EFFECT OF TWO NON-STEROIDAL ANTIFERTILITY AGENTS ON PREGNANCY IN MICE

II. EFFECTS ON TUBAL TRANSPORT RATE AND IMPLANTATION

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(Received 25th July 1967)

Summary. The oral administration of clomiphene or U-11555A to mice appears to block pregnancy primarily through an effect on the maternal environment, rather than by direct action on the cleaving embryos. If given during the period of tubal transport, both agents cause a delay in the passage of the embryos into the uterus. When the compounds are given after the embryos have reached the uterus, implantation fails to occur, and the blastocysts pass out of the uterus. No evidence was found to suggest that pregnancy-blocking levels of the agents have a direct effect on the embryos.

INTRODUCTION

The effects of two non-steroidal antifertility agents, clomiphene (MRL-41, The Merrell Company) and U-11555A (The Upjohn Company), have been investigated in this laboratory, using the mouse as the experimental animal. The results with mice and with rats have shown that oral or injected doses of the compounds are effective if administered during the first few days of pregnancy. Within this period of time, the zygotes are undergoing cleavage and blastocyst formation, as well as being transported from the upper oviduct to the uterus, in preparation for implantation. It is not known whether the agents act directly on the embryo, alter the rate of tubal transport or interfere with implantation.

In a previous study (Thomson, 1968) it was found that, for the mouse, the doses of the compounds required to block in-vitro development of embryos were roughly comparable to those which prevented pregnancy *in vivo*. Thus the possibility of a direct deleterious effect on the embryos *in vivo* could not be eliminated. The present study was undertaken in order to determine whether the agents were able to change tubal transport rates or to interfere with implantation, as well as to investigate the condition and stage of embryos taken from treated mothers.

MATERIALS AND METHODS

Swiss female mice were given 2 i.u. of pregnant mare serum gonadotrophin (Gestyl, Organon), followed 48 hr later by 2 i.u. of human chorionic gonadotrophin (Pregnyl, Organon), in order to synchronize ovulation. The females were placed with Swiss males after the second injection and were checked for vaginal plugs on the following morning. Using this method of preparation two different types of experiments were carried out.

Effect of clomiphene and U-11555A on tubal transport rate

For each compound, 120 pregnant mice were randomized into twelve groups of ten mice. Using doses determined in previous experiments (10 mg/kg of clomiphene and 20 mg/kg of U-11555A), six of the groups were given one of the antifertility agents, by gavage with 0.1 ml aqueous solution, on Day 1 of pregnancy; mice in the other six groups were given 0.1 ml of water, by gavage, on the same day. This experimental plan was used twice, once for each of the two compounds.

Twenty mice, ten treated and ten control, were killed on each of the first 5 days of pregnancy; the last twenty mice were killed on Day 10. On each of the days, the oviducts and the uterine horns were removed from the twenty mice, and the contents of each oviduct and each horn were flushed out separately with culture medium. The embryos which were thus obtained were scored according to location, number, stage and condition. For each day and each treatment, the total numbers of embryos recovered from control and from treated animals were compared using a *t*-test.

Effect of clomiphene and U-11555A on implantation

Pregnant mice were randomized into six groups of ten mice, and, on Day 4 of pregnancy, all of the mice were given 0.1 ml of fluid by gavage. Twenty mice received 10 mg/kg clomiphene, twenty received 20 mg/kg of U-11555A, and twenty were given distilled water. Ten mice from each of the three groups were killed on Day 5 of pregnancy, while the remaining thirty were killed on Day 8. The oviducts and uterine horns of each mouse were flushed separately, and the contents were scored as in the preceding section. The experiment was done three times, in three different weeks, so that there was a total of thirty mice per treatment. After conversion of percentage values to angles and determination of the mean of three experiments for each treatment, a *t*-test was used to compare the effect of the compounds on the number of mice having late blastocysts in the uterus on Day 5.

