FURTHER STUDIES ON THE PHARMACOLOGY OF CLOMIPHENE

A THESIS

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INTRODUCTION

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Unexpected and interesting biological activity in the field of reproductive physiology, was encountered while evaluating clomiphene (MRL 41), an analogue of the synthetic, non-steroidal estrogen compound chlorotrianisene (TACE) (Greenblatt et al.,1961). In the rat, clomiphene has been shown to have pituitary-gonadotrophin inhibiting and antifecundity properties (Holtkamp et al., 1960), while in the human it was found to possess a surprising potential for the induction of ovulatory type cycles in amenorrheic patients (Greenblatt et al., 1961). A related compound, ethamoxytriphetol (MER-25) had also been reported to inhibit ovulation (Kistner and Smith, 1961).

Although the induction of ovulation with clomiphene is an important breakthrough in this defficult field of endevour, the advances in this field dated back with the studies of the compound chlorotrianisene (TACE) (Thompson and Werner, 1951). This weakly estrogenic compound unlike most estrogens was noted not to cause enlargement of the pituitary when given in rats in high doses (Segal and Thompson, 1956) i.e. while estradiol normally causes pronounced enlargement

of the pituitary gland, its effect was greatly reduced when chlorotrianisene was given concurrently with it.

Modification of the chemical structure of chlorotrianisene (TACE), had led to a varied spectrum of biological activities . Several related compounds have been investigated in Merrel laboratories. The specific biological effects produced by those modified substances are inhibition of cholesterol synthesis by Triparanol (MER-29) (Blohm et al. 1959) and estrogen antagonism by ethamoxytriphetol (MER -25) (Lerner et al. 1958) . Ethamoxytriphetol (MER.25) is triphenyl ethanol derivative of chlorotrianisene (1 " P - 2 - diethyl aminoethoxyphenyl" 1 - phenyl - 2 -P anisyl ethanol) . It was found to be strongly antiestrogenic (Lerner et al. 1958). MER - 25 inhibits endogenous estrogens as well as estrogen given in the form of natural compounds, or diethyl stilbesterol or chlorotrianisene. This inhibition was seen in a variety of effects as well as in several species (Meyerson and Lindstrom 1968). When female rats are treated for a brief post-copulatory period with an adequate dose of this compound, implantation never occurs. These animals enter a period of pseudoprognancy characteristic of rats following sterile mating and upon resumption of estrous cycles, are completely capable of having normal litters(Segal and

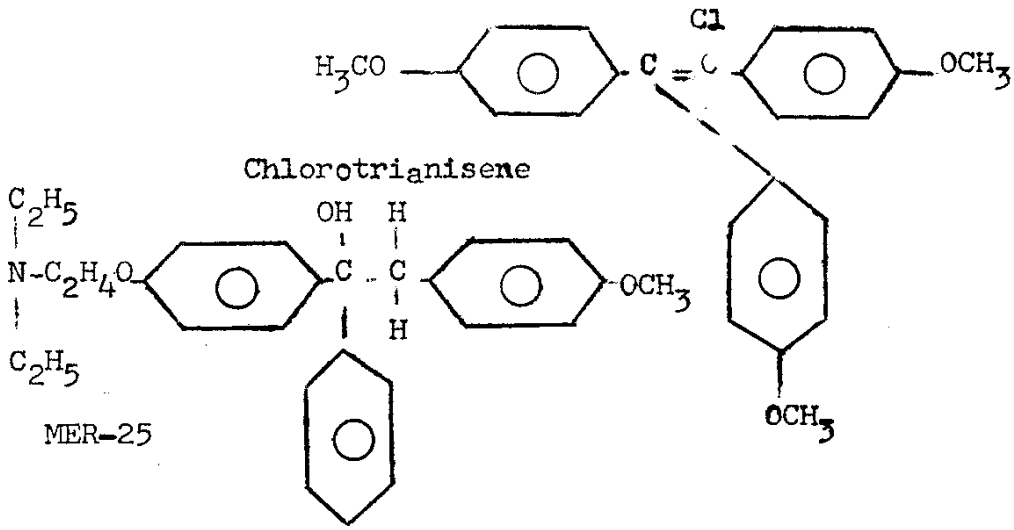
Nelson 1958) . Estrogen antagonism activity of MER-25 had been demonstrated in intact and castrated rats , mice and monkeys and in chicks and rabbits (Lerner et al. 1958). The estrogen target organs employed for the measurement of the estrogen antagonism activity of MER-25 , were the uterus, vagina and pituitary gland in the mammals, and the oviduct in chicks. An additional end point employed was the chick plasma phospholipids(Lerner et al.1958) .

