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A Paradoxical Effect of an Antiestrogen, CI-628, on Implantation in the Mouse (42780)

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Abstract. Some triphenylethylene compounds (TPE), commonly known as antiestrogens, have been reported to interfere with pregnancy. This effect of these TPE compounds was attributed to their antagonistic effects to estrogen in the uterus and/or the embryo. Paradoxically, recent evidence suggests that several TPE compounds act as estrogen agonists in inducing embryo implantation in the uterus of ovariectomized, progesterone-treated, delayed-implanting mice. The present study was undertaken to determine the effect of CI-628, a TPE compound, on normal pregnancy and its site of action. Mice were given a single injection of CI-628 (1.5, 5, or 15 μg/mouse, ip) on Day 3, Day 4, or both days and killed on Day 8 for examination of implantation sites or unimplanted embryos. CI-628 at 5 and 15 μg reduced the implantation rate when injected on Day 3 or Days 3 and 4, but not when administered only on Day 4. The lower dose of the compound (1.5 μg) was ineffective in altering implantation. The implantation failure was overcome to various degrees by simultaneous injection of progesterone (2 mg/mouse, sc), but not by estradiol-17β (20 ng/mouse, sc). These results suggest that Day 3 is critical for CI-628 to exert its inhibitory effect and that this compound appears to affect ovarian progesterone synthesis and/or secretion. This suggestion is consistent with our findings that animals that showed implantation following injection of 5 or 15 μg of CI-628 had higher serum progesterone levels than those without implantation. Serum progesterone levels of animals bearing implantation sites following 5 μg of CI-628 on Day 3 or Day 4 were comparable to those of vehicle-treated controls, whereas the level of this steroid in animals treated with 15 μg of CI-628 and having implantation sites was relatively lower. However, a small number of animals that had implantation following treatment with 5 μg of CI-628 on Days 3 and 4 showed lower progesterone levels compared to those of the controls, but not different from those of some of the treated animals without implantation. Treatment with estrogen could not restore progesterone levels or implantation. In summary, the results suggest that CI-628 acts as an estrogen antagonist in interfering with implantation at the ovarian level in intact, pregnant mice.

Normally, implantation in the mouse requires progesterone and estrogen. Progesterone in the absence of estrogen induces blastocyst dormancy and implantation failure (1, 2). The mechanism by which estrogen functions in this system remains an enigma. Although estrogenic action is commonly associated with phenotypic cellular changes secondary to altered gene expression, several other, more rapid effects are expressed in the uterus (3–6). We have recently shown that early effects of estrogen are essential for implantation in the mouse (7). Some triphenylethylene (TPE) compounds, commonly known as nonsteroidal antiestrogens, compete with estrogens for binding to estrogen receptors and have been shown to inhibit estrogen-stimulated tissue growth and differentiation in the uterus (8, 9). Some TPE compounds also exhibit estrogen agonistic activity, the degree of which is tissue and cell specific (10, 11). These compounds have been used to interrupt pregnancy, a result thought to be due to their estrogen antagonism in the uterus and/or embryo (12, 13). However, our recent work shows that most TPE compounds can initiate implantation in ovariectomized, progesterone-treated, delayed-implanting mice. This implantation-inducing effect has been correlated with the ability of the TPE compound to exhibit early estrogenic effects (7). Therefore, in the delayed-implanting uterus, some TPE compounds act as estrogen agonists in initiating implantation, but not antagonists as believed earlier (7). In the present study, we have at-

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tempted to determine whether a TPE compound, CI-628, can interfere with implantation in intact mice and if so, its site of action.

Materials and Methods. Charles River (CD-I), virgin, female mice (48 days old, 20–25 g) were mated with fertile males of the same strain. Day 1 of pregnancy was designated as the day of finding a vaginal plug. The first several groups of pregnant mice were injected intraperitoneally with CI-628 (Warner–Lambert/Parke Davis, Ann Arbor, MI) as a single injection (1.5, 5, or 15 μg/0.1 ml saline/mouse) on Day 3 or Day 4, or on both days. The next groups of mice received CI-628 (5 or 15 μg) and a subcutaneous injection of progesterone (2 mg/0.1 ml sesame seed oil/mouse, Sigma Chemical Co., St. Louis, MO) on Day 3 or Days 3 and 4 of pregnancy. The last groups of mice received a single subcutaneous injection of estradiol-17β (20 ng/0.1 ml oil/mouse, Sigma) with CI-628 (5 μg) on Day 3 or Days 3 and 4 of pregnancy. Implantation was determined on Day 8 of pregnancy by an intravenous injection of 0.1 ml of 1% Chicago Blue B (Sigma), 5 min before killing (14). If no implantation was observed, uteri were flushed with 0.9% NaCl to recover embryos. A small number of plug-positive mice always fails to show implantation or recoverable embryos under no treatment conditions. Therefore, these animals in vehicle-treated control and certain experimental groups were excluded from the experiment. However, when the number of these animals was large under certain treatment conditions, they were included in the experiment in order to avoid bias of the treatment effects. Serum progesterone level was determined by radioimmunoassay using a kit from Diagnostic Systems Inc. (Webster, TX).

