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Long-term Colchicine Prophylaxis on Operative Adhesion Formation in Embryo Transfer Donor Ewes and the Cytogenetic Evalution of Therapy

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Embryo Transfer Donor Ewe에 생기는 수술상의 Adhesion 형성에 대한 장기간의 Colchicine 치료와 그에 따른 세포유전학적 분석

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요 약

30마리의 암양에게 surgical embryo collection과정을 통해 oviduct와 uterine horn에 trauma를 생성시켰다. 수술시 절개부위를 봉합하기 직전 노출된 abdominal tissue에 irrigigant로 10% dexamethasone을 사용하였다. 처리군에게는 17mg의 colchicine(1ml/ewe)를 투여했으며, 대조군에게는 1.0ml의 placebo를 처리하였다. 처음 17mg/im의 colchicine를 처리한 15마리 양은 colchicine독성증세를 2~5일 내에 보였기 때문에 본 연구에서 제외되었다. 17mg에서의 colchicine독성 때문에 colchicine수준은 8, 4 그리고 2mg으로 낮추어졌다. 8mg group에 있던 또 하나의 양은 5일째에 독성증세를 보였기 때문에 나머지 양들은 4와 2mg의 수준으로 이틀에 한번씩 처리되었다. 두번째 laparotomy는 첫번째 처리로부터 9주후에 실시되었다. 두번째 laparotomy후 처리군은 4mg의 colchicine을 이틀에 한번씩 14일 동안 처리되었는데 아무런 독성증세를 나타내지 않았다. 세번째 laparotomy는 마지막 처리 5주후에 실시되었고 adhesion score로 계산하였다. Adhesion grading은 0~4의 분포에 근거하여, 4는 가장 심한 adhesion을 나타낸다. 두번째 laparotomy 결과 adhesion grading(≥ 3)은 두 group 사이에 큰 차이가 없었다($P>0.05$). 세번째 laparotomy결과는 처리군에서 약간 낮은 수치를 보였지만, 통계적으로 두 group간에는 큰 차이는 없었다($P>0.05$). 10마리의 양(5마리는 대조구, 5마리는 처리구)들은 처리후 5일경에 bone marrow analysis를 통해 cytogenetically분석되었다. 두 group간 염색체수와 구조에 있어서 차이가 없었다($p>0.05$).

Key Words : Colchicine, Adhesion, Embryo, Ewe, Chromosome

I. INTRODUCTION

Peritoneal adhesions are the consequence of an inflammatory reaction and a healing process following irritation or trauma to the peritoneum during surgery(Grosfeld et al., 1973). Many studies aimed at preventing and managing peritoneal adhesion have been done by animal and medical researchers. Minimizing or reducing postoperative adhesion formation requires the use of surgical techniques that reduce the effects of

factors associated with adhesion formation (Holtz, 1984). Microsurgical techniques and antiadhesive agents have been shown to be effective in reducing the severity of postoperative adhesions(Utian et al., 1979 ; Pfeffer, 1980).

Minimal manipulation of tissue is essential to reducing surgical trauma and its associated inflammatory processes(Holtz, 1984). However, even with the best procedures, a certain amount of tissue irritation and trauma occur with surgery(Goldberg et al., 1980 ; Larsson et al., 1977). Therefore, the administration of several different types of prophylactic agents have been used to inhibit the inflammatory process, thus affecting the degree of adhesion formation(Holtz, 1984).

Peritoneal adhesion formation/reformation limits the repeatability of embryo transfer in small domestic livestock. Adhesion development commonly limits the number of surgical embryo collections in donor sheep and goats to 3~5 collections (personal observation).

The first objective of this study was to determine the dosage of colchicine that could be safely administered to the ewe. The second objective was to determine the efficacy of long-term prophylaxis of colchicine in reducing adhesion formation following embryo transfer collection procedures in the ewe. The third objective was to determine whether colchicine treatment causes abnormal chromosomal numbers or chromatid breaks. The Western white-faced Targhee-type ewe was used in this study as an animal model.

Ⅱ. MATERIALS AND METHODS

Development of adhesions

Thirty ewes weighing an average of 150 lbs were divided into two groups for surgery. Ewes were subjected to surgical embryo transfer twice with their oviducts and uterine horns mechanically manipulated to collect embryos. Under general anesthesia using steril procedures, the abdominal wall was clipped and covered with povidone-iodine(Betadine). The reproductive tract was exteriorized by midline laparotomy and 20~30ml of Dulbecco's Phosphate Buffered Saline(PBS) supplemented with 1g glucose/L, 0.036g Na pyruvate/L and 10% Fetal Calf Serum (FCS) was retroflushed from the tip of the uterine horn through the oviduct. Medium was forced through the utero-tubal junction and collected via a cannula inserted 2~3cm into the lumen of the ampullae. Both uterine horns were flushed with media. One-half of the ewes were randomly selected for treatment and the other half served as controls. The reproductive tract was not traumatized beyond that which is encountered in routine embryo-recovery procedures.

