FERTILITY AND STERILITY
Vol. 42, No. 4, October 1984
Printed in U.S.A.

Evaluation of progestogens for postoperative adhesion prevention*

Pedro J. Beauchamp, M.D.†
Martin M. Quigley, M.D.‡
Berel Held, M.D.

Department of Obstetrics, Gynecology and Reproductive Sciences, The University of Texas Health Science Center, Houston, Texas

Progesterone (P) has been shown to have potent antiinflammatory and immunosuppressive properties. Previous reports have suggested that the use of P decreases postoperative adhesion formation. To further evaluate the role of pharmacologic doses of progestogens in adhesion prevention, 42 mature New Zealand White rabbits underwent standardized injuries to the uterine horns, fimbriae, and pelvic peritoneum and received one of six treatments. Group S had intraperitoneal placement of normal saline (0.9%); group H received intraperitoneal placement of 32% dextran 70; group IM-P received intramuscular P-in-oil 10 days before and after laparotomy in addition to intraperitoneal saline; group IP-P had intraperitoneal placement of an aqueous P suspension; group DP received medroxyprogesterone acetate intraperitoneally; and group C received no intramuscular or intraperitoneal adhesion-prevention agents. The animals were sacrificed 6 weeks after laparotomy, and the adhesions were scored. Intraperitoneal saline (group S) significantly reduced the amount of adhesions when compared with the control group (C) ($P < 0.05$). No significant difference was observed when group S was compared with group H. Intramuscular P added to saline (group IM-P) did not cause further reduction in adhesions when compared with group S. Both group IP-P and group DP had more adhesions than did group S ($P < 0.01$). These data fail to support previous claims regarding adhesion prevention by the use of locally or parenterally administered progestogens. Fertil Steril 42:538, 1984

The development of adhesions following pelvic surgery is one of the leading causes of infertility and failure of reconstructive tubal surgery.[1-4] Adhesions usually result from trauma to tissue, accompanied by an inflammatory reaction, exudation of fibrinogen, fibroblastic proliferation, and organization of the fibrin matrix into scar tissue.[4, 5] Both blood (fibrin) and peritoneal trauma are necessary elements for adhesion formation, because neither independently causes adhesions.[6, 7]

The pharmacologic basis for adhesion prophylaxis includes agents that decrease the inflammatory reaction, prevent blood coagulation and fibrin deposition, inhibit fibroblastic proliferation, promote fibrinolysis, or mechanically separate the injured tissues. Multiple studies comparing the clinical efficacy of these agents have been

Received April 27, 1984; revised and accepted June 19, 1984.

*Presented in part at the Fortieth Annual Meeting of The American Fertility Society, April 2 to 7, 1984, New Orleans, Louisiana.

†Reprint requests: Pedro J. Beauchamp, M.D., Department of Obstetrics, Gynecology and Reproductive Sciences, 6431 Fannin, Suite 3270, Houston, Texas 77030.

‡Present address: The Cleveland Clinic Foundation, Cleveland, Ohio.

reviewed recently.[3] The available studies do not clearly establish the efficacy of any one class of agents, method, or regimen.

Progesterone (P) has been shown to have potent antiinflammatory and immunosuppressive properties.[8-11] Siiteri et al.[8] studied the inflammatory and foreign tissue reaction in rats induced by subcutaneous placement of Silastic implants wrapped with cotton thread or hamster skin. Animals in which the Silastic implants did not contain P exhibited marked inflammatory reaction with granuloma and adhesion formation, compared with those animals with P-containing implants. Systemic administration of P did not prevent this inflammatory reaction. Local P therapy also prevented rejection of transplanted hamster skin for up to 35 days, as opposed to systemic or no therapy, with which the graft did not survive beyond 10 days.[8, 10]

P has also been shown to enhance survival of xenogeneic tumor cells implanted in hamster uteri.[12] Rejection occurred promptly after cessation of P therapy. Similarly, Clemens et al.[13] demonstrated a marked lymphocytic invasion of the rat placenta within 4 hours after oophorectomy, which can be prevented by local administration of P. Inhibition of lymphocyte migration,[14] T-cell activation,[15] lymphocyte deoxyribonucleic acid synthesis,[16] and humoral antibody production[17, 18] characterize the significant immunosuppressive properties of P. P decreases local vascular permeability and transudation volume.[9] It does not appear to influence fibroblastic proliferation or collagen degradation.

