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CONTRACEPTIVE EFFICACY OF DEPOT PROVERA JET-INJECTED INTO THE CERVIX

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Depot-medroxy progesterone acetate (DMPA) was jet deposited into the uterus/cervix of rats, rabbits and rhesus monkeys with the help of a modified jet injection apparatus. Since the drug was delivered under pressure, it was distributed deep into the muscular layers around the place of deposition. After one deposition the drug acted as an effective contraceptive for a period of three months. The merits of this delivery technique are discussed.

Key words : medroxyprogesterone acetate cervix jet-injection

INTRODUCTION

Medroxyprogesterone acetate (DMPA), the long acting injectable steroid contraceptive supresses ovulation by inhibiting pituitary secretion of LH and FSH which in turn inhibits ovarian steroidogenesis, though the progestogen may also directly interfere with the synthesis and metabolism of gonadal progesterone (1). In addition, it also turns the cervical mucus unfavourable to spermatozoal migration. Though the contraceptive effects of a single intramuscular dose are maintained for about three months, the progestogen causes significant amenorrhea, breakthrough bleeding and other deleterious metabolic effect (2). To minimize the side-effects progestogens have been tried locally in the form of a vaginal ring or intra-uterine device but the problems like vaginitis, expulsion, interference with coitus, difficulty of insertion and occasional break-through bleeding could not be avoideo (3,4). In the present study, we have tried to deposit the drug locally into the cervix by using a jet injection technique with an idea to minimise the side effects.

METHODS

Hypodermic jet-injection apparatus (Vermitron Medical Products New Jersey), commonly used for mass vaccination, was modified and used in this study. The instrument shoots the drug with a great force (8.97×10^4 newton/m^2). The system was adapted to a needle whose tip was blocked and there were 2-3 microholes near its tip (5). This system was able to achieve side-shooting with a great force resulting in deep drug penetration and formation of a drug depot at the site of the microholes. For rats and rabbits, a smaller needle, (Gauge No. 23) was used, whereas in monkeys a longer needle (Gauge No. 13) was required to deposit the drug into the cervix (Fig. 1). The dose of the drug to be delivered, could be adjusted by changing the position of the plunger of the apparatus. DMPA as Depot Provera (Upjohn, USA) was used for the study.

Sixteen regularly cycling albino rats were used in this study. The procedure of jet injection has been described in our previous study (5). 0.6 mg of DMPA in 0.2 ml saline was jet deposited into the cervical end of uterine horn. Another batch of four animals was used as control or sham operated and 0.2 ml of saline was used. In two rats, a higher dose of DMPA (2.5 mg) was deposited.

After a rest for ten days, the animals in pro-estrous, were mated with fertile males. The

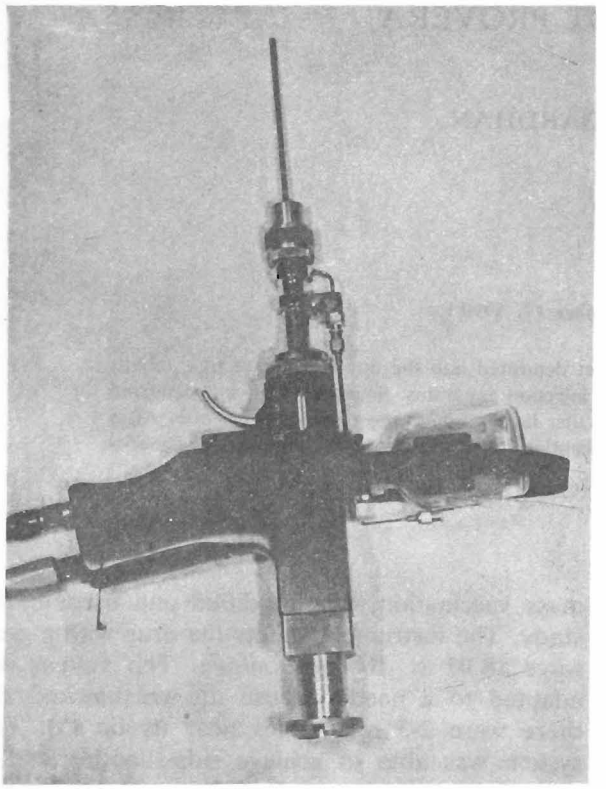

Fig. 1 : Jet injection apparatus adapted to a 10 cm long needle with microholes near its tip.

animals were opened on day-13 of expected pregnancy, the number of implantations and corpora lutea were counted. Two animals from the experimental group after 23 days of jet injection were sacrificed and their uteri, oviducts and ovaries were preserved in Bouin's fluid for histological studies. Since none of the experimental animals showed implants, these animals were left with males (2:1) for a period of three months after jet injection and the animals were regularly examined for pregnancy.