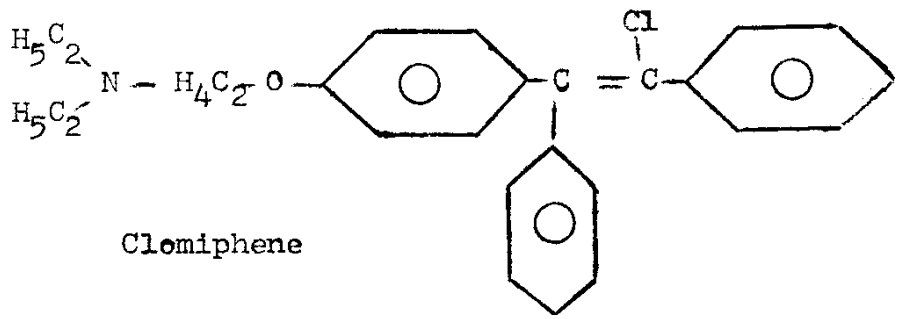
Ethamoxytriphetol (MER 25) antagonises the activity of both endogenous and exogenous estrogens, steroidal and non-steroidal (Meyerson and Lindstrom 1968) . The pituitaries of rats to which MER-25 was administered were smaller than those of control animals and it prevented estrogen and castration induced pituitary hypertrophy (Lerner et al. 1958). Weak gonadotrophin inhibition was evident in the treated male rats and in the parabiotic female rats. MER-25 interfered with normal pregnancy in the rat, The male offsprings of animals to which the drug was administered during lactation were fertile but the female offsprings were infertile. On the other hand, MER-25 does not have estrogenic, androgenic, antiandrogenic, progestational, antiprogestational or gonadotrophin-like activities (Lerner et al. 1958) . When MER-25 was adminis-

tered orally to female rats on the day of mating and continued throughout the normal period of oviductal passage of ova, implantation did not occur. Restricting the treatment period to only one day post-coitus was also effective in preventing nidation (Segal and Nelson 1958). The antifertility effects of MER-25 according to Segal and Nelson (1958) appear to be exerted prior to exit of ova from the oviducts. The compound does not act by preventing decidua development, nor by interfering with post-copulatory maintenance of luteal body function. Histologic evidence indicates that ova of females treated with MER-25 undergo degeneration after extrusion of second polar body (Segal and Nelson 1958).

Soon MER-25 was removed from further clinical trials because of its toxicity. However, successful induction of ovulation in six out of 18 anovulatory women was reported with MER-25 by Tyler and Coworkers (1960) . Kistner and Smith (1961) reported on 4 patients with Stein-Leventhal syndrome who were treated with MER-25. All of them ovulated and 2 became pregnant, This compound led to the introduction of clomiphene.

Clomiphene (MRL-41) is (1-" P B diethyl amino ethoxy" phenyl) 1-2 diphenyl - 2 - chloroethylene).





Clomiphene

Animal tests with clomiphene showed very slight estrogenic activity and moderate antiestrogenic activity (Murad and Gilman 1975) . Also , the striking effect was inhibition of the pituitary gonadotrophin formation (Holtkamp et al. 1960). Small doses stopped the estrous cycle of normal rats, reduced the size of the ovary. Somewhat larger doses, inhibited spermatogenesis also by inhibition of gonadotrophins. Thus in

both sexes, the compound initially was a potent contraceptive (Murad and Gilman 1975). Holtkamp et al. (1960) started the work in animals. They concluded that the major biological activity refers to pituitary gonadotrophin inhibition and antifecundity. Clomiphene produced a dose dependent reduction in the weight of reproductive organs of intact rats: ovaries and uteri in females and testes, seminal vesicles and ventral prostate in males (Holtkamp et al. 1960). It prevented the ovarian hypertrophy induced by joining castrate and intact rats in parabiosis. Such results are indicative of pituitary gonadotrophin inhibition. Clomiphene ,however, did not change pituitary gonadotrophin content and produced no change or a slight decrease in pituitary weight. Estradiol, on the other hand, produced pituitary hypertrophy (Veraldo 1958). Administration of clomiphene to mature female rats interrupted normal estrous cycles and produced a loss of fecundity (Holtkamp et al. 1960).Both these effects were reversible after drug administration was stopped, while administration of clomiphene to immature female rats gave a doubling of uterine weight indicative of a weak estrogen-like activity. However, it antagonised the indirect uterotrophic effect of PMS which suggests an antiestrogen action of clomiphene (Holtkamp et al. 1960).