Maurer and Bonaventura[19] evaluated the efficacy of high doses of intraperitoneal (IP) and intramuscular (IM) aqueous P in preventing surgically induced adhesions in the guinea pig. Adhesion formation was significantly reduced in the P-treated animals compared with control animals. The reduction in adhesion formation achieved with P was comparable to that achieved with 32% dextran 70. On the other hand, Holtz and co-workers[18] could not demonstrate decreased adhesion formation in rabbits treated with IM medroxyprogesterone acetate (MPA) beginning 2 days prior to surgery and continued daily for a total of six doses. Reduced adhesion formation has also been reported in monkeys undergoing wedge resection of the ovaries containing a corpus luteum,[18] where, presumably, a high local tissue concentration of P may have exerted a beneficial effect.

This study was designed to evaluate the effect of local and systemic aqueous P and local MPA on postoperative adhesion formation. Injectable MPA (Depo-Provera, The Upjohn Company, Kalamazoo, MI) also contains polyethylene glycol 4000, which has been claimed to reduce surgically induced adhesions.[20] IP saline[3, 21] and 32% dextran 70[22-24] have been shown to reduce adhesion formation, and were used for comparative purposes.

MATERIALS AND METHODS

Forty-two adult, virgin, female, New Zealand White rabbits weighing 2.5 to 3.5 kg were used. The animals were individually caged to avoid pseudopregnancy. All the animals received standard care and were kept on a 14-hour light/10-hour dark cycle. Anesthesia was induced with a 0.5 ml/kg IM injection of a solution containing equal parts of ketamine (900 mg/ml, Parke-Davis, Morris Plains, NJ), xylazine (180 mg/ml, Haver-Lockhart Laboratories, Shawnee, KS), and acepromazine maleate (30 mg/ml, Fort Dodge Laboratory, Fort Dodge, IA); and maintained with 2% halothane by mask with an oxygen flow of 2 l/minute. The abdomen was shaved, prepared with povidone-iodine and alcohol, and draped in a sterile fashion; and laparotomy was performed through a lower midline incision.

Six standard injuries were made to induce pelvic adhesions: (1) abrasion and scraping the left fimbria with a scalpel blade until punctate bleeding was observed; (2) abrasion and scraping of a 1.5-cm length of the serosa of the left uterine horn with a blade until punctate bleeding and a cyanotic appearance were observed; (3) a full-thickness 1.5-cm long longitudinal incision in the antimesenteric border of the right uterine horn to expose the endometrium; (4) a crushing injury of the right fimbria performed with a hemostat until punctate bleeding and tissue disruption were noted; and 1.5-cm lacerations of the (5) right and (6) left gutter peritoneum (Fig. 1). The pelvic organs and sites of injuries were copiously irrigated with sterile saline solution to remove any blood clots and debris. Meticulous hemostasis was achieved with bipolar coagulation prior to abdominal closure. The abdominal incision was closed in three layers with a running suture of 3-0 chromic catgut. All animals received 0.5 ml Flo-cillin (Bristol Laboratories, Syracuse, NY; containing 75,000 U each of benzathine penicillin G and procaine pen-

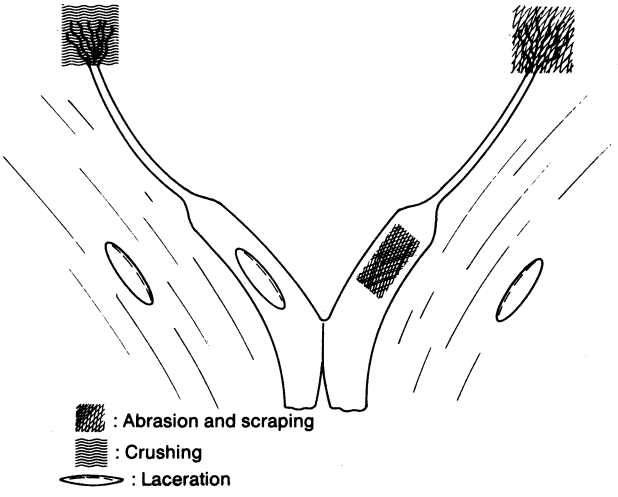

Figure 1
Diagram showing standard injury site and type.

icillin G) as one IM dose after the operation. All the surgical procedures were performed by the same surgeon (P. J. B.) in an identical fashion.