Results. As shown in Fig. 1, CI-628 given at 5 or 15 μg on Day 3 reduced the implantation rate to 43 and 38%, respectively. When given on Days 3 and 4, implantation was further reduced to 29% by 5 μg and completely inhibited by 15 μg. The mean number of implantation sites was also lower under

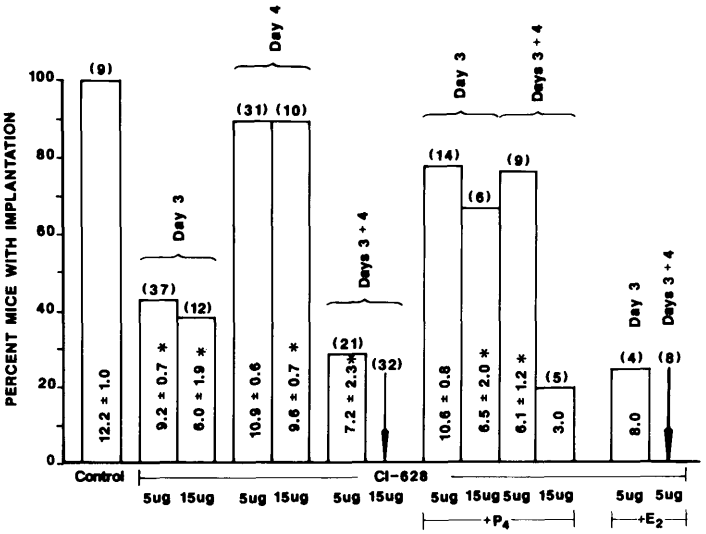

FIG. 1. Effects of vehicle (0.1 ml), CI-628, CI-628 + estradiol-17β (E_2, 20 ng/mouse/day), or CI-628 + progesterone (P_4, 2 mg/mouse/day) on implantation in the mouse. Numbers in parentheses represent the number of animals with implantation sites and/or embryos with the exception of groups treated with 15 μg of CI-628 on Day 3, Days 3 + 4, or CI-628 + E_2 on Days 3 + 4 which include animals without embryos. Values within the bars indicate mean (±SEM) number of implantation sites for animals with implantation. *$P < 0.05$ compared to the number of implantation sites of vehicle-treated controls. No statistical analysis could be done for animals receiving CI-628 + E_2 on Day 3, Days 3 + 4, or CI-628 at 15 μg + P_4 on Days 3 + 4 due to lack of sufficient numbers of animals with implantation sites.

these conditions. In addition, a considerable number of animals treated with 15 μg of CI-628 on Day 3 (33%) or Days 3 and 4 (66%) had no recoverable embryos. In contrast, CI-628 administered on Day 4 at either 5 or 15 μg was not very effective in reducing implantation rates; only 10% of the animals had implantation failures. Implantation rates were restored to approximately 78% when progesterone was administered simultaneously with 5 μg of CI-628 on Day 3, or Days 3 and 4. Although progesterone was quite effective in restoring implantation rates to 67% in animals treated with 15 μg of CI-628 on Day 3, it only restored implantation in 20% of the animals when treated on Days 3 and 4. CI-628 when administered at 1.5 μg on Days 3 and 4 was totally ineffective in interfering with implantation (mean number of implantation sites in 6 animals was 9.3 ± 0.7).

As shown in Table I, serum progesterone levels in animals with implantation following treatment with CI-628 were higher than those without implantation. Animals showing implantation after treatment on Day 3 or Day 4 with 5 μg of CI-628 had progesterone levels comparable to those of vehicle-treated controls. In contrast, the animals receiving 15 μg of CI-628 on the same days had relatively lower levels of this steroid. However, a few animals treated with 5 μg of CI-628 on both days and having implantation sites had progesterone levels that were not different from those of some treatment groups without implantation. Injection of estrogen with CI-628 did not restore implantation or serum progesterone levels. In addition, 7 of 8 animals treated with CI-628 and estradiol-17β on Days 3 and 4 lacked recoverable embryos.

Discussion. Our previous work and the results of our present study indicate that although CI-628, a TPE compound, acts as an estrogen agonist in initiating implantation in ovariectomized, progesterone-treated, delayed-implanting mice (7), it interfered with implantation in intact animals. Several modes and sites of action could be considered. Estrogen has been shown to induce progesterone receptors in the uterus (15). It is possible that CI-628 interfered with progesterone receptor synthesis and thus with progesterone action and implantation (16). This argument is probably not valid, because exogenous administration of progesterone

TABLE I. EFFECT OF ADMINISTRATION OF CI-628 DURING THE PREIMPLANTATION PERIOD ON SERUM PROGESTERONE LEVELS (ng/ml) IN MICE KILLED ON DAY 8

Treatment		With implantation sites	Without implantation sites
CI-628 (μg)			
Day 3	5	76.6 ± 7.9 (6)	48.4 ± 6.8 (8)[a]
	15	60.7 ± 3.4 (3)*	49.4 ± 5.6 (7)[b,*]
Day 4	5	79.6 ± 3.5 (9)	48.5 ± 0.1 (2)[a,*]
	15	59.5 ± 5.2 (8)*	—
Day 3 + 4	1.5	64.8 ± 4.9 (6)	—
	5	44.3 ± 6.9 (5)*	31.7 ± 3.7 (13)[a]
	15	—	37.7 ± 4.0 (19)[b,*]
CI-628 (5 μg) + estradiol-17β (20 ng)			
Days 3 + 4		—	13.0 ± 2.3 (7)[b,*]
Vehicle		77.0 ± 3.8 (12)	—

Note. Results shown are means (±SEM) for the number of animals in parentheses.