Similarly, a dose of 0.9 mg DMPA suspended in saline was jet-injected into the cervix of eight rabbits. The animals were mated after 10 days rest and implants if any, were examined by palpation on day 10 of expected pregnancy. Two animals were sacrificed on day 17 and their uteri preserved in Bouin's fluid and processed for histological examination. The remaining six experimental animals were left with males for a period of 90 days after deposition of the drug and regularly examined for pregnancy. Another batch of rabbits (control) were sham operated by jet-injection of saline and bred as in case of experimental animals. Reyersibility of fertility was evaluated in six rabbits after 130 days of jet-injection of DMPA. After mating, the number & condition of implants were counted on day 10 of expected pregnancy.

Jet-deposition in monkeys: 7 adult regularly cycling rhesus monkeys weighing 5-6 kg were used for this study. The cervical mucus was collected by means of a long needle attached to a syringe on day 6-16 of the menstrual cycle. The quantity of the mucus was graded as, + for scanty, ++ for copious and +++ for abundant. A drop of cervical mucus was tested for spinnbarkeit, the cervical hostility was evaluated by mixing a drop of semen with mucus on a slide. A cover slip was

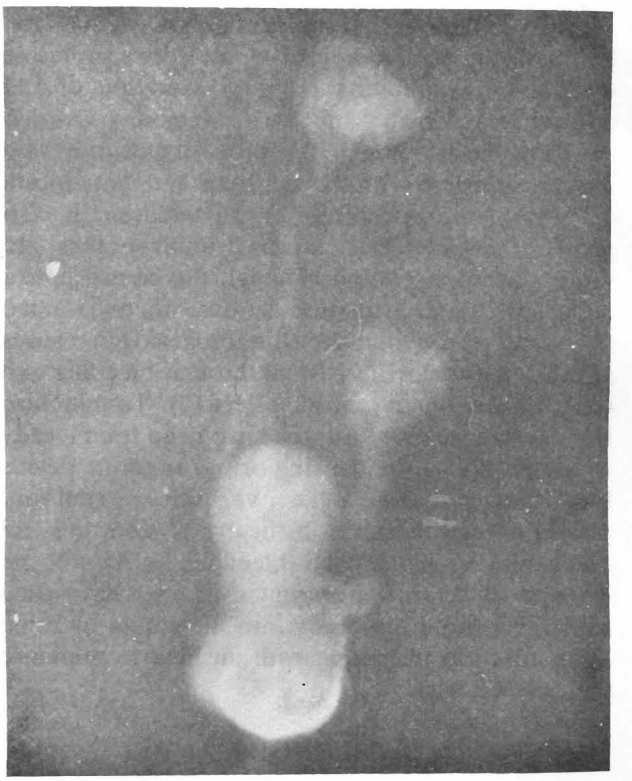

Fig. 2 : Uterus of monkey jet injected with 0.5 ml of 60% Urographin dye. X-Ray photograph.

placed and cervical hostility was graded as complete if none of the spermatozoa migrated into the crevices of the mucus. This test was done on the days of suspected ovulation only. A record of the length of the cycle and the amount of bleeding in each cycle was kept. Vaginal smears were studied on day 6-16 of the cycle. A comparison between epithelial cells and leucocytes was made in each slide. On these days, rectal temperature was also recorded with the help of a clinical thermometer.

Before DMPA was deposited one animal was sacrificed, its uterus removed and 0.5 ml of 60% Urographin dye was jet-injected into the cervix and an X-ray was taken to know the distribution of the dye. In another animal, 0.5 ml of Imferon (containing 25 mg of iron) was jet injected into the cervix, animal sacrificed, sections of uterus and cervix cut and stained for iron with potassium ferrocyanide and then with HE method. The distribution of the dye after jet deposition in cervix is shown in Fig. 2.