RESULTS

Effect of clomiphene and U-11555A on tubal transport rate

The results of the studies on tubal transport rates are presented in Tables 1 and 2 and are shown graphically in Text-fig. 1. In control mice, gavaged with water, the cleaving embryos pass from the oviducts into the uterine horns between the 3rd and 4th days of pregnancy. In mice which received clomiphene on Day 1, nearly all of the embryos were found in the oviducts on Day 4, and,

even on Day 5, over half of the embryos were recovered from the oviducts. None of the clomiphene-treated mice had implanted foetuses on Day 10, although blastocysts were found in the oviducts and uteri at this time. In the

TABLE 1
EFFECT OF ORAL ADMINISTRATION OF 10 mg/kg GLOMIPHENE ON PREGNANCY IN MICE
(TEN MICE/GROUP)

Day of pregnancy when opened	Normal stage present	Treatment on Day 1	Total No. of embryos recovered from ten mice	No. of embryos recovered from oviducts of ten mice		No. of embryos recovered from uteri of ten mice	
				Total	Normal stage	Total	Normal stage
1	1-cell	Clomiphene	113	113	113	0	0
		Water	129	128	128	1	1
2	2-cell	Clomiphene	141	141	126	0	0
		Water	125	125	104	0	0
3	8-cell	Clomiphene	123	122	81	1	0
		Water	125	125	96	0	0
4	Morula or blastocyst	Clomiphene	56	55	49	1	1
		Water	88	4	3	84	81
5	Late blastocyst	Clomiphene	72	46	16	26	21
		Water	52	0	0	52	40
10	Foetus	Clomiphene	42	13*	0	29*	0
		Water	88	0	0	88	87

* Late blastocysts.

TABLE 2
EFFECT OF ORAL ADMINISTRATION OF 20 mg/kg U-11555A ON PREGNANCY IN MICE
(TEN MICE/GROUP)

Day of pregnancy when opened	Normal stage present	Treatment on Day 1	Total No. of embryos recovered from ten mice	No. of embryos recovered from oviducts of ten mice		No. of embryos recovered from uteri of ten mice	
				Total	Normal stage	Total	Normal stage
1	1-cell	U-11555A	114	114	114	0	0
		Water	143	143	143	0	0
2	2-cell	U-11555A	192	192	191	0	0
		Water	100	100	95	0	0
3	8-cell	U-11555A	64	60	43	4	3
		Water	126	107	88	19	19
4	Morula or blastocyst	U-11555A	48	48	48	0	0
		Water	84	7	5	77	75
5	Late blastocyst	U-11555A	38	37	19	1	0
		Water	38	4	0	34	31
10	Foetus	U-11555A	38	38*	0	0	0
		Water	124	0	0	124	124

* Thirty-eight were late blastocysts.

mice gavaged with U-11555A on Day 1, none of the embryos were found in the uterus on Day 4 or Day 5, and, even on Day 10, embryos were found only in the oviducts.

The possibility that vaginal loss of embryos may occur as the result of treatment was tested by comparing the total numbers of embryos recovered from treated and control groups on each of the days. For clomiphene, although

TEXT-FIG. 1. Effect of oral administration of clomiphene and U-11555A on the number and distribution of embryos in the reproductive tracts of treated females. Standard errors of the means are represented by the vertical lines.

more embryos were found in the control mice on Days 4 and 10, the differences were not significant. The relatively small number of embryos recovered from control mice on Day 5 is probably due to the fact that implantation is beginning

at this time, so that some blastocysts can not be flushed from the uterine horns. With U-11555A, more embryos were recovered from control mice than from those treated on Days 3, 4 and 10, and the difference was found to be significant ($P < 0.05$) for Days 3 and 10.

Effect of clomiphene and U-11555A on implantation

The results of three identical experiments in this section are summarized in Table 3, where each treatment group consists of thirty mice. The mice were gavaged on Day 4, when nearly all embryos have already reached the uterus. When the treated and control groups were examined on Day 5, all of the embryos were late blastocysts, and all but a few were found in the uterus. The number of mice in the control group having uterine blastocysts was higher than the number in the treated groups, and the difference was significant ($P < 0.05$) in the case of U-11555A.