Preoperatively, the animals were divided into six groups of seven animals each. Group S received 20 ml of sterile normal saline (0.9%) solution IP prior to abdominal wall closure. Group H received 10 ml of 32% dextran 70 (Hyskon, Pharmacia, Piscataway, NJ) IP. Group IP-P received 500 mg of P powder suspended in 10 ml of sterile normal saline (0.9%) solution IP. Group DP received 500 mg of MPA IP (100 mg/ml; total volume, 5 ml). Group IM-P received 10 mg of P-in-oil (Eli Lilly and Company, Indianapolis, IN) IM daily for 10 days before and after the laparotomy, as well as 20 ml normal saline solution IP as in group S. Group C received no IM or IP medication and served as a control group. In this group, peritoneal fluid and saline irrigation were completely aspirated prior to abdominal wall closure. In all other groups, IP fluid was not aspirated; and saline or medication-containing solution was instilled immediately prior to peritoneal closure.

The animals were sacrificed 6 weeks after laparotomy. The adhesions were scored by an observer (M. M. Q.) with no prior knowledge of the treatment regimen given to any particular animal.

The following point system classification was utilized: 0, absence of adhesions; 1, mild, filmy, avascular adhesions; 2, moderate, thick, avascular adhesions; 3, severe, dense, vascular adhesions. Each injury site was scored separately; and the total adhesion score for each animal was determined by adding the scores for each of the six sites (minimum score, 0; maximum score, 18).

In three animals each from the IP-P and IM-P groups, blood was collected from the ear vein prior to the operation, as well as 6, 12, and 24 hours after treatment. The serum samples were subsequently analyzed for P in a radioimmunoassay with no cross-reactivity with MPA.

Statistical analysis of results was accomplished with Wilcoxon's rank sum test for independent (unpaired) samples.

RESULTS

All postoperative courses were uncomplicated except for one animal from group DP that died without explanation 9 days after the operation. That animal's carcass was destroyed before an autopsy could be performed.

The adhesion scores are summarized in Table 1. Each group had seven animals scored for adhesions except group DP, in which six animals were scored. IP saline (group S) significantly reduced the number of adhesions, compared with the number in the control group ($P < 0.05$). No significant difference in adhesion scores was observed among group S, group H, and group IM-P. The numbers of adhesions formed in group IP-P and group DP were similar to the number in the control group but significantly higher than those of group S, group H, and group IM-P (Table 2).

All animals in group DP had a marked vascular engorgement evident throughout the mesentery of the uterus, fallopian tubes, and intestines. In addition, deposits of a white material were present throughout the pelvis.

Preoperative serum P levels were undetectable in all the animals tested. Peak circulating P levels measured 6 to 24 hours after IP or IM injection

Table 1. *Summary of Adhesion Scores in the Control and Treatment Groups*

Group	Therapy	Adhesion scores[a]
C	None	4, 6, 7, 8, 9, 9, 11
S	20 ml normal saline IP	0, 0, 2, 4, 4, 5, 7
H	10 ml 32% dextran 70 IP	2, 3, 4, 4, 7, 8, 9
IM-P	10 mg P-in-oil IM for 20 days plus 20 ml saline IP	1, 2, 5, 6, 6, 8, 9
IP-P	500 mg P suspension in 10 ml saline IP	8, 8, 9, 9, 11, 12, 13
DP	500 mg MPA (5 ml) IP	7, 9, 9, 9, 10, 10

[a]Each value represents the total adhesion score for each animal. Minimum score, 0; maximum score, 18.

Table 2. *Statistical Comparison of the Six Treatment Groups*

	Treatment groups					
	C	S	H	IM-P	IP-P	DP
C	—					
S	$P < 0.05$	—				
H	$P < 0.05$	NS[a]	—			
IM-P	$P < 0.05$	NS	NS	—		
IP-P	NS	$P < 0.01$	$P < 0.01$	$P < 0.01$	—	
DP	NS	$P < 0.01$	$P < 0.01$	$P < 0.01$	NS	—

[a]NS, not significant.

ranged from 25 to 60 ng/ml. Normal pregnancy P values in rabbits range from 15 to 30 ng/ml.[25]

