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The effect of aqueous progesterone on operative adhesion formation

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Progesterone (P) has been shown to have antiinflammatory and immunosuppressive properties. This study was designed to evaluate these effects on operative adhesion formation. Forty guinea pigs received standardized injuries to their uterine horns. Four groups were examined. Normal saline was used as an irrigant in the first, or control, group. Aqueous P (50 mg or 1 ml) was dripped over the injured site and instilled intraperitoneally in the second group. The third group received intramuscular aqueous P (3.3 mg/kg body weight) 1 day postoperatively, the day of surgery, and either 6 or 13 days postoperatively until reexploration. In the fourth group 1 ml of 32% dextran 70 (Hyskon) was administered in the same manner as aqueous P in the second group. The animals in all groups were reexplored 1 or 2 weeks after the initial surgical procedure, and the adhesions were scored. Adhesion formation was significantly reduced ($P < 0.001$) in all treatment groups when compared with the control group. Aqueous P may have a role in the prevention of adhesion formation associated with pelvic surgery and, in particular, microscopic tubal and ovarian surgery. Fertil Steril 39:485, 1983

The importance of adhesion formation to the gynecologic surgeon is well recognized. Pelvic surgery is one of the most frequent causes of postoperative adhesion development, which can lead to small bowel obstruction and infertility and can be a major factor preventing the success of reconstructive tubal surgery.

The pathogenesis and prevention of postoperative adhesion formation has been the subject of a substantial amount of research. Yet despite all the work that has been done in this area, much controversy still exists, and the search for a reliable prophylactic agent or regimen continues.

The basis for our investigation of progesterone (P) as an antiadhesive agent originates primarily from research performed by Siiteri et al.,[1] who have emphasized that the role of P in maintaining pregnancy may be to prevent immunologic destruction of the fetus. They took Silastic tubing filled with P and wrapped with either surgical cotton thread or hamster skin and implanted these subcutaneously in the flanks of rats. Similar capsules without hormone (controls) were also implanted. Examination after 1 week revealed the control implants to be surrounded by granuloma formation and adhesions that anchored them to the underlying fascia. The P-containing capsules, however, elicited little, if any, inflammatory response. Hamster skin adjacent to P-con-

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taining tubes remained healthy up to 35 days following transplantation, whereas none of the control skins survived beyond 10 days. Measurements of local tissue P concentrations were not made, but the release rates for the P-filled Silastic tubes were noted to be 170 μg/cm/day. Further work with P implants showed that granuloma response to cotton thread was blocked by the use of P capsules with release rates of 10 μg/cm/day or only 2 μg/cm/day. These latter experiments were performed in adrenalectomized rats, suggesting that the P effect is independent of corticosteroids.[2]

Other related research is also of interest. Results of a study done by Moriyama and Sugawa[3] also suggested an important role for P in the development of a state of immunologic tolerance. Xenogeneic cultured cells such as human endometrial and ovarian cells implanted and proliferated in the uteri of hamsters receiving intramuscular (IM) estradiol, 2 μg/day, and IM P in a dose ratio of 20 mg/kg body weight/day. No cell transplantation was established in those animals that received estradiol alone.

Hulka et al.,[4] evaluated the effect of several steroids on humoral antibody production. Rabbits weighing between 3 and 4 kg were immunized with bovine serum albumin, and the antibody responses were measured 2 weeks later. P-treated animals received 20 mg IM three times weekly for a period of several weeks. Antibody responses measured as milligrams of antibody per milliliter of serum were significantly reduced, approximately threefold, in the P-treated group, as compared with a control group that received no treatment.

Inhibition of human mixed lymphocyte cultures (MLCs) and leukocyte migration by high local concentrations of P has also been demonstrated.[1, 5, 6] Significant suppression was observed in the range of 1 to 10 μg/ml, and essentially complete inhibition (95% to 98%) was found at 20 μg/ml when P was added to human MLCs. In pregnant rats undergoing bilateral oophorectomy on the 11th day of pregnancy, marked leukocytic infiltration into placental vessels was observed as early as 4 hours after ovariectomy. Placental P levels fell from 266 to 78 ng/gm of tissue between 4 and 12 hours after ovariectomy. In addition, in vitro experiments found that P directly inhibited the migration of human leukocytes placed in 1% agar gels containing P at a concentration of 20 μg/ml.