TABLE 3
SUMMARY OF IMPLANTATION EXPERIMENTS (THIRTY MICE/TREATMENT)

Treatment on Day 4	Day opened	Implanted		Late blastocysts in oviducts		Late blastocysts in uterus		Ovulated	
		% mice	No. of embryos	% mice	No. of embryos	% mice	No. of embryos	% mice	No. of ova
Water	5	0	0	3.33	2	83.3	123	0	0
Clomiphene (10 mg/kg)	5	0	0	3.33	4	63.3	110	0	0
U-11555A (20 mg/kg)	5	0	0	3.33	1	60.0	112	0	0
Water	8	36.7	190	6.67	19	20.0	31	10.0	24
Clomiphene (10 mg/kg)	8	0	0	0	0	0	0	33.3	55
U-11555A (20 mg/kg)	8	0	0	3.33	6	0	0	50.0	98

On Day 8, over a third of the control mice contained implanted foetuses, while none of the treated animals was pregnant. One fifth of the control mice had delayed blastocysts in the uterus, but no embryos were recovered from the uterine horns of the treated mice. In addition, one-third of the clomiphene-treated and one half of the U-11555A-treated mice had begun cycling again, as indicated by the presence of recently ovulated eggs in the oviducts.

DISCUSSION

The results of the experiments described above indicate that the two anti-fertility agents affect both the rate of transport of embryos through the Fallopian tubes and implantation of blastocysts in the uterus. It is also apparent, judging by the stage and condition of embryos taken from treated females, that the in-vivo doses used have no effect either on the cleavage process or on the viability of embryos in treated mothers.

Although the two agents were not identical in their effect on tubal transport,

both resulted in a delay in the passage of most embryos from the oviduct to the uterus. After treatment with U-11555A, embryos were recovered almost entirely from the oviducts on each of the days studied. However, since there were significantly fewer embryos recovered from treated mice than from controls on Days 3 and 10, the possibility exists that some embryos may pass rapidly through the uterus and be expelled from the vagina. With clomiphene, all embryos appeared to be retained in the tubes through Day 4, although over a third were found in the uterus on Day 5 and over half were in the uterus on Day 10. These results are in agreement with those obtained by Chang (1964) in rabbits, where certain levels of the compounds, especially U-11555A, caused retention of embryos in the tubes. On the other hand, Davidson, Schuchner & Wada (1965) found that clomiphene caused an acceleration of tubal passage in the rat.

The administration of the two antifertility agents on Day 4 of pregnancy, when most embryos have already passed from the oviducts to the uterus, resulted in the loss of all embryos from the reproductive tracts by Day 8, with the exception of one mouse treated with U-11555A, which had six late blastocysts in the oviducts. The fact that, on Day 5, the number of late blastocysts recovered from the uteri of control mice was greater than the number from the clomiphene group, and significantly greater than the number from the U-11555A group, suggests that the agents not only interfere with implantation, but cause expulsion of embryos from the uterus. This is further supported by the data from Day 8, when the control group had both implanted fetuses and delayed uterine blastocysts, while the two treated groups had no embryos in the uterine horns. In addition, the presence of newly ovulated ova in the oviducts on Day 8 indicated that many of the treated mice had begun cycling again.

The implantation-inhibiting effects of U-11555A in rats was demonstrated by Duncan & Forbes (1965) by transferring delayed blastocysts from treated mothers into new hosts, where they implanted normally, indicating an interference with the maternal environment, rather than an effect on the blastocyst. Using clomiphene treatment, Staples (1966) found that blastocysts from treated female rats implanted normally in new hosts, while blastocysts from untreated rats failed to implant in treated hosts. Morris, van Wagenen, McCann & Jacob (1967) studied histological sections of implantation sites from rabbits treated with low doses of the compounds and noted that degenerative changes in the sites were evident after treatment with both agents.

In conclusion, these studies in mice suggest that clomiphene and U-11555A administration result both in a delayed tubal transport rate and in an interference with implantation. No evidence was found to support the idea that pregnancy-blocking levels of the compounds exert their primary effect on the cleaving embryo.

ACKNOWLEDGMENTS

This investigation was supported by a grant from the National Institutes of Health (D-02315-01).

Clomiphene was donated by Dr Dorsey E. Holtkamp of The William S. Merrell Company, and U-11555A was a gift from Dr Gordan W. Duncan of The Upjohn Company.

The excellent technical assistance of Mrs Peggy Earnest, Mrs Mary Reale and Miss Mary Ryans is gratefully acknowledged.

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