The reproductive tract of all ewes undergoing surgery was rinsed with 10% Dexamethasone. The abdominal incision was closed with monofilament nylon sutures in two layers. All surgery was performed by the same operators.

Colchicine administration

Following the first surgery the treatment groups received 17mg/im colchicine [suspended in 1ml physiological saline(PSS)] and the control groups were administered a 1.0ml(PSS/im) placebo. The treatment group were to receive the same daily level of colchicine during the entire treatment period(14 days)unless they developed clinical symptoms of colchicine toxicity. Then the level of colchicine would be adjusted based upon the tolerance of the animals.

The ewes were placed into two groups for surgery. Surgery was performed on the second group approximately 24 hr after the first group. Ewes were examined for number and degree of adhesions following two surgical procedures and

two treatments with colchicine. In Group (8 treatment and 7 control), all eight ewes treated with 17 mg colchicine developed hemorrhagic entero-colitis and typhlitis within 48 hr and had to be removed from the study. They received two treatments of colchicine prior to toxicity. The treatment for Group II (7 treatment and 8 control) was altered based upon the severity of colchicine toxicity in Group 1. The treatment ewes (4 ewes/group) were placed on 8, 4 and 2mg colchicine for the remainder of the treatment period. The lower levels were chosen because of the severe acute toxicity at the initial high level of colchicine treatment. Treaments consisted of daily injections of colchicine. One ewe in the 8mg group showed colchicine toxicity by day 5 of treatment and was removed from the study. The remaining ewes in the 8mg group were randomly placed in the 4 (n=6) and 2mg (n=5)groups. Ewes were than administered colchicine every other day to day 14 postsurgery.

After a safe level of colchicine administration was determined, then the final treatment groups consisted of two groups of three animals each treated at 4 and 2 mg, respectively. Following the second surgery, the two treatment groups were combined and colchicine was administered at 4mg every day for 14 days. This treatment was performed with 13(5 treatment and 8 control) of 15 ewes remaining on the study. Two ewes were excluded from the study because they were found to be pregnant at the time of second surgery.

Evaluation of adhesions

At the second(1st surgery evaluation)and third(2nd surgery evaluation)laparotomy, photographs were taken, and the degree and extent of adhesions within the region of the oviduct and uterine horn were recorded according to the following classification(Granat et al, 1983) : grade 0, no adhesions ; grade 1, thin, filamentous, easily separated adhesions ; grade 2, thick adhesions in a limited area ; grade 3, widespread thick adhesion ; grade 4, grade three plus adhesions to abdominal wall.

Culturing of bone marrow

To determine whether colchicine treatment causes abnormal chromosomal number or morphology, five treatment and five control ewes were screened for cytogenetic aberrations. Cytogenetic analysis was performed after the second surgery, five days following the last treatment with colchicine. Bone marrow was taken by sternal puncture and drawn into a syringe containing 0.15ml sodium heparin(20,000 IU/ml). Two to 2.5ml of bone marrow was aspirated from each ewe. The method used to culture bone marrow was a modification of the procedure described by Priest(1977)

Evaluation of chromosomes

Individual chromosome spreads were observed at 1000×. Slides from ten ewes were evaluated for chromosomal numeric and structural changes. The number of spreads evaluted per ewe varied between 10 and 25

Analysis of data

The Fisher's exact test for equality of two binomial proportions was utilized to test for significance of differences between groups as to the prevalence of ewes with three or four grade adhesions, and student's t-test was employed for statistical testing of mean scores from each laparotomy and of numeric expression according to surgical period. Chi-square analysis was used for the analysis of the cytogenetic data.

Table 1. Summary of Adhesion Grading at Second Laparotomy

Group	Number of Adhesions	Adhesion gradings[a]			
		1	2	3	4
Colchicine(n=3)	6	2(33%)	4(67%)	0	0
Control(n=8)	16	3(19%)	12(75%)	0	1(6%)

[a]Grade 1, grade 2, grade 3, grade 4, especially grades 3 and 4 represent severe adhesions($P>0.05$)

Table 2. Summay of Adhesion Mean Scores[a] from Second Laparotomy

Group	Grading of Adhesions	No.of Adhesions	Numeric[b] Expression
Colchicine (n=3)	1.67	2.0	3.33
Control(n=8)	1.94[c]	2.0	3.88

[a] $P>0.05$ for all column means.

[b] Numeric expression, number of adhesions times grading.

