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CONTRACEPTION BY INTRAUTERINE RELEASE OF PROGESTERONE—EFFECTS ON ENDOMETRIAL TRACE ELEMENTS, ENZYMES AND STEROIDS

KERSTIN HAGENFELDT and BRITT-MARIE LANDGREN

Reproductive Endocrinology Research Unit and Department of Obstetrics and Gynecology, Karolinska Sjukhuset, Stockholm, Sweden

SUMMARY

Endometrial biopsies were obtained in the proliferative phase (days 10–12) and in the secretory phase (days 20–23) in two consecutive cycles before insertion and after 2 and 6 months use of the ALZA-T device, which releases 65 μg progesterone/24 h and a Placebo-T of exactly the same shape and size and manufactured by the same material. Twenty-five women were studied with the ALZA-T and 20 women with the Placebo-T.

The endometrial concentrations of copper, zinc and manganese were determined as well as the endometrial protein concentration and the activity of alkaline and acid phosphatase, betaglucuronidase and total lactic dehydrogenase. The endometrial concentration of progesterone and estradiol were determined using a radioimmunoassay. The insertion of the progesterone releasing device induced major changes in the morphological picture of the endometrium with a decidual transformation and atrophy of the glands. The zinc concentration of the secretory endometrium was decreased while the manganese concentration was increased, thus abolishing the physiological difference between the phases. Both the ALZA-T and the Placebo-T increased the endometrial protein concentration. There was a significant decrease in alkaline phosphatase and betaglucuronidase activity while the acid phosphatase increased and the total lactic dehydrogenase showed a biphasic variation with an increase in the proliferative and a decrease in the secretory phase. No effect on endometrial enzymes was induced by the Placebo-T.

Finally, the presence of the ALZA-T in utero increased endometrial progesterone levels and decreased estradiol levels without any concomitant change in the circulating levels of the same steroids.

INTRODUCTION

The purpose of the study reported in this communication was to investigate the influence of progesterone released from an intrauterine device on the enzyme activity, trace element and steroid pattern of the human endometrium. Among the endometrial enzymes the changes in the activity of alkaline and acid phosphatase, β-glucuronidase and total lactic dehydrogenase will be discussed, and among the trace elements the endometrial concentrations of copper, zinc and manganese will be described. Finally, a preliminary report will be given on the endometrial levels of progesterone and estradiol.

In order to evaluate the effect of progesterone released *in utero*, the results obtained in women using the progesterone device were compared with those obtained in a group of women using an inert device of exactly the same shape and size, which was manufactured by the same material.

EXPERIMENTAL

Intrauterine devices

The two devices used in this study were the ALZA-T device releasing 65 μg of progesterone per day and a Placebo-T, also manufactured by ALZA Corp. Palo Alto, California, U.S.A.

Clinical material

Fifty healthy normally menstruating women volunteered for the study of the ALZA-T device and 25 women for the study of the Placebo-T. The age of the women was between 21 and 35. As the contraceptive effect of the Placebo device could not be guaranteed, these couples were free to use a mechanical contraceptive (condomes) during the study.

The women were randomly assigned to the three groups, that is the study of trace elements, enzymes and steroids. The study will extend to 1 yr, however, only the results for the first 6 months will be reported in this communication. The number of women who have completed the 6 months period is shown in Table 1.

Plan of study

Two control biopsies were taken, the first one in the secretory phase (days 20–23) and the second

Table 1. Number of volunteers who participated in the study

Assays	ALZA-T	Placebo-T
Trace elements	10	8
Enzymes	8	5
Steroids	7	7

one in the proliferative phase (days 10–12) of the next cycle. Immediately after the last biopsy, an intrauterine device was inserted. The next sampling was done in the secretory phase of the second cycle and in the proliferative phase of the third cycle after the insertion of the device. The next two biopsies were taken in the secretory phase of the sixth and in the proliferative phase of the seventh cycle after the insertion of the device.