In remaining 6 animals, on the day the menstrual bleeding stopped, a bifid vaginal speculum was passed, exocervix cleaned and tip of the injection needle was inserted into the cervix so that the micro-holes were inside the exocervix. 12.5 mg of DMPA suspended in 0.5 ml of saline was jet injected. The cervical mucus and other parameters were studied for a period of three consecutive cycles. The animals were mated every alternate day between day 6 to 16 of each cycle and pregnancy if any, diagnosed. Similarly two more injections of DMPA were given at an interval of 90 days each and the fertility was estimated. Side by side, a batch of 5 control monkeys in whom saline was injected into the cervix, were mated and their pregnancy diagnosed.

Blood samples were drawn on every 2-3 days interval in the control cycle i.e. before drug treatment and after different days of DMPA administration. Serum was used for the estimation of LH, FSH and progesterone by radio-immunoassay (6). LH and FSH levels in monkey serum were estimated using heterologus RIA. Circulatory levels of progesterone were measured by the RIA technique of Orcezyk et al 1979 (7).

RESULTS

Histological examination of rat uterus after 0.6 mg of DMPA injection revealed mild reduction in epithelial cell height and loss of secretory activity of uterine glands. The animals were found to be regularly cycling, but when mated with males there were no implantations whereas controls showed normal pregnancy. These antifertility effects of DMPA were studied for 3 months after jet injection. In rats when the dose of DMPA was increased to 2.5 mg, though the animals exhibited normal cylces, they did not mate.

In rabbits, 0.9 mg of DMPA jet injected into the cervix or base of the uterine horn, was able to prevent pregnancy when studied for 90 days. Histological examination of the area of jet injection revealed mild changes suggestive of progestational influence. After 130 to 140 days of jet-injection of DMPA in rabbits, there was a complete return of fertility. Normal implantations occurred. The pups born were all healthy and their number was comparable to the controls. In control animals no sign of preudopregnancy was observed after jet injection into the cervix. All the animals were allowed to mate and delivered normal pups.

The jet deposition of radio opaque dye and imferon in rhesus monkeys revealed that the material was distributed uniformally all round and there was a deep penetration right upto the sub-epithelial stromal layers of the cervix. Thus a drug depot with an even distribution throughout the tissue i.e. cervix, is developed. This drug is likely to be released slowly into the lumen and act on mucus or the epithelium.

After the jet-deposition of DMPA into the cervix, there was no change in the menstrual flow but the cycle length became irregular. The change was more prominent immediately after DMPA deposition but showed partial recovery towards the third cycle (Table I). The structure of the uterus

or cervix showed decreased glandular secretory activity (Fig 3), the amount of cervical mucus decreased after DMPA with reduced elasticity. The pH of the mucus showed no change. When tested for cervical hostility on day 6-16 the cervical mucus did not allow the spermatozoa to penetrate into the crevices and was completely hostile. Similar result were obtained in next schedule of DMPA injection (Table I).

After single DMPA injection, pregnancy was prevented in all the animals when mated with fertile males. Out of 5 control monkeys 2 animals conceived during the first cycle and 2 in the second cycle.

Alterations in circulatory levels of LH, FSH and progesterone in five animals during the control and treatment cycles are presented in Fig. 4,4a. All the five animals had ovulatory cycles as confirmed by progesterone levels being greater than 3 ng/ml in the luteal phase and mid-cycle LH surge in animal No. 2 and 4. Similarly, animal number 5 showed a FSH peak on day 11 of the cycle. Since daily blood samples were not withdrawn in our study, LH and FSH peaks could not be detected in all the animals.

Fig. 3 : Section of cervix of monkey jet injected with 0.5 ml of suspension containing 12.5 mg of DMPA. Note glands showing almost no secretion. Interstitial edema with mainly round cell infiltrate × 234.

