments, the researchers gave the drug to three women who later had their ovaries removed. After giving the women the clomiphene over a ten-day period, each woman experienced an elevation in estrogen levels but no change in gonadotropin levels. After two of the three women developed cysts in their ovaries, the researchers excised all of the women's ovaries. While examining the contents, the researchers found that each ovary contained high concentrations of estrogen. They also found developing follicles in the first patient's ovaries, meaning that the woman was about to ovulate when her ovaries were removed. Based on those results, the researchers concluded that clomiphene directly impacts estrogen production of the ovary, unlike MER-25 which does so by first affecting the pituitary gland.

Lastly, the researchers performed two experiments using one woman. The researchers gave that woman both drugs at different times. Prior to the experiments, the woman had a complete hysterectomy, meaning her entire reproductive tract had been removed, including her uterus and ovaries. Therefore, the woman required her estrogen-levels to be artificially supplemented because estrogen cannot be created without ovaries. For this reason, she was a control due to her lack of ovaries. The researchers gave each drug to the woman separately to study the individual effects of the drugs on a woman without ovaries. Neither drug was capable of stimulating any estrogen production in the woman in the first experiment.

In the second experiment on the control woman, the researchers gave the woman MER-25 while simultaneously giving her oral estrogen supplements. The researchers observed a significantly heightened urinary estrogen output and high gonadotropin levels in the woman. The author's note that the estrogen levels found in the urine were significantly higher than what the woman was given orally, meaning the MER-25 had increased the levels of estrogen in the woman. The researchers concluded that MER-25 affects the pituitary secretion of gonadotropins, which in turn causes ovarian secretion of estrogen, but that the process can only occur when a certain amount of estrogen is present in the body.

Based on all of their experiments, the researchers concluded that MER-25 acted on the anterior pituitary gland in the brain while clomiphene citrate appeared to act on the ovaries. In women who had a sufficient amount of estrogen, MER-25 caused the pituitary gland to produce gonadotropins. Therefore, the researchers suggested that the MER-25 influences the pituitary gland to secrete gonadotropins, which produces a response in the ovaries. Clomiphene citrate produced estrogen in every woman except the woman without ovaries and therefore the researchers concluded that clomiphene citrate likely acts on the ovaries.

Following the experiments, more clinical trials indicated that MER-25 produced too many dangerous central nervous system side effects, including hallucinations and psychotic episodes. The drug was never approved for use in the US. However, following Smith, Smith, and Kistner's article, clomiphene citrate became a prominent fertility aid.

Sources

1. Garcia, Jairo, Georgeanna Seegar Jones, and Anne C. Wentz. "The use of clomiphene citrate." *Fertility and Sterility* 28 (1977): 707-17.
2. Kistner, Robert W., and Olive W. Smith. "Observations on the use of a non-steroidal estrogen antagonist: MER-25." *Surgical Forum* 10 (1960): 725.
3. Koninckx, Philippe R., Christel Meuleman, Stephan Demeyere, Emmanuel Lesaffre, and Freddy J. Cornillie. "Suggestive evidence that pelvic endometriosis is a progressive disease,

- whereas deeply infiltrating endometriosis is associated with pelvic pain." *Fertility and Sterility* 55 (1991): 759-65. <http://europepmc.org/abstract/med/2010001> (Accessed July 13, 2017).
4. Smith, Olive W., George V. Smith, and Robert W. Kistner. "Action of MER-25 and of Clomiphene on the Human Ovary." *The Journal of the American Medical Association* 11 (1963): 878-86.