Thus the vaginal smear picture, the mild uterotrophic effect and antagonism to uterine weight increase , indirectly induced by PMS, indicate that clomiphene has a secondary mild or atypical estrogen like and estrogen antagonising effects. Treatment during co-habitation produced a loss of fecundity which was reversible after cessation of treatment in rats . Also clomiphene failed to block exogenous gonado - trophins (Holtkamp et al. 1960).

In the human, unexpected and astonishing results were obtained . Greenblatt et al. (1961) were the first to report that clomiphene was responsible of inducing ovulation in a high percentage of amenorrheic women. Although induction of ovulation in the human has been of considerable research interest for several decades, for the clinician, however, the problem of ovulatory failure remains a frustrating dilemma. Clomiphene is the most welcome development in this field .
Of the methods adopted, administration of human follicle stimulating hormone (FSH) when followed by human chorio-nic gonadotrophin (HCG) has been known to induce ovulation (Gemzell et al. 1958) . However, this method is not likely to come into general use because of the scarcity of human FSH and adverse reactions (Jones et al. 1953 , Buxton and Herman 1961 ; Greenblatt et al. 1961).

Furthermore, large follicular cysts and cystic corpora lutea are frequently produced by these gonadotrophins. Glucocorticoids have also some merit in anovulatory women particularly in certain hirsute amenorrheic women (Greenblatt et al. 1953) . Similarly, wedge resection of the ovaries has proved a valuable procedure in the management of the Stein-Leventhal syndrome (Stein and Leventhal 1953) through the reduction of the ovarian mass, (Greenblatt et al 1961). Occasionally, cyclic estrogen and progesterone have been used successfully in functional amenorrhea to restore pituitary -ovarian balance. Also, striking results are frequently obtained with thyroid medication in the subclinical hypothyroid patients with ovulatory failure (Greenblatt et al 1961). In, addition, radiation therapy of the pituitary gland and the ovaries has its protagonists, successes have been reported with stimulating doses but fear of genetic mutations restricts its use (Rakoff 1953), .

How clomiphene came to induce ovulation, was reported in Greenblatt own words (Greenblatt 1977):- Clomiphene was first given to 4 people in the United States as a contraceptive agent because in the experimental animals it suppressed ovulation and spermatogenesis(Greenblatt et al. 1961), This was true for the experimental animals as rat and the dosage

was too high and perhaps it had a suppressive action. When tried as a contraceptive in the human , starting with 10 mg a day from day 5 to day 25 of the menstrual cycle, it did not inhibit ovulation. Dosage was increased from 10 to 25 to 50 to 100 mg a day. After 50 mg per day for 20 days a month, many of the patients have prolonged luteal phase of 20-35 days. We said"Ah, we have a luteotrophic agent, something that maintains the corpus luteum ! " . And, whether our thinking was correct or not, we said, " Let's try it in the amenorrheic patient". Lo and behond , the first patients we give it to, had been amenorrheic for a year. We gave her 100 mg a day for 20 days. She developed two massive ovaries and endometrial biopsy revealed a secretory endometrium. The next time we gave a smaller dose and we were again able to stimulate ovulation. We tried it in 36 patients with ovulatory failure and induced ovulation in 27 patients .

Our report in 1961 in the Journal of the American Medical Association was not beleived by most gynecologists, who remained skeptical.2 years later we published our results in more than 250 cases with an ovulation rate of about 70% . Sence that time, I think that there have been a thousand articles in the world literature supporting the concept that clomiphene can stimulate ovulation in the

proper type of patients ". (Greenblatt 1977).

Those observations have been confirmed by many groups of investigators (Charles et al. 1962, 1966 ; Kistner et al. 1962, 1965; Southam et al. 1962 ; Pouble and Greenblatt 1964 ; Roy et al. 1964; Thompson & Mellinger 1965; Hammerstein1967; Igarashi et al, 1967 and Jacobson, Marshal & Ross 1968) .

Greenblatt et al. (1962) and Roy et al. (1963) also reported an increase in urinary excretion of gonadotrophins and estrogens in patients treated with clomiphene. Excessive enlargement of ovaries and the formation of ovarian cysts were common features of the treatment when doses of 100-200 mg daily were given for 2-3 weeks but with doses of 50-75 mg daily, this complication was loss frequent and the ovaries returned to normal size after treatment has been completed (Murad & Gilman 1975).

The substance gave evidence of antiestrogenic effects. Greenblatt et al (1961) tried the drug in precocious-puberty in three girls. They received clomiphene daily for 4-5 months. They ultimately showed lessening in the amount of the menstrual flow, regression of the vaginal cytologic response and some decrease in breast size. An antiestrogenic property of MRL-41 is suggested also by hot flashes in several cases which is due to competotion with estrogen receptors at the hypothalamus resulting in disturbance of autonomic activities.