An experimental model, known as the carrageenan air pouch method, encompassing both the acute exudative and chronic proliferative stages of the inflammatory process, was utilized by Nakagawa et al.[7] to examine the antiinflammatory action of P. Air was injected subcutaneously into the backs of male rats to form a pouch, after which carrageenan was introduced into the air pouch to induce inflammation. In the early phase of inflammation, day 0 to day 4 (day 0 representing carrageenan injection), P-treated animals received 1 mg/kg body weight IM every 12 hours for 4 consecutive days, starting on day 0. Wet weight granulation tissue, exudative volume in the pouch, and local vascular permeability as measured by radioiodinated human serum albumin (^{131}I-HSA) uptake were all significantly reduced in comparison with a control group not receiving P. Similar results were obtained in evaluating the effect of P on the chronic phase of inflammation (after day 4), in which the dose of P was administered as above but treatment was not initiated until 7 days after carrageenan injection. The authors[7] stated it was evident that P enhanced the involution of preexisting granulation tissue by means of an increased degradation of noncollagen protein. However, P did not have an effect on collagen degradation or on the incorporation of proline into collagen.

It is on the basis of these many observations that it can be stated that P seems to have significant antiinflammatory and immunosuppressive properties. In view of these findings, the present study was designed to evaluate the effect of aqueous P on operative adhesion formation.

MATERIALS AND METHODS

Forty nonpregnant albino guinea pigs weighing between 300 and 500 gm were selected for the study. All were individually housed and received standardized care. The animals were weighed prior to surgery and anesthetized with approximately 0.5 ml of ketamine (80 mg/ml) and acepromazine (2 mg/ml) solution given IM. Neither preoperative nor postoperative antibiotics were administered. After an abdominal preparation using chlorhexidine gluconate (Hibitane, Stuart Pharmaceuticals, Wilmington, DE), laparotomy was performed through a lower midline incision. Sterile techniques were utilized throughout the operation. All animals received standardized injuries. One uterine horn in each guinea pig was

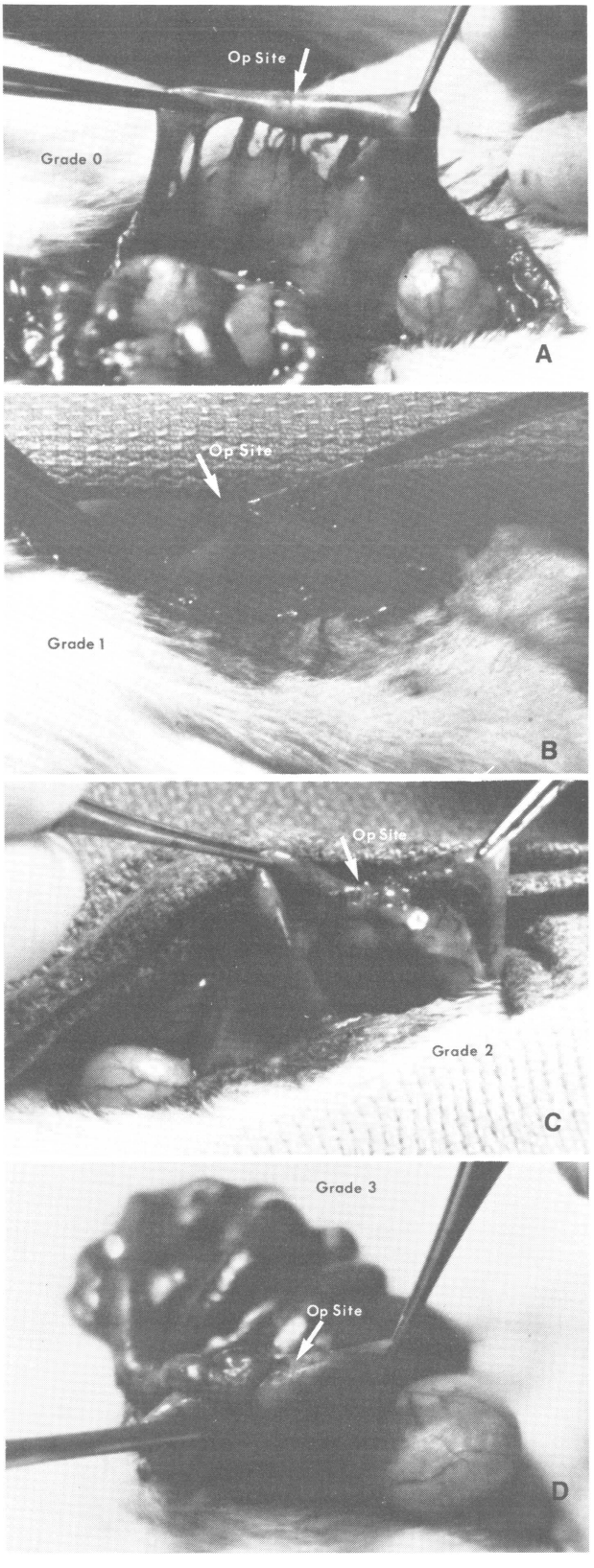

Figure 1
Examples of adhesion formation.