[c] 1.94, based upon n=4

Ⅲ. RESULTS

A safe level of colchicine treatment was selected at 4mg/ewe/day after the first treatment period. Levels of 17 mg/ewe/day resulted in acute toxicity. Ewes exhibiting acute toxicity were removed from the study. Three other ewes that where initially placed on the study were also removed for other reasons. At second surgery, two of the ewes were pregnant and one was removed because she exhibited numerous abdominal adhesions at first laparotomy. All remaining sheep were observed six days after first treatment for external wound healing. The process of wound healing was similar in both colchicine-treated and control ewes.

Ewes(n=16) that developed clinical symptoms of acute colchicine toxicity were necropsied. All exhibited hemorrhagic entro-colits and typhlitis. Ewes(n=8) that received their first dosage at 17mg colchicine and lowered to either 8, 4 or 2 mg and then developed acute colchicine toxicity had a hemorragic abomasitis.

The results of colchicine treatment from second laparotomy are shown in Table 1 and 2. Table 1 shows the percentage of adhesion gradings of each group.

Among a total of six adhesions in the colchicine group, 33% had mild grade 1 adhesions and four were moderate grade 2 adhesions. In the control group, one adhesion among sixteen was grade 4 and fifteen adhesions were grade 1 or 2 Four of the eight control ewes did not have any adhesions while all in the treatment group had adhesions.

However, both groups were not different significantly($P>0.05$). Table 2 shows the mean values of adhesion scores at the second surgery. Adhesion grading in the colchicine group showed a slightly, but nonsignificant, lower score than the control group.

As previously noted, the animals were retreated after second laparotomy with a level of 4 mg colchicine for 14 days. During this treatment, there were no symptoms of colchicine toxicity(i. e. gastroenteritis, muscular weakenss and collapse).

The third laparotomy was performed by the same operators five weeks after final treatment of cochicine. As shown in Table 3, summation of the data on both number, grading of adhesions and numeric expression showed less adhesion formation in the colchicine group, as compared with the control group. However, comparison of two groups revealed no statistically significant

Table 3. Summary of Adhesion Mean Scores[a] from Third Laparotomy

Group	Grading of Adhesions	No. of Adhesions	Numeric[b] Expression
Colchicine (n=3)	1.45	3.67	5.33
Control(n=8)	1.72	5.57	9.88

[a] P>0.05 for all collumn. means.

[b] Numeric expression, number of adhesions times grading.

difference(P>0.05).

Gradings of 3 and 4 are indicative of marked adhesion formation. In the control group, 5% of the adhesions fell in that category and involved five of eight ewes. The colchicine group did not have any 3 and 4 graded adhesions. A total of 11 adhesions were observed in the colchicine group and are mainly distributed in mild grade 1 and moderate grade 2 categories(Table 4).

Table 5 incorporates the data from Tables 2 and 3 to show the cumulative colchicine effect based upon numeric expression and surgical periods. The colchicine treated animals showed a

Table 4. Summary of Adhesion Grading at Third Laparotomy

Group	Number of Adhesions	Adhesion Gradings[a] 1	2	3	4
Colchicine(n=3)	11	6(55%)	5(45%)	0	0
Control(n=8)	46	21(46%)	18(39%)	6(13%)	1(2%)

[a] Grade 1, grade 2, grade 3, grade 4, especially grades 3 and 4 represent severe adhesions(P>0.05).

Table 5. Comparison of Numeric Expression According to Surgical Period

Treatment	Surgical Period	No. of Animals	Numeric[a] Expression	Difference
Colchicine	1	15	0	
	2	3	3.33	3.33
	3	3	5.33	2.00
Control	1	15	0	
	2	8	3.88	3.88
	3	8	9.88	6.00

[a] Numeric expression, number of adhesions times grading.

Table 6. Results of Chromosome Evaluations from Bone Marrow Cultures 5 Days Post-Treatment

Group[a]	No. of Spreads	Chromosome No.(2n) 48	49	50	51	52	53	54	55	56	Chromatid Breaks S[b]	D[c]
Cochicine	50	1	0	0	1	0	0	48	0	0	0	0
Control	56	1	1	1	1	1	0	51	0	0	0	0

[a] Five ewes were sampled in each group.

[b] S, single.

[c] D, double.

difference of 2.00 between the two surgeries while untreated animals showed differences of 6.00. Due to the small number of animals in the treated group and large number of variability in the untreated group, the difference of numeric expression between two groups was not statistically significant($P>0.05$)

There was no correlation between length of time the reproductive tract was exteriorized and the ease of flushing the oviduct on the incidence of severity adhesion formation.

Table 6 shows the results of day 5 post-treatment effects of colchicine on chromosome number and morphology. In the colchicine group, 96% of the mitoses had normal diploid numbers of 54 and 91% in the control group. The statistical comparison between groups was not significant($P>0.05$). There was no difference in the incidence of altered chromosome number and structure between the colchicine treated ewes and the controls.