[a] Only animals with nonimplanting embryos.

[b] Animals with and without nonimplanting embryos. *$P < 0.05$ compared to normal Day 8 serum values (ANOVA). The progesterone values of animals treated with CI-628 at 1.5 or 5 μg on Days 3 and 4 with implantation sites were different from each other ($P < 0.05$), as were those treated at 5 or 15 μg on Day 4. All animals treated either with the vehicle or with the CI-628 at 1.5 μg on Days 3 and 4 had implantation, while none of the animals treated with CI-628 (15 μg) or CI-628 (5 μg) + estradiol-17β (20 ng) on these days had implantation.

overcame the implantation inhibiting effect of CI-628 to a considerable extent. Impaired function of progesterone due to an inadequate population of progesterone receptors should not be corrected by exogenous progesterone administration. However, ligand–receptor interaction could be adequate in the presence of excess ligand even when the receptor population remains low. Other possible targets could be the pituitary and/or the ovary. Whether CI-628 is acting on the pituitary cannot be ascertained from the present investigation.

The probable target for CI-628 during the preimplantation period in intact animals appears to be the ovary. Day 3 of pregnancy, but not Day 4, appears to be the critical day for CI-628 to be maximally effective in interfering with implantation in the mouse. In the mouse, plasma progesterone levels are low during the first two days of pregnancy and steadily increase from Day 3 until Day 5 (17). The results of our present investigation indicate that interference with ovarian progesterone synthesis and/or secretion between Days 3 and 4 appears to be critical for implantation. This explanation is consistent with our finding that implantation rates and the mean number of implantation sites were lower when CI-628 (5 or 15 μg) was given on Day 3, and Days 3 and 4, but not on Day 4. Improvement of implantation rates by progesterone replacement further lends support to this suggestion. Our observation of lower progesterone levels in animals without implantation following CI-628 treatment suggests that progesterone may have been the missing ingredient resulting in implantation failure. Indeed, intraluteal estrogen has been suggested to be necessary for luteal progesterone synthesis in the rat (18). Reduction of plasma progesterone levels by CI-628 on Day 3, plus the low levels on Days 1 and 2, may not provide adequate priming of the uterus for implantation. In the rodent, 48 hr of progesterone priming is required for implantation (14). It is to be noted that animals having implantation after receiving 15 μg of CI-628 consistently showed lower progesterone levels than those treated with 5 μg. Thus, it appears that these relatively low progesterone levels were sufficient to induce implantation. However, in 5 of 21 animals that received 5 μg of CI-268 on Days 3 and 4, implantation occurred even though plasma progesterone was not different from some of the CI-628 treated animals without implantation sites. We cannot offer any explanation for this discrepancy at this time.

Although the effect of CI-628 appears to be mainly due to the interference of ovarian progesterone synthesis and/or secretion, other nonspecific effects cannot be disregarded. This is particularly worth considering for animals treated with 15 μg of CI-628 where large numbers of animals not only failed to show implantation but also had no embryos. This could result from toxic effects of the drug on embryos and/or the uterus making the environment hostile for implantation.

Now the question arises as to the mechanism of action of CI-628 in interfering with ovarian progesterone synthesis and/or secretion. CI-628 may behave as an estrogen antagonist in the ovary, because of its inherent property of competing for estrogen receptors or it may interfere with intraovarian estrogen synthesis. In the rat, tamoxifen, another TPE compound, has been shown to interfere with estradiol synthesis in the ovary (19). Alternatively, some TPE compounds may have a direct effect on progesterone synthesis and/or secretion in the ovary.

If estrogen is necessary for ovarian secretion of progesterone, then administration of estrogen with CI-628 should have overcome the implantation inhibiting effect of CI-628. The majority of animals given both estradiol-17β and CI-628 on Day 3 and all animals treated on Days 3 and 4 lacked implantation sites or recoverable embryos. This suggests that the exogenous estrogen rendered the uterus nonreceptive. Therefore, the absence of embryos is not surprising because embryos degenerate in a nonreceptive uterus.

In summary, it would seem that CI-628 in the intact pregnant mouse acts as an estrogen antagonist, probably at the ovarian level. However, in the ovariectomized, progesterone-treated, delayed-implanting mouse, this compound functions as an estrogen agonist in the uterus to initiate implantation (7).

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