The endometrial biopsies were always taken without anesthesia and without dilatation of the cervix. Specimens for trace element analyses were taken by a curette manufactured from pure nickel (AB Stille-Werner, Stockholm, Sweden). The specimens were immediately frozen and kept at −20°C until processing. A piece of the endometrium was always fixed in Bouin's solution for the histological examination of the tissue. At each occasion a cubital venous blood sample was taken for the assay of circulating progesterone and estradiol.

METHODS

Copper, zinc and manganese in the endometrial specimens were determined with atomic absorption spectrophotometry using a Varian–Techtron AA-5 apparatus with a carbon rod attachment after drying the endometrial specimens in a vacuum desiccator and hydrolysing the dried sample with concentrated nitric acid (Pro Analysi; E. Merch, Darmstadt, BRD) and H_2O_2.

The assay of endometrial enzymes, protein and DNA was performed after homogenizing the tissue in water with a Potter–Elvehjem homogenizer, centrifuging for 10 min at 800 *g* at +4°C. The supernatant was used for enzyme assays and protein determination and the pellet for the determination of DNA. Enzyme assays were performed using a microscale modification of the enzyme kits of Boehringer & Sons. Protein was measured according to Lowry *et al.*[1] and DNA according to Burton *et al.*[2].

The histological examination of the endometrial specimens was kindly performed by Dr. Manuel Maqueo-Topete, Mexico City.

The steroid assays of circulating hormones were done using a RIA method by Brenner *et al.*[3]. The steroids in the endometrium were determined by Dr. Guerrero and co-workers in our laboratory using a method recently developed for this purpose (Guerrero *et al.* to be published).

Statistical methods

For the purpose of this study we have assumed that the individual assay results follow a lognormal rather than a normal distribution as suggested by Gaddum many years ago [4]. The results obtained in the two groups, e.g. ALZA-T and Placebo-T respectively have been submitted to a complete analysis of variance including comparisons of selected effects according to Snedecor and Cochran[5]. The difference between the effects of the active and the Placebo device were compared with the *t*-test.

RESULTS

The histological examination of the endometrial biopsies revealed a decidual transformation with atrophic glands in the ALZA-T group and slight changes of the type usually seen with inert devices in the Placebo-group. The plasma levels of progesterone and estradiol indicated normal cycles in both groups and were always in agreement with the normal values for the two steroids according to the day of the cycle.

The results of the trace element determinations are presented in Table 2 and it can be seen from the figures that the insertion of the ALZA-T device did not induce any significant change in the endometrial concentration of copper, neither in the proliferative nor in the secretory phase. The copper levels in the biopsies obtained in women using the Placebo-T did not differ from those using the ALZA-T. However, the insertion of the progesterone releasing device caused a significant decrease in the zinc concentration of the secretory endometrium and abolished the previously [6] established physiological difference between the proliferative and secretory endometrium.

Table 2. Copper, zinc and manganese concentration (μg/g dry tissue) in endometrial biopsy specimens before and during the use of an intrauterine device (geometric mean values)

	Control		IUD *in situ* 2 Months		IUD *in situ* 6 Months	
	Prolif.	Secret	Prolif.	Secret	Prolif.	Secret
Copper						
ALZA-T	4·8	5·0	4·2	4·7	4·5	4·7
Placebo-T	4·3	4·8	4·2	4·3	4·4	4·3
Zinc						
ALZA-T	63	96	55	57	64	78
Placebo-T	63	86	80	96	85	82
Manganese						
ALZA-T	1·2	0·9	1·3	1·1	1·4	1·3
Placebo-T	1·4	1·0	1·3	0·8	1·3	0·8

Table 3. Enzyme activity and protein concentration in endometrial biopsy specimens obtained before and during the use of an intrauterine device (geometric mean values)