DISCUSSION

The most effective way to reduce postoperative adhesion formation is to minimize surgical trauma. In addition, adjuvant measures may assist in minimizing this complication.[3]

This study demonstrated a significant reduction in postoperative adhesions in the saline group and 32% dextran 70 group, compared with the control group. The "flotation effect" observed with IP fluids prevents tissue apposition and keeps raw surfaces separated. Furthermore, dextran (and possibly saline) covers the peritoneal surfaces and provides a fine, protective coat, thus hindering adhesion formation. IP fluids could have diluted or washed away local fibrin and clotting factors attached to the injured tissue. In the absence of clotted blood and a fibrinous matrix, tissue repair by fibroblastic proliferation from adjacent tissues might have been limited, resulting in less scarring and adhesion formation. The relative pelvic dryness in the control group may have contributed to increased adhesion formation. Therefore, we recommend not aspirating all the peritoneal and irrigant fluid at the completion of pelvic surgery, but, instead, instilling an amount of fluid in the pelvis prior to peritoneal closure.

The type of fluid used IP seems to be of secondary importance. Our studies show comparable adhesion scores in the saline and dextran groups. Contrary to previous reports,[23, 24] this study did not demonstrate improved adhesion prophylaxis with dextran, compared with saline. Furthermore, the total adhesion score in the saline group (22 points) was lower than that of the dextran group (37 points, no statistical difference). Electrolyte imbalance due to rapid IP fluid shift, infection, wound dehiscence, and anaphylactic reactions have been reported in patients following the use of IP dextran.[3, 21, 24] If further studies confirm our findings, the potential risks associated with the use of IP dextran may not be justified.

This study was designed to evaluate the use of progestational agents for the prevention of postoperative adhesion formation. Preoperative and postoperative IM P therapy, when added to IP instillation of saline, did not cause further reduction in adhesion scores. In spite of circulating P values two to four times higher than the levels observed in rabbits during pregnancy, perioperative systemic P therapy does not appear to further reduce adhesion formation. Similar results were obtained by Siiteri et al.[8] and Holtz and co-authors.[18]

IP placement of an aqueous P suspension (group IP-P) did not decrease adhesion formation when compared with adhesion formation in the control group. Adhesion scores in group IP-P were significantly higher than those of group S, group H, and group IM-P. Our data fail to support previous claims regarding reduced adhesion formation by local P.[8, 19]

P powder is insoluble in normal saline, partly soluble in oil, and completely soluble in alcohol and other organic solvents. We chose not to dissolve the P powder in an oily or alcohol solution because of the potential local irritative effect and foreign body reaction but, instead, an aqueous P suspension was utilized. In spite of the insolubility of P in saline, serum P levels obtained 6 to 24 hours after IP placement reassured us that the medication was readily absorbed from the peritoneal surface and thus was locally available to the injured tissues.

The poor results obtained with IP P were not due to an insufficient dosage. The IP dose administered corresponds to 10 gm of P for a 60-kg human female. P absorption from the peritoneal surface resulted in circulating levels two to four times higher than those observed during rabbit gestation.

The animals that received IP instillation of an aqueous suspension of P had increased tissue irritation, inflammation, and adhesion formation, compared with the saline-treated animals, perhaps due to undissolved P powder in the saline suspension.

The rationale for the IP use of MPA was that progestational agents and polyethylene glycol 4000 both have been claimed to reduce adhesion formation.[19, 20] In our study, IP MPA resulted in increased adhesion scores and increased vascular

engorgement in the pelvis. The local irritant effect of Depo-Provera was evident, especially in the areas surrounding the deposits of white material. The commercial preparation of Depo-Provera contains, in addition to MPA and polyethylene glycol 4000, many components and preservatives which may have been responsible for these effects.

The increased adhesion scores in this study, compared with previous studies, may have been due to the considerably more severe injuries which were inflicted in our animals, compared with those of the previous reports.[8, 19]

In summary, our results demonstrate that IP fluids (saline or 32% dextran 70) significantly reduce adhesion formation when compared with the control group. No difference in adhesion scores between the saline and the 32% dextran 70 groups could be demonstrated. Perioperative IM P did not cause further reduction in adhesion formation when compared with IP saline or dextran. Both IP P and MPA groups had more postoperative adhesions than the saline and 32% dextran 70 groups.