Ⅳ. DISCUSSION

Very few studies have been conducted using colchicine as a prophylactic agent in treating adhesion formation. The reports of Shapiro et al.(1982) and Granat et al.(1983) demonstrated a significant reduction of adhesion formation from colchicine administration. In the study of Shapiro et al.(1982) 2ml colchicine in a concentration of 0.2mg/g body weight saline was administered daily for three weeks postsurgery to rats.

Granat and associates(1983) reported that rats were treated with 50 μg/g body weight of colchicine every day for 14 days after surgery.

On the basis of these studies, 17 mg colchicine was chosen to treat adhesion development in sheep weighing an average 150lbs. This level, however, resulted in acute colchicine toxicity. To reduce the risk of colchicine poisoning, dosages were lowered. To further avoid potential additive effects of daily administration of colchicine during the first treatment period, colchicine was administered on an every other day basis. The two week treatment period used in this study was based on the data of Granat et al. (1983)

The high level treatment with colchicine resulted in hemorrhagic entero-colitis and typhlitis. All sheep that were initially treated with 17mg colchicine showed a loss of epithelial cells of the intestinal mucosa and exhibited mitotic figures in the remnant epithelial cells. A well developed array of microtubules is normally centered around the centrioles in the mast cells. The microtubules were completely lost upon exposure to high concentrations of colchicine(Lagunoff and Chi, 1976). Also, when a cell enters mitosis under the influence of colchicine, the spindle fails to form (Lagunoff and Chi, 1976). Chromosome division is then temporarily arrested at metaphase, a phenomenon which was observed in the remnant epithelial cells. During the second treatment period, 4 mg colchicine was administered per day for 14 days and did not induce toxicity. Colchicine administered intramuscularly at the 4 mg level was a safe dosage and although there were some numeric expression reduction, the differences were not statistically significant. The results might be considered to be consistent with the reports of Shapiro et al.(1982) and Granat et al.(1983) in reducing severity of adhesion formation in the treated group.

Cytogenetic analysis of colchicine therapy indicated that long-term treatment did not cause an increase in either numerical or structural chromosome abnormalities. No apparent differences were discernible between the colchicine treated ewes and the controls. These results

suggest that continuous long-term colchicine treatment does not induce chromosomal irregularities. Cohen and his associates(1977) performed a cytogenetic evaluation of long-term colchicine therapy in FMF(Familial Mediterranean Fever) patients. There were no adverse effects with respect to fertility, teratogenicity, and chromosomal aberrations. The present study showed similar results in terms of chromosomal aberrations.

The results of this study show that colchicine can be safely administered to sheep at levels of 4mg /lb of body weight but is toxic at levels of 8mg or higher. One of four ewes treated at the 8mg level showed acute toxicity during first treatment. However, none of the ewes showed toxicity at the 4mg level during the second treatment period. A higher level (>4, <8), if nontoxic, might be more effective for adhesion therapy than the 4 mg level. It might be possible that 6 mg could be administered as a safe level either daily or every other day for 14days. The optimal level of colchicine administration and its efficacy as a prophylactic agent warrants further investigation.

V. ABSTRACT

Thirty ewes received typical trauma to their oviducts and uterine horns from surgical embryo collection procedures. Ten percent Dexamethasone was used as an irrigant on the exposed abdominal tissue prior to closing the incision. The treatment group received 17mg colchicine (1ml /ewe) and the control group was administered a 1.0ml placebo(PSS). Fifteen ewes that were initially treated with 17mg /im colchicine showed acute colchicine toxicity within 2~5 days after initial treatment and were removed from the study. Due to acute colchicine toxicity at 17mg, the colchicine level was lowered to 8, 4 and 2mg(4 ewes /group). Treatments consisted of daily injections of colchicine. One ewe in the 8mg group developed toxicity on day 5. Therefore, ewes were then administered colchicine every other day from day 6 to day 14 postsurgery at 4 and 2 mg. the second laparotomy was performed 9 weeks after first treatment. Following second laparotomy, the treatment group(n=5) received 4 mg colchicine every day for 14 days and there was no clinical symptoms of colchicine toxicity. The third laparotomy was performed by the same operators 5 weeks after final treatment and the adhesions scored. Adhesion grading was based on a scale of 0-4, with 4 being the most severe. The results of adhesion grading(> 3) at second laparotomy were not significantly different($P>0.05$)between the two groups. Adhesion formation observed at third laparotomy showed a reduced, but not significant reduction ($P>0.05$) in the colchicine-treated ewes when compared with the controls. Ten ewes(5 control and 5 treatment)were examined cytogenetically by bone marrow analysis five days post-treatment. There was no difference($P>0.05$)in the incidence of numerical or structural aberrations between the two groups.

(Key words : Colchicine, Adhesion, Embryo, Ewe, Chromosome)

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