	Control		IUD *in situ* 2 Months		IUD *in situ* 6 Months	
	Prolif.	Secret	Prolif.	Secret	Prolif.	Secret
Alkaline phosphatase (mIU/mg DNA)						
ALZA-T	94	207	32	32	71	96
Placebo-T	110	175	122	232	135	187
Acid phosphatase (mIU/mg DNA)						
ALZA-T	122	160	115	177	220	265
Placebo-T	213	245	270	273	238	267
Beta-Glucuronidase (μIU/mg DNA)						
ALZA-T	2030	2975	1200	1270	1326	1470
Placebo-T	1634	2550	1830	1760	1710	2170
Lactic dehydrogenase (IU/mg DNA)						
ALZA-T	15·7	40·1	27·3	25·7	28·5	29·8
Placebo-T	20·9	27·8	22·8	38·7	23·9	37·5
Protein (mg/mg DNA)						
ALZA-T	8·1	6·9	11·7	11·0	14·9	13·9
Placebo-T	9·5	8·0	13·1	13·3	11·5	14·9

The zinc concentration of the secretory endometrium at two and six months after insertion of the device was significantly lower than that of the control biopsy ($P < 0.001$).

The effect of the Placebo device was a slight increase in the zinc concentration of the proliferative endometrium ($P < 0.05$) both at 2 and 6 months after the insertion of the device.

The insertion of the ALZA-T induced an increase in the manganese concentration of the secretory endometrium ($P < 0.05$) both at 2 and 6 months, while the Placebo-T had no effect on endometrial manganese levels.

The results of the enzyme assays and protein determination are presented in Table 3 and as can be seen from the figures the effect of the ALZA-T and the Placebo-T on the endometrial *protein* levels were the same; a significant increase ($P < 0.001$) both in the secretory and proliferative phase, both at 2 and 6 months after the insertion of the devices. The progesterone releasing device induced a significant decrease in the *alkaline phosphatase* activity of both the proliferative and secretory endometrium at 2 months ($P < 0.01$) while at 6 months only the secretory endometrium showed a significant decrease ($P < 0.05$). The Placebo-T had no effect on the endometrial activity of alkaline phosphatase.

The endometrial activity of *acid phosphatase* at two months did not change in the ALZA-T group, at 6 months however, there was a significant increase both in the proliferative and secretory endometrium ($P < 0.01$). No effect was seen in the Placebo-group.

Endometrial *beta-glucuronidase* activity was significantly decreased in the ALZA-T group both in the proliferative ($P < 0.05$) and in the secretory phase specimens ($P < 0.001$) at 2 as well as 6 months after the insertion. The insertion of the Placebo-T had no effect on endometrial betaglucuronidase activity.

The insertion of the ALZA-T induced a biphasic effect on the endometrial *lactic dehydrogenase* activity. There was an increase in the proliferative phase ($P < 0.01$) and a decrease in the secretory phase ($P < 0.01$) both at 2 and 6 months, while the Placebo-T had no effect.

The results of the steroid analyses of the endometrial specimens are indicated in Table 4. As can be seen from the figures the insertion of the ALZA-T resulted in a significant increase in the endometrial concentration of progesterone, both in the proliferative and the secretory phase specimens. The progesterone concentration in biopsies obtained from women using the Placebo-T did not differ from the control values.

Table 4. Progesterone and estradiol concentration in endometrial biopsy specimens before and during the use of an intrauterine device (geometric mean values)

	Control		IUD *in situ* 2 Months		IUD *in situ* 6 Months	
	Prolif.	Secret	Prolif.	Secret	Prolif.	Secret
Progesterone (ng/g fresh tissue)						
ALZA-T	15·1	30·3	135·7	152·3	205·8	114·9
Placebo-T	5·3	12·0	8·1	8·6	4·4	13·2
Estradiol (ng/g fresh tissue)						
ALZA-T	1·5	0·6	0·4	0·3	0·8	0·3
Placebo-T	1·0	0·5	0·9	0·4	1·2	0·8

The endometrial levels of estradiol were significantly depressed by the presence of the progesterone releasing device while they remained unchanged after the insertion of the Placebo-T.