transected approximately 1.5 cm from the bifurcation. Each horn was subsequently reconstructed by a microsurgical technique. One-layer closure through the serosa and muscularis was accomplished by placement of six to seven interrupted 8-0 nylon monofilament sutures. Following reanastomosis, the adjacent area on each side of the anastomotic site was crushed for 10 seconds with a hemostat, and the serosa was abraded until macroscopic bleeding was noted. The peritoneal cavity was then irrigated with normal saline to remove blood clots and debris. The abdominal incision was closed in two layers with the use of a running 3-0 chromic catgut stitch for the peritoneum-rectus muscle layer and simple interrupted 3-0 silk stitches for the skin. All surgery was performed by the same operator.

The animals were divided into four groups. The operator was aware which animals would be receiving what particular treatment regimen prior to surgery. Group 1 (8 animals) served as the control group and received no further treatment; in group 2 (14 animals), aqueous sterile P suspension (Elkins-Sinn, Inc., Cherry Hill, NJ) was dripped over the injured site and instilled intraperitoneally (IP), and a total of 50 mg or 1 ml was used in each animal; in group 3 (9 animals), IM aqueous P 3.3 mg/kg body weight was given 1 day preoperatively, the day of surgery, and either 6 or 13 days postoperatively until reexploration; and in group 4 (9 animals), 32% dextran 70 in dextrose (Hyskon, Pharmacia, Piscataway, NJ) was dripped over the injured site and instilled IP, for a total of 1 ml in each animal.

The dose of aqueous P given IM to the animals in group 3 is equivalent to a 200-mg/day dose in a 60-kg human female. Comparable doses of medication given to groups 2 and 4 would be 200 ml (10 gm) of aqueous P suspension instilled IP and 200 ml of 32% dextran 70 instilled IP in a 60-kg human female.

One or 2 weeks after the first operation, a second laparotomy was performed, at which time adhesion formation was scored and photographed.

Adhesions were scored by the author, who had no prior knowledge of which particular group was being evaluated. The following classification was utilized: grade 0, absence of adhesions; grade 1, filmy adhesions (mild); grade 2, dense adhesions (moderate); grade 3, dense adhesions with bowel adherent to the injured site (severe). Figure 1 A to D represents examples of each grade of adhesion formation.

Table 1. *Summary of Adhesion Scores*

Group	Total no.	Adhesion score			
		0	1	2	3
1. Control	8	—	—	3 (37%)	5 (63%)
2. Intraperitoneal P[a]	14	11 (79%)	2 (14%)	1 (7%)	—
3. Intramuscular P[a]	9	5 (56%)	2 (22%)	2 (22%)	—
4. 32% Dextran 70[a]	9	5 (56%)	2 (22%)	2 (22%)	—

[a]$P < 0.001$ for groups 2, 3, and 4 when compared with the control group.

In addition, serum P levels were measured with a radioimmunoassay technique.[8-10] Representative samples were obtained from control animals and from both P-treated groups via intracardiac aspiration. Serum levels were measured in group 2 24 and 48 hours after after instillation of aqueous P into the abdominal cavity and measured after 8 or 15 days of daily IM injection of aqueous P in group 3.

Analysis of variance and Student's *t*-test were employed for statistical testing of the data.

RESULTS

All animals survived. The adhesion scores are summarized in Table 1. The animals in the control group uniformly demonstrated high-grade adhesions, 100% having severe grade 3 or moderate grade 2 adhesions. Adhesion formation in all treatment groups was significantly reduced ($P < 0.001$) when compared with the control group. In particular, the fewest adhesions were seen in group 2 animals, which received IP aqueous P. Seventy-nine percent had no adhesions, 14% had mild grade 1 adhesions, in only one animal were moderate grade 2 adhesions found. Adhesion formation in group 3 animals, which received IM aqueous P and in group 4 animals, which received 32% dextran 70, were similar. However, comparison of the three treatment groups revealed no statistically significant difference.