TABLE I : Effect of DMPA on menstrual cycle and cervical mucus in rhesus monkeys.
n = 5

	Control cycle (Before DMPA)	*1st cycle*	*2nd cycle*	*3rd cycle*	*1st cycle*	*2nd cycle*	*3rd cycle*	*1st cycle*	*2nd cycle*	*3rd cycle*
		DMPA			DMPA				DMPA	
Menstrual cycle length (days)	27 (22.33)	21 (16-26)	23.7 (20-27)	25 (21-28)	20.6 (12-28)	21.5 (10-24)	32 (27-36)	27.3 (25-29)	26.6 (24-28)	26.6 (24-28)
Cervical mucus (amount)	+++	+	+	+	+	+	+	+	+	+
Cervical mucus hostility	receptive	←		Completely	hostile					→
Spinnbarkeit (length cms)	8.0	0.5	0.6	0.4	0.7	0.4	0.3	0.4	0.5	0.6

+++ abundant
+ scanty
Cervical mucus hostility and SPK studied between days 6-16 of each cycle.
DMPA dose 12.5 mg once between days 6-16

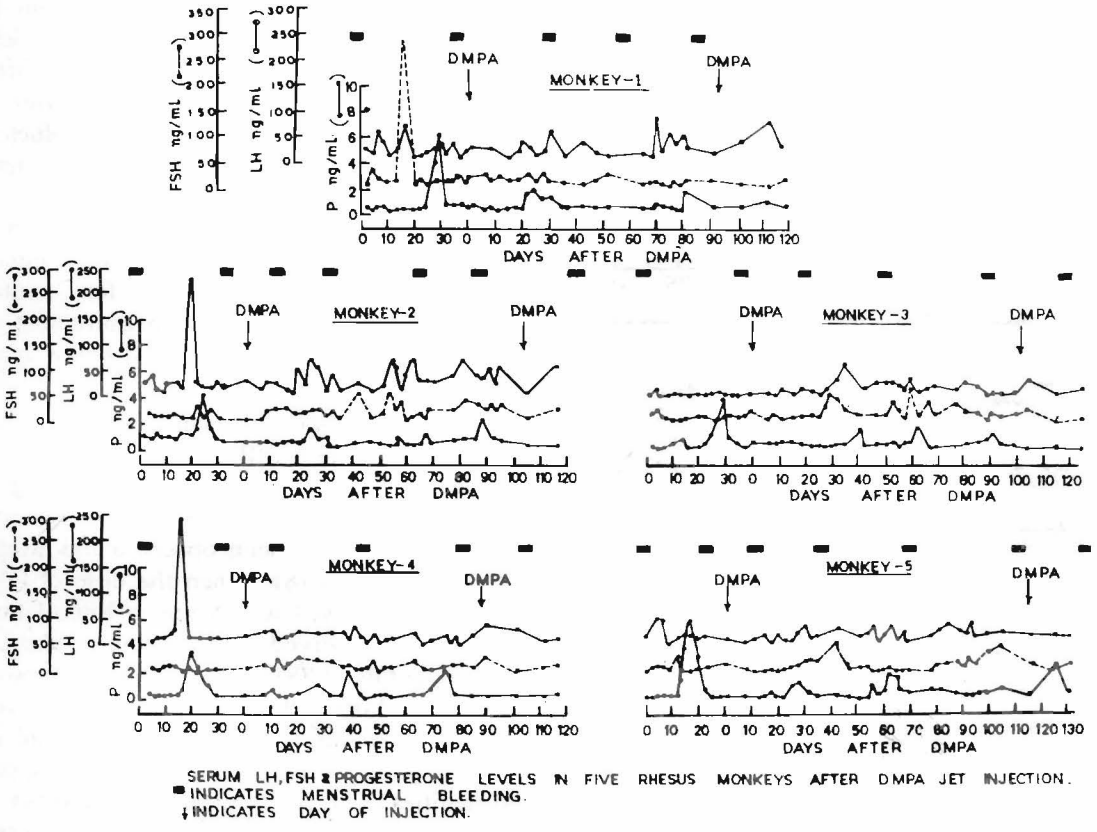

Fig. 4 : Serum LH, FSH and progesterone levels in five monkeys after DMPA jet-injection.

Well defined LH or FSH peaks as seen in some of the animals in control cycles were not observed during DMPA administration. Basal LH and FSH levels did not show any change during the treatment cycles. In each of the three treated cycles progesterone level failed to rise above 2 ng/ml in marked contrast to elevated levels during the luteal phase of the control cycle.