DISCUSSION

The data presented in Tables 1–3 indicate major differences in the parameters studied between the tissue specimens obtained from women using the so called inert device, the Placebo-T, and the progesterone releasing ALZA-T. The steroid assays of the control samples both in the ALZA-T group and in the Placebo-T group indicated progesterone levels which did not differ significantly between the proliferative and secretory phases. It should be noted that the endometrial concentration of progesterone in the proliferative phase of the control cycle greatly exceeds that of systemic blood, which invariably is less than 1 ng/ml. These data indicate that circulating steroid levels may not always reflect the levels in the target organ. The insertion of the ALZA-T gave a significant, 5–10-fold increase in endometrial progesterone levels, both in the proliferative and the secretory endometrium. The Placebo-T had no effect on endometrial progesterone levels. The strongly elevated levels in the ALZA-T group suggest that the endometrial reductases could not cope with all the progesterone released by the device.

Following the insertion of the ALZA-T, there was a significant depression of the estradiol levels both at 2 and 6 months. These findings seem to suggest that the continuous release of progesterone in the endometrial cavity interfered with the synthesis of the high affinity estradiol receptor. As in the case of progesterone, the Placebo-T had no influence on the estradiol levels of the endometrium.

The morphological evaluation of the endometrial specimens showed a normal cyclic development in the specimens obtained from women using the Placebo-T with slight changes of the type known from studies with other so called inert devices; an increased oedema in the stroma and sometimes inflammatory cells. On the other hand, the specimens obtained from women using the progesterone releasing device showed a specific picture which could be attributed to the influence of the progesterone delivered to the tissue: a decidual transformation with a depression of the glandular development.

These major differences in morphological development of the endometrial tissue in the two groups studied could obviously result in a different metabolic behaviour of the tissues. Looking at the results of the protein and enzyme assays one can conclude that the increase in the protein content, expressed per mg DNA, was the same for the two types of devices. This may therefore be a result of the foreign body *per se*. The same increase in uterine protein has been reported by us earlier in a study of the effect of the copper-T on the endometrium [7].

The effect on the four enzymes studied seemed to be induced by the release of progesterone in the uterine cavity, as there was no significant change elicited by the Placebo-T. The main effect of the ALZA-T was a decrease in endometrial alkaline phosphatase and β-glucuronidase activity, an increase in acid phosphatase activity and a biphasic effect on the total lactic dehydrogenase activity with an increase in the proliferative and a decrease in the secretory phase.

Of the trace elements studied two were affected by the presence of the ALZA-T namely zinc and manganese. The zinc concentration of the secretory endometrium was depressed and the physiological difference between the phases abolished. These data are in agreement with unpublished data from our laboratory where consistently low zinc values were found in human decidua obtained from the 12th to 18th week of gestation. The slight increase in the zinc concentration of the proliferative endometrium in biopsies obtained from women using the Placebo-T needs confirmation. The ALZA-T induced an increase in the manganese concentration of the secretory endometrium, thus abolishing the physiological difference between the phases.

As a conclusion it can be stated that the progesterone releasing intrauterine device induces major changes in the endometrial milieu. At the present state of incomplete knowledge we would not dare to speculate about which one of the demonstrated changes, if any, could be associated with the mechanism of action of the progesterone releasing device. All we can do is to humbly collect more data, in the hope that the accumulated information finally will enable us to reveal and understand the mechanism of action.

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REFERENCES

1. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: *J. biol. Chem.* **193** (1951) 265–275.
2. Burton K.: *Biochem. J.* **62** (1956) 315–323.
3. Brenner P. F., Guerrero R., Cekan Z. and Diczfalusy E.: *Steroids* **22** (1973) 775–794.
4. Gaddum J. H.: *Nature* **156** (1945) 463–466.
5. Snedecor G. W. and Cochran W. G.: *Statistical Methods* (6th edn.). The Iowa State University Press, Ames, Iowa, U.S.A. (1967).
6. Hagenfeldt K., Plantin L. O. and Diczfalusy E.: *Acta endocr., Copenh.* **65** (1970) 541–551.
7. Hagenfeldt K.: *Contraception* **6** (1972) 191–206.