As noted previously, the animals were reexplored 1 or 2 weeks after the initial procedure, and the adhesions were scored. Comparison of the severity of adhesion formation as indicated by the average adhesion score and the number of animals in which adhesions formed within each group showed no statistically significant difference between animals reoperated upon at 1-week or 2-week intervals (Table 2).

Serum P levels are shown in Table 3. The average serum level in six control animals was 1.5 ng/ml. The levels in both P-treated groups were three to four times higher. Normal serum P levels in the guinea pig range from undetectable levels at the time of ovulation to a peak level of 2.8 ng/ml 5 days after ovulation.[11]

DISCUSSION

In this study, aqueous P was found to be effective in preventing and reducing the severity of postoperative adhesion formation. Extended IM administration of aqueous P over a 1-week or 2-week period did not further improve adhesion scores (Table 2). A one-time IP instillation of aqueous P was found to be just as effective and represents a more attractive route of utilization in terms of ease of administration and less discomfort when compared with receiving an injection every day.

Large doses of aqueous P were utilized, especially when one considers dosage in terms of a milligram-per-kilogram ratio (see Materials and Methods). The use of progestins, however, even in large concentrations, has not been associated with serious side effects.[12, 13] In addition, when considering the doses used in the present study, serum P levels were only minimally elevated. Measured levels were 6.2 ng/ml and 5.1 ng/ml in animals receiving IP and IM aqueous P, respectively. These values represent approximately a 2-fold increase over a peak value of 2.8 ng/ml in the nonpregnant guinea pig and are over 50-fold

Table 2. *Summary of Adhesion Formation in Relation to Postoperative Time Interval*

Group	Operative interval	Animals operated upon	Average adhesion score[a]	Animals forming adhesions
	days			
1. Control	7	5	2.8 ± 0.20	5
	14	3	2.3 ± 0.33	3
2. Intraperitoneal P	7	7	0.29 ± 0.18	2
	14	7	0.29 ± 0.29	1
3. Intramuscular P	7	4	0.75 ± 0.48	2
	14	5	0.60 ± 0.40	2
4. 32% Dextran 70	7	5	0.60 ± 0.40	2
	14	4	0.75 ± 0.48	2

[a]Values are mean ± standard error.

Table 3. *Serum Progesterone Levels*[a]

	Control	Intraperitoneal P[b]		Intramuscular P[c]	
		24 hours	48 hours	8 days	15 days
No. of samples	6	5	5	5	3
Average serum levels (ng/ml)	1.5 ± 0.19	6.0 ± 0.19	6.2 ± 0.40	5.1 ± 0.97	5.0 ± 0.47

[a]Values are mean ± standard error.
[b]Hours after instillation of 50 mg into the abdominal cavity.
[c]3.3 mg/kg body weight given for 8 or 15 consecutive days.

less than peak values, which approach 330 ng/ml in the pregnant guinea pig.

The properties of P have already been discussed. Yet a proposal concerning its mechanism of action in preventing adhesion formation is of interest. The series of events leading to peritoneal adhesions begins when peritoneal injury initiates an inflammatory reaction and injured mesothelial cells release vasoactive substances such as histamine. The increase in vascular permeability promotes an exudation of protein-rich material containing fibrinogen, which is rapidly converted to fibrin. The accumulation of this fibrin-laden exudate causes fibrinous adhesions to form; and in time fibroblasts deposit collagen, which is organized into fibrous adhesions. P reduces vascular permeability[7] and thus may reduce histamine release and the subsequent accumulation of fibrin. It causes increased degradation of protein and helps involute granulation tissue,[7] which may retard later fibrous adhesion development. Its immunosuppressive properties, in particular, the inhibition of human MLC, the inhibition of leukocyte migration, and the suppression of humoral antibody responses may also contribute to reducing adhesion formation.

32% Dextran 70 is currently one of the more common substances used for adhesion prophylaxis. As noted in direct comparison of the groups of animals treated with IP 32% dextran 70 or IP aqueous P, a larger percentage of animals failed to develop adhesions with the utilization of aqueous P. This trend was not statistically significant but could become more apparent in a larger-based study. In addition, although 32% dextran 70 has been reported to be effective in reducing adhesion reformation,[14] it has not been shown to be effective in preventing the reformation of adhesions after surgical lysis.[14] This is a critical area in which we are currently conducting a study evaluating the effect of aqueous P on adhesion reformation after surgical lysis.

Clinically, aqueous P could be used as an intraoperative irrigant in a concentration of 50 mg/ml. P, as has been demonstrated, has a role in the prevention of adhesion formation associated with pelvic surgery, in particular, microscopic tubal and ovarian surgery.

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