DISCUSSION

The present technique of jet injection, has shown some major advantages. The drug depot is evenly distributed in the cervix and there is a deep drug penetration from where it is slowly released into the lumen, affecting the mucus for a long time. Using same technique of jet-injection earlier in rats and rabbits (5) we found that the material injected (iron or copper) remained restricted to the area of deposition, without spillage to other organs like fallopian tubes or ovaries.

The antifertility effects of jet deposited DMPA have been 100% when studied for a period of 3 months after single injection. Nine cycles were studied in monkeys where three such injections were given and complete fertility inhibition was achieved. The jet injected DMPA was able to alter the properties of cervical mucus completely. This change in cervical mucus rheology seems to be one of the mechanisms by which the pregnancy could be avoided. However, the principle mode of action of DMPA appeared to be due to its inhibitory effect on ovulation. Although we did

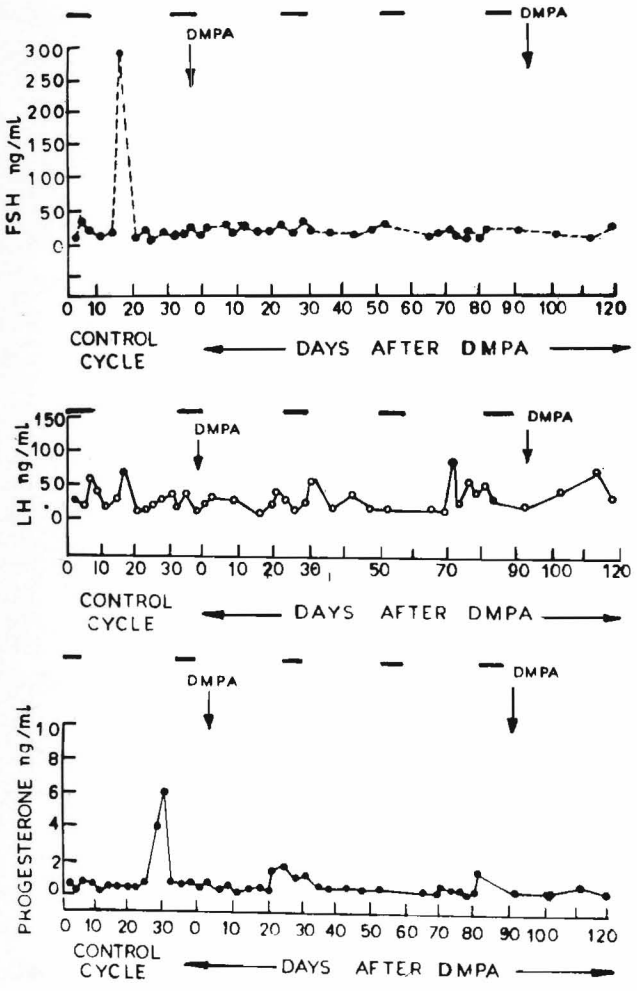

Fig. 4a : Serum LH, and progesterone levels in one representative monkey after DMPA jet-injection.

not estimate hormone levels daily, none of the cycles treated with DMPA showed LH or FSH surges. Hence it is logical to assume that treatment with DMPA inhibited mid cycle LH and FSH peaks but did not alter basal gonadotropin levels. Confirmation of the pituitary suppressing activity was obtained by examination of the ovaries after DMPA treatment. The ovaries had follicles in various stages of maturation but no luteal activity could be found on histological examination. This indicates that though DMPA in cervix *per se* did not effect development of follicles but it inhibited ovulation. Additional evidence for ovulation inhibition included the measurement of progesterone levels which remained below 2 ng/ml in the DMPA treated cycles.

Though there was no change in estrous cycle pattern of rats after DMPA, the normal menstrual cycles in monkey were disrupted. This change was more apparent in the first cycle following injection. Similar changes have been observed following i/m injection of DMPA (8). When the dose of DMPA in rats was increased to 2.5 mg, a loss of mating behaviour was observed inspite of the regular estrus cycle. In rabbits, 130-140 days after jet deposition of 0.9 mg DMPA, there was a complete return of fertility. The implants were normal and none of the pups born showed any apparent abnormalities. The delayed return of fertility after oral or i/m use could be avoided by intracervical deposition of the progestogen.

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