REFERENCES

1. diZerega GS, Hodgen GD: Prevention of postoperative tubal adhesions: comparative study of commonly used agents. Am J Obstet Gynecol 136:173, 1980
2. Ellis H: The cause and prevention of postoperative intraperitoneal adhesions. Surg Gynecol Obstet 133:497, 1971
3. Pfeffer WH: Adjuvants in tubal surgery. Fertil Steril 33:245, 1980
4. Holtz G: Prevention of postoperative adhesions. J Reprod Med 24:141, 1980
5. Levinson C, Swolin K: Postoperative adhesions: etiology, prevention, and therapy. Clin Obstet Gynecol 23:1213, 1980
6. Ryan GB, Grobety J, Majno G: Postoperative peritoneal adhesions. Am J Pathol 65:117, 1971
7. Nisell H, Larsson B: Role of blood and fibrinogen in development of intraperitoneal adhesions in rats. Fertil Steril 30:470, 1978
8. Siiteri PK, Febres F, Clemens LE, Chang RJ, Gondos B, Stites D: Progesterone and maintenance of pregnancy: is progesterone nature's immuno-suppressant? Ann NY Acad Sci 286:384, 1977
9. Nakagawa H, Min KR, Nanjo K, Tsurufuji S: Anti-inflammatory action of progesterone on carrageenin-induced inflammation in rats. Jpn J Pharmacol 29:509, 1979
10. Stites DP, Siiteri PK: Steroids as immuno-suppressants in pregnancy. Immunol Rev 75:117, 1983
11. Turcotte JG, Haines RF, Brody GL, Meyer TJ, Schwartz SA: Immuno-suppression with medroxyprogesterone acetate. Transplantation 6:248, 1968
12. Moriyama I, Sugawa T: Progesterone facilitates implantation of xenogeneic cultured cells in hamster uterus. Nature (New Biol) 236:150, 1972
13. Clemens LE, Contopoulos AN, Ortiz S, Stites DP, Siiteri PK: Inhibition of progesterone (P) of leucocyte migration in vivo and in vitro. In Abstracts of the Fifty-Ninth Annual Meeting of the Endocrine Society, June 8 to 10, 1977, Chicago, Illinois. Abstract 237, p 175
14. Contopoulos AN, Clemens LE, Ortiz S, Stites DP, Siiteri PK: Inhibition of leucocyte migration by progesterone in vivo and in vitro. Soc Gynecol Invest 8:110, 1977
15. Mori T, Kobayashi H, Nishimoto H, Suzuki A, Nishimura T, Mori T: Inhibitory effect of progesterone and 20 alpha-hydroxypregn-4-en-3-one on the phytohemagglutinin-induced transformation of human lymphocytes. Am J Obstet Gynecol 127:151, 1977
16. Clemens LE, Siiteri PK, Stites DP: Mechanism of immunosuppression of progesterone on maternal lymphocyte activation during pregnancy. J Immunol 122:1978, 1979
17. Hulka JF, Mohr K, Lieberman MW: Effect of synthetic progestational agents on allograft rejection and circulating antibody production. Endocrinology 77:897, 1965
18. Holtz G, Neff M, Mathur S, Perry LC: Effect of medroxyprogesterone acetate on peritoneal adhesion formation. Fertil Steril 40:542, 1983
19. Maurer JH, Bonaventura LM: The effect of aqueous progesterone on operative adhesion formation. Fertil Steril 39:485, 1983
20. Punnonen R, Viinamäki O: Polyethylene glycol 4000 in the prevention of peritoneal adhesions. Fertil Steril 38:491, 1982
21. Gomel V: Recent advances in surgical correction of tubal disease producing infertility. Curr Probl Obstet Gynecol 1:1, 1978
22. Neuwirth RS, Khalaf SM: Effect of thirty-two percent dextran 70 on peritoneal adhesion formation. Am J Obstet Gynecol 121:420, 1975
23. Rosenberg SM, Board JA: High-molecular weight dextran in human infertility surgery. Am J Obstet Gynecol 148: 380, 1984
24. Utian WH, Goldfarb JM, Starks GC: Role of dextran 70 in microtubal surgery. Fertil Steril 31:79, 1979
25. Kendall JZ, Liggins GC: The effect of dexamethasone on pregnancy in the rabbit. J Reprod Fertil